NEURAL REGULATION OF RHYTHMIC GROWTH HORMONE SECRETION IN THE RAT BY SOMATOSTATIN AND CATECHOLAMINES

by

© L. CASS TERRY

Submitted in partial fulfillment of the requirements for the Degree

DOCTOR OF PHILOSOPHY

Supervised by Joseph B. Martin, M.D., Ph.D.

Department of Medicine

Division of Experimental Medicine

McGill University

Montreal, Quebec, Canada

Table of Contents

ABSTRACT

The functions of somatostatin, norepinephrine and epinephrine in regulation of rhythmic growth hormone secretion were evaluated in male Sprague-Dawley rats. Three experimental strategies were used: (1) the chronic cannulation model to assess the effects of stress, lateral hypothalamic stimulation, monosodium glutamate, and pharmacologic agents on growth hormone dynamics, (2) biochemical mapping of central somatostatinergic pathways involved in growth hormone regulation, and (3) an *in vitro* perifusion system to study somatostatin release.

It is shown that exfctatory and inhibitory neural inputs must be intact to maintain normal rhythmic growth hormone secretion. Growth hormone rises are generated by hypothalamic neurons that liberate a growth hormone releasing factor. These neurons are activated by adrenergic (and probably noradrenergic) inputs. The periventricular and amygdalofugal somatostatinergic systems control ebbs in plasma growth hormone and stress- or lateral hypothalamic stimulation-induced growth hormone suppression. Catecholaminergic regulation of somatostatin release is not defined clearly.

Resume

Les fonctions de la somatostatine, de la norépinéphrine et de l'épinéphrine dans la régulation de la sécrétion rythmique de l'hormone de croissance ont été étudiées sur des rats mâles de Sprague-Dawley. Trois approaches expérimentales ont été développées: 1) à l'aide d'une cannule chronique: études des effets du stress, de la stimulation de l'hypothalamus latéral, du monosodium glutamate et d'agents pharmacologiques sur la dynamique de l'hormone de croissance, 2) localisation biochimique des faisceaux centraux somatostatinergiques impliqués dans la régulation de l'hormone de croissance, et 3) étude de la sécrétion de somatostatine par périfusion in vitro.

Les résultats montrent que l'intégrité des afférences nerveuses excitatrices et inhibitrices est essentielle au maintien de la sécretion rythmique normale de l'hormone de croissance. L'accroissement de l'hormone de croissance est déclenché par des neurones hypothalamiques libérant un facteur de relâche ("releasing factor") pour l'hormone de croissance. Ces neurones sont activés par des afférences adrénergiques (et probablement noradrénergiques). Les systèmes somatostatinergiques périventriculaires amygdalofuges contrôlent la diminution plasmatique de l'hormone de croissance et la supression de l'induction par le stress obtenu par stimulation de l'hypothalamus latéral. La régulation catécholaminergiques de la production de somatostatine n'est pas clairement définie.

ACKNOWLEDGEMENTS

Dr. Joseph B. Martin is acknowledged for his observations and concepts that led to many of the experiments described herein. These studies would not have been possible without support from grants awarded to him by the Medical Research Council of Canada. His guidance, encouragement and ambition resulted in a unique environment that promoted intellectual honesty and independence.

Dr. William R. Crowley and I collaborated in the biochemical mapping of somatostatinergic systems and studies on the role of central adrenergic systems in growth hormone regulation. Dr. Crowley taught me the microdissection technique and performed the radioenzymatic assays of tissue catecholamines. Dr. John O. Willoughby collaborated with me in studies describing the effects of stress on growth hormone secretion. Dr. Otto Rorstad taught me the affinity chromatography technique and performed somatostatin bioassays. Ms. Adah Saunders showed me the cannulation technique and conducted the experiments involving the effects of pregnancy, parturition and suckling on growth hormone. I enjoyed working with these people and gratefully acknowledge their contributions to these studies.

Dr. Albert Aguayo is especially acknowledged for providing my first opportunity in neurobiologic research. Donald Baxter, Leo Renaud, Garth Bray, Gloria Tannenbaum and Jacques Epelbaum are recognized for their support and friendship. Dr. Epelbaum performed the somatostatin radioimmunoassays in the monosodium glutamate studies. Paul Brazeau supervised many of the

radioimmunoassays of prolactin and somatostatin. Yogesh Patel provided one of the somatostatin antisera used in the stress study. Norman White taught me the principles and methods of intracranial self-stimulation.

Special acknowledgement is given to John Whitaker, Sol Solomon and Andrew Kang. They provided a superb intellectual environment and critical reviews of several experiments and manuscripts. Through their support and guidance, I was able to continue these studies at the University of Tennessee. Seymour Reichlin provided encouragement and a critique of the experiments involving epinephrine synthesis inhibitors.

Without the technical help of Judy Audet, Wendy Gurd and Gayle Faille, in Dr. Martin's laboratory, and Georgia Householder and Lee Brinkley, in my laboratory, this work would still be in progress. Special thanks to Cecil Lynch and Chuck Longserre in my laboratory for their sincere dedication and countless hours of radioimmunoassay and sampling. Cecil Lynch wrote the computer programs that saved several hours in data reduction.

Excellent secretarial help was contributed by S. Harrington, A. D'Amato, I. Shestowsky, S. Ellis, L. Figgins, T. Hampton, A. Bobrowski, B. Chandler, and my daughter, Kristin.

Mr. Artinian, E. Sweezy and P. McDougall provided excellent photographic and artistic work. I thank Dr. Martin for his gift of somatostatin antiserum, Dr. Parlow and the National Pituitary Agency for pituitary hormone radioimmunoassay kits, and R. Pendleton of Smith, Kline and French and R. Fuller of Lilly Research Laboratories for their gifts of norepinephrine-N-methyltransferase inhibitors.

These studies were supported by grants from the Medical Research Council of Canada, National Institutes of Health, Veterans Administration and the University of Tennessee.

Special acknowledgement is given to Corinne Johnson at the Ann Arbor Veterans Administration Medical Center for typing this thesis.

Finally, I wish to acknowledge Suzanne Terry for love and understanding. Her constant encouragement and strong sense of responsibility helped to put this thesis in the state of completion.

This entire thesis is a compilation of manuscripts published in refereed journals (see pp. 237-239) over the last seven years. Articles in which Dr. Martin was not an author resulted from experiments in the candidate's laboratory where he was the principal investigator funded by grants from the Veterans Administration and National Institutes of Health.

SECTION I.

INTRODUCTION AND BACKGROUND

There is compelling evidence to support the hypothesis that rhythmic growth hormone secretion is regulated by discrete neuronal structures and systems. These systems have both stimulatory and inhibitory actions on growth hormone secretion. Their effects are thought to be mediated by the release of hypothalamic hormones from nerve endings into the capillaries of the hypothalamic-adenohypophyseal portal circulation thereby regulating the synthesis and secretion of growth hormone by pituitary somatotropes. This control is achieved by at least two hypothalamic hormones (neuropeptides or factors), somatotropin release inhibiting factor (somatostatin, SRIF), a tetradecapeptide that inhibits growth hormone release (Brazeau et al., 1973), and growth hormone releasing factor (GRF), the structure of which is unknown. Secretion of these hypothalamic hormones is, in turn, believed to be regulated (modulated) by second order neuronal systems that project to or originate within the hypothalamus and release aminergic neurotransmitters.

The following pages review experimental observations supporting the above concepts and establish the background upon which the hypotheses in this dissertation were developed and tested.

A. PITUITARY GROWTH HORMONE

Human growth hormone is a single-chain polypeptide (molecular weight approx. 21,500) containing 190 amino acid residues (Merimee, 1979). Growth hormone accounts for 4-10 percent of the wet weight of the adult human pituitary. It is secreted and synthesized by specific cells in the anterior

pituitary, the somatotropes. The majority of these cells are eosinophilic, as determined by conventional staining techniques. Histochemical and immunofluorescent methods have demonstrated that these cells are distinct from those that synthesize other pituitary tropic hormones. Somatotropes are best recognized by electron microscopy. The typical somatotropic cell possesses numerous, large, round secretory granules that are 300-400 mu in diameter.

At the cellular level, release of growth hormone appears to involve exocytosis or fusion of secretory granules within the plasma membrane, followed by solubilization and diffusion of the granule content into the circulation. Membrane fusion must be preceded by movement of secretory granules from their site of formation, the Golgi area, to their site of exocytosis, the plasma membrane. Studies with vincristine indicate that the microtubular system is involved in both basal and induced secretion of growth hormone. The exact role of cyclic AMP and cyclic GMP is not as yet clarified in this process.

Growth hormone circulates unbound in the plasma. The half-life of disappearance is between 17 and 45 minutes and the estimated rate of secretion is approximately 17 μg/hour or 400 μg/day (Martin, 1977). Growth hormone does not act on a specific target organ, but exerts effects at many different sites. It has both an insulin-like and anti-insulin effect on glucose metabolism, a powerful anabolic action promoting enhanced incorporation of amino acids into protein, and lipolytic effects (Merimee, 1979). However, the insulin-like activity may be a pharmacologic effect. The biologic actions of growth hormone are thought to be mediated by a class of substances, somatomedins (MW 7000-9500), that are synthesized by hepatic, and

possibly renal cells. Somatomedins stimulate sulfate incorporation into cartilage (sulfation factor) and thymidine uptake in liver, and also have insulin-like effects (Martin, et al. 1977).

B. PATTERNS OF SPONTANEOUS GROWTH HORMONE SECRETION

Rhythm is defined as "nonrandom variation, especially uniform or regular variation, of any quantity or condition characterizing a process" (Websters, 1977). It denotes "regular patterned flow, the ebb and rise, of sounds and movement in natural phenomenon, speech, music, writing, dance, and other physical activities." One who is expert in, studies or produces, or has a keen sense of rhythm is defined as a rhythmist.

Divers rhythmists have shown that plasma levels of growth hormone rise and fall at frequent intervals in man and experimental animals. This pattern of secretion has been described as rhythmic, episodic, or pulsatile.

Basal levels of plasma growth hormone in resting, nonstressed human adults range from 1-5 ng/ml (Martin et al., 1977). However, sequential determinations at frequent intervals throughout the day and night show significant oscillations. Individual surges of growth hormone reach plasma levels of 20-60 ng/ml, the largest peaks usually occur during the first two hours of nocturnal sleep (Martin et al., 1977; Boyar, 1978). There is a close correlation between age and 24 hour secretory rate of growth hormone with highest levels during adolesence, followed by a decline in young adults (Finkelstein, 1972).

The metabolic basis of these physiologic oscillations in growth hormone secretion in man has been the subject of numerous investigations. Physiologic changes in plasma amino acids or free fatty acids have minimal effects on the

pattern of growth hormone secretion (Martin et al., 1977; Reichlin, 1974a,b). Hyperglycemia temporarily suppresses daytime growth hormone surges and fasting increases their frequency. However, nocturnal growth hormone secretion is not affected by either fasting nor hypoglycemia (Martin, 1976). These data indicate that sudden changes in plasma growth hormone levels are not the result of variations in metabolites in the peripheral circulation.

Similar profiles of growth hormone secretion have been documented in experimental animals. Chair-adapted rhesus monkeys have episodic secretion of growth hormone unrelated to caloric intake, stress, or blood glucose levels (Martin, 1976). Akin to humans, the baboon shows a significant elevation in plasma growth hormone during sleep (Parker et al., 1972). Episodic release of growth hormone is also observed in the unanesthetized rabbit (McIntyre and Odell, 1974).

In the rat, growth hormone secretion is characterized by high amplitude secretory episodes that reach levels greater than 800 ng/ml (Tannenbaum and Martin, 1976). The rises in growth hormone occur rapidly and terminate abruptly. The rate of decline in plasma growth hormone levels after a pulse is consistent with the half-life of the hormone. Secretory episodes of growth hormone in the male albino rat occur rhythmically every 3-4 hours and this rhythm is entrained to the light-dark cycle. Since it is not consistently affected by feeding or glucose infusions, episodic growth hormone secretion in the rat, as in man and other animals, is not primarily determined by requirements for glucose homeostasis (Tannenbaum and Martin et al., 1976).

In comparison to other hormonal systems, there is no apparent relationship between rises and ebbs of growth hormone release in the rat and other pituitary hormones such as prolactin and thyrotropin, adrenal corticosterone

(an indication of ACTH secretion), and the pancreatic hormones, glucagon and insulin (Martin, 1976; Martin et al.; 1978a). Mechanisms responsible for the generation of rhythmic growth hormone secretion are specific and independent of other hormonal "pulse generators."

The amplitude, frequency and entrainment of growth hormone secretory episodes and their independence of demands for metabolic homeostasis indicate that the rises and ebbs of growth hormone are generated and controlled primarily by neural mechanisms. This concept is supported further by the observation that anterior pituitaries incubated in vitro fail to secrete growth hormone in a similar episodic manner (Carlson et al., 1974).

C. HYPOTHALAMIC STRUCTURES: THEIR ROLE IN REGULATION OF GROWTH HORMONE SECRETION

A large number of experimental observations support the concept that specific hypothalamic structures are involved in the regulation of rhythmic growth hormone secretion (Martin et al., 1978a). Data from these experiments are derived from measurements of pituitary and plasma growth hormone levels after ablation or activation of discrete hypothalamic regions.

An understanding of intrahypothalamic connections is essential in the interpretation of hypothalamic function. The classic Golgi studies have called attention to the abundant internal connectivity of the hypothalamic nuclei. The increasing wealth of data suggest that the individual hypothalamic nuclei cannot be regarded clearly as functional entities. It has also become clear that the hypothalamus is an integral part of both the central nervous system and the neuroendocrine control mechanism.

Several thousands of neural inputs go to certain hypothalamic neurons; at least this order of magnitude of synaptic terminals can be revealed on their surface (Leranth et al., 1975). A similar number of intranuclear connections can be found organizing the cell groups as a nucleus. At the same time, the axons of the hypothalamic neurons are connected partly with other hypothalamic cells groups, and partly with extrahypothalamic brain regions, presumably by means of numerous axon collaterals.

One can conclude from electrophysiological, autoradiographic, and electron microscopic studies that almost all hypothalamic nuclei are connected with each other. Based on the pattern of axonal arborization, one can also conclude that even a single neuron can make contact with the neurons of several hypothalamic nuclei.

Nevertheless, one can distinguish between two essential functional groups within the hypothalamus. The first group is represented by connections with the medial hypothalamus including the preoptic area. The second is formed by connections between the medial and lateral hypothalamus. These two systems differ from each other in both their anatomical structure and their function. The lateral hypothalamus, which contains mainly the ascending and descending fibers of the medial forebrain bundle and its cells, represents a link between the medial hypothalamus and the central nervous system, mainly the limbic system. The different pathways not only pass through the lateral hypothalamus but also arise and terminate there. The neural information reaching the hypothalamus is at least partially relayed in the lateral hypothalamus and enters the medial hypothalamus through neurons of the lateral hypothalamus.

1. Internuclear Connections of the Hypothalamus

a. Preoptic Region

The preoptic region can be divided into medial and lateral regions. The medial one contains three nuclei: medial preoptic, suprachiasmatic, and periventricular preoptic. The lateral preoptic region corresponds to the preoptic part of the medial forebrain bundle, it contains numerous fibers of passage. Its medial part is called the lateral preoptic nucleus, its dorsal part is the substantia innominata; and its lateral most part is termed the magnocellular preoptic nucleus (Palkovits and Zaborszky, 1979).

Autoradiographic studies have revealed the medial preoptic nucleus has neural connections with practically all hypothalamic nuclei and the median eminence (Conrad and Pfaff, 1976a; Swanson, 1976). Direct connections have been shown between the medial preoptic nucleus and the arcuate and ventromedial neclei (Dyer and Cross, 1972; Halasz et al., 1975; Koves and Rehthelyi, 1976). The periventricular preoptic nucleus has similar hypothalamic connections (Conrad and Pfaff, 1976a; Swanson, 1976). The preoptic suprachiasmatic nucleus has contacts with the hypothalamic suprachiasmatic nucleus (Swanson, 1976). The efferent connections of the lateral preoptic area are of local origin only to a small extent.

Dopaminergic cells (A 14 cell group) can be found in the periventricular preoptic nucleus (Bjorklund and Nobin, 1973; Bjorklund et al., 1975). These cells are believed to innervate the preoptic region (Palkovits et al., 1977).

b. Anterior Hypothalamic Nucleus

The axons or axon collaterals of the anterior hypothalamic nucleus distribute diffusely to the hypothalamus (Conrad and Pfaff, 1976b). Axons arising from different parts of this nucleus descend to the median eminence, arcuate, ventromedial and dorsomedial nuclei.

c. Suprachiasmatic Nucleus

The regulatory role of this nucleus in the light-dark entrained rhythm of growth hormone (Martin, 1978) as well as its connection with the retina create a special interest in studying the efferent connections of this nucleus (Moore, 1978). Simple electrolytic lesion techniques cannot be used for the revelation of the efferent connections of the suprachiasmatic nucleus because of the great number of pathways within or in the immediate vicinity of the nucleus (especially the stria terminalis and medial corticohypothalamic tract). The suprachiasmatic nucleus has connections with the periventricular nucleus, median eminence, medial forebrain bundle, and ventromedial nucleus (Krieg 1932; Szentagothai et al. 1968; Swanson and Cowan, 1975a).

Neurons localized in the retrochiasmatic area are connected mainly with the arcuate nucleus and the median eminence (Szentagothai et al., 1968; Swanson and Cowan, 1975a).

d. Periventricular Nucleus

This thin nucleus is localized on both sides of the third ventricle and consists of a delicate fiber meshwork of extreme density and several layers of nerve cells. Some of the cells contain dopamine (part of the A 12 cell group) (Dahlstrom and Fuxe, 1964; Bjorklund and Nobin, 1973; Hokfelt, 1978). More importantly, they contain a heavy concentration of

somatostatin-positive cell bodies (see section I,E,1). Microlesions of this nucleus cause degeneration in the arcuate nucleus and both layers of the median eminence (Zaborszky and Makara, 1979).

e. Ventromedial Nucleus

The ventromedial nucleus, which consists of six subdivisions, is surrounded by a uniform capsule formed by the dendrites of the ventromedial neurons as well as the nerve terminals of numerous pathways (Millhouse, 1973). Fibers of the ventromedial nucleus go to the median eminence and the arcuate nucleus.

f. Arcuate Nucleus

The main projection field of the arcuate neurons is the median eminence (Szentagothai, 1964; Fuxe and Hokfelt, 1966; Szentagothai et al., 1968, Yagi and Sawaki, 1970; Makara et al., 1972; Bjorklund et al., 1973). More recent electrophysiological and anatomical studies show that the arcuate nucleus innervates the medial preoptic, suprachiasmatic, ventromedial, paraventricular, and premammillary nuclei (Harris and Sanghera, 1974; Makara et al., 1972; Makara and Hodacs, 1975; Moss et al., 1975; Renaud, 1976b, Zaborsky and Makara, 1979). It is assumed that axons arborize in several directions i.e., their axons run into the median eminence, but their axons collaterals reach different hypothalamic regions. It is known that the arcuate nucleus contains dopaminergic (Dahlstrom and Fuxe, 1964; Fuxe and Hokfelt, 1966; Hokfelt et al., 1978a) and cholinergic (Meszaros et al., 1969) cells taking part presumably in the innervation of not only the median eminence but also of certain other hypothalamic cell groups. This is

indicated by the observation that the dopamine content and choline acetyltransferase activity are unchanged in the medial hypothalamus after complete hypothalamic deafferentation (Brownstein et al., 1976b).

g. Paraventricular, Supraoptic and Dorsomedial Nuclei

The neurosecretory connections of the magnocellular part of the paraventricular nucleus with the pituitary have long been known and discharge of neurohypophyseal peptides into the portal circulation have been described by several investigators. The projections of the pars parvocellularis are still unknown. Relatively little is known about the efferent connections of the supraoptic nucleus except for the supraopticohypophyseal tract. Similarly, only a few data are known about the intrahypothalamic connections of the dorsomedial nucleus.

h. Neural Interconnections Between the Medial and Lateral Hypothalamus

In addition to the ascending and descending fibers of the medial forebrain bundle, the lateral hypothalamus contains a large number of neurons. The perikarya probably form a relay system between the medial hypothalamus and other brain areas. The axons of neurons localized in the medial forebrain bundle proceed in the medial forebrain bundle proper. Their collaterals may also reach the medial hypothalamic nuclei. Conversely, efferent fibers of the medial basal hypothalamus may terminate on cells in the medial forebrain bundle, which connect it with the other regions of the central nervous system. Axons arising from the medial basal hypothalamus (especially from the anterior hypothalamic and ventromedial nuclei) terminate on cells in the medial forebrain bundle.

A significant portion of the fibers ascending from the brainstem pass through the medial forebrain bundle. Terminal degeneration can be observed in all hypothalamic nuclei after damage of these fibers (Zaborszky and Palkovits,

1978). Consequently, a lesion of the medial forebrain bundle proper (Morin, 1950; Guillery, 1957; Eager et al., 1971) cannot give information regarding the existance of fibers connecting the medial forebrain bundle with the medial hypothalamus.

2. Fiber Connections of the Median Eminence

The fine structure of the median eminence is well known. According to their origin, the fibers can be divided into intrahypothalamic and extrahypothalamic groups. Some of the fibers terminate in the pituitary, others in the median eminence itself. These later can be of two kinds: those terminating on the portal vessels and those displaying axo-axonal contacts. The existence of the axo-axonal contacts is supported only by rather circumstantial evidence.

a. Intrahypothalamic Connections

Axons enter the median eminence, arising from the hypothalamic nuclei, especially from neurons of the medial basal hypothalamus. Their presence has been established by several methods (Palkovits and Zaborszky, 1979). Most of the axons that enter the median eminence are of intrahypothalamic origin (Halasz et al., 1962; Szentagothai, 1964; Szentagothai et al., 1968; Rethelyi and Halasz, 1970; Makara et al., 1972), however, many extrahypothalamic brain regions also project directly into the median eminence.

Neurochemical micromethods have revealed dozens of neurotransmitters, enzymes, hormones and other chemical substances (Palkovits, 1977b) which are probably localized either in the capillaries or in the axons, since the median eminence contains a negligible number of cells. One cannot exclude that further substances will be identified in the median eminence. The verification of such pathways will be imperative to revise current views on the physiological role of the median eminence.

Before elucidation of the hypothalamic dopaminergic cells (A 12 groups) projecting into the median eminence, axons arising outside the magnocellular neclei were termed parvicellular neurosecretory fibers (Szentagothai, 1964). The application of fluorescence techniques permitted a distinction between dopaminergic and nondopaminergic neurons.

Currently, the intrahypothalamic connections can be divided into peptidergic and aminergic fibers.

i. Peptidergic fibers

The majority of parvicellular fibers that do not contain monoamines are classified as peptidergic. Most of these fibers are of medial hypothalamic origin (Halasz et al., 1962; Szentagothai et al., 1968; Rethelyi and Halasz, 1970). As determined by Golgi impregnations, these axons arise mostly from the arcuate nucleus, the ventral part of the periventricular nucleus, and the cells of the retrochiasmatic area (Szentagothai, 1964; Szentagothai et al., 1968). The axons originating in the arcuate nucleus can be found in both layers of the median eminence by electron microscopy (Zaborszky and Makara, 1979). Golgi impregnations and light microscopic studies following electrolytic lesions have not provided convincing proof as

to whether or not the ventromedial nucleus projects to the median eminence. This connection is assumed on the basis of electron microscopic observations (Zaborszky and Makara, 1979); however, it was not confirmed by autoradiography (Saper et al., 1976b). Contrary to the light and electronmicroscopic observations following lesions (Rethelyi and Halasz, 1970; Halasz et al., 1975; Koritsanszky and Koves, 1976), autoradiographic studies revealed axons projecting into the median eminence from other hypothalamic nuclei (preoptic medial, anterior hypothalamic, and suprachiasmatic nuclei) (Swanson and Cowan, 1975a; Conrad and Pfaff, 1976a,b; Swanson 1976).

The axons of the cells localized in the medial basal hypothalamus terminate in the external layer of the median eminence; therefore, these cells are termed tuberoinfindibular neurons. The neurons are connected not only with the median eminence but also with preoptic and other hypothalamic regions, presumably by axon collaterals (Makara et al., 1972; Harris and Sanghera, 1974; Makara and Hodacs, 1975). These areas, in turn, send fibers to the median eminence and arcuate nucleus, as shown by autoradiography. Tuberoinfindibular cells project to the outer, lateral part of the external layer, where they come into close contact with the capillaries of the primary portal plexus (Szentagothai, 1964).

The chemical identification of the axons terminating in the median eminence has not been carried out unequivocally. Some of the tuberoinfindibular cells are dopaminergic; others are cholinergic (Meszaros et al., 1969). However, they represent only a low percentage of the neurons in the arcuate nucleus.

The localization of somatostatin within the central nervous system is reviewed in Section III, B, 1.

ii. Aminergic fibers

Biochemical methods have shown that the median eminence contains a large amount of dopamine (Palkovits et al., 1974a) and significant amounts of other biogenic amines (Palkovits et al., 1974a; Saavedra et al., 1974; Brownstein et al., 1976b; VanderGugten et al., 1976). Most of them are from afferent connections of the hypothalamus (extrahypothalamic). However, the major portion of dopamine derives from the dopaminergic cells of the hypothalamus.

The dopaminergic fibers terminating in the median eminence arise from the cells of the arcuate and periventricular nuclei (Fuxe, 1965; Fuxe and Hokfelt, 1966; Bjorklund et al., 1970, 1973, 1974; Jonsson et al., 1972b; Smith and Fink, 1972; Bjorklund and Nobin, 1973; Ajika and Hokfelt, 1973, 1975). Biochemical and electron microscopic studies (Kizer et al., 1976b; Palkovits et al., 1977b) also suggest that axons or axon collaterals of the A9 and A10 dopaminergic cell groups of the mesencephalon, may project to the median eminence. Using fluorescence microscopy, Bjorklund et al. (1973) showed that the rostral and central parts of the arcuate nucleus send their axons into the pituitary, whereas the caudal part, being close to the infindibulum, sends terminals to the median eminence.

Most of the monoamine-containing axons terminate in the external layer of the median eminence, especially close below the surface in its lateral aspect. They comprise about one-third of all axons in this region. Norepinephrine - containing fibers are observed in both the external and

internal layers (Bjorklund et al., 1970, 1973, 1974). However, it is thought that most of them terminate in the internal layer (Fuxe, 1965, Jonsson et al., 1972b; Swanson and Hartman 1975).

A detailed description of the norepinephrine- and epinephrine-containing fibers that project to hypothalamus and median eminence is provided in Section I,F,1,b,c.

b. Extrahypothalamic Connections

Most of the extrahypothalamic axons of the median eminence are of aminergic nature (Palkovits and Zaborszky, 1979). With this exception, relatively little is known regarding the extrahypothalamic cells projecting into the median eminence. As determined by autoradiographic studies, several fibers of the nucleus interstitialis striae terminalis reach the median eminence (Conrad and Pfaff, 1976a).

3. Structure-Function Relationships

Lesions of the median eminence and mediobasal hypothalamus suppress hypoglycemia- (Abrams et al., 1966) and stress-induced (Brown et al., 1971) growth hormone release in the monkey. Lesions of the ventromedial nucleus cause a fall in plasma and pituitary growth hormone levels in young female rats (Frohman and Bernardis, 1968; Frohman et al., 1972); whereas, lesions outside the ventromedial arcuate nuclei do not cause growth hormone deficiency (Martin, 1976). In adult male rats, electrolytic lesions in the ventromedial nucleus also block episodic growth hormone release (Martin, 1976).

In contrast, small, discrete lesions of hypothalamic periventricular nuclei increase growth hormone levels (trunk blood) for up to two weeks postoperatively; and inhibit stress-induced growth hormone suppression in male

albino rats (Critchlow et al., 1981). This effect is most likely caused by ablation of somatostatinergic pathways that originate in the periventricular nuclei and project to the mediobasal hypothalamus (see Section I,E, 1 and III, B,1). Data from these experiments favor this hypothesis, since somatostatin levels in the median eminence were reduced by 83 percent (Critchlow et al., 1981).

Growth hormone release can be induced by stimulation of the ventromedial nucleus in unanesthetized rabbits (McIntyre and Odell, 1974). Either unilateral (Frohman et al., 1968) or bilateral (Martin, 1972, 1976) activation of the ventromedial-arcutate region with square wave pulses also results in growth hormone release in pentobarbital-anesthetized rats. However, growth hormone release occurs after cessation of the stimulus, possibly as a postinhibitory rebound effect. Similar postinhibitory rebound surges have been described after hypothalamic stimulation in the sheep (Malven, 1974) and canine (Martin et al., 1978a).

Stimulation sites effective in releasing growth hormone are confined to the ventromedial-arcuate nuclei. Stimulation of the lateral or anterior hypothalamus (Frohman et al., 1972, Martin, 1972), supraoptic or paraventricular nuclei, mammillary bodies, or locus ceruleus has no effect on plasma growth hormone levels (Martin 1972, Cheng et al., 1972, Terry, unpublished observations). Electrical stimulation of the anterior or posterior hypothalamus (excluding the ventromedial nucleus) of the cat has no effect on growth hormone either (Kokka et al., 1972b).

Although the frequency, ebbs and light-entrainment of the growth hormone secretory rhythm are altered in rats with complete hypothalamic

deafferentation, periodic rises of plasma growth hormone persist (Willoughby et al., 1977). These data suggest a neural mechanism for episodic release of a growth hormone releasing factor exists within the mediobasal hypothalamus, and that the mediobasal hypothalamus does not require afferent hypothalamic connections for the release of such a stimulus.

Clinical studies support the importance of particular hypothalamic areas in growth hormone regulation. Hypothalamic destruction and pituitary stalk section result in reduced plasma growth hormone levels in man and in suppression of responses to insulin-induced hypoglycemia (Martin, 1976, 1978, b; Reichlin 1974b; Brown and Reichlin, 1972, Muller, 1973). Hypothalamic lesions also partially inhibit sleep-associated growth hormone release (Krieger and Glick, 1974).

Taken together, these data indicate that specific neural systems within the hypothalamus are responsible for the regulation of rhythmic growth hormone secretion. Furthermore, there appears to be a dual regulatory system for generation of growth hormone bursts (the ventromedial-arcuate nuclei) and ebbs (the hypothalamic periventricular nucleus) with entrainment to the light-dark cycle mediated through retino-suprachiasmatico-hypothalamic connections.

D. AFFERENT CONNECTIONS OF THE HYPOTHALAMUS AND GROWTH HORMONE REGULATION

Afferent connections of the hypothalamus were first implicated in pituitary growth hormone regulation by Elefteriou et al., (1969) who reported that lesions of the amygdala in the deermouse increase pituitary growth hormone and hypothalamic growth hormone releasing activity. Lesions of the amygdala and pyriform cortex also reduce plasma growth hormone levels in rats (Newman et al., 1967).

Electral stimulation of the amygdala, hippocampus, and ventral mesencephalic tegmentum influence growth hormone secretion in the rat (Martin, 1972). Furthermore, hypothalamic deafferentation studies, in addition to confirming the importance of the mediobasal hypothalamus in growth hormone secretion, show that the frequency and ebbs in plasma growth hormone are altered with loss of light-dark entrainment (Willoughby et al., 1977). Anterior and posterior deafferentation have no apparent effect on the normal 3 hour frequency of growth hormone rises, but light-dark entrainment is extinguished by anterior deafferentation.

Afferent fibers enter the hypothalamic nuclei from numerous brain regions. In many cases, the exact site of origin of the fibers is not known, nor their site of termination. Numerous pathways passing through the hypothalamus further complicate the study of these pathways. The afferent connections of the hypothalamus that have potential roles in regulation of growth hormone secretion are listed below. Ascending pathways from the brainstem to the hypothalamus are discussed in section I,F,1.

1. Corticohypothalamic Fiber Connections

a. Neocortical- and limbic cortical-hypothalamic connections

Several data indicate possible neural connections; however, there is no direct evidence proving this assumption (Palkovits and Zaborszky, 1979).

b. Olfactory

A direct link exists between the olfactory tubercle and the medial hypothalamus as indicated by degenerated boutons in the ventromedial and arcuate nuclei and in the retrochiasmatic area (Palkovits and Zaborszky,

1979). Terminal degenerations are also found in the supraoptic nucleus after a rostral forebrain section including the olfactory regions (Zaborszky et al., 1979).

c. Hippocampal connections (Palkovits and Zaborszky, 1979)

The afferent hypothalamic fibers of hippocampal origin enter the septal area dorsally through the fornix superior and fimbria hippocampi and terminate in the ventromedial, paraventricular, supraoptic, perifornical and dorsal premammillary nuclei and the mammillary body. These pathways form direct hippocampal-hypothalamic connections. Another large bundle of hippocampal efferent fibers toward the hypothalamus is the medial cortico-hypothalamic tract. The majority of these fibers terminate in the rostral aspect of the arcuate nucleus.

2. Septohypothalamic connections

Autoradiographic studies have revealed the presence of fibers of septal origin (Conrad and Pfaff, 1976c) in numerous hypothalamic nuclei (arcuate, periventricular, anterior hypothalamic, dorsal premammillary, dorsomedial, posterior hypothalamic and medial mammillary nuclei). On the other hand, Meibach and Siegel (1977), using autoradiography, could not reveal any projection from the septum toward the medial hypothalamic nuclei. Electronmicroscopic studies are required to determine whether the silver grains found in different regions indicate fibers of passage or terminals. The supraoptic nucleus has been studied by electron microscopy. According to these studies, 13 percent of the supraoptic afferents are of septal origin (Zaborszky et al., 1975).

3. Amygdalar connections

The amygdala has close neural connections with the hypothalamus. Fibers reach the hypothalamus through two major pathways: the stria terminalis and the ventral amygdalofugal pathway. The fibers connecting these two areas are the integral parts of a polysynaptic system between the neuroendocrine hypothalamus and the limbic system.

a. Stria terminalis

Fibers coursing a loop-shaped pathway from the amygdala form a compact bundle only up to their entrance to the hypothalamus. In the nucleus interstitialis striae terminalis, and also in the hypothalamus proper, one can find only a fine, topographically diffuse system of stria fibers.

The nucleus interstitialis striae terminalis represents one of the largest diencephalic nuclei in rats; it is localized in the angle of the septum, the preoptic region, the globus pallidus, and the anterior hypothalamus, penetrating deeply into the preoptic region.

Neurons originating in the nucleus interstitialis striae terminalis are also connected with hypothalamic nuclei; namely, the preoptic, ventromedial, premammillary and supramammillary nuclei (Valverde, 1965; Millhouse, 1969; Turner, 1974; Conrad and Pfaff, 1976a).

The topography and nature of the stria terminalis fibers have been studied by several investigators. Considering the results of a systematic study in rats, the classification of De Olmos and Ingram (1972) seems to be logical and the simplest. These authors divide the stria terminalis into three components, namely dorsal, ventral, and commissural.

The dorsal component runs through the anterior hypothalamic nucleus and terminates around the ventromedial neucleus. A direct neural connection between the amygdala and the ventromedial nucleus has been described by both degeneration (Ban and Omukai, 1959; Lundberg, 1960; Hall, 1963; Knook, 1965; Ishikawa et al., 1969; Heimer and Nauta, 1969; De Olmos and Ingram, 1972) and electrophysiological studies (Gloor, 1955, 1960; Gloor et al., 1969). Electron microscopic studies reveal (Raisman, 1970; Field, 1972) that the nerve terminals of strial origin end on dendritic spines of neurons in the ventromedial nucleus. A few axons also end in the arcuate nucleus (Palkovits and Zaborszky, 1979).

Accounts of the distribution of the ventral stria terminalis fibers offer varied statements derived from studies carried out in several species with numerous methods. These data need to be verified with electron microscopic studies. The data agree that fibers terminate in the nucleus striae terminalis (Ishikawa et al., 1969; De Olmos and Ingram, 1972), the preoptic region (Adey and Meyer, 1952; Nauta 1956, 1961; Morgan 1958; Valverde, 1965; Ishikawa et al., 1969; De Olmos and Ingram, 1972), the anterior hypothalamic nucleus (Adey and Meyer, 1952; Nauta, 1956, 1961; Knook, 1965; Valverde, 1965; Morgan, 1968; De Olmos and Ingram, 1972), and the ventromedial nucleus (Adey and Meyer, 1972; De Olmos and Ingram, 1972).

The fibers running in different components of the stria terminalis arise from various cell groups of the amygdala. Fibers reaching the hypothalamus originate from the cortical and medial amygdalar nuclei. The fibers arising from the caudal third of those nuclei proceed within the dorsal component,

whereas those originating in the rostral two-thirds of amygdala enter the ventral component (De Olmos and Ingram, 1972). The other amygdalar nuclei probably have projections through other structures or none at all.

It must be kept in mind that the stria terminalis also contains fibers of brainstem origin. These fibers are mostly aminergic and reach the amygdala after passing through the lateral hypothalamus (Jacobowitz, 1975).

b. Ventral amygdalofugal pathway

This pathway is less well defined. Opinions are divergent not only regarding the origin and course of the axons but also whether this pathway contains any amygdalar fibers innervating the hypothalamus. The existence of such fibers in the rat is denied (Leonard and Scott, 1971; De Olmos and Ingram, 1972; Heimer, 1975). There are data showing a direct neural connection between the central amygdalar nucleus and the lateral hypothalamus (Valverde, 1963; De Olmos, 1972). However, in contrast to earlier observations (Szentagothai et al., 1968; Millhouse, 1969), more recent investigations could not verify the existence of neural input of the medial hypothalamic nuclei from the amygdala through the ventral amygdalofugal pathway. Electrophysiological studies show the absence in rats and the presence in cats of fibers originating in the amygdala and reaching the hypothalamus via the ventral amygdalofugal pathway (Renaud, 1976a). Further studies on the amygdalofugal fibers are of importance in order to get a better understanding of the amygdalar fibers efferent to the hypothalamus.

4. Hypothalamic Afferent Fibers from the Basal Ganglia

Numerous connections between the hypothalamus and the basal ganglia have been described.

a. Nucleus Accumbens

The nucleus accumbens is a relatively large cell group in the rat, whereas it is less significant in higher animals. Although this structure has been thought to belong to the limbic system, its development and structure is close to that of the striatum. In monkey, axons arising from this nucleus have been described in the paraventricular and suprachiasmatic nuclei (Powell and Leman, 1976). In rat, a projection was found to the medial preoptic, anterior hypothalamic and dorsomedial nuclei (Conrad and Pfaff, 1976c).

b. Nucleus caudatus and putamen

There are no data indicating direct connections of the caudate and putamen with the hypothalamus (Palkovits and Zaborszky, 1979).

c. Pallidohypothalamic connections

Although authors have described direct neural connections between the globus pallidus and the hypothalamus (Bard and Rioch, 1937; Papez 1938, 1942; Ranson and Ranson, 1939, 1941; Vidal, 1940; Ranson et al., 1941; Mettler, 1945; Woodburne et al., 1946; Laursen, 1955; Johnson and Clemente, 1959), there are data contradicting those findings (Nauta and Mehler, 1966). These studies require electron microscopic verification.

d. Thalamohypothalamic connections

There is no direct electron microscopic evidence for the existence of direct neural connections between any thalamic nuclei and the hypothalamus (Palkovits and Zaborszky, 1979).

e. Subthalamus (Incertohypothalamic connections)

It is known that catecholaminergic neurons (cell group A 13) (Bjorklund and Nobin, 1973; Jacobowitz and Palkovits, 1974; Bjorklund et al.,

1975) form the incertohypothalamic dopaminergic system. Fibers from this system may terminate in the dorsomedial and paraventricular nuclei (Palkovits et al., 1977a).

5. Retinohypothalamic Connections

Connections between the retina and hypothalamus have been described in all classes of mammals (Conrad and Stumpf, 1975a). However, there are strong contraindications as regards the termination of the retinohypothalamic fibers within the hypothalamus. If rigid criteria are applied to the methods of identification of such pathways, only two hypothalamic nuclei have direct neural input from the retina, namely the suprachiasmatic nucleus and the anterior hypothalamic nucleus (Palkovits and Zaborszky, 1979). Neither have the studies been supported according to which fibers of the optic nerve might terminate in other hypothalamic nuclei (Hayhow, 1959; Hayhow et al., 1960; O'Steen and Waugham, 1968; Sousa-Pinto and Castro-Correia, 1970, Printz and Hall, 1974).

The retinohypothalamic fibers are of obvious physiological significance in the regulation of growth hormone secretion, especially light-dark entrainment of rises and ebbs (Tannenbaum and Martin, 1976; Willoughby et al., 1977).

6. Structure-Function Relationships

Electrical stimulation of the hippocampus causes growth hormone release, whereas stimulation of the amygdala elicits either a rise or fall in plasma growth hormone depending on the exact location of the electrode tip (Martin, 1974a,b). Activation of the basolateral amygdala releases growth hormone (Martin, 1974a,b). This response is blocked by bilateral lesions of ventromedial nuclei, suggesting that these effects are mediated via the

mediobasal hypothalamus. In contrast, stimulation of the corticomedial amygdalar region causes a fall in plasma growth hormone levels. A similar response occurs after medial preoptic stimulation (Martin, 1976). It is possible the amygdalar efferents course in the stria terminalis and activate the inhibitory somatostatinergic medial preoptic-periventricular system that projects to the median eminence (see section III,B,1). Moreover, it is relevant that coronal cuts through the anterior hypothalamus increase growth in the rat (Mitchell et al., 1973) and elevate plasma growth hormone levels (Collu et al., 1973). Destruction of preoptic area also blocks stress-induced growth hormone suppression in the rat (Rice and Critchlow, 1976). Taken together, these observations suggest that afferent connections of the hypothalamus have both excitatory and inhibitory effects on rhythmic growth hormone secretion. Furthermore, the retinohypothalamic system is responsible for light-dark entrainment of this rhythm.

E. HYPOTHALAMIC PEPTIDES AND GROWTH HORMONE SECRETION

1. Somatostatin

Somatostatin was discovered by its ability to inhibit the release of growth hormone from monolayer cultures of dispersed anterior pituitary cells (Brazeau et al., 1973). It was first isolated from chromatographic fractions of sheep hypothalamic extracts. Somatostatin is a cyclic tetradecapeptide with a disulfide bond between the third and fourteenth amino acids. Chemical synthesis of somatostatin (Rivier et al., 1974) led to several studies, using various experimental models, that confirmed the inhibitory effects of somatostatin on the secretion of growth hormone by the pituitary.

Somatostatin inhibits growth hormone secretion in the rat induced by electrical stimulation (Martin, 1974a), pentobarbital (Brazeau et al., 1974), morphine (Martin et al., 1975), and chlorpromazine (Kato et al., 1974). It prevents growth hormone release in response to L-dopa in man (Siler et al., 1973), baboon (Ruch et al., 1974), and dog (Lovinger et al., 1974), and growth hormone stimulated by insulin-induced hypoglycemia (Hall et al., 1973; Yen et al, 1974) and arginine infusions in man. Sleep-related growth hormone secretion is also suppressed by somatostatin (Parker et al., 1974). Somatostatin prevents thyrotropin releasing hormone-induced thyrotropin release (Siler et al., 1974), but not prolactin release. It also suppresses plasma growth hormone levels in acromegaly and diabetes.

After intravenous infusion, somatostatin has a rapid onset and a short duration of action in terms of its ability to lower plasma growth hormone levels. Growth hormone levels increase significantly after cessation of somatostatin infusions. However, such a postinhibitory rebound of growth hormone is observed only at times spontaneous surges of growth hormone should occur (Martin et al., 1978a). Administration of somatostatin during ebbs of growth hormone secretion or to rats with hypothalamic ventromedial nuclear lesions (which abolish rhythmic growth hormone release) is not followed by growth hormone rebound, presumably excluding a direct pituitary effect. In contrast, studies by Stachura (1976) using isolated rat pituitaries do show postinhibitory rises in growth hormone release after cessation of exposure to somatostatin. Taken together, these data do not resolve the issue of whether the rises and ebbs in growth hormone secretion require the presence of a releasing factor.

Although simplistic, it is possible that growth hormone rises occur as postinhibitory rebound surges resulting from episodic somatostatin release, and that a releasing factor is not a requisite. Several observations argue against this theory (Martin et al., 1978a). First, ablation or interuption of somatostatinergic systems would abolish rhythmic release and cause persistently high growth hormone levels. This is not the case. Albeit altered, episodic growth hormone secretion continues after depletion of hypothalamic somatostatin (Willoughby et al., 1977; Critchlow et al., 1981). Secondly, stimulation of the preoptic and corticomedial amygdalar nuclei, areas rich in somatostatinergic neurons and fibers, causes growth hormone inhibition without rebound. Thirdly, lesions of the ventromedial nucleus extinguish episodic growth hormone release and result in low levels (Martin et al., 1974). Fourthly, activation of specific afferent hypothalamic connections such as the hippocampus and basolateral amygdala cause a rapid rise in plasma growth hormone that is stimulus-entrained and cannot be attributed to a postinhibitory rebound effect. Finally, somatostatin infusions do not cause postinhibitory growth hormone release when administered (a) during a growth hormone ebb in normal rats, or (b) to animals with ventromedial nuclear lesions.

The availability of specific antisera against somatostatin provides another method whereby one can study the function of this tetradecapeptide in regulation of episodic growth hormone secretion. Effective neutralization of circulating somatostatin can be accomplished by passive immunization with the antiserum. This technique was used in the present studies to elucidate the role of somatostatin in growth hormone regulation under various experimental conditions (see sections III, A,1 and II,A,2).

The distribution of somatostatin has been described in several regions of the nervous system. Numerous immunohistochemical studies, including some ultrastructural studies, deal with the localization of somatostatin in the nervous system (Hokfelt et al., 1978; Elde et al., 1978; Rorstad et al., 1980). Somatostatin is also present in the circumventricular organs, in the pineal gland, and in endocrine-like cells in different tissues including pancreas, gastrointestinal tract and thyroid gland (Hokfelt et al., 1978). Consideration of the ramifications of these later observations is beyond the scope of this dissertation.

Subcellular distribution studies indicate that somatostatin in the hypothalamus, preoptic area, and amygdala is localized predominantly in synaptosomes (nerve terminals) (Terry and Martin, 1978b). The precise role of somatostatin in neuronal function is not clear (Rorstad et al., 1980). Somatostatin depresses electrical activity in neurons (Renaud et al., 1975), inhibits calcium release from synaptosomes (Tan et al., 1977), and elicits behavioral effects (Terry et al., 1978b; Rorstad et al., 1980). These properties are consistent with a function of the peptide as a synaptic neurotransmitter or modulator.

It is obvious that determination of the site of origin of somatostatin in the hypothalamus is important to the understanding of its function in regulation of rhythmic growth hormone secretion. A detailed description of somatostatin-containing perikarya, nerve terminals, and pathways is presented in sections II,B,1,2,3.

2. Peptides with Growth Hormone Releasing Activity

a. Growth Hormone Releasing Factor

There are several experimental observations that support the

existence of a growth hormone releasing factor. Evidence derived from the effects of stalk section and hypothalamic lesions indicates a predominant stimulatory influence of the hypothalamus in the generation of growth hormone secretory bursts. Crude and semipurified extracts of hypothalamus are effective in stimulating growth hormone release both in vivo and in vitro (Frohman et al., 1971; Malacara et al., 1973; Szabo and Frohman, 1975; Wilber et al., 1971; Sandow et al., 1973; Machlin et al., 1974; Peake et al., 1973). However, all of these later studies do not exclude the possibility that growth hormone releasing activity may be due to contamination with other peptides which may cause growth hormone release, but which are not believed to be the specific growth hormone releasing factor. Thus, although there is a considerable body of evidence to support the existance of a hypothalamic growth hormone releasing factor, its structure has yet to be identified.

b. Peptides with growth hormone releasing activity

The peptides that are reported to release growth hormone under several different experimental conditions are vasopressin, thyrotropin releasing hormone, luteinizing hormone releasing hormone, alpha-melanocyte stimulating hormone, substance P, neurotensin, myelin basic protein, cholera enterotoxin and opioids (enkephalins and β-endorphin) (Martin et al., 1978). Many of these observations were recorded in urethane-anesthetized rats and their interpretations are open to question. Furthermore, not all of these peptides have a direct stimulatory effect on the pituitary to cause growth hormone release. For example, morphine and opioids cause release of growth hormone in intact animals, but this effect is blocked by pretreatment with pharmacological agents that inhibit noradrenergic and/or adrenergic

neurotransmission (see section IV,B). Therefore, they appear to act on the hypothalamus or its afferent connections. The role of these peptides in the generation of growth hormone secretory bursts requires further study.

F. CATECHOLAMINERGIC SYSTEMS: THEIR ROLE IN GROWTH HORMONE REGULATION

The concentrations of catecholamines and serotonin (not reviewed in this dissertation) in the hypothalamus are higher than most other regions of the brain (Hokfelt et al., 1978; Iversen et al., 1978; Moore and Bloom, 1978, 1979), and they are thought to be the primary central monoaminergic neurotransmitters that regulate growth hormone secretion (Krulich, 1979; Lal and Martin, 1980; Martin, 1980; Muller et al., 1977; Terry 1982; Weiner and Ganong, 1978). The high levels of hypothalamic catecholamines and the proximity of catecholaminergic nerve terminals to hypothalamic peptidergic neurons and their terminals in the median eminence emphasizes the possibility that the function of these systems is to regulate the production and release of hypothalamic peptides and pituitary hormones.

To understand how the neurotransmitters can control release and inhibition of the hypothalmic hormones, it is worthwhile to review briefly their synthesis and metabolism (Cooper et al., 1978). Tyrosine is transported actively into catecholaminergic neurons and hydroxylated by tyrosine hydroxylase to L-dopa. L-dopa is then decarboxylated by a nonspecific enzyme, L-amino acid decarboxylase, to dopamine which, in turn, is hydroxylated by the enzyme dopamine-β-hydroxylase to norepinephrine. Norepinephrine can be methylated to form epinephrine by phenylethanolamine-N-methyltransferase (also called neorpinephrine-N-methyltransferase). After their synthesis, dopamine,

norepinephrine, and epinehphrine are stored in cytoplasmic granules within nerve terminals. In response to neuronal depolarization, these granules are extruded into the synaptic cleft. It is assumed that specific postsynaptic binding sites (receptors) are present on hypothalamic peptidergic neurons. It is postulated that there are two classes of adrenergic receptors on hypothalamic neurons, one corresponding to alpha-receptors, and the other to β-receptors. Dopamine receptors are also believed to exist on certain hypothalamic neurons. It is possible that some hypothalamic neurons may have more than one type of receptor. After catecholamines are released, unbound neurotransmitter in the synaptic cleft can be taken up into the presynaptic nerve ending and reincorporated back into storage granules. Catecholamines are vulnerable to destruction by monoamine oxidase and catechol-O-methyl transferase.

1. Sites of Origin of Catecholamines in the Hypothalamus and Its Afferent Connections

a. Dopamine

High concentrations of dopamine are found in the hypothalamic nuclei (Palkovits et al., 1974; Versteeg et al., 1976). A large group of dopaminergic cells is located in the arcuate and the periventricular nuclei (A 12 cell group). (Dahlstrom and Fuxe, 1964). A smaller cell group (A 14) is formed by a few cells in the periventricular preoptic nucleus (Bjorklund et al., 1973; Bjorklund and Nobin, 1973). These two dopaminergic cell groups probably innervate the other hypothalamic nuclei. The dopamine concentrations of the nuclei do not change after complete hypothalamic deafferentation (Weiner et al., 1972; Brownstein et al., 1976; Palkovits et al., 1977a).

Dopamine-containing cells are found in the subthalamus (A 13 cell group) in the medial part of the zona incerta. Their axons form the incertohypothalamic dopaminergic system (see section I,E,4,e).

A large group of dopaminergic cells is found in the mesencephalon, mostly substantia nigra and ventral tegmental area (A 9, A 10, and a portion of A 8) (Dahlstrom and Fuxe, 1964). The fibers arising from the nigrostriatal dopaminergic pathway innervate the telecephalic and diencephalic nuclei as well as cortical regions (Moore and Bloom, 1978). Lesions to this region result in a decrease of dopamine in the ventromedial nucleus and median eminence (Kizer et al., 1976b).

Dopamine-containing cells in the rostral part of the central gray matter of the midbrain (A 11 cell group) are also believed to innervate certain hypothalamic nuclei (Bjorklund et al., 1975)

b. Norepinephrine

The norepinephrine-containing cells are known to form several groups within the brainstem (Dahlstrom and Fuxe, 1964). The largest is the locus ceruleus (A 6 cell group). In the ascending pathways, the noradrenergic cells of the following areas make contributions: nucleus tractus solitarii (A 2 cell group); lateral reticular nucleus (A 1 cell group); cells between the superior olive and the intracranial protion of the facial nerve (A 5 cell group); and regions in the pontine and midbrain reticular formation (A 7 and A 8 cell groups). Axons arising from these cell groups form two ascending bundles, called the dorsal and ventral noradrenergic bundles (Moore and Bloom, 1979; Palkovits and Zaborszky, 1979).

Locus ceruleus fibers contribute to both bundles, although mainly to the dorsal one, whereas the axons of the other cell groups run mainly within the ventral bundle. Both bundles course to the lateral hypothalamus and enter the medial forebrain bundle where they can no longer be traced as individual tracts.

The norepinephrine-containing fibers innervating the medial hypothalamic nuclei reach them laterally. Rostral or caudal surgical deafferentation of the medial basal hypothalamus does not result in changes in the norepinephrine content of the nuclei in the region. However, complete deafferentation decreases hypothalamic norepinephrine (Palkovits et al., 1977a).

Based on fluorescent microscopic observations, the periventricular, medial preoptic, anterior hypothalamic, paraventricular, retrochiasmatic, dorsomedial, ventrotuberal and tuberomammillary and supraoptic regions have the most dense noradrenergic innervations. The arcuate nucleus receives only a moderate innervation (Moore and Bloom, 1979).

Outside the hypothalamus, the central amygdala, hippocampus and septum receive an extensive input of noradrenergic fibers (Moore and Bloom, 1979).

c. Epinephrine

Epinephrine is not distinguishable from other catecholamines using standard fluorescence histochemical techniques and a large, conflicting body of literature on its existence and localization has arisen. With the development of sensitive and specific techniques of gas chromatography-mass spectrometry, radioenzymatic assay, and immunohistochemical localization of norepinephrine-N-methyltransferase, more accurate determinations of the regional distribution of epinephrine have been made.

The highest concentrations of epinephrine are found in the periventricular nucleus, the arcuate nucleus and the paraventricular nucleus (Moore and Bloom, 1979). Few or no epinephrine fibers are found in the median eminence or the ventromedial, preoptic or anterior hypothalamic areas. The percentage of epinephrine as a function of norepinephrine in different brain regions (hypothalamic regions are grouped) varies from 1-14 percent (Palkovits and Zaborszky, 1979). The telecephalon contains no measurable epinephrine with the exception of low concentrations in septal nuclei, the basal amygdala and rostral medial forebrain bundle.

Hypothalamic deafferentation causes a decrease in the activity of norepinephrine-N-methyl transferase, indicating the extrahypothalamic origin of hypothalamic epinephrine (Brownstein et al., 1976b). So far, norepinephrine-N-methyltransferase-positive cell bodies have been identified only in the medulla oblongata in the lateral reticular nucleus (A 1 cell group) and in the nucleus tractus solitarii area (A 2 cell group). Thus, these groups can be considered to be the probable origin of the hypothalamic epinephrine nerve terminals (Hökfelt et al., 1978). However, since total hypothalamic deafferentation only reduces levels by 60 percent (Brownstein, et al., 1976), more epinephrine-containing cell bodies may be undiscovered.

Immunofluoresence micrographs of consecutive sections of the anterior hypothalamic periventricular area after incubation with antiserum to norepinephrine-N-methyltransferase and somatostatin show the presence of epinephrine nerve terminals in close contact with somatostatin cell bodies, suggesting they may innervate somatostatin neurons (Hökfelt et al., 1978).

2. Potential Sites of Action

There are several potential loci at which growth hormone secretion could be influenced by catecholamines. One possibility is direct axoaxonic synapses between catecholaminergic and peptidergic nerve terminals. Secondly, direct axodendritic or axosomatic contacts between neural elements, or multisynaptic connections through monoaminergic neurons distant to the peptidergic neuron, are also possible. Thirdly, catecholamine release into the portal circulation could stimulate or inhibit directly growth hormone release or alter pituitary sensitivity to hypothalamic peptides. There is no convincing evidence to indicate that catecholamines act at the pituitary somatotrope level, either directly or in synergism with hypothalamic hormones (Martin et al., 1978b). Alternatively, catecholamines in the peripheral circulation might affect pituitary release indirectly by affecting peripheral nerve pathways communicating with the central nervous system. Finally, neurons may contain both monoamines and peptides, with the former affecting secretion of the later.

There is little direct evidence of the site of action of catecholamines in growth hormone control. Electrical stimulation studies in the rat show that agents that interfere with catecholaminergic neurotransmission do not block growth hormone release induced by stimulation of the ventromedial nucleus (Martin, 1976). In contrast, growth hormone release induced by amygdalar and hippocampal stimulation are prevented by pretreatment with alpha-methyl-para-tyrosine, an amino acid analogue that blocks catecholamine biosynthesis by inhibiting tyrosine hydroxylase (Martin, 1976). These results are consistent with the interpretation that catecholamines may function as neurotransmitters

in the relay of information from higher neural centers to hypothalamic peptidergic neurons. Such information could modulate the frequency and entrainment of growth hormone secretory episodes.

There is general agreement that the effects of catecholamines on growth hormone release are mediated by hypothalamic peptides. More recent data suggests that neurotransmitters may have a direct pituitary action. Acetylcholine receptor sites on rat and sheep anterior pituitaries have been reported (Schaeffer and Hsuch, 1980; Tolliver et al., 1981). Also, $alpha_2$-adrenergic receptors in cultures of bovine anterior pituitary cells show close correlation with ACTH secretion (Beaulac Baillargeon et al., 1980). Furthermore, low doses of gamma-aminobutyric acid inhibit prolactin release from isolated rat pituitaries (Schally et al., 1977; Enjalbert et al., 1979; Grandison and Guidotti, 1979). The physiological significance of these findings requires further study.

The majority of evidence implicating catecholamines in growth hormone regulation derives from experiments in which pharmacological agents were administered systematically or intracerebroventricularly. These data are reviewed in Sections IV, A, B.

Because the effects of central neurotransmitters on growth hormone secretion are believed to be mediated by hypothalamic peptides, the effects of central neurotransmitters on somatostatin release in vitro were also investigated (see section V).

G. FEEDBACK CONTROL OF GROWTH HORMONE SECRETION

The acute rises and ebbs in plasma growth hormone levels are most likely the result of neural effects. However, the degree of growth hormone responses to neural stimuli may be determined by circulating levels of growth hormone and/or somatomedins (see section II, B, 2). Three types of feedback control mechanisms can be postulated. The first is a long-loop mechanism whereby high levels of somatomedins, induced by elevated plasma growth hormone, act at pituitary and/or hypothalamic sites to reduce growth hormone secretion directly or by inhibition of releasing factors (or stimulation of somatostatin). The second, ultrashort-loop feedback, could occur when increased levels of hypothalamic peptides inhibit their own secretion. The third type, short-loop feedback, results from growth hormone inhibiting the release of growth hormone releasing factor and/or stimulating somatostatin release. It is known that growth hormone stimulates somatostatin release from isolated hypothalami (Sheppard et al., 1978; Patel, 1979) and that intracerebroventricular injections of growth hormone inhibit episodic growth hormone release (Tannenbaum, 1981).

H. RESEARCH PLAN

The experimental strategy described herein was devised to test the hypothesis that rhythmic growth hormone secretion is regulated by the complex interaction of specific inhibitory (somatostatin) and excitatory (growth hormone releasing factor) hypothalamic neuronal systems that are, in turn, regulated by central aminergic neurotransmitter systems. The overall objective of the research plan was to define the role of somatostatin and the catecholamines, norepinephrine and epinephrine, in regulation of episodic growth hormone secretion. The specific aims of studies designed to accomplish this objective are outlined below.

1. Selection of the animal model most suitable with respect to constancy in the amplitude, frequency and entrainment of its growth hormone secretory episodes. The chronically cannulated male albino rat was selected for experiments hereinafter because it fulfilled these criteria and allowed sequential blood sampling without disturbing the animal. The female albino rat was also studied to assess the effects of the estrus cycle, pregnancy, parturition, and suckling on episodic growth hormone. In this and subsequent studies, prolactin, thyrotropin, and/or corticosterone were measured concomitantly with plasma growth hormone in an effort to show specificity of hormonal responses to various stimuli (section II,A).

2. Determine the effect of stress on the dynamics of episodic growth hormone secretion. Previous studies showed that various types of stressful stimuli suppressed plasma growth hormone levels, however, the effects of stress on the rises and ebbs of growth hormone anxiously awaited elucidation.

Swimming stress in a constant temperature bath was selected as a model because it yielded reproducible results and reduced the number of independent variables to a minimum (section II,A).

3. Assess the effects of intracranial self-stimulation on growth hormone dynamics. These experiments were based on the hypothesis that positive reinforcing effects of lateral hypothalamic intracranial self-stimulation could be mediated by specific neural pathways, and therefore result in a growth hormone response distinct from stress. The results were compared with the effects of forced stimulation in positive- and non-positive-reinforcing sites. The growth hormone response could not be clearly distinguished from the effects of stress. These findings provided the substrate for further studies to examine the role of central catecholamines and peptides in growth hormone responses to stress and electrical stimulation of the lateral hypothalamic-medial forebrain area (section II,B).

4. Determine the role of somatostatin in rhythmic growth hormone secretion. This study was based on the hypothesis that episodic rises in plasma growth hormone are due to the intermittent release of a growth hormone releasing factor, rather than a postinhibitory rebound effect resulting from episodic somatostatin release. To accomplish this goal, circulating somatostatin was inactivated by passive immunization with antisera to somatostatin (section III,A,2).

5. Elucidate the role of somatostatin in stress- and lateral hypothalamic stimulation-induced growth hormone suppression. As above, animals were passively immunized against somatostatin and then subjected to swimming stress or electrical stimulation of the lateral hypothalamus (sections III,A,1 and II,A,2). Lateral hypothalamic stimulation was delivered during both ebbs and

rises of growth hormone to determine if the effects of such activation were dependent on the timing of delivery of the stimulation in relation to the spontaneous episodic growth hormone bursts (III,A,2).

6. Define central somatostatinergic pathways involved in the regulation of growth hormone secretion. To accomplish this objective three separate experiments were performed. The first was to define more precisely the site of origin of somatostatin in the hypothalamus by ablation of somatostatin-positive cell bodies in the anterior periventricular hypothalamic and medial-basal amygdalar nuclei (section III,B,1). The second was to observe the effects of hypophysectomy on somatostatin in discrete hypothalamic nuclei, based on the hypothesis that growth hormone may exert a feedback effect on somatostatinergic systems (section III,B,2). The third was to investigate the effects of swimming stress on somatostatin levels in discrete hypothalamic and extrahypothalamic nuclei (section III,B,3).

7. Investigate the role of the hypothalamic arcuate nucleus in the generation of spontaneous growth hormone secretory episodes. To accomplish this objective, attempts were made to selectively lesion perikarya in the arcuate nucleus with the neurotoxin monosodium glutamate. The acute and chronic effects of monosodium glutamate on growth, rhythmic growth hormone secretion, and brain somatostatin levels were investigated (section III,C).

8. Ascertain the role of central noradrenergic systems in the regulation of episodic growth hormone secretion. Previous studies showed that dopamine plays a relatively minor role in the generation of growth hormone pulses. The first objective of the present studies was to produce a selective blockade of central norepinephrine biosynthesis with the dopamine-β-hydroxylase inhibitor, FLA-63, and observe the effects of this state on growth hormone secretion. The

second aspect was to stimulate central alpha-adrenergic receptors in animals pretreated with FLA-63 in an attempt to restore pulsatile growth hormone secretion (section IV,A).

9. Search for a role of the central adrenergic system in growth hormone regulation. Previous attempts to study the role of epinephrine in growth hormone regulation produced inconclusive results primarily because specific drugs were not available. With the development of analogues that inhibit norepinephrine-N-methyltransferase, it became possible to selectively block epinephrine biosynthesis without affecting brain levels of dopamine or norepinephrine. The effects of these analogues on spontaneous, morphine-, and clonidine-stimulated growth hormone release was investigated (section IV,B).

10. Study the release of somatostatin from hypothalamic fragments in vitro. The first goal of these experiments was to determine if somatostatin could be released by membrane depolarization and whether release was calcium-dependent, properties attributed to classic neurotransmitters. The second goal was to determine the site of action of central neurotransmitters that alter growth hormone release. To this end, hypothalamic fragments were perifused with physiologic concentrations of several putative neurotransmitters in an effort to determine their effects on somatostatin release (section V).

II. DYNAMICS OF GROWTH HORMONE SECRETION: RESPONSES TO EXERCISE STRESS AND INTRACRANIAL SELF-STIMULATION

A. PHYSIOLOGIC SECRETION OF GROWTH HORMONE AND PROLACTIN IN MALE AND FEMALE RATS*

ABSTRACT

Growth hormone and prolactin are secreted episodically in man and experimental animals. To investigate physiologic mechanisms of GH and PRL secretion, a series of experiments were performed in individual, unanaesthetized male and female rats.

GH secretion in the male rat is characterized by intermittent surges that occur approximately every 3 h and are entrained to the light-dark cycle. Peaks reach 200-400 ng/ml and troughs are unmeasurable. PRL is secreted in more frequent episodes with a pattern distinct from GH.

In the female rat, GH surges occur more frequently--approximately once each hour. PRL levels are low (<15 ng/ml) except on the afternoon of pro-oestrous when they surge to levels of 100-300 ng/ml. Prolactin rises 4-6 h before delivery. Levels decline rapidly at the onset of parturition and surge with each episode of suckling in the post-partum period. Growth hormone and corticosterone rise during delivery and remain elevated for several hours after delivery. Reinstitution of suckling after removal of pups causes an

*Terry et al., 1977d.

immediate rise in PRL and GH. The PRL response is sustained for 3-4 h, whereas the GH response is brief with return to baseline within 1 h. The time courses of the two responses are clearly independent.

Stress in the male rat causes a rapid rise in PRL and suppression in GH. The PRL surge to stress is brief with return to baseline by 1 h. GH pulses are suppressed for up to 5 h after stress.

These studies indicate that separate neuroendocrine control mechanisms exist for regulation of the episodic release of GH and PRL in the rat.

INTRODUCTION

Anterior pituitary hormones are secreted intermittently, rather than continuously. Both growth hormone (GH) and prolactin (PRL) secretion are characterized by marked fluctuations in circulating levels in man and experimental animals. The undisturbed male rat secretes GH in episodic bursts with intervals between surges of 3.0-3.5 h (Tannenbaum and Martin, 1976). GH surges continue throughout the 24 h period and are entrained to the light-dark cycle. Fluctuations of PRL also occur in the male rat but a full description of such surges has not been reported. There is little available evidence of secretory profiles of GH and PRL in the female rat.

Stress in the rat results in the inhibition of GH secretion and release of PRL (Schalch and Reichlin, 1966; Takahashi et al., 1971; Kokka et al., 1972; Krulich et al., 1972; Collu et al., 1973; Brown et al., 1973; Brown and Martin, 1974). The mechanism of GH suppression and PRL elevation during stress is unknown, although it is hypothesized to be mediated by the hypothalamus. There is evidence that circulating somatostatin (SRIF) may have a role in stress-induced GH suppression in the rat (Arimura et al., 1976; Terry et al., 1976). In the present studies, longitudinal profiles of GH and PRL secretion were obtained in cannulated unanaesthetized male rats following exposure to stress. Similar methods were used to study female rats during the oestrous cycle, pregnancy, parturition and suckling to determine secretory dynamics of the two hormones.

METHODS

Male and female Charles River Sprague-Dawley rats weighing 300-350 g were prepared with indwelling intraatrial cannulae using methods previously described (Martin et al., 1974; Tannenbaum and Martin, 1976). Animals were individually adapted to sampling boxes until body weight had returned to preoperative levels. The light-dark cycle was regulated with lights on from 06:00 to 18:00 hours and animals were given free access to lab chow and tap water. Blood samples (0.4 ml) were removed every 15 min for periods of 2-6 h. In one experiment, samples were removed from male rats at 5 min intervals from 10:00 to 12:00 hours to determine more accurately the size and duration of surges of PRL occurring between regular 15 min periods of sampling. Plasma was separated, frozen and stored at -20°C until assayed. Red blood cells were resuspended in normal saline and returned to the animal at the time of removal of the next sample. Plasma GH and PRL were measured by radioimmunoassay using materials supplied by NIAMDD and results are expressed in terms of the appropriate reference preparation.

To obtain secretory profiles of GH and PRL in the basal state, samples were removed from males and from cycling females during various phases of the oestrous cycle. To assess the effects of stress in the male, baseline blood samples were obtained from 09:30 to 10:00 hours; each animal was then removed from its isolation cage, placed in a large water bath at 37°C and forced to swim for 30 min. The rat was then returned to its isolation cage and sampled

for 3.5-5.0 h. To compare the amounts of GH secreted during stress with that of undisturbed animals, the areas encompassed by single GH secretory episodes were calculated by planimetry. In order to standardize determinations of GH secreted, periods of 3.5 h were measured from the end of stress.

Pregnant rats were cannulated on days 10-14 of gestation and blood samples taken during the last week of pregnancy and during delivery of pups. Each litter was reduced to eight pups on the third post-partum day and the effects of suckling were assessed by the return of pups to the mother after removal for a period of 3-6 h. A minimum of six rats was studied in each group.

RESULTS

GH and PRL in normal males

Non-stressed, freely behaving male rats showed a normal pulsatile pattern of GH release. Plasma GH levels frequently exceeded 400 ng/ml during major secretory episodes (Fig. 1). PRL was secreted episodically with one to four bursts occurring during a 5 h sampling session, peak values occasionally reaching 70 ng/ml. Prolactin bursts were usually brief (<15 min) and approximately 60 percent coincided temporally with a GH peak although there was no regular pattern. Profiles obtained during a 5 min sampling frequency indicated that PRL fluctuates more rapidly than a 15 min sampling frequency would detect (Fig. 2). Occasionally, animals had no PRL secretory bursts during a 5 h sampling period.

GH and PRL in stressed male rats

In each rat, stress caused release of PRL and prevented pulsatile release of GH for up to 5 h (Fig. 3). The mean integrated GH level expressed as ng/ml was 105.6± 12.5 (SE) for unstressed animals compared to 7.6±1.3 for the stressed group (Table 1).

Stress caused a rapid rise in PRL to levels within 15 min after the termination of the stress. Prolactin pulsatile secretion failed to recommence during the remainder of the sampling period.

GH and PRL during the oestrous cycle

The patterns of GH secretion were similar during all phases of the oestrous cycle. Episodic surges of GH occurred with a mean inter-peak interval of 70.8±7.8 min. There were no significant differences between the frequency of amplitude of the secretory bursts in diestrous (Fig. 4a-c), pro-oestrous (Fig. 5) or oestrous (not shown) rats. The mean amplitude of the GH peaks was 73.8±6.3 ng/ml and maximal levels usually did not exceed 100 ng/ml. In some animals, secretory bursts occurred at approximately 2 h intervals (Fig. 4c). Trough values between peaks declined to less than 10 ng/ml in most rats.

Plasma PRL values showed minor fluctuations during dioestrus (Fig. 4) and metoestrus with mean values between 10 and 20 ng/ml; occasional surges reached levels of 30-35 ng/ml. There was a marked rise in PRL during the afternoon of pro-oestrus (Fig. 5a-d) which occurred as a series of individual bursts of secretion resulting in elevation of PRL to levels of 100-300 ng/ml.

GH and PRL during pregnancy and parturition

Episodic secretion of GH was evident during the fourtheenth to eighteenth day of gestation with amplitudes and inter-peak intervals between pulses similar to those of non-pregnant rats (Fig. 6). There was a tendency for interpeak trough levels to remain higher than in non-pregnant rats. GH secretion increased on days 18-21 of pregnancy with increased amplitude of individual pulses and elevated trough values (Fig. 7). Plasma PRL levels remained low during days 14-21 of gestation, with plasma levels less than 20 ng/ml. However, PRL levels increased dramatically 4-6 h before delivery, reaching concentrations of 300-400 ng/ml (Fig. 8). PRL declined rapidly at the onset of or during parturition. Plasma GH levels were low immediately before but rose to unusually high concentrations, often greater than 400 ng/ml during delivery. The elevation in plasma GH persisted for several hours.

Effects of suckling on GH and PRL

Removal of the pups from the lactating mother resulted in low baseline levels of both GH and PRL with minimal fluctuations. Return of the pups and onset of suckling resulted in an immediate surge in both GH and PRL (Fig. 9). Plasma GH increased to levels of 75-200 ng/ml. The duration of the GH surge was brief and, despite continuous suckling, GH levels returned to baseline within 60-90 min. PRL rose with suckling to concentrations exceeding 300 ng/ml; the rise was rapid and sustained. In some animals the surge in PRL was associated with several distinct pulses.

Table 1

Experimental group	N	Mean serum GH (ng/ml)*
Non-stressed	6	105.6 ± 12.5†
Stressed	6	7.6 ± 1.3

*Derived by calculating area under secretory curve by planimetry.

†Mean ± SEM; $P < 0.005$ compared to stressed rats using Students t-test for unpaired samples.

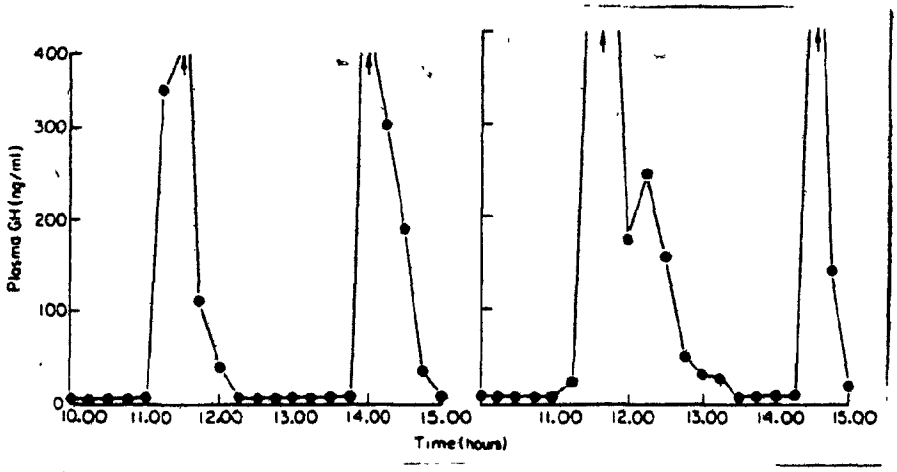

Fig. 1. Plasma GH secretion in two normal male rats.

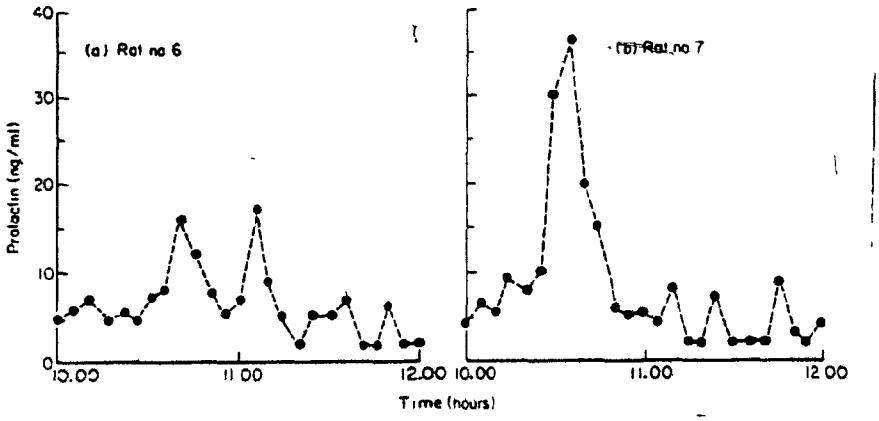

Fig. 2. Plasma PRL levels in two normal male rats sampled every 5 min shows rapid fluctuations.

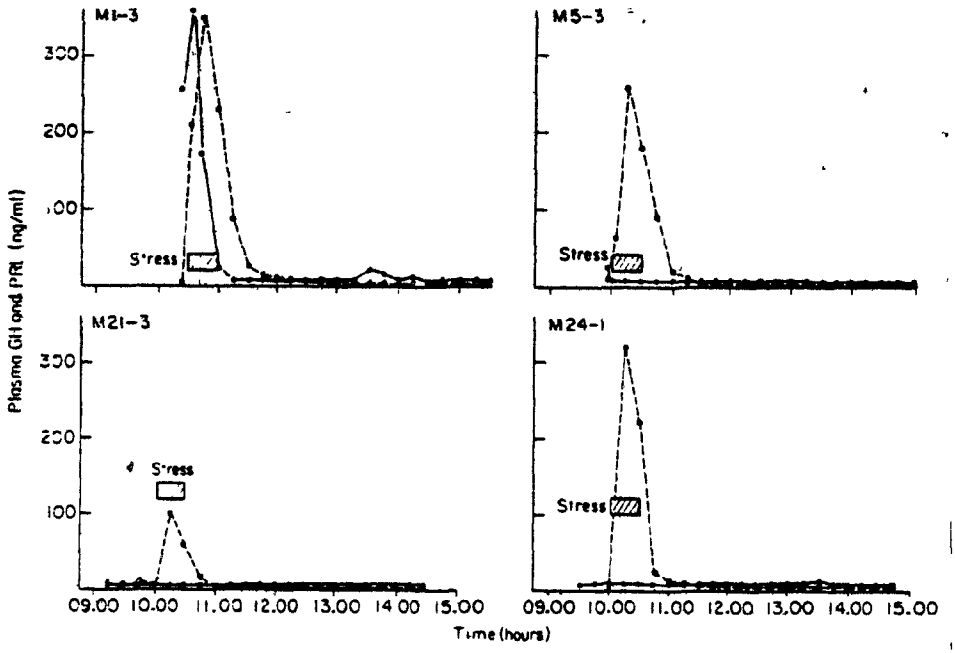

Fig. 3. GH (•) and PRL (○) responses to stress in four male rats. Stress caused a rapid rise in PRL levels with return to baseline within 15 min after end of stress. Pulsatile release of GH was prevented for up to 4 h after stress.

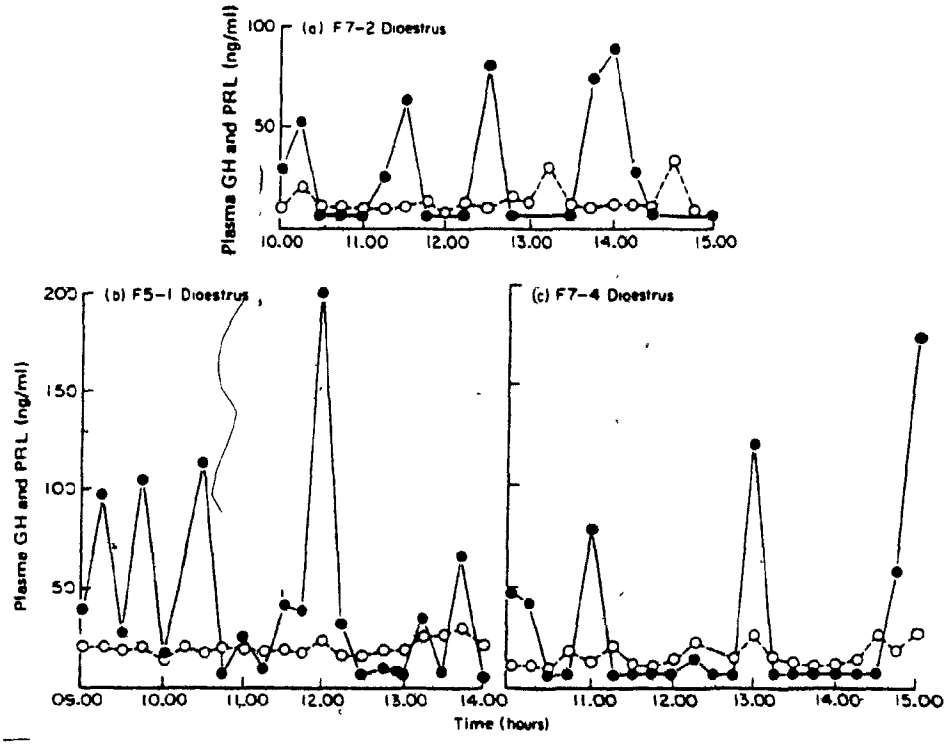

Fig. 4. Plasma GH (___) and PRL (- - -) secretion in three normal female rats sampled during dioestrus.

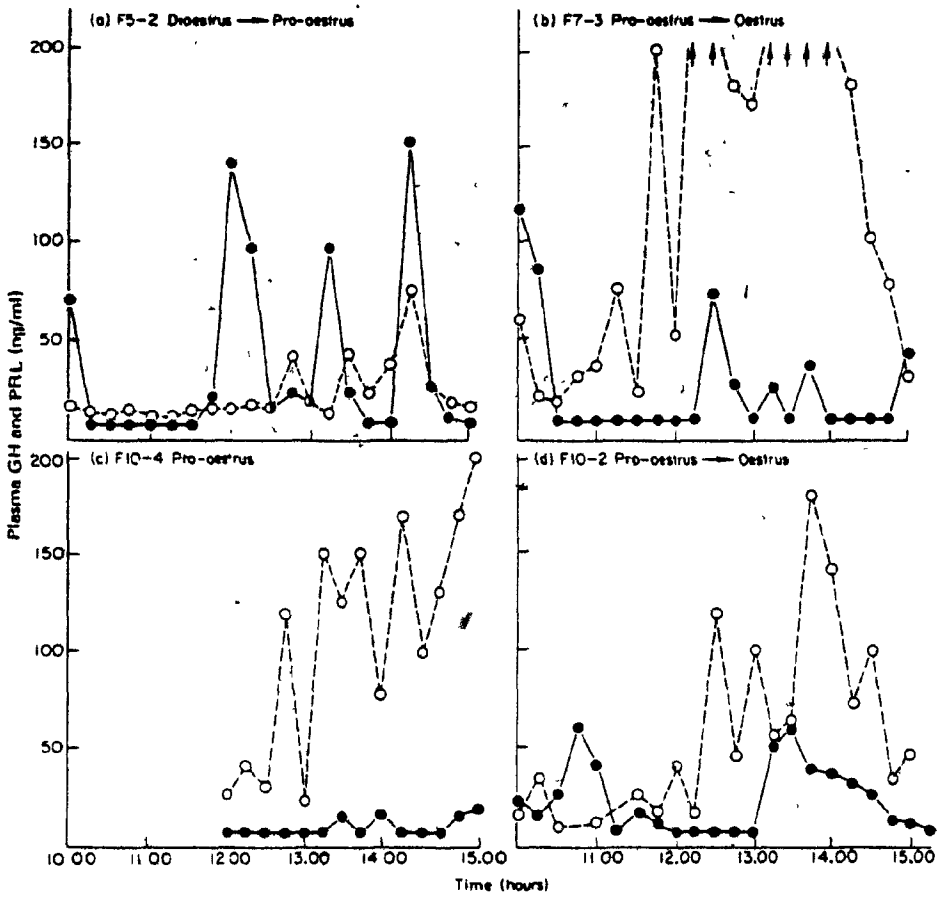

Fig. 5. Plasma GH (—) and PRL (---) secretion during pro-oestrus. Episodic GH secretion persists as PRL rises during the afternoon from 12.00 to 15.00 hours. PRL is secreted in a series of bursts.

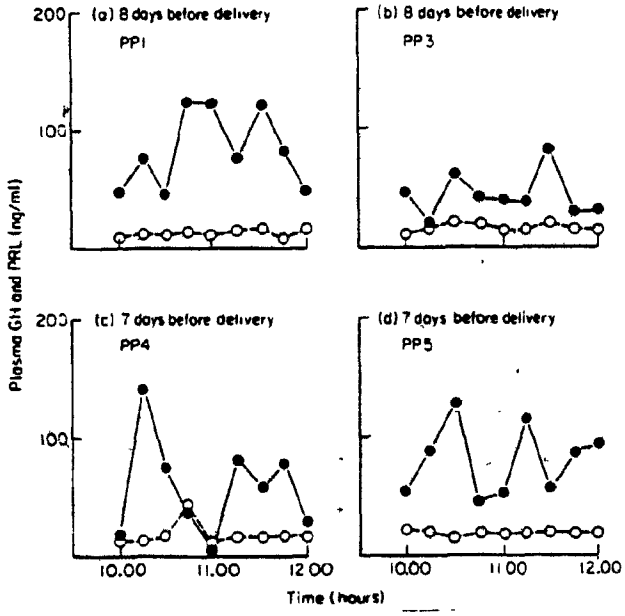

Fig. 6. GH (●) and PRL (○) secretion in four pregnant rats 7-8 days prior to delivery. Trough values are elevated in three of the four rats. PRL levels remain low.

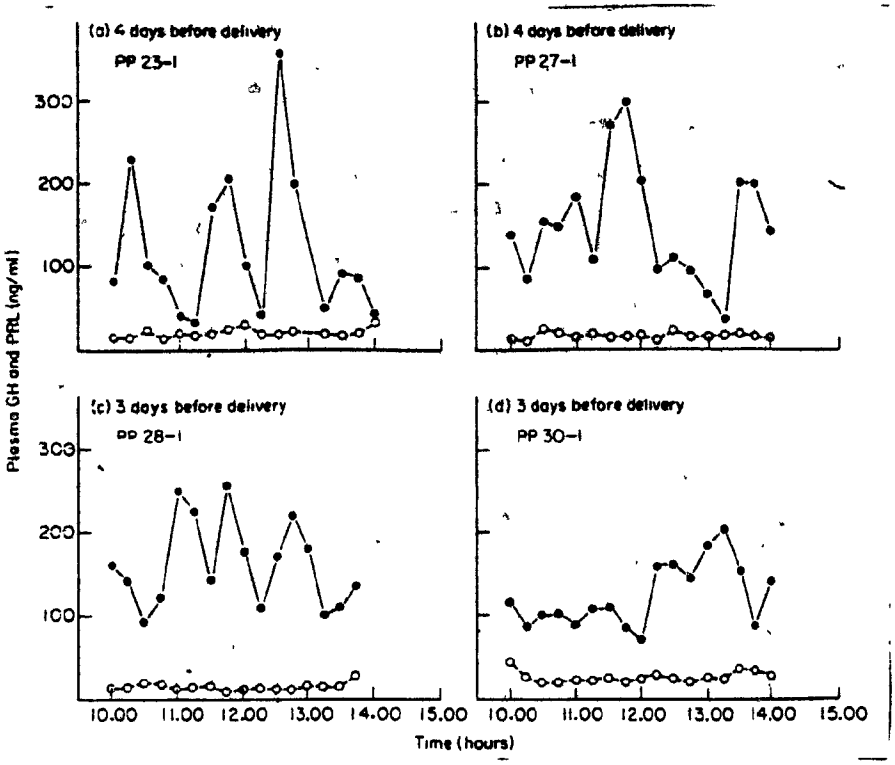

Fig. 7. GH (•) and PRL (o) secretion in four rats on days 18-21 of gestation. GH surges reach higher peaks and GH levels in troughs are 25-100 ng/ml.

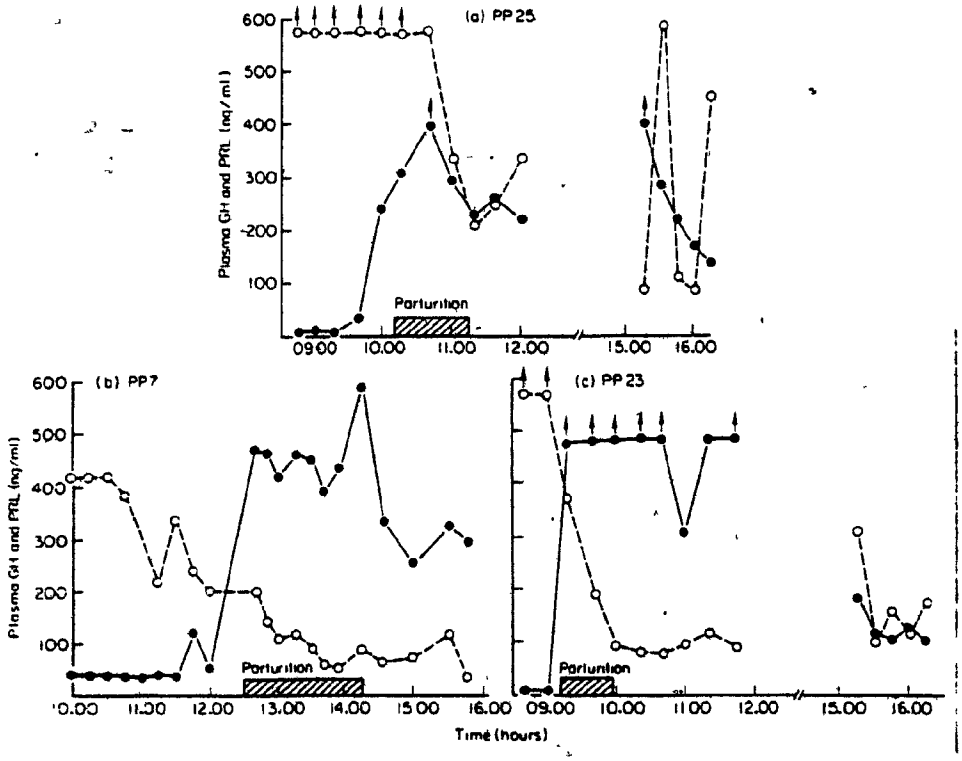

Fig. 8. GH (•) and PRL (o) secretion during parturition. PRL levels are high before delivery and fall at onset or during delivery. GH levels are low before parturition and increase sharply at onset of labour. Fluctuations in GH and PRL after parturition occur with suckling.

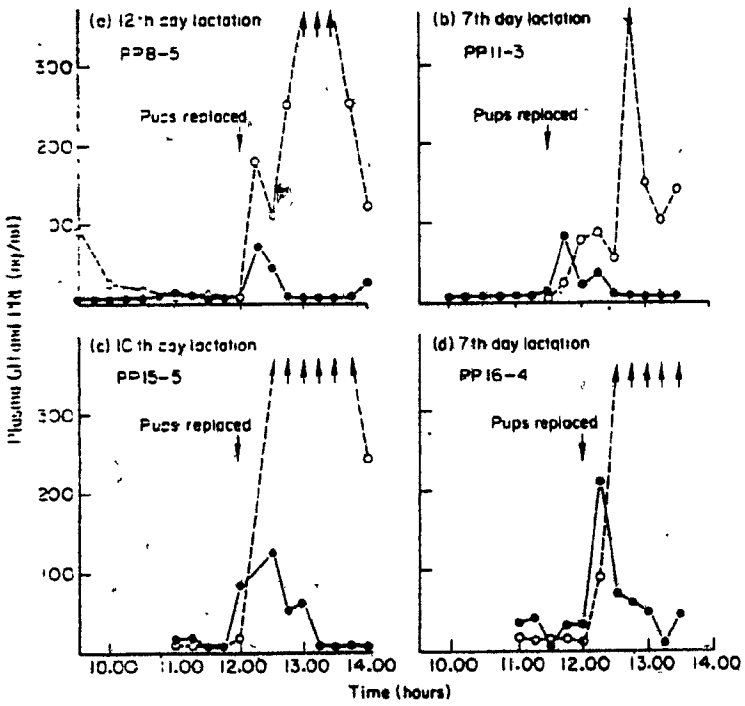

Fig. 9. GH (•) and PRL (○) response to suckling in four rats. Both hormones show elevation in blood after suckling but the time of onset, magnitude and duration of the two responses are different.

DISCUSSION

The present studies show that female rats, like males, secrete GH entirely in episodic bursts; the female, however, shows significant differences in the pattern of secretion, the pulses occurring more frequently and reaching lower peak values. There were no differences in the secretory pattern of GH during different phases of the oestrous cycle, although samples were only obtained during the daytime. In late pregnancy, peak values of GH were increased and trough values were elevated. GH rose during delivery and was continually elevated for several hours after completion of parturition. The significance of this sustained surge of GH is unknown. Elevation of GH during parturition does not occur in the human (G. Tolis, personal communication).

Suckling causes a significant and reproducible elevation of plasma GH confirming data of others (Chen et al., 1974). GH returned to basal levels by 1 h.

Stress in the male rat causes complete suppression of pulsatile GH secretion for up to 4 h (Terry et al., 1976). Antisera to SRIF will partially restore pulsatile GH secretion after stress (Terry et al., 1976). Circulating SRIF therefore probably plays a role in stress-induced GH suppression.

PRL is secreted episodically, but in a pattern distinct from GH. In the male rat, PRL bursts are brief, may reach plasma concentrations of 70 ng/ml, and do not follow a regular temporal sequence. They frequently coincide with GH peaks and it is therefore unlikely that PRL bursts result from stress. Plasma PRL levels show small fluctuations during the oestrous cycle with the exception of the rise during the afternoon of pro-oestrus. This previously

documented increase is the result of the cumulative effect of a series of surges in PRL. PRL secretion also increased prior to parturition as previously reported (Grosvenor and Turner, 1960: Amenori et al., 1970), and declined during or immediately after delivery concurrent with GH elevation. This is the reverse of what is observed during stress in the rat even though corticosterone is increased simultaneously (Saunders and Martin, unpublished observations).

Suckling induced a rise in PRL as well as GH but with different times of onset and duration indicating separate neural regulatory mechanisms. The rise in both hormones is dependent on actual breast stimulation as neither hormone is released if the pups are returned to sampling box but suckling is prevented by a barrier (Saunders and Martin, unpublished observations). Olfactory and visual stimulation are therefore inadequate to stimulate release of either hormone.

The dissociation of PRL and GH secretion under a variety of physiologic conditions provides a valuable model for further studies of the neural regulation of these two hormones.

II. B. HYPOTHALAMIC-PITUITARY RESPONSES TO INTRACRANIAL SELF-STIMULATION IN THE RAT*

ABSTRACT

The effects on pituitary and adrenal hormones of intracranial self-stimulation (ISS) and involuntary stimulation in rewarding (PS) and non-rewarding (NSS) sites were investigated in male rats. Growth hormone (GH) was suppressed during periods of ISS and PS and rose sharply to a peak within 15 min of cessation. The interval between GH secretory episodes was significantly shortened during all 3 types of stimulation when compared to the normal rhythmic discharge observed in freely behaving baseline (BL) sampled rats. ISS, PS and NSS resulted in a rapid rise in prolactin (Prl), which returned to normal by the end of each hourly period of stimulation. There was a significant reduction of Prl elevation in the second and third periods of stimulation, suggesting that the first exposure to ISS had a greater stimulatory effect than subsequent stimulations. There was a rapid and sustained release of corticosterone (CS) during ISS and PS. As with Prl, the initial period of either ISS or PS caused a greater effect than subsequent periods.

These studies provide data to compare the relationship between ISS and neuroendocrine responses. The hormonal responses, with minor exceptions, were similar under all experimental conditions, and could not be clearly dissociated from previously described stress responses. Neural pathways and

*Terry and Martin, 1978a.

substrates involved in GH, Prl and CS secretion are discussed in relation to pathways activated by LH-MFB stimulation. Potential differential functions of monoamines and hypothalamic neuropeptides in behavioral and neuroendocrine regulation are hypothesized.

INTRODUCTION

Discovery of the positive reinforcing effects of intracranial self-stimulation (ISS) by Olds and Milner (1954) has led to numerous studies aimed at elucidation of the neural mechanisms involved (Hall et al., 1977; Olds 1977; Wauquier and Rolls, 1976). Although these studies have implicated selective neural pathways and putative neurotransmitters that enhance or reduce ISS, there is little information available by which to accurately assess the internal activation process(es) that accompanies ISS.

Characterization of neuroendocrine responses to stress provide baseline data by which the response to ISS can be compared (Terry, et al.,1976b; 1977 a,b). A variety of conditions described as stressful cause corticosterone (CS) and prolactin (Prl) release and growth hormone (GH) inhibition in the rat, effects that are mediated by the hypothalamus (Allen, et al., 1973; Brown and Martin 1974; MacCleod, 1976; Martin, 1976; Martin, et al., 1977).

In the present experiments, we hypothesized that the positive reinforcing effects of lateral hypothalamic (LH) ISS could be mediated by specific neural pathways, and therefore result in a neuroendocrine response distinct from stress. To test this hypothesis, plasma GH, Prl and CS were determined in chronically cannulated rats before, during and after episodes of ISS. The results were compared with the effect of forced stimulation of both ISS and non-self-stimulating (NSS) animals. The hormonal responses, with some minor exceptions, were similar under all experimental conditions and could not be clearly dissociated from the effects of stress. These results provide a baseline for further studies to examine the effects of pharmacologic agents and centrally active peptides in the mediation of both ISS and neuroendocrine responses.

(1) Catheterization and electrode implantation

Male Sprague-Dawley (Charles River) rats weighing 310-410 g were housed at constant temperature (22± 1°C) in individual cages with free access to laboratory chow and water during all experiments. The light-dark cycle was maintained at 12:12 h with lights on at 06:00. All animals were observed for 1 week prior to surgery. Indwelling cannulae were placed in the right atrium via the right external jugular vein and secured to the skull with screws and acrylic cement. Monopolar nichrome electrodes (0.2 mm diameter) with tapered exposed tips were stereotaxically implanted in the right LH-medial forebrain bundle (MFB) using deGroot coordinates: anterior, 4.4; lateral, 1.4; depth (-)2.2 mm (DeGroot, 1959). One skull screw served as an indifferent electrode. Animals were observed daily postoperatively and attempts to self-stimulate were not initiated until each had attained presurgical body weight, usually 3-5 days.

(2) Procedure for ISS

A Model 7150 Nuclear Chicago constant current generator was used for all stimulations and the pulses delivered were monitored on a Tektronix oscilloscope. Stimulation parameters consisted of symmetrical, biphasic square waves delivered in 0.2 sec trains at 400 pulse pairs/sec. High frequency stimulation (400 Hz) was used because higher rates of bar pressing (BP) were elicited compared to lower frequencies (50-100 Hz) (Carter and Phillips, 1975). Each pulse pair was 0.5-1.0 msec in duration and optimum currents for ISS ranged from 50-180 μA. Only animals that demonstrated steady

rates of BP at least 3 consecutive periods of 60 min on different days were used. After preliminary testing, each rat was placed in an isolation box and electrical connectors and venous cannulae were fed through a swiveled coiled spring fastened to the top of the cage. Stimulating wires were attached to a mercury commutator to allow 360^0 of rotation without entanglement of wires and catheter. This assembly allowed complete freedom of movement inside the cage during stimulation and blood sampling. The ISS cage was made of open wire mesh with dimensions 28.0 x 20.5 x 20.5 cm. The isolation box (BRS, LVE, Beltsville, Md, U.S.A.) containing the ISS cage was 40.5 x 50.8 x 40.5 cm. The bar press (4 x 8 cm) was placed at the end of the cage and fastened to a microswitch which activated the stimulator. The numbers of BP and stimulations delivered were counted electronically.

(3) Procedure for blood sampling

Blood samples (0.4 ml) were removed every 15 min from 10.00 to 15:30 h in order to encompass two GH secretory episodes (Willoughby, et al., 1977). The plasma was separated and frozen: red blood cells were resuspended in physiologic saline and returned to the rat after removal of the next sample. This technique minimized the fall in hematocrit and allowed multiple samples to be removed without hemodynamic disturbance.

(4) Determination of GH, Prl and CS

Plasma GH and Prl were measured in dupblicate samples by radioimmunoassay using kits supplied by the NIAMDD. Hormone data are presented in terms of the respective reference preparations. Corticosterone was determined by a competitive protein binding assay (Brown and Martin, 1974; Murphy, 1967).

(5) Experimental design

Seven ISS rats were sampled for 5.5 h on 4 or 5 separate occasions on different days. The first period of sampling was a baseline (BL) and the second and third consisted of ISS each alternate hour with reversal of the pattern of the following day to prevent confusion between a physiological and stimulus-induced hormonal response. During the fourth experimental day, each rat was passively (involuntarily) stimulated (PS) every other hour at approximately the same rate as had been attained with ISS: during such stimulation the microswitch was disconnected so that BP was ineffective in delivering a stimulus. To compare neuroendocrine responses to brain stimulation under non-reinforcing conditions, 6 rats were implanted with electrodes in the posterior hypothalamus (anterior, 4.6; lateral 0.9; depth (-)2.2 mm). These rats (NSS) were first screened for ISS and subsequently stimulated each alternate hour at approximately 4000 stimulations/h using conditions identical to those effective in eliciting ISS. None of the NSS rats demonstrated ISS.

(6) Histological verification of electrode placement

At the end of the experiment, rats were anaesthetized with pentobarbital (50 mg/kg b.w.) and perfused with 10 percent formalin through the left ventricle. The brains were removed and fixed in 10 percent formalin containing 10 percent sucrose for a minimum of 24 h. Brain sections (40 μm) were cut on a cryostat, stained with a cresyl violet/thionin solution and examined microscopically for electrode path and tip location.

(7) Analysis of data

Each 15 min blood sample supplied a data point for GH, Prl and CS. Plasma values for each hormone at every 15 min segment were tabulated and mean ± S.E. was plotted on the ordinate with its respective time on the abscissa. ISS, PS and NSS rates were averaged and mean ± S.E. calculated for every category. The mean level of each hormone was determined for the first, second and third hourly periods of ISS, PS, NSS and corresponding baseline periods. Statistical comparison was done using the two-tailed Student's test. A P value of < 0.05 was considered significant.

RESULTS

Rates of ISS, PS and NSS

The mean rate of ISS was 4394 ± 277 BP/h with a range of 2111-6467. All animals were passively stimulated at 3533 ± 165 stimulations/h. Hourly segments of stimulation beginning at either 10:00 or 11:00, 12:00 or 13:00, and 14:00 or 15:00 h were defined as the first, second and third periods, respectively.

Baseline hormone secretion --GH, Prl and CS

GH secretion in the male rat is characterized by intermittent surges that occur approximately every 3 h and are entrained to the light-dark cycle (Tannenbaum and Martin, 1976; Willoughby, et al., 1977). GH peak levels reach 200-800 ng/ml and troughs are unmeasurable. Individual animals in the present experiments had a normal secretory profile of GH (Fig. 10). Mean GH rose to

279.2 ± 52.7 and 287.5 ± 56.3 ng/ml in the first (12:30 h) and second (15:15 h) peaks, respectively (Fig. 11). The mean GH interpeak interval (Table I) was similar to that reported preveiously by Tannenbaum and Martin (1976). Individual animals had trough levels of 6.2 ng/ml or less. Averaged trough levels were 49.9 ± 18.8 ng/ml, which reflected slight variation in the timing of GH peaks and troughs for each animal. There was no significant difference in mean GH between the first, second and third periods (Fig. 14).

No specific pulsatile pattern of Prl release was observed in baseline studies, although random sporadic bursts occurred in the afternoon (Fig. 10). Mean Prl for the first period was less ($P<0.025$) than the second and third (Fig. 14). The lowest mean level was 2.2 ± 0.3 (10:30h) and the highest 33.1 ± 19.3 ng/ml (15:30 h), suggesting an afternoon Prl elevation (Fig. 12).

The trend toward a late afternoon CS elevation was similar to that observed with Prl, confirming a diurnal variation (Figs. 10 and 13). The lowest mean CS level was 2.0 ± 0.4 (10:14 h) and the highest 10.5 ± 2.7 µg/100 ml (13:45 h) (Fig. 13). Mean CS for the first and second periods was less ($P< 0.025$) than the third (Fig. 14).

(3) Effects of self-stimulation

Growth hormone was suppressed during periods of ISS and rose sharply to a peak within 15 min after cessation, a phenomemon which occurred consistently after each hour of ISS between 10:00 and 15:30 h (Fig. 10 and 11). Mean GH was less than controls during all 3 periods of stimulation (Fig. 14). The mean interpeak GH interval was decreased compared to baseline periods, which indicated a marked disruption of normal rhythmic pulsatile secretion (Table II).

Self-stimulation resulted in a rapid rise in plasma Prl, which had returned to normal by the end of each hourly period of stimulation (Figs. 10 and 12). The first and second hour of ISS resulted in elevation of Prl above control values, and the first was greater than the second ($P < 0.005$) (Fig. 14).

There was a rapid and prolonged elevation of CS during all ISS periods (Figs. 10 and 13). A decrease ($P < 0.01$) in the CS response from the first/second and second/third periods was observed, but levels were consistently higher than controls (Fig. 14).

Effects of passive stimulation of ISS animals

Mean GH was suppressed during all PS periods (Figs 10 and 14). Suppression of GH was less ($P < 0.05$) than ISS only during the second hour of stimulation. The mean interpeak interval was not different from ISS rats (Table II).

Prolactin was elevated above baseline during the first and second, but not the third period of PS (Figs. 10 and 14). There was no difference between ISS and PS.

Mean CS was elevated above control values for all periods of PS and there was no difference between the first, second and third periods (Figs. 10 and 14). Passive stimulation had a similar effect on CS as ISS, and the only difference occurred during the third period, where mean CS was higher ($P < 0.05$) during PS (Fig. 14).

Effects of passive stimulation of NSS animals

Although there was a trend toward GH suppression during PS in NSS rats,

the effects were not significant (Fig. 14). However, the mean GH interpeak interval was reduced, as occurred in both ISS and PS groups (Table II).

The first and third periods of NSS resulted in Prl elevation (Fig. 14). The pattern of Prl release was similar to that observed during PS and ISS.

Corticosterone was not determined for this group.

Rate of ISS and hormone levels

There was no correlation between the rate of BP and the elevation or reduction in GH, Prl or CS.

Histological verification of electrodes

Electrode tips of ISS rats were located in the LH-MFB region (Fig. 15). NSS animals had electrodes in the LH-MFB and posterior hypothalamic region.

Table II

Mean interpeak intervals ± S.E. between successive GH pulses in BL, ISS, PS and NSS groups

Numbers in parentheses indicate numbers of animals in each group.

	h ± S.E.
Baseline control	3.2 ± 0.1 (7)
Self-stimulation	1.8 ± 0.3 (7)*
Passive stimulation	2.0 ± 0.2 (7)*
Non-self-stimulation	1.6 ± 0.2 (6)*

*Less than baseline control, $P < 0.001$.

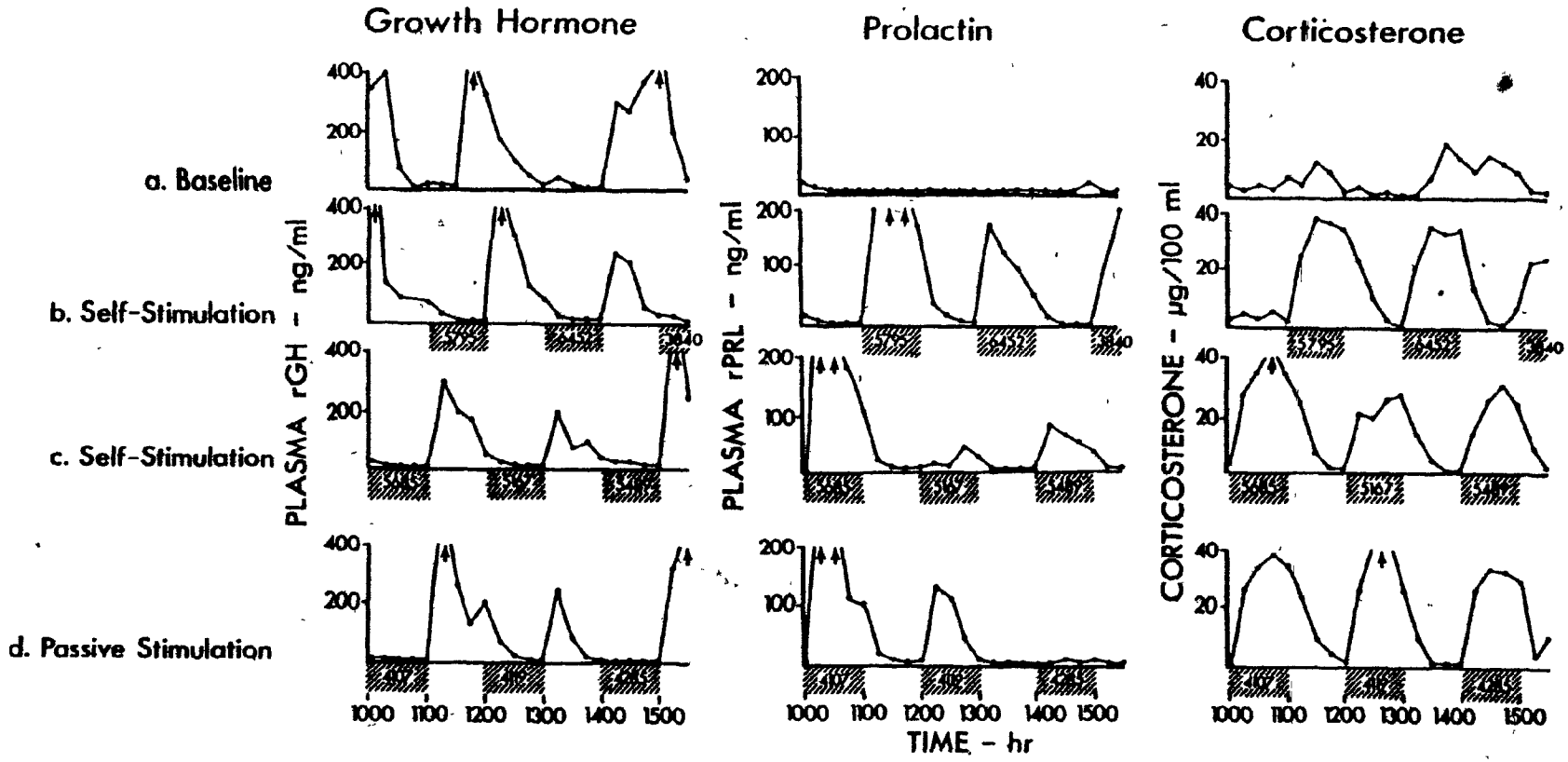

Fig. 10. Plasma GH, Prl and CS of a representative individual rat during BL (a), ISS (b and c) and PS (d) periods. In this and subsequent figures, the cross-hatched squares indicate hourly periods of stimulation and the enclosed number indicates the number of BP/h for ISS or stimulation/h for PS. ISS and PS result in GH suppression and Prl and CS elevation.

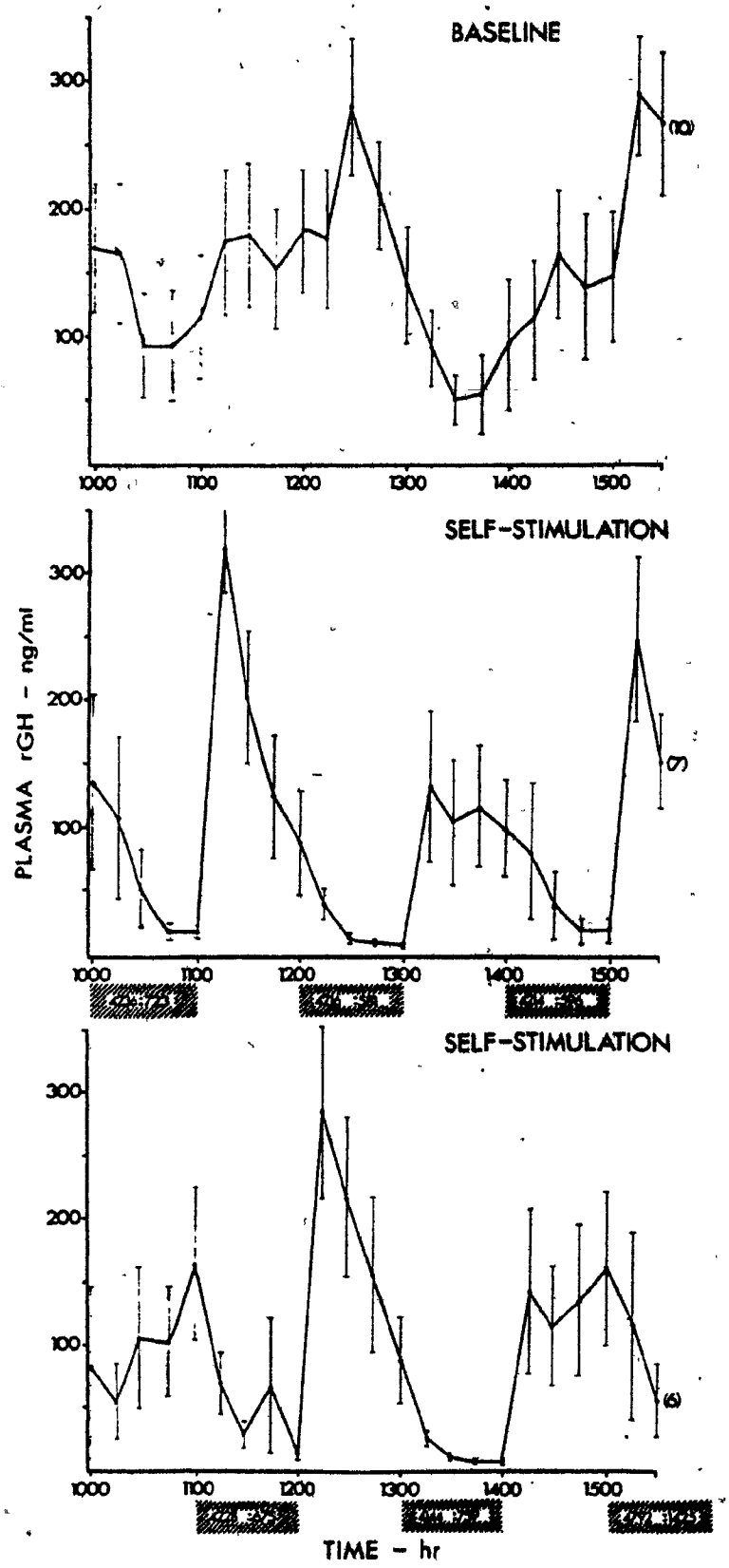

Fig. 11. Mean plasma GH ± S.E. during BL and ISS periods. In this and subsequent figures the numbers in parentheses indicate the number of animals in each experiment.

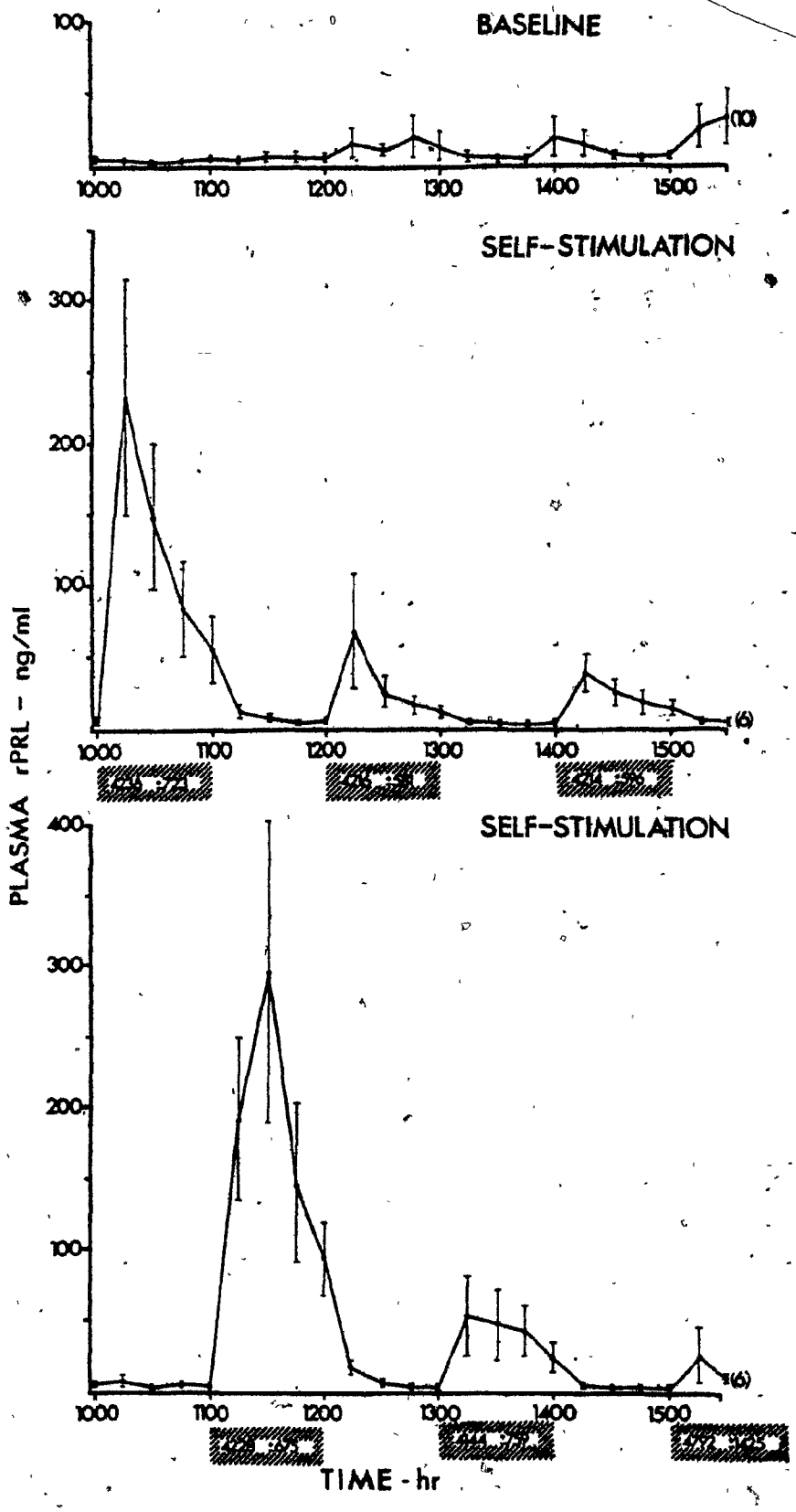

Fig. 12. Mean plasma Prl ± S.E. during BL and ISS periods.

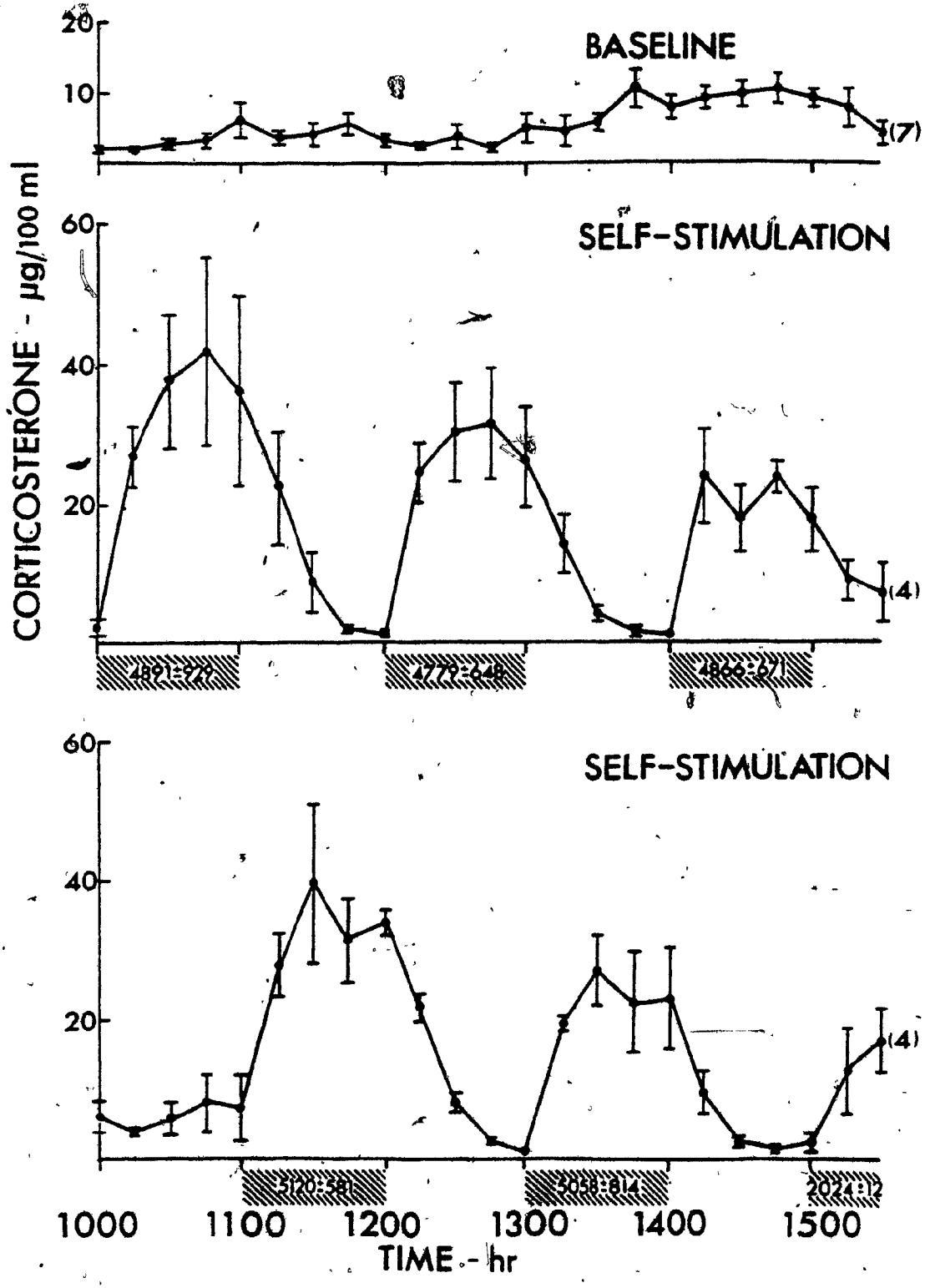

Fig. 13. Mean plasma CS ± S.E. during BL and ISS periods.

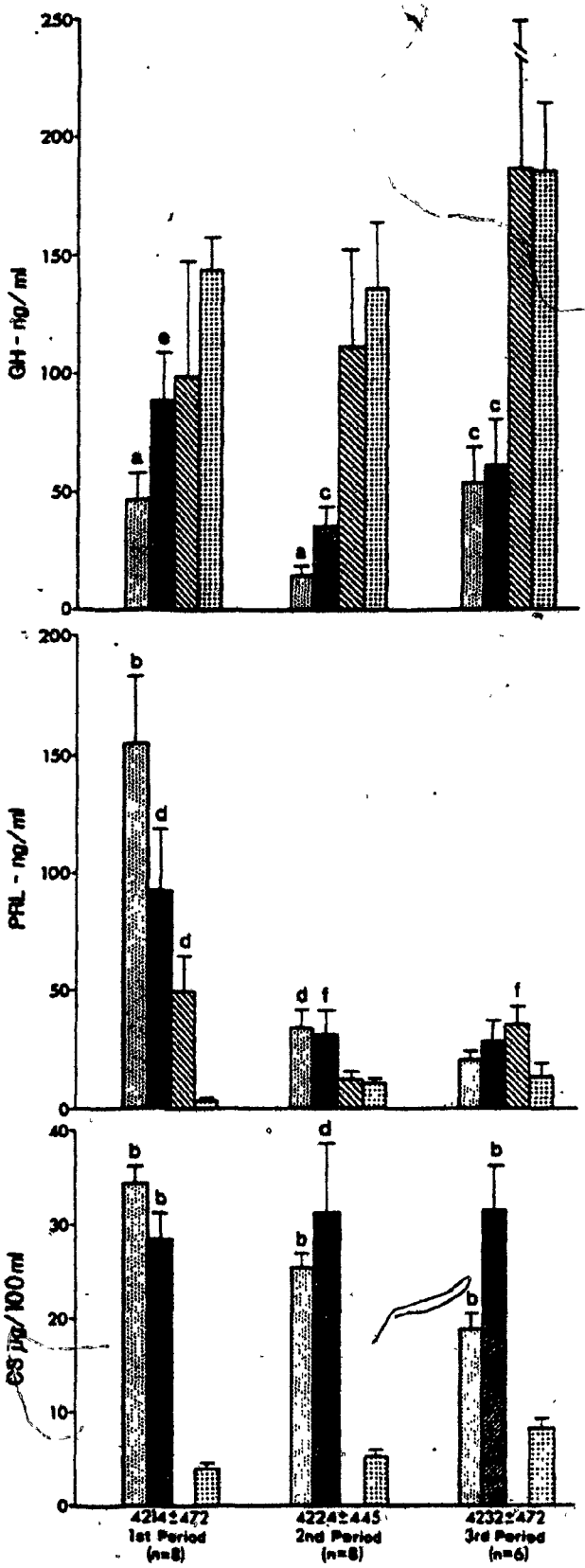

Fig. 14. Mean ± S.E. plasma GH (upper), Prl (middle) and CS (lower) during the first, second and third periods of ISS, PS, NSS and BL groups. Numbers below each bar graph represent mean BP/h ± S.E. and numbers in parentheses indicate number of animals per group. a and b: ($P < 0.001$) less or greater than BL respectively; c and d: ($P < 0.005$) less or greater than BL respectively; e: ($P < 0.025$) less than BL; f: ($P < 0.05$) greater than BL.

Fig. 15. Schematic diagrams of coronal sections of the rat brain showing locations of electrode tips of self-stimulating (SS) and non-self-stimulating (NSS) animals. Numbers to the side of sections indicate anterior-posterior plane of section from DeGroot atlas. Abbreviations: CI, capsula interna; CL, nucleus subthalamicus; FX, fornix; LM, lemniscus medialis; MM, nucleus mamillaris medialis; MFB, medial forebrain bundle; ML, nucleus mamillaris lateralis; MT, tractus mamillo-thalamicus; OT, tractus opticus; PC, peduncularis cerebri; PH, nucleus posterior hypothalami; PMD, nucleus premamillaris dorsalis; PMV, nucleus premamillaris ventralis; RE, nucleus reuniens thalami; RH, nucleus rhomboideus thalami; TT, tractus mamillotegmentalis; VM, nucleus ventralis thalamis, pars medialis; ZI, zona incerta; LHA, lateral hypothalamic area.

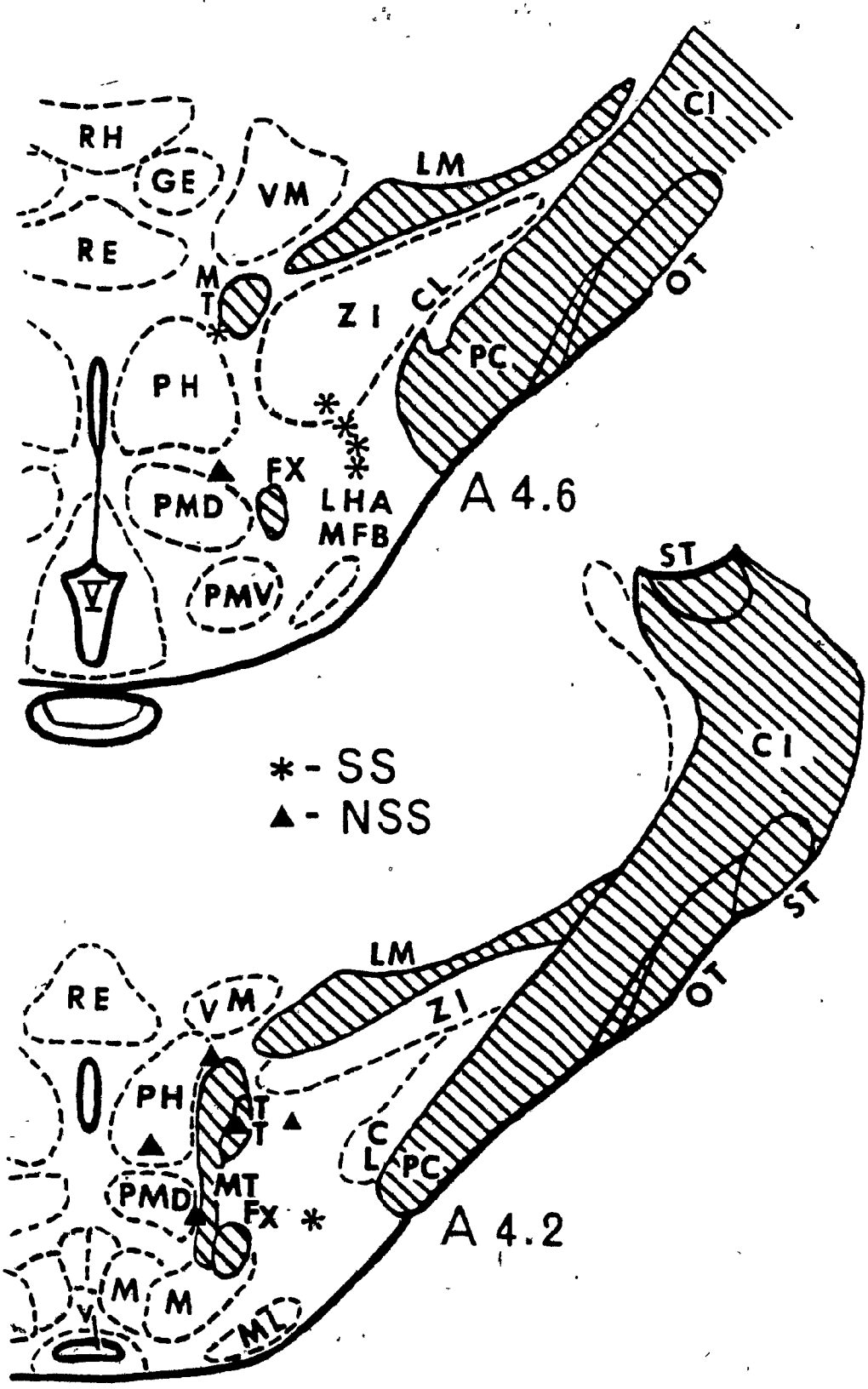
RH
GE
VM
RE
LM
CI
MT
ZI
CL
OT
PC
PH
FX
PMD
LHA
MFB
A 4.6
PMV
V
ST
*- SS
▲- NSS
CI
ST
LM
RE
VM
ZI
OT
PH
CL
PC
PMD
MT
FX
A 4.2
M
M
V
ML

Fig. 15

DISCUSSION

The results of these experiments indicate that the neuroendocrine responses elicited by ISS, PS and stimulation of adjacent non-reinforcing sites (NSS) are similar. These observations are reviewed in the context of information available for each hormone examined.

Hypothalamic-pituitary-adrenal axis

The present studies show that ISS results in rapid activation of the hypothalamic-pituitary-adrenal axis. There was no significant difference in mean CS levels in ISS compared to PS rats. The elevation during successive periods of stimulation on the same day was significantly less than the preceding period, suggesting either that the initial period of stimulation has a greater effect or that the CS response varies diurnally. It has been reported that the CS response to minor stresses such as handling varies in relation to peak and trough of the adrenal diurnal cycle (Brown and Martin, 1974; Zimmerman and Critchlow, 1967).

Several previous studies have examined the hormonal concomitants of ISS with respect to the question of whether the behavioral response is an emotionally quiescent or excited state. Adrenal steroids are elevated during ISS in most animal species (McHugh, et al., 1966; Natelson, et al., 1977; Sadowski, 1972). The cat appears to be an exception (Endroczi, et al., 1967).

Although ISS activates neuroendocrine pathways involved in the secretion of adrenal steroids, the question of whether the response is either specific to or required for ISS remains unclear. McHugh et al. (1966) showed that

lateral preoptic stimulation in the monkey, which was previously demonstrated to be reinforcing, resulted in endocrine changes like those which occurred after ISS, whereas electrical stimulation of non-reinforcing loci did not increase plasma corticoids. In the experiments reported by Sadowski et al. (1976), forced stimulation in rewarding sites led to a lesser activation of corticosteroid synthesis than when the animals actively pressed on the lever. In the rat, ISS with electrodes in the MFB caused similar elevations of adrenal steroids whether PS was given when awake or anaesthetized (Uretsky et al., 1966). In one study it was shown that hypothalamic ISS, forced stimulation and escape avoidance induced by midbrain tegmental stimulation each resulted in similar elevations of adrenal steroids (Olds and Yuwiler, 1972). Since the MFB and mammillary peduncle are reported to mediate adrenal responses to photic, acoustic and sciatic nerve-stimulation (Feldman et al., 1971, 1972a, 1972b), it seems likely that the CS response is the result of direct stimulation of pathways involved in hypothalamic control of ACTH secretion.

Growth hormone

Numerous studies in humans and animals have demonstrated that physiologic GH secretion occurs in an episodic pattern (Martin, 1976, Martin et al., 1977; 1978; Stewart et al., 1977). In the rat, postpubertal males exhibit a 3.3 h rhythmic discharge of GH (Tannenbaum and Martin, 1976) with levels rising within a 15 min period from less than 1 to greater then 400 ng/ml.

The final common pathway for the regulation of GH secretion lies within the hypothalamic ventromedial nucleus (VMN)-arcuate region (Rice et al., 1976;

Willoughby et al., 1977). Bilateral ablation of the hypothalamic VMN reduces plasma GH levels and stimulation results in release (Frohman and Bernardis, 1968; Frohman et al., 1968; Martin, 1976). Disconnection of specific inputs to the medial basal hypothalamus in the rat has differential excitatory of inhibitory effects on GH secretion (Krey et al., 1975; Mitchell et al., 1973; Willoughby et al., 1977). Stimulation of the medial preoptic area results in inhibition of GH secretion (Martin et al., 1976; Martin et al., 1978a)

Stress alters GH secretion in man and animals, but the response is species-dependent. In man and primates, GH is secreted in response to a variety of stressful stimuli (Reichlin, 1974), whereas, in rodents, GH secretion is inhibited (Martin et al., 1978a; Terry et al., 1976, 1977). Ablation of the medial preoptic area prevents the stress-induced suppression of GH in the rat (Martin et al., 1975; Rice and Critchlow, 1976; Rice et al., 1975).

In the present study, ISS from the LH-MFB region caused inhibition of pulsatile GH secretion. PS had the same effect, but NSS did not. These results suggest that stimulation of rewarding sites causes a significantly greater alteration of neural mechanisms controlling GH release than does stimulation through non-rewarding sites. The timing of GH secretory bursts (mean peak-to-peak interval) was significantly shortened in all stimulated animals compared to the normal 3.1 h rhythmic discharge in baseline sampled rats. These findings are consistent with previously reported findings on effects of stress on GH secretion in the rat (Terry et al., 1976b, 1977d), although we have not applied intermittent stress for similar periods of time.

Stimulation in the LH-MFB region could influence ascending and/or descending fibers from limbic structures known to have an effect on GH secretion. It is unlikely that the elevations in plasma CS directly inhibit GH secretion since administration of dexamethasone has no effect on pulsatile GH release (Terry and Martin, unpublished observations).

Prolactin

Basal Prl secretion is characterized by a series of bursts of release (Sassin et al., 1972; Saunders et al., 1976; Terry et al., 1976b). Although the neural substrate mediating pulsatile basal Prl secretion is not well established, the hormone has been shown to be secreted in response to stress in both man and animals (Bohnet and Friesen, 1976; Terry et al., 1976b). Hypothalamic deafferentation attenuates the Prl response to stress in rats (Krulich et al., 1975).

Blood Prl was reported to rise significantly during low-level LH-MFB ISS in rats, but failed to rise further when the current and operant rate were increased (Wanquier and Rolls, 1976). The present results indicate that ISS causes a rapid and significant elevation of PRL, which returns to baseline levels at the end of each period of stimulation. The significant reduction in Prl levels between the first and second periods of stimulation suggests that the first exposure to ISS in the day has a greater stimulatory effect than subsequent stimulations, a finding that could imply either exhaustion of pituitary Prl stores, adaptation of a behavioral response or depletion of CNS neurotransmitters or peptides that mediate Prl release. The Prl response to PS, although less, was not significantly different from ISS. Forced

stimulation of NSS animals also resulted in Prl release, but the response was significantly less than occurred during stimulation of rewarding sites. Thus, as observed with CS and GH, the Prl response to ISS is similar to that which occurs in other stressful conditions.

Catecholaminergic involvement in ISS and neuroendocrine regulation

There is substantial evidence that catecholamines are important in both regulation of hypothalamic-pituitary function and in the mediation of ISS. Several reports have documented a role of norepinephrine (NE) and dopamine (DA) in the physiologic regulation of GH, Prl and CS secretion (Fuxe et al., 1973; Jones et al., 1975; MacCleod, 1976; Martin, 1976; Martin et al., 1978a; Van Loon, 1973). Major catecholaminergic pathways are also hypothesized to be involved in ISS; these include the mesolimbic and nigrostriatal DA and the ascending dorsal NE systems (Antelman and Caggiula, 1977; Hall et al., 1977; Olds, 1977; Wanquier and Rolls, 1978). There is a considerable degree of overlap between terminations of the catecholaminergic systems that support ISS and those involved in the regulation of hormone secretion (Hokfelt et al., 1978). It is likely that the neuroendocrine responses which occur during ISS are also dependent upon central catecholaminergic systems.

Potential role of hypothalamic neuropeptides

Measurement of anterior pituitary hormones in the peripheral blood indicates that intense activation of the hypothalamic-pituitary axis occurs during ISS. This does not imply that pituitary hormones are required for ISS, but raises the possibility that hypothalamic releasing or inhibiting factors

are involved in mediation of the response. Infusion of somatostatin (SRIF) into normal rats results in an abrupt fall in plasma GH, which is followed by rebound GH secretion after cessation of the infusion (Martin, 1976). This rapid suppression and rebound pattern is similar to that observed during ISS, and suggests that SRIF release is induced by electrical stimulation of the MFB. We have shown that passive immunization of rats with antisera to SRIF prevents the suppression of GH caused by stress (Terry et al., 1976b, 1977d) or LH-MFB stimulation (Terry and Martin, 1981b). These findings support the hypothesis that SRIF is released and responsible for GH suppression in both experimental situations. The rapid surge in Prl during ISS is presumably the result of release of prolactin releasing factor (PRF) (Terry and Martin, 1978a; Vale et al., 1977). Preliminary studies (not shown) show that ISS is not accompanied by release of thyroid stimulating hormone (TSH), supporting the concept of a physiologic PRF separate from TSH releasing factor (TRF). Similarly, CS responses are presumably the result of release of corticotropin releasing factor (CRF).

A number of studies have shown that systemic, cerebroventricular or local injection of TRF or SRIF induces a variety of motor, behavioral and electrophysiological changes (Brown and Vale, 1975; DeWied and Gispen, 1977; Renaud et al., 1975; Vale et al., 1977). The possible interaction of CNS neurotransmitters and hypothalamic peptides in the mediation of ISS concomitant with activation of the hypothalamic-pituitary axis merits further consideration (Terry and Martin, 1978b).

Conclusion

It is evident that stimulation of the LH-MFB during ISS results in perturbation of GH, Prl and CS secretion. As PS in rewarding sites elicits similar neuroendocrine effects, it is possible that the responses observed are not the consequence of positive reinforcement, but rather result from either (1) direct stimulation of neural pathways that regulate the secretion of hypothalamic release and/or release-inhibiting factors or (2) aversive effects, as has been suggested by Steiner et al. (1969). The responses to ISS, PS and NSS are qualitatively and quantitatively similar to those known to occur with a variety of noxious stimuli, i.e. 'stress'. Our experiments do not resolve the central issue of what ISS represents but do indicate that, from a neuroendocrine standpoint, the effects of centrally reinforced behavioral activation cannot be readily separated from stress.

C. CONCLUSIONS

Results of these studies confirm earlier observations that male albino rats secrete growth hormone in a rhythmic manner with rises occurring approximately every 3.3 hours and reaching levels greater than 800 ng/ml. This rhythmic pattern was similar in all rats. Animals maintained on the same light-dark cycle did not have growth hormone rises at exactly the same time, but there was usually not more than a one hour difference in timing between animals, or in the same animal sampled on different days. These data suggest that the rhythm was light-dark entrained, although longer sampling periods with alteration of the light-dark cycle would be required to substantiate this conclusion.

Female albino rats also secreted growth hormone rhythmically, however, the rises occurred more frequently than males and were of lower amplitude. The pattern of growth hormone pulses was more eratic than males and there was no clear indication of entrainment to the estrus nor the light-dark cycle. Pregnancy, parturition and suckling significantly altered the frequency and amplitude of growth hormone rises and ebbs. Thus, the female rat was rejected as a suitable model for further experiments because it did not fulfill the criteria of constancy in the amplitude, frequency and entrainment of its growth hormone secretory rhythm.

Swimming stress suppressed pulsatile growth hormone secretion for up to 5 hours. Self-stimulation and passive-stimulation through electrodes in the lateral hypothalamic-medial forebrain area also inhibited growth hormone release, but levels rose quickly after cessation of stimulation. The growth hormone responses to lateral hypothalamic activation could not be clearly distinguished from the response to stress.

Growth hormone suppression resulting from stress or lateral hypothalamic stimulation could be due to release of somatostatin, inhibition of a growth hormone releasing factor, disruption of catecholaminergic neurotransmission, or alterations in other peptidergic and/or aminergic systems. The purpose of the next series of experiments (section III,A,1,2) was to determine (1) the role of somatostatin in spontaneous rhythmic growth hormone secretion i.e. are the rises and ebbs due to intermittent release of somatostatin followed by postinhibitory rebound or episodic release of a growth hormone releasing factor, and (2) whether suppression of this rhythm induced by swimming stress and lateral hypothalamic stimulation is mediated by somatostatin. To this end, male rats were passively immunized with antiserum to somatostatin and their growth hormone responses to the aforementioned stimuli were observed.

III. ROLE OF SOMATOSTATIN IN REGULATION OF GROWTH HORMONE SECRETION

A. EFFECTS OF PASSIVE IMMUNIZATION AGAINST SOMATOSTATIN ON GROWTH HORMONE SECRETION

1. ANTISERUM TO SOMATOSTATIN PREVENTS STRESS-INDUCED INHIBITION OF GROWTH HORMONE SECRETION IN THE RAT*.

ABSTRACT

Plasma growth hormone levels fall and remain low for several hours after stress in the rat. When antisera to somatostatin are administered to rats prior to stress, growth hormone secretory pulses are partially restored. The results provide evidence that circulating somatostatin plays a prominent role in stress-induced inhibition of growth hormone secretion in the rat.

INTRODUCTION

Growth hormone (GH) secretion in the rat is characterized by pulsatile or episodic release (Martin et al., 1974; Martin, 1976). In freely-behaving, non-stressed male rats surges of GH secretion occur at intervals of 3.2 to 3.4 hours, reaching plasma concentrations of 300 to 400 ng/ml (Tannenbaum and Martin, 1976; Martin et al., 1975). Between bursts, levels of GH are undetectable (< 10 ng/ml). The surges in GH are entrained to the light-dark cycle with major secretory episodes occurring at 1030 to 1230 hours and at 1330 to 1500 hours when lights turn on at 0600 hours (Tannenbaum and Martin, 1976; Martin et al., 1975; Willoughby and Martin, 1977). Pulsatile GH

*Terry et al., 1976b.

surges are blocked by administration of somatostatin (GH-release inhibiting hormone), a tetradecapeptide isolated from the hypothalamus (Brazeau et al., 1973), and by bilateral lesions placed in the hypothalamic ventromedial nuclei (Martin et al., 1974; Martin, 1976).

Stress in the rat results in acute inhibition of GH secretion (Schalch and Reichlin, 1966; Takahashi et al., 1971; Kokka et al., 1972; Krulich et al., 1972; Collu et al., 1973; Brown et al., 1974). We now report that stress suppresses pulsatile GH release for at least 5 hours and that antiserum to somatostatin administered prior to stress partially restores the GH secretory pattern.

METHODS AND RESULTS

Male Sprague-Dawley rats (300 to 350 g) were prepared with permanent indwelling intra-atrial catheters and adapted to small isolation boxes to permit repeated blood sampling without disturbance to the animal (Martin et al., 1974; Martin, 1976). This experimental procedure is important since even minor stress, such as handling, inhibits GH secretion (Brown and Martin, 1974; Martin et al., 1974; Martin, 1976). Cage adaptation was carried out by housing the animals in the isolation chambers for a period of 48 to 72 hours prior to sampling. All animals had reached their presurgical body weight before study. Each rat was given free access to water and Purina rat chow; the light-dark (LD) cycle was 12:12, with lights on from 0600 to 1800 hours. Blood samples (0.4 ml) were taken every 15 minutes from 0930 to 1500 hours to encompass two GH secretory episodes. The plasma was separated and frozen; red

blood cells were resuspended in physiologic saline and returned to the animal after the removal of the next sample. This technique prevented a fall in hematocrit and allowed multiple samples to be taken without hemodynamic disturbance. Plasma GH was measured in duplicate samples by radioimmunoassay using materials supplied by the National Institute of Arthritis, Metabolism, Diabetes and and Digestive Diseases.

To develop a model to study the effects of stress on GH secretion, two different stressful conditions were used. In the first, the effect of an intraperitoneal injection was evaluated. Six normal rats were sampled for 5 hours (1000 to 1500 hours) on two separate occasions. Three of the animals were first sampled without handling and three were injected with 0.5 ml of physiological saline intraperitoneally at 0830 hours. The sequence was reversed 2 to 4 days later. The area encompassed by a single GH secretory episode was calculated by planimetry. In order to standardize the determination, a period of 3.5 hours was taken from the onset of each secretory episode. Both saline-injected (Fig. 16A) and control groups had a normal pulsatile pattern of GH release. However, animals that were injected had a significantly lower, mean integrated GH level ($P < .05$) compared to the noninjected group (62.9 ± 7.2 compared to $105.6 + 12.5$ ng/ml per 3.5 hours)* (Table III). These results demonstrate that a minor stress can alter the subsequent secretion of GH without completely abolishing the pulsatile pattern.

In a second experiment, the effect of a more severe stress on GH secretion was assessed. After the baseline blood samples were obtained, each animal was removed from its isolation cage at 1000 hours, placed in a large water bath at

*Data represented are means + SEM, statistical evaluation was performed with student's t-test for paired and unpaired samples.

37°C and forced to swim for 30 minutes. Each rat was then returned to the isolation cage and sampled for 3.5 to 5.0 hours. Six control rats showed complete suppression of pulsatile GH secretion for up to 5.0 hours after stress, confirming previous reports (Schalch and Reichlin, 1966; Takaheshi et al., 1971; Kokka et al., 1972; Krulich et al., 1972; Collu et al., 1973; Brown et al., 1974; Brown and Martin, 1974) that acute stress causes inhibition of GH in the rat and demonstrating that this suppression is due to abolition of pulsatile GH release.

If the GH suppression to stress is mediated by somatostatin, we hypothesized that its effects might be blocked or diminished by administration of a large dose of antiserum to somatostatin. Two different antisera to somatostatin were used. One antiserum (AS 1) was generated in normal rabbits by injecting somatostatin conjugated to thyroglobulin, and the second (AS 2) by injecting somatostatin conjugated to bovine serum albumin. At a dilution of 1:2000 both antisera bound more than 50 percent of ^{125}I-labeled tyrosine-1 somatostatin and at a dilution of 1:300 they bound more than 95 percent. The antisera were specific for somatostatin and showed no cross-reactivity to thyrotrophin releasing hormone, luteinizing hormone releasing hormone, and various other small peptides. Three rats were given 1.0 ml each of AS 1 and three were given 1.0 ml of AS 2 intravenously through the cannulae at 0830 hours. Control rats received normal rabbit serum. Each animal was placed in the swimming tank from 1000 to 1030 hours and blood samples were taken every 15 minutes from 0930 to 1500 hours.

Plasma GH levels were low (< 10 ng/ml) in all animals prior to stress. Each of the six animals given antisera showed a partial restoration of pulsatile GH secretion to above baseline levels within 15 minutes to 2.5 hours

after stress (Fig. 16C). In contrast, the serum control group showed complete suppression of GH throughout the sampling period (Fig. 16B). Over the 3.5-hour period, the mean integrated GH levels of animals given the antiserums were significantly greater ($P < .01$) than that of the control serum group (25.5 ± 5.5 as compared to 7.6 ± 1.3 ng/ml). The mean integrated GH levels of the group treated with antiserums to somatostatin were significantly lower ($P < .005$) than those of the saline-injected group (Table III).

Table III. Integrated plasma GH levels in nonstressed, saline-injected animals and in stressed rats injected with antiserum to somatostatin.

Experimental group	N	Serum GH (ng/ml per 3.5 hours)*
Nonstressed	6	105.6 ± 12.5†
Saline-injected	6	62.9 ± 7.2‡
Stressed + normal rabbit serum	6	7.6 ± 1.3
Stressed + antiserum to somatostatin	6	25.5 ± 5.5§

*Derived by calculating area under secretory curve by plainmetry. †Mean ± S.E.M. ‡$P < .05$ compared to nonstressed rats. §$P < .01$ compared to controls treated with normal rabbit serum; $P < .005$ compared to saline-injected rats.

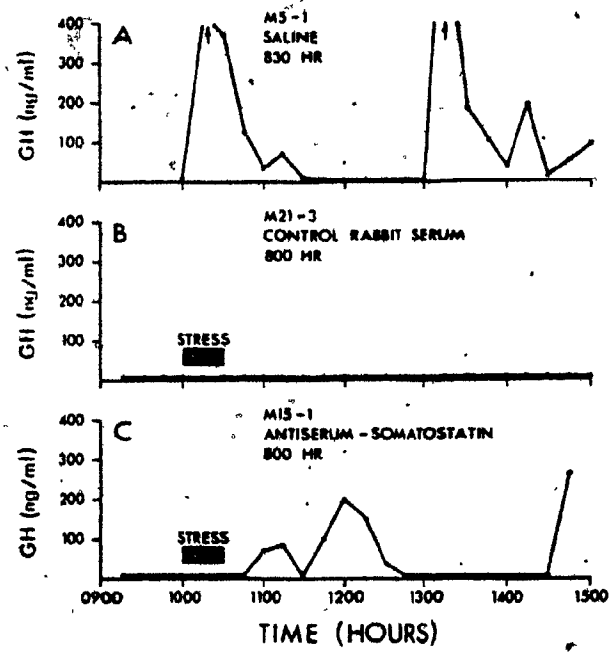

Fig. 16. Growth hormone secretion in individual unanesthetized rats. (A) Plasma GH levels show typical pulsatile pattern in saline-injected rat. (B) Stress causes complete suppression of pulsatile GH secretion in animals first treated with normal rabbit serum. Growth hormone levels remain low (< 10 ng/ml) for 4.5 hours after stress. (C) Partial restoration of GH pulses in rats treated with antiserum to somatostatin. Two distinct GH surges are evident: 15 minutes and 225 minutes after stress. Values of plasma GH are expressed in terms of the rat GH reference preparation No. 1 supplied by National Institute of Arthritis, Metabolism, and Digestive Diseases.

DISCUSSION

Somatostatin is present not only in the hypothalamus, but also in extrahypothalamic brain regions and in pancreas, stomach, and intestine (Brownstein et al., 1975; Patel et al., 1975). Administration of pharmacologic doses of somatostatin inhibits GH in animals (Brazeau et al., 1974; Lovinger et al., 1974; Martin, 1974; Ruch et al., 1974) and man (Siler et al., 1973; Besser et al., 1974; Yen et al., 1974) and also suppresses glucagon, insulin, secretin, and gastrin secretion (Gerich et al., 1974; Bloom et al., 1974; Unger, 1974). To qualify as a hormone, the peptide must be demonstrated to have physiologic effects and to circulate in the blood. Our findings that two different antisera partially restore the pulsatile pattern of GH secretion after stress provide compelling evidence that somatostatin plays a prominent role in stress-induced inhibition of GH in the rat and suggest that this effect is due to circulating somatostatin*. The fact that plasma GH rises before stress were not increased by the antiserum indicates that the episodic pattern of GH secretion is not due solely to somatostatin. We believe this suggests that normal pulsatile surges of GH are due to hypothalamic release of GH releasing factor.

*Two short communications that also indicate an effect of antiserums to somatostatin on GH secretion appeared after submission of this report. Ferland et al. (1976) reported that plasma GH levels in the rat rose two to threefold after injection of antiserum to somatostatin without any effect on the pulsatile pattern. Arimura et al. (1976) found significantly higher levels of plasma GH 30 minutes after electroshock in animals first treated with antiserum to somatostatin.

III. A. 2. THE EFFECTS OF LATERAL HYPOTHALAMIC-MEDIAL FOREBRAIN STIMULATION AND SOMATOSTATIN ANTISERUM ON PULSATILE GH SECRETION IN FREELY-BEHAVING RATS: EVIDENCE FOR A DUAL REGULATORY MECHANISM*

ABSTRACT

The purpose of this investigation was to examine the role of somatostatin (SRIF) and electrical stimulation of the lateral hypothalamic-medial forebrain bundle (LH-MFB) on dynamics of pulsatile GH secretion in freely-behaving, chronically cannulated male rats with implanted brain electrodes. The effects of administration of antisomatostatin serum (AS-SRIF) on pulstile GH and on TSH and PRL secretion was also studied. The results may be summarized as follows: 1) circulating AS-SRIF increases trough levels of GH in freely-behaving rats, but has no significant effect on the amplitude of GH secretory bursts or mean GH levels; 2) LH-MFB excitation can stimulate GH release if delivered when circulating GH levels are low; 3) LH-MFB stimulation inhibits secretion of GH if given at the time of a spontaneous GH burst; 4) stimulation-induced GH inhibition is prevented by pretreatment with AS-SRIF, suggesting that this response is mediated by endogenous SRIF; and, 5) AS-SRIF increases TSH secretion, but has no effect on Prl.

These results provide evidence to support the hypothesis that pituitary GH secretion is regulated by a combination of excitatory and inhibitory influences, the inhibitory component of which is mediated by SRIF.

*Terry and Martin, 1981b.

INTRODUCTION

Somatostatin (SRIF) has been shown to inhibit GH and TSH secretion in a variety of species (Vale et al., 1977; Schally et al., 1978). Previous studies in rats have shown that passive immunization with antisera to SRIF (AS-SRIF) prevented a decrease of GH secretion by stress (Arimura et al., 1976; Terry et al., 1976b), reversed the starvation-induced depression in plasma GH (Tannenbaum et al., 1978), increased basal or trough serum GH and TSH levels (Ferland et al., 1976, 1977; Terry et al., 1977b; Arimura and Schally, 1976; Gordin et al., 1976; Chihara et al., 1978), and enhanced the TSH response to cold exposure (Ferland et al, 1976) or to TRH (Arimura and Schally, 1976; Gordin et al., 1976). Antisomatostatin serum also increased basal GH levels in dogs (Schusdziarra et al., 1978). Active immunization against SRIF in baboons (Steiner et al., 1978) or sheep ((Varner et al., 1980) resulted in elevated baseline GH secretion. Chihara et al., (1978) reported that both GH and TSH responses to AS-SRIF required an intact medial basal hypothalamus and that the TSH response to AS-SRIF was mediated by hypothalamic TRH. Although there appears to be general agreement that systemic administration of AS-SRIF results in elevation of baseline or "trough" GH levels, reports differ as to what effect AS-SRIF has on the amplitude and frequency of GH secretory bursts and on overall mean plasma GH levels (Ferland et al., 1976; Steiner et al., 1978).

It has been suggested that SRIF is involved in the physiological control of basal GH secretion and that episodic bursts of GH release may be due to the intermittent release of a GH-releasing hormone (GHRH). Ehle et al. (1977)

reported that electrical stimulation of the amygdala in conscious monkeys produced an increase in plasma GH when the levels were low; when levels were high, GH decreased as a result of stimulation. These results suggest that amygdalar stimulation delivered during periods of episodic GH release results in suppression of GH secretion, possibly due to SRIF release, an effect potentially reversible by pretreatment with AS-SRIF. Conversely, stimulation during a trough period, when GH levels are low, increases GH secretion. This effect could be due to GHRH release or to inhibition of SRIF release and should not be affected by AS-SRIF.

The present study was designed to determine the role of SRIF in pulsatile GH secretion in the rat under conditions known to elicit either a stimulation or inhibition of GH secretion. Electrical stimulation of the lateral hypothalamic-medial forebrain bundle (LH-MFB) was previously shown by us to cause inhibition of GH secretion when delivered during a spontaneous surge of secretion (Terry and Martin, 1978a). The present results indicate that the effects of such stimulation are dependent on the timing of delivery of the stimulation in relation to the spontaneous, episodic GH secretion.

MATERIALS AND METHODS

1. Characterization of antiserum of SRIF

Antiserum to SRIF was generated in sheep and characterized by methods previously described (Rorstad et al., 1979b).

2. Animals and experimental design

Male Sprague-Dawley (Charles River) rats weighing 300-400g were housed at constant temperature (22 ± 1°C) in individual cages with free access to laboratory chow and water during all experiments. The light-dark cycle was maintained at 12:12h with lights on at 0600h. All animals were observed for 1 week prior to surgery.

Experiment 1. Animals, implanted with chronic indwelling cannulae placed in the right atrium via the right external jugular vein were adapted to isolation test chambers, using methods previously described (Terry and Martin, 1978). After recovery of preoperative body weight (usually 1 week postoperatively) blood samples (0.4 ml) were withdrawn every 15 minutes for periods of 6h (1000-1600h). Each animal was sampled in the basal state (BL) 2 days before receiving 1 ml of antiserum to SRIF (AS-SRIF) or preimmune normal sheep serum (NSS) iv at 0900h. This procedure allowed 2 sets of control data, NSS and BL. All blood samples were immediately centrifuged, and the plasma was separated and stored at -20°C for subsequent assay.

Experiment 2. Animals were maintained and implanted with indwelling cannulae as described in experiment 1. Monopolar nichrome electrodes (0.2 mm diameter) with tapered exposed tips were sterotaxically implanted in the right lateral hypothalamic-medial forebrain bundle (LH-MFB) using deGroot

coordinates: anterior, 4.4; lateral 1.4; depth (-)2.2 mm, as previously described (Terry and Martin, 1978a). One skull screw served as the indifferent electrode. Animals were observed daily postoperatively and blood sampling was not initiated until each had attained presurgical body weight, usually 7d. Blood samples were withdrawn every 15 minutes for periods of 6 1/2h (0930-1600h) in the basal state without stimulation (nonstimulated baseline control).

A model 7150 Nuclear Chicago constant current generator was used for all stimulations and the pulses delivered were monitored on a Tektronix oscilloscope. Stimulation parameters consisted of symmetrical, biphasic square waves delivered in 0.2 sec trains at 400 pulse pairs/sec. Each pulse pair was 0.5-1.0 m sec in duration with a current of 100 μA. Individual rats were placed in an isolation box and electrical connectors and venous cannulae were assembled as described elsewhere (Terry and Martin, 1978a). The number of stimulations delivered was counted electronically. Three days after the non-stimulated baseline control, two blood samples were removed at 0930 and 0945 followed by administration of 1 ml of NSS iv at 0950. All animals were stimulated at approximately 3800 stimulations/h, similar to nonreinforcing conditions, as previously described (Terry and Martin, 1978a). One hour periods of stimulation were delivered at 1030, 1230, and 1430h. On day 6, 1 ml of AS-SRIF was followed by electrical stimulation delivered in an identical manner.

3. Antibody titers of rat plasma after administration of SRIF-AS

Antibody titers in plasma of SRIF-AS-treated rats from experiment 1 were assessed by determining the capacity of aliquots of rat plasma obtained 1h and

7h after SRIF-AS administration to bind ^{125}I [Tyr^{1}] SRIF. Plasma samples from each rat were diluted 1:20 in the RIA buffer (Rorstad et al., 1979a,b). Binding to labeled SRIF was determined under the conditions used in the RIA for SRIF (Rorstad et al., 1979a,b).

4. Hormone assays

Plasma GH, PRL and TSH from experiment 1 were measured in duplicate by RIA using materials kindly supplied by the National Pituitary Agency. Results are expressed in terms of the respective NIAMDD reference preparation. In experiment 2, only plasma GH was measured. Aliquots of AS-SRIF were assayed for GH, PRL and TSH. To examine possible interference in the double antibody RIA's by AS-SRIF or NSS, which was administered to the animals, hypophysectomized rats were injected iv with 1 ml of AS-SRIF or NSS and bled 1h later. The serum obtained was tested to determine any effect on the standard curves for GH, TSH and PRL. There is no cross-reactivity with ovine GH.

5. Histological verification of electrode placement

At the end of the experiment 2, rats were anesthetized with pentobarbital and perfused with 10 percent formalin through the left ventricle. The brains were removed and fixed in 10 percent formalin and sucrose for 24h. Brain sections (40 μm) were cut on a cryotome, stained with a cresyl violet/thionin solution and examined microscopically for electrode path and tip location.

6. Analysis of data

Experiment 1. The unpaired Student's t-test was used to compare mean 6h plasma levels of GH, PRL and TSH, mean peak and trough levels of GH pulses between treatment groups, and mean SRIF binding capacity of the plasma.

Experiment 2. The mean level of GH was determined for the first (1030-1130), second (1230-1330) and third (1430-1530) periods of stimulation in AS-SRIF- and NSS-treated rats or non-stimulated baseline controls. Statistical comparison was done using the unpaired Student's t-test.

RESULTS

Effect of AS-SRIF and NSS on pituitary hormone RIA's

Sheep AS-SRIF contained a small amount of material reacting in the rGH assay (3.6 ± 0.1 ng/ml). Rat TSH and PRL were undetectable in the AS-SRIF. The RIA standard curves for GH, TSH, and PRL were not displaced by serum from hypophysectomized rats that received AS-SRIF or NSS, indicating that the sheep sera did not cause spurious values in these RIA's.

Effects of AS-SRIF on GH, TSH and PRL secretory profiles: Experiment 1.

Figure 17 shows the mean plasma GH levels of the AS-SRIF and NSS-treated groups of rats. Both groups exhibited a pulsatile pattern of GH secretion, with most peak GH values > 200 ng/ml and trough values < 7 ng/ml. GH profiles of animals administered AS-SRIF indicated that surges of secretion occurred earlier than in NSS-treated animals (1000 and 1300h vs 1100 and 1400h, respectively) and mean GH trough values in AS-SRIF treated rats did not fall below 50 ng/ml. Although AS-SRIF administration resulted in an elevation of the mean 6h GH level, the difference was not significant when compared to BL and NSS controls (122.4 ± 21.4 vs 85.6 ± 9.5 and 77.2 ± 10.8 ng/ml respectively; Figure 18). The mean peak GH level after AS-SRIF was 404.2 ± 43.6 ng/ml, not significantly higher than NSS and BL controls, 359.2 ± 62.3

and 434.4 ± 39.2, respectively (Fig. 19). However, the mean trough level of GH was significantly elevated in AS-SRIF-treated animals compared to controls (16.5 ± 5.3 vs 6.6 ± 0.4 and 6.8 ± 0.3 ng/ml; $p < .005$, Fig. 19). No significant difference was observed in the GH secretory pattern between BL and NSS rats.

The mean plasma TSH levels of AS-SRIF and NSS-treated animals are shown in Figure 20. The mean 6h TSH level was increased significantly in AS-SRIF treated animals compared to BL and NSS controls (241.8 ± 0.8 vs 136.2 ± 4.2 and 148.1 ± 7.5 respectively; $p < .001$) as illustrated in Figure 18.

In contrast, no difference was observed in the secretory profiles or mean plasma PRL levels of AS-SRIF and control groups (Fig. 21). The mean 6h PRL level of SRIF-AS-treated rats was 4.9 ± 0.8, not significantly different from BL and NSS animals, 4.3 ± 0.6 and 3.3 ± 0.3 ng/ml, respectively (Fig. 18).

Effects of LH-MFB stimulation and AS-SRIF on GH secretion: Experiment 2

Typical episodic secretion of GH was observed in the non-stimulated baseline control group with peaks at 1115 and 1430 and a trough at approximately 1300h (Fig. 22). LH-MFB stimulation in NSS-treated animals at the initiation of or during a GH peak (1030-1130h and 1430-1530h) resulted in the suppression of GH secretion to trough levels; whereas, stimulation during the trough period (1230-1330h) resulted in the release of GH (Fig. 22). Pretreatment with AS-SRIF antagonized the inhibitory effect of LH-MFB stimulation on GH during peaks but had no effect on stimulation-induced GH release during trough periods (Fig. 22). These results are summarized in Figure 23. Mean plasma GH was significantly lowered below the baseline

control by LH-MFB stimulation during secretory bursts at 1030 and 1430h, an effect that was prevented by AS-SRIF administration. Conversely, mean plasma GH was elevated significantly above baseline by stimulation during the trough period at 1230h in NSS- and AS-SRIF-treated animals with no significant difference between the two treatment groups.

Antiserum to SRIF in plasma

The mean plasma binding capacity of ^{125}I [Tyr^1] SRIF by a 1/20 dilution of plasma from experiment 1 at 7h post-AS-SRIF treatment was 56.5 $\pm$ 1.5 percent, significantly less than at 1h, 64.0 $\pm$ 1.4 percent ($p < .01$, $n=6$). When compared to titers of antiserum used in the RIA, the binding capacities of these samples were equivalent to 1:200 at 1h and 1/620 at 7h. Although the binding capacity at 7h was significantly less, it remained high.

Histological verification of electrodes

Electrode tips were located in LH-MFB region similar to studies previously reported (Terry and Martin, 1978a).

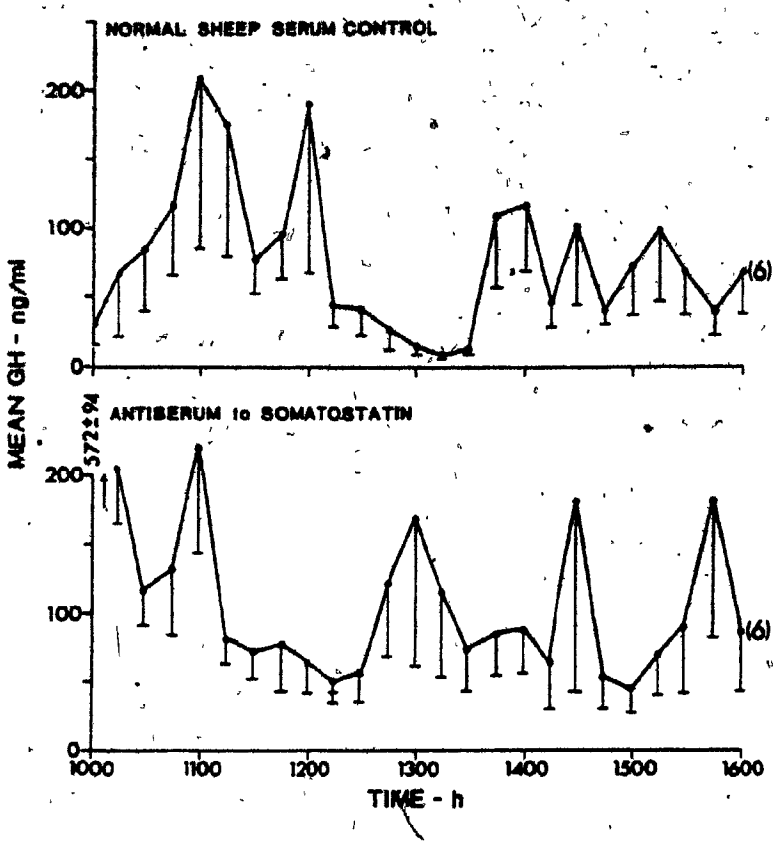

Fig. 1F. Effects of an iv injection of AS-SRIF at 0900h on mean plasma GH in freely-behaving, chronically cannulated male rats. Top, Typical pulsatile GH secretion is evident in NSS-treated rats. Bottom, AS-SRIF results in elevated trough levels. Vertical lines represent SEM. Number of animals in each group is shown in parentheses.

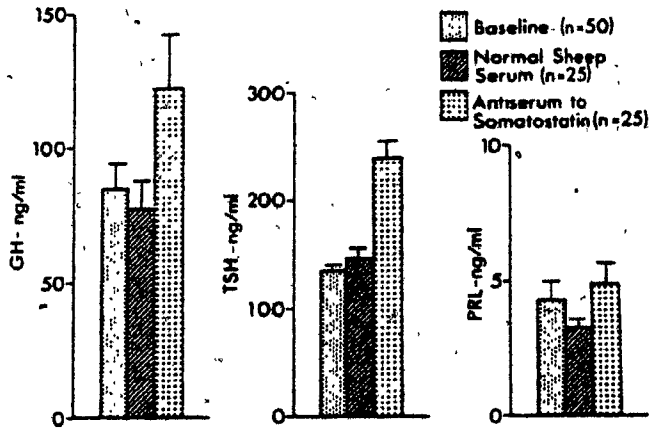

Fig. 18. Mean 6h plasma GH, TSH, and PRL levels in untreated (baseline), NSS-, and AS-SRIF-treated rats. The mean 6h plasma TSH (middle) was increased significantly ($p < .001$) in AS-SRIF-treated animals. Although mean plasma GH (left) was higher after AS-SRIF, the increase was not significant. Mean plasma PRL (right) was unaffected. Numbers of samples in each group are shown in parentheses. Vertical bars represent SEM.

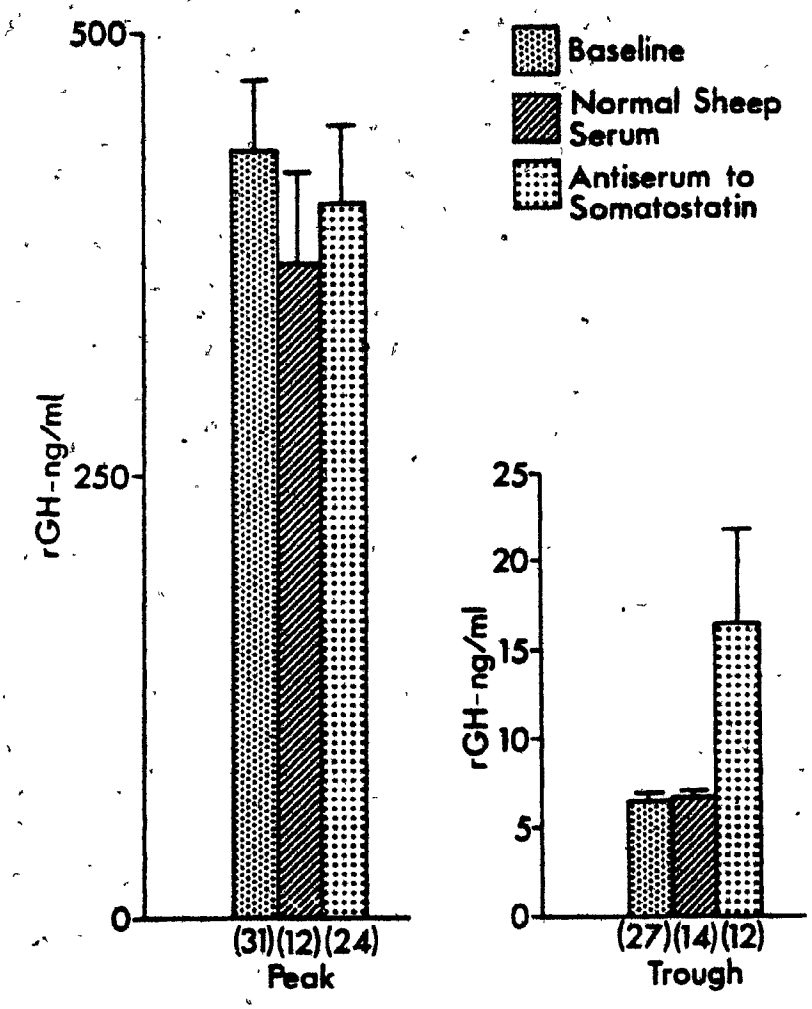

Fig. 19. Effect of AS-SRIF on mean peak and trough GH levels. Left, The mean peak level of GH was not affected by AS-SRIF. Right, The mean trough GH level was increased significantly ($p < .005$) by administration of AS-SRIF. Vertical lines represent SEM. Number of samples in each group is shown in parentheses.

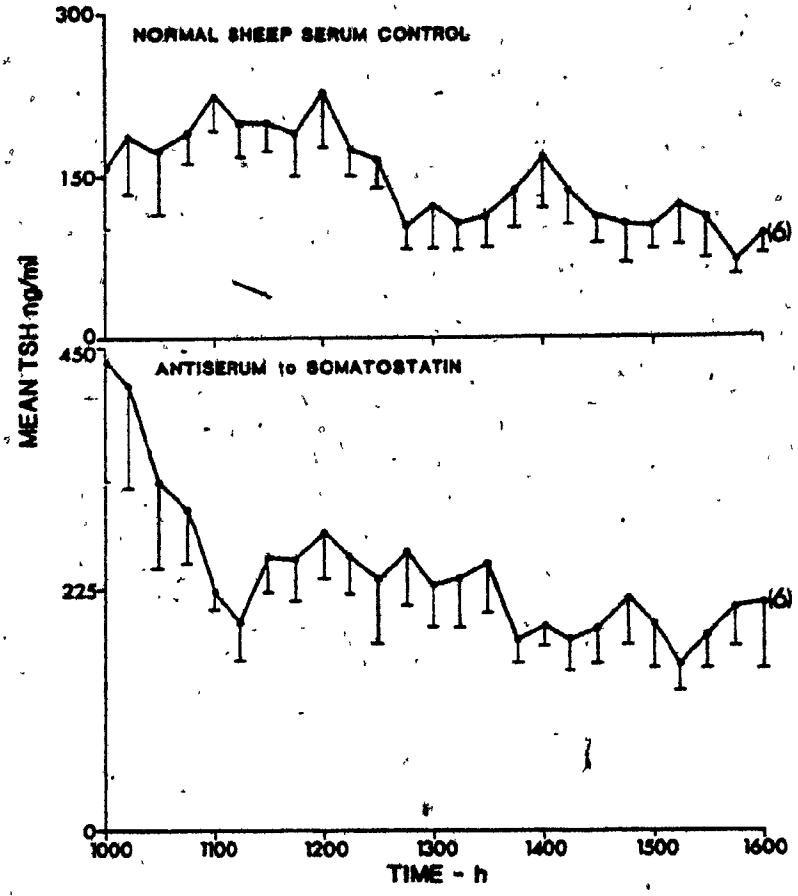

Fig. 20. Effects of an iv injection of AS-SRIF at 0900h on mean plasma TSH in freely-behaving, chronically cannulated rats. Top, Normal 6h TSH secretory pattern in NSS-treated rats. Bottom, Increased TSH secretion in AS-SRIF-treated animals, an effect that is most pronounced 1h after injection. Numbers of animals in each group are shown in parentheses. Vertical bars represent SEM.

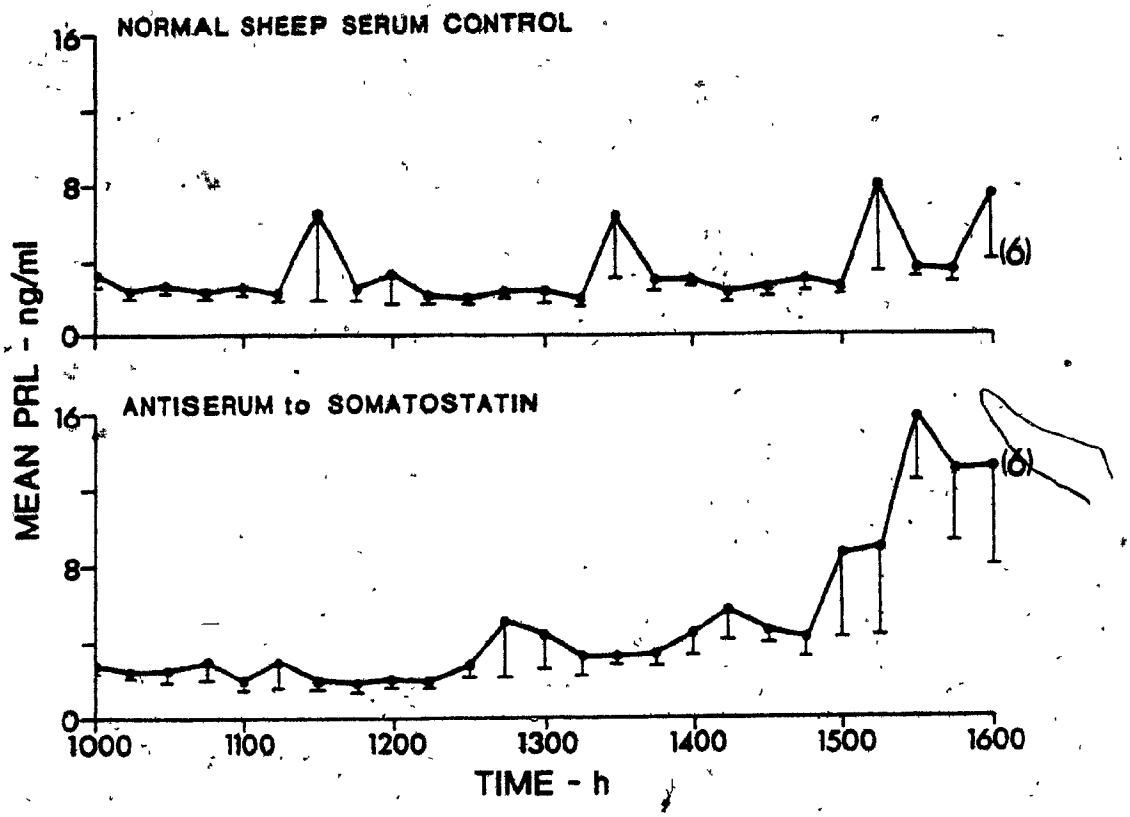

Fig. 21. Effects of an iv injection of AS-SRIF on mean plasma PRL in freely-behaving, chronically cannulated male rats. Top, Normal 6h PRL secretory profile in the NSS-treated group; Bottom, AS-SRIF had no significant effect on the 6h PRL secretory pattern. Vertical bars represent SEM. Numbers in parentheses indicate the number of animals in each group.

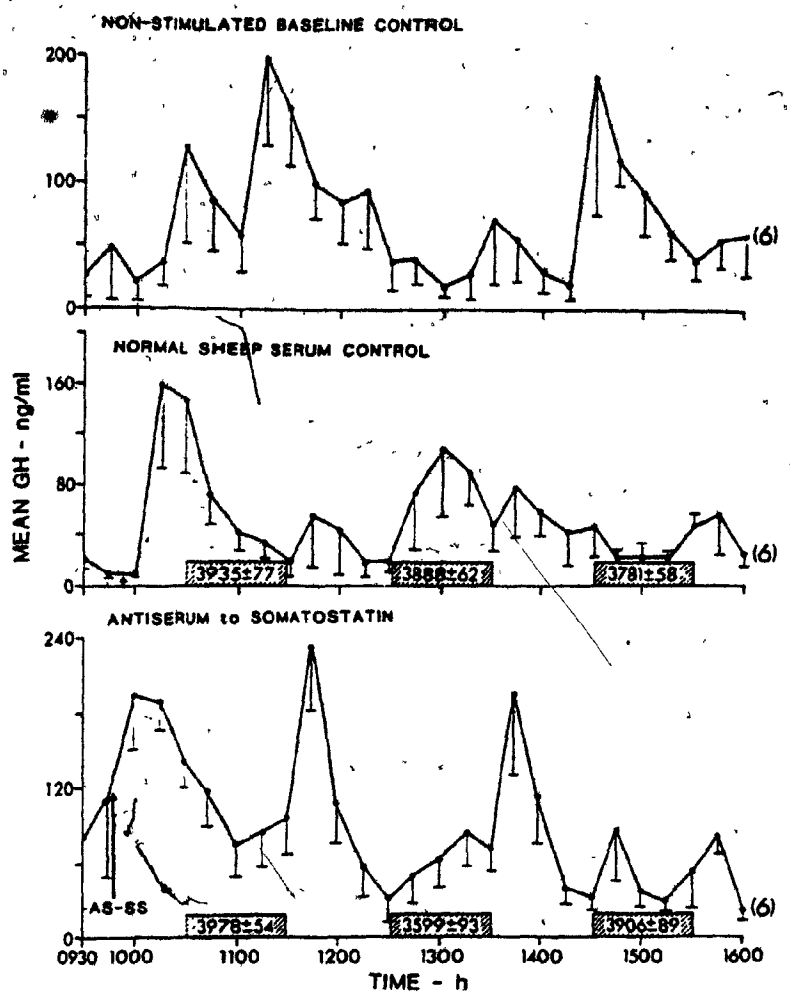

Fig. 22. Effects of LH-MFB stimulation and AS-SRIF on mean plasma GH in freely-behaving, chronically cannulated male rats. Top, Typical pulsatile GH secretion is evident in the non-stimulated baseline control group. Middle, stimulation at 1030 and 1430h suppresses episodic GH; whereas, stimulation at 1230h results in GH release above trough levels. Bottom, pretreatment with AS-SRIF antagonizes stimulation-induced GH suppression at 1030 and 1430h, but has no effect on increased GH secretion resulting from stimulation at 1230h. Arrows indicate the administration of NSS or AS-SRIF at 0950h. Cross-hatched squares represent hourly periods of stimulation and the enclosed number indicate the number of stimulations/h. Vertical bars represent SEM and numbers in parentheses indicate the number of animals in each group.

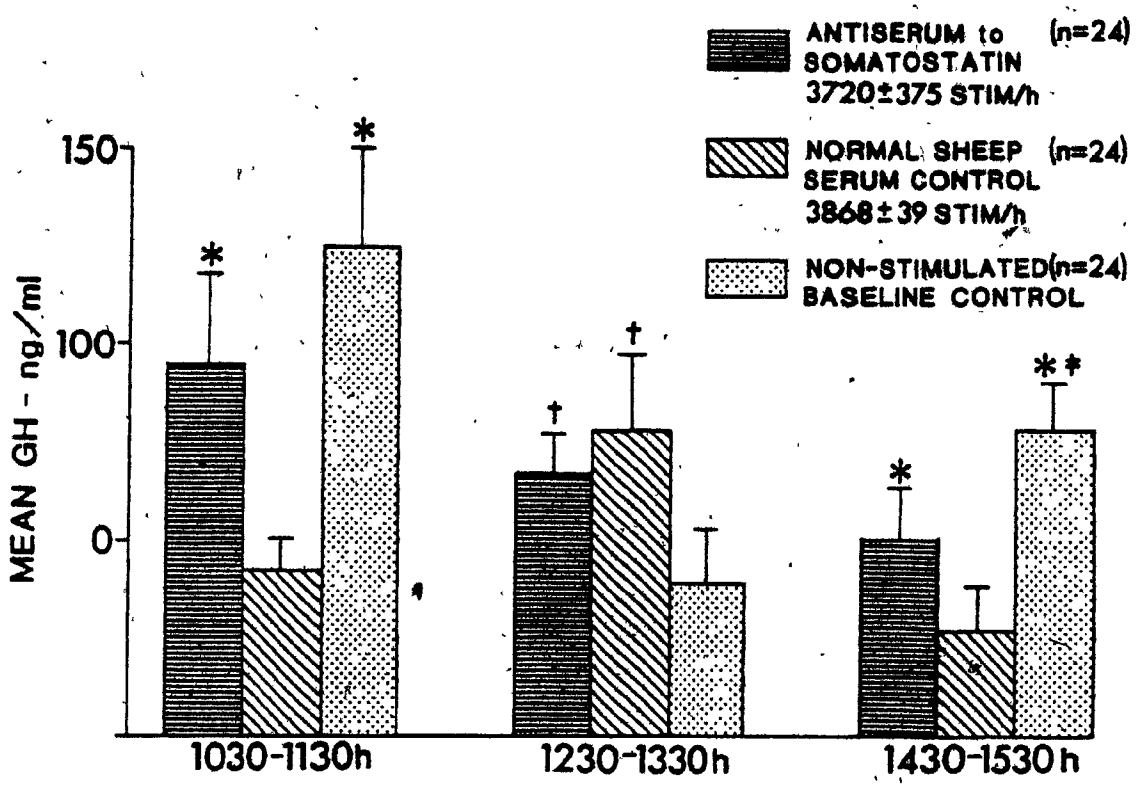

Fig. 23. Effects of AS-SRIF on mean plasma GH during hourly periods of LH-MFB stimulation. Stimulation during secretory episodes (1030-1130 and 1430-1530h) in NSS-treated animals results in suppression of GH (*$p < .001$), an effect reversible by AS-SRIF. The antagonistic effect of AS-SRIF on stimulation-induced GH suppression was less pronounced (ǂ$p < .02$) during the second secretory episode. LH-MFB excitation during a trough period (1230-1330h) induced GH secretion (†$p < .05$); this effect was not influenced by AS-SRIF. Vertical bars represent SEM and numbers in parentheses indicate number of samples.

DISCUSSION

The present study indicates that passive immunization with AS-SRIF increases baseline or trough GH levels in freely-behaving rats without significantly affecting the amplitude of GH secretory episodes and overall mean plasma GH. Several investigators have reported increased basal serum GH in response to circulating AS-SRIF (Ferland et al., 1976, 1977; Terry et al., 1977b; Chihara et al., 1978a), and our results confirm these studies. GH and TSH responses to AS-SRIF require an intact medial basal hypothalamus (Chihara et al., 1978b); therefore, it is most likely that the GH response to AS-SRIF is due to the binding of SRIF released into the portal circulation from the median eminence. Reports differ as to the effect of AS-SRIF on GH secretory bursts and mean plasma GH level. Ferland et al. (1976) observed a 2-3 fold increase in the amplitude of GH secretory peaks after AS-SRIF administration to unanesthetized rats. It is possible that in these experiments sufficient time had not elapsed from surgery to sampling to permit recovery of normal episodic GH secretion. Varner et al. (1980) reported similar effects on GH secretory spikes and an increase in the overall serum GH in sheep immunized against SRIF. In contrast, Steiner et al. (1978) found a marked diminution in episodic GH bursts and no difference in the total integrated 12h GH secretion in baboons after active immunization against SRIF. These conflicting results could be explained by the confounding effects of fasting, cannulation stress, anesthetics, passive (acute) vs active (chronic) immunization against circulating SRIF, species differences in the temporal pattern of GH secretion,

or the variability in GH levels and limited number of animals. Data from the present experiments support the hypothesis that trough periods of diminished GH secretion are the result of episodic SRIF release.

Previous studies from this laboratory showed that electrical stimulation of the LH-MFB delivered by intracranial self-stimulation in the rat resulted in suppression of GH (Terry and Martin, 1978a). The results of the present study suggest that activation of the LH-MFB, an area known to contain ascending monoaminergic pathways (Moore and Bloom, 1978, 1979; Hokfelt et al., 1978), causes rapid inhibition of GH secretion when levels are high. A similar effect on GH was observed after amygdalar stimulation in conscious monkeys (Ehle et al., 1977) and in anesthetized rats (Martin et al., 1972). This effect may have been the result of the stimulation-induced GHRH release and/or inhibition of SRIF release and was not altered by administration of AS-SRIF. Taken together, these results provide evidence to support the hypothesis that GH is regulated by the episodic secretion of at least two hypothalamic bypophysiotropic hormones, one inhibitory and the other excitatory, the inhibitory component of which is mediated by SRIF. The possibility also exists that circulating GH may have feedback effects to regulate cyclicity and that this might influence responses to stimulation of the same brain region.

In this study we also observed that the iv injection of AS-SRIF caused a significant increase in the mean 6h TSH level. There was no discernible effect on episodic TSH secretion. This suggests that SRIF exerts a physiologically important inhibitory effect on TSH secretion. It has been shown that hypothalamic destruction or pretreatment with anti-TRH abolished

the TSH response to AS-SRIF (Chihara et al., 1978); therefore, it appears that the inhibitory effect of SRIF on TSH is dependent on stimulation by TRH. Serum PRL was unaffected by AS-SRIF, suggesting that SRIF is not involved in the regulation of PRL secretion.

It is possible that the decline in both GH and TSH after AS-SRIF was due to a decrease in the neutralizing effectiveness of AS-SRIF after 6 hours of multiple sampling. To effect complete immunoneutralization of circulating SRIF for extended periods, small amounts of AS-SRIF could be administered after removal of each blood sample.

3. CONCLUSIONS

Results of these experiments support the hypothesis that spontaneous rhythmic growth hormone secretion is determined by a combination of excitatory and inhibitory influences, the inhibitory component of which is mediated by somatostatin. Circulating somatostatin antiserum increased the amplitude of growth hormone ebbs, but had no significant effect on the magnitude of growth hormone rises or mean levels. Furthermore, stress- and stimulation-induced growth hormone suppression was prevented by pretreatment with somatostatin antisera, suggesting that this response is mediated by endogenous somatostatin. Interestingly, lateral hypothalamic stimulation during growth hormone ebbs caused growth hormone release. This effect was not augmented by somatostatin antiserum, suggesting it was due to a growth hormone releasing factor or stimulation of such by enhanced catecholamine release.

Although these studies indicate that somatostatin has an important function in growth hormone regulation, they do not define the pathways involved in this process. Hence, the next sequence of experiments (section III,B,1,2,3) were devised to define the site of origin of hypothalamic somatostatin and determine the effects of stress and hypophysectomy on levels of this peptide in discrete hypothalamic and extrahypothalamic sites.

CENTRAL SOMATOSTATINERGIC PATHWAYS INVOLVED IN REGULATION OF GROWTH HORMONE SECRETION

III. B. 1. BIOCHEMICAL MAPPING OF SOMATOSTATINERGIC SYSTEMS IN RAT BRAIN: EFFECTS OF PERIVENTRICULAR HYPOTHALAMIC AND MEDIAL BASAL AMYGDALOID LESIONS ON SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN DISCRETE BRAIN NUCLEI*

ABSTRACT

Immunohistochemical studies have demonstrated the presence of somatostatin (growth hormone release-inhibiting factor)-positive cell bodies in the periventricular nucleus of the hypothalamus and in the medial-basal amygdala. In order to map biochemically the projections of these cell groups, electrolytic lesions were made in these structures and somatostatin was measured by radioimmunoassay in microdissected brain nuclei. Bilateral destruction of the periventricular nucleus significantly decreased somatostatin-like immunoreactivity (SLI) in the median eminence, and in the rostral periventricular, medial preoptic and arcuate nuclei. Bilateral lesions placed in the medial-basal amygdala significantly decreased SLI in the median eminence and suprachiasmatic nucleus. Similar depletions were observed following lesions of the stria terminalis. These results suggest that both the periventricular and amygdaloid somatostatin systems may participate in the regulation of growth hormone secretion via their projections to the median eminence and other hypothalamic nuclei.

*Crowley and Terry, 1981a

INTRODUCTION

There is substantial evidence that somatostatin, (growth hormone release-inhibiting factor) a tetradecapeptide present in brain and in several peripheral tissues, constitutes an important regulatory influence on the secretion of growth hormone (GH) from the anterior pituitary gland. Somatostatin suppresses the normal pulsatile release of GH (Martin et al., 1974) and antagonizes the discharge of GH elicited by a variety of experimental manipulations in rats and other species (Martin, 1976; Martin et al., 1978a). It is likely that somatostatin interacts with other neural and hormonal systems in the overall physiological control of GH secretion.

There are numerous reports on the regional localization of somatostatin within the central nervous system, using immunocytochemical techniques (Alpert et al., 1976; Baker and Yu, 1976; Dube et al., 1975; Elde and Parsons, 1975; Hokfelt et al., 1975, 1978; Parsons et al., 1976; Setalo et al., 1975) and bioassay or radioimmunoassay of microdissected brain areas (Browstein et al., 1975; Epelbaum et al., 1975; Palkovits et al., 1976; Rorstad et al., 1979b). With these approaches, the highest concentrations of somatostatin-like immunoreactivity (SLI) are observed in the median eminence, and lower, but significant, amounts are also present within the preoptic area, medial basal hypothalamus and amygdala. Immunohistochemical studies have shown that these regions predominantly contain somatostatin-positive fibers and nerve terminals, while SLI-containing cell bodies appear to be clustered within the periventricular hypothalamic nucleus, medial, cortical and basomedial amygdaloid nuclei, zona incerta, interstitial nucleus of the stria terminalis

and hippocampus (Alpert et al., 1976; Baker and Yu, 1976; Dube et al., 1975; Elde and Parsons, 1975; Hokfelt et al., 1975, 1978; Parsons et al., 1976; Setalo et al., 1975).

It has been suggested that the somatostatin in the median eminence derives from cells located more rostrally because large lesions of the preoptic area and anterior hypothalamus markedly decrease somatostatin in this structure (Critchlow et al., 1978; Epelbaum et al., 1977b). Moreover, total or frontal deafferentation of the medial basal hypothalamus reduces SLI within this fragment (Brownstein et al., 1977; Epelbaum et al., 1977b). It is unclear at present whether these effects are due to destruction of the periventricular hypothalamic somatostatin system or to interruption of somatostatin-containing fibers from other forebrain structures, or both.

The objective of the present investigation was to define more precisely the specific projection patterns of the anterior periventricular hypothalamic and medial-basal amygdaloid somatostatin-positive cell groups by employing the biochemical mapping approach (Kobayashi et al., 1974; O'Donohue et al., 1979). To this end, discrete lesions were placed in these areas, according to stereotaxic somatostatin mapping (Hokfelt et al., 1978), and SLI was determined in individual brain nuclei that were microdissected from the hypothalamus and other forebrain structures.

METHODS

Animals

Adult, male Sprague-Dawley rats, 200-250 g body weight, were obtained from Harlan Industries (Indianapolis, Ind.). Animals were housed in groups of 5 and maintained on a 14h light, 10h dark schedule and ad lib food and water.

Surgery

Animals were anesthetized with chloral hydrate and placed in a Stoelting stereotaxic apparatus, with the top of the incisor bar 23 mm from the surface of the animal platform. In one experiment, bilateral electrolytic lesions were placed in the periventricular hypothalamic nucleus at the level of the periventricular nucleus. This corresponds to the location of periventricular cells that display somatostatin-like immunofluorescence (Hokfelt et al. 1978). The coordinates were 6.8 mm anterior to the interauricular line; 7.0 mm ventral to the dural surface, and the electrode was lowered in the mid-line. The lesions were made by passing 2 mA current from a Pulsar 6bp stimulator (Frederick Haer, Brunswick, Me.) for 5 sec to a 24-gauge platinum-iridium electrode that had a 1 mm tip exposed. For sham-operated animals, the electrode was lowered to 4 mm beneath the dural surface and no current was passed.

In a second experiment, bilateral electrolytic lesions were placed either in the medial-basal amygdaloid area or in the stria terminalis. The amygdaloid placement corresponds to the location of cells showing somatostatin-like immunofluorescence (Hokfelt et al., 1978), while lesions of the stria

terminalis interrupt a major amygdaloid efferent pathway. For the amygdaloid placement, the coordinates were 6.0 mm anterior; 3.3 mm lateral and 8.0 mm ventral. For the stria terminalis placement, the coordinates were 6.0 mm anterior, 3.4 mm lateral and 3.8 mm ventral. For each lesion, 8 mA current was passed for 5 sec. Sham-operated animals had the electrode lowered just dorsal to the medial amygdala, and no current was passed.

Microdissection of brain regions

After one week's recovery from surgery, all animals were decapitated and their brains removed rapidly and frozen with powdered dry ice. Serial coronal sections, 300 μm thick, were cut in a microtome cryostat at -7°C and frozen onto microscope slides. Sections of 50 μm thickness were cut through the extent of the lesion and stained with thionin for histological evaluation. Only animals bearing correctly placed lesions were used for further study. Individual brain nuclei were removed with stainless steel cannulae as described previously (Palkovits et al., 1975). Table IV provides further details of the microdissection procedure. Tissue specimens were expelled into 50 μl of ice cold 1 N HCl and sonicated for approximately 3 sec. Aliquots of 5-10 μl were removed for analysis of protein content (Lowry et al., 1951). The remaining homogenate was stored at -90°C until somatostatin was measured.

Somatostatin radioimmunoassay (RIA)

The somatostatin content of tissue samples was determined by a specific RIA as described elsewhere (Rorstad et al., 1979a,b). Synthetic cyclic somatostatin and Tyr^{11}-somatostatin were purchased from Bachem (Torrance,

Calif.). The purity of each peptide was verified by high pressure liquid chromatography (courtesy of Dr. D. Desiderio) and amino acid analysis (courtesy of Dr. A. Kang). Tyr^{11}-somatostatin was radioiodinated by a modification (Rorstad et al., 1979) of the method of Greenwood et al. (1963) and stored in aliquots at -90°C. The radioiodinated product was purified by ion-exchange chromatography on a column of carboxymethyl cellulose (CM-52, Whatman, Clifton, N.J.) by a previously described method (Arimura et al., 1975; Rorstad et al., 1979a,b). Supernatants of the homogenates were neutralized with 1 N sodium hydroxide and 0.1 M sodium phosphate. Median eminence extracts were diluted 1:15 in the assay buffer, and the other regions were measured undiluted. Samples of 10 µl were assayed for SLI in triplicate. Individual samples from the same region of lesioned and sham-operated animals were measured for SLI in the same assay to eliminate between-assay variation.

Sheep B-antisomatostatin (Rorstad et al., 1979a,b) bound 32.8 ± 2.1% of ^{125}I-labeled Tyr^{11} somatostatin at a final dilution of 1:150,000. The minimal detectable concentration of the RIA was 10.2 pg/tube. The within-assay coefficients of variation for samples that contained means of 78 and 242 pg/tube somatostatin were, respectively, 8.1 (n = 12) and 12.8 (n =12). The between-assay coefficients of variation of samples that contained means of 52, 106 and 194 pg/tube were 12.4, 10.6 and 11.8, respectively (23 assays). There was no interference by other hypothalamic peptides (Rorstad et al., 1979). The displacement curve of median eminence extracts was shown to be parallel to the standard curve, indicating immunologic similarity. It has been shown previously that SLI from acid extracts of median eminence, anterior hypothalamus, and amygdala are biologically active (Rorstad et al., 1979a,b).

RESULTS

Experiment 1: effects of periventricular hypothalamic lesions

Table V presents somatostatin concentrations in 12 brain nuclei from sham-operated animals and animals with periventricular lesions. These data were analyzed by unpaired Student's t-tests. Bilateral destruction of the periventricular nucleus significantly decreased the levels of somatostatin-like immunoreactivity in the median eminence (-72 percent), in the arcuate (-50 percent) and medial preoptic nuclei (-33 percent), and in the periventricular nucleus immediately rostral to the lesion (-44 percent). No changes in somatostatin concentration were noted in the ventromedial nucleus, or in the other telencephalic and mesencephalic structures dissected.

Fig. 24A depicts a typical anterior periventricular lesion. In the animals used in this study, damage was largely limited to the periventricular nucleus, with little or no involvement of the adjacent medial preoptic, paraventricular or anterior hypothalamic nuclei or the median eminence.

Experiment 2: effects of medial-basal amygdaloid and stria terminalis lesions

Table VI presents somatostatin concentrations in 8 brain nuclei after medial-basal amygdaloid, stria terminalis or sham lesions. Lesions of the medial amygdala significantly reduced somatostatin-like immunoreactivity in the median eminence (-36 percent) and in the suprachiasmatic nucleus (-28 percent). These effects were mimicked by transection of the stria terminalis (Table VI). However, neither lesion affected the somatostatin content of the

caudate, interstitial stria terminalis, medial preoptic, periventricular, ventromedial or arcuate nuclei.

Fig. 24b,c shows the extent of tissue damage following typical medial-basal amygdaloid and stria terminalis lesions. The amygdaloid lesions usually destroyed most of the medial, basomedial and cortical amygdaloid nuclei and occasionally damaged parts of the central and lateral amygdaloid nuclei. Lesions of the stria terminalis tended to be smaller, and usually involved the adjacent fimbria, internal capsule and dorsolateral thalamus.

Table IV

Microdissection of discrete brain regions

Brain Region	No. Punches/brain	Cannula size (mm)	Approximate coordinates (μM)*
N. caudatus	2	1.0	A8920
N. accumbens	4	0.5	A8920
N. interstitialis striae terminalis	4	0.5	A6860
N. preopticus medialis	2	0.75	A6860
N. periventricularis	4	0.5	A6360, A6060
N. suprachiasmaticus	4	0.3	A6360, A6060
N. ventromedialis	4	0.5	A4620
N. arcuatus	6	0.3	A4620-A4110
Median eminence	3	0.5	A4620-A4110
N. amygdaloideus medialis	4	0.5	A4620, A4380
N. amygdaloideus centralis	4	0.5	A4620, A4380
N. interpeduncularis	2	0.75	A1.8, A1.4
Central gray	4	0.5	A1.8, A1.4

*(Based on refs.: Jacobowitz and Polkovits 1974; Palkovits and Jacobowitz 1974)

Table V

Effects of lesions in the periventricular nucleus on somatostatin-like immunoreactivity (SLI) in selected brain nuclei in male rats

Brain region	SLI (pg/µg protein ± S.E.M.)	
	Sham (n = 9)	Lesion (n = 9)
N. caudatus	2.4 ± 0.5	3.0 ± 0.25
N. accumbens	4.4 ± 0.9	5.3 ± 0.7
N. interstitialis striae terminalis	15.1 ± 1.4	13.3 ± 1.2
N. preopticus medialis	14.5 ± 2.0	9.7 ± 1.3*
N. periventricularis	21.2 ± 2.7	11.8 ± 1.8**
N. ventromedialis	25.1 ± 5.1	18.6 ± 2.4
N. arcuatus	81.2 ± 13.1	41.0 ± 3.8**
Median eminence	599.2 ± 98.6	169.5 ± 45.8***
N. amygdaloideus medialis	12.3 ± 1.3	9.2 ± 1.4
N. amygdaloideus centralis	10.4 ± 1.5	11.4 ± 1.0
N. interpeduncularis	1.3 ± 0.1	2.0 ± 0.6
Central gray	5.5 ± 0.7	6.5 ± 0.8

*P < 0.05; **P < 0.01; ***P < 0.001 vs sham, based on Student's t-tests.

Table VI

Effects of medial-basal amygdaloid or stria terminalis lesions on somatostatin-like immunoreactivity (SLI) in selected brain nuclei in male rats

Brain region	SLI (pg/µg protein ± SEM)		
	Sham (n = 10)	Amygdala lesion (n = 13)	Stria terminalis lesion (n = 7)
N. caudatus	6.4 ± 0.7	6.9 ± 0.5	6.3 ± 1.0
N. interstitialis stria terminalis	20.8 ± 1.0	19.6 ± 1.5	18.2 ± 2.5
N. preopticus medialis	20.9 ± 4.8	28.3 ± 4.5	19.4 ± 4.7
N. periventricularis	46.6 ± 4.6	46.8 ± 4.5	40.5 ± 4.6
N. suprachiasmaticus	18.1 ± 2.0	13.0 ± 1.4*	12.7 ± 1.1*
N. ventromedialis	64.5 ± 7.3	53.4 ± 5.9	61.6 ± 8.9
N. arcuatus	223.1 ± 18.1	181.6 ± 24.4	266.8 ± 10.0
Median eminence	899.4 ± 89.9	575.1 ± 79.4*	584.4 ± 74.7*

*P < 0.05 vs sham, based on single factor analysis of variance and Newman-Keuls tests.

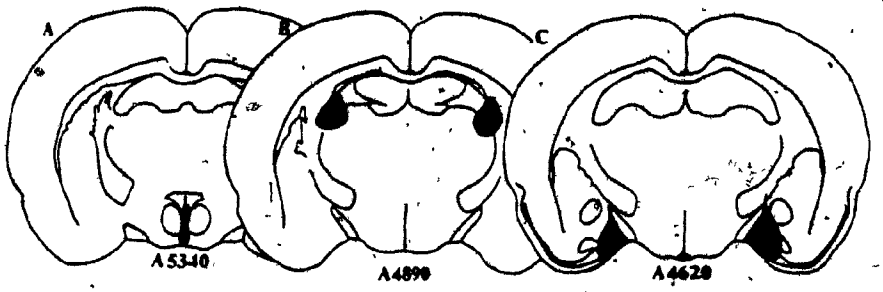

Fig. 24. Schematic representations of typical lesions placed in the periventricular hypothalamic nucleus (A), stria terminalis (B) and medial-basal amygdala (C). Coronal sections were redrawn from Jacobowitz and Palkovits (1974).

DISCUSSION

The results of the present study indicate that the hypothalamic somatostatin-positive cell group, demonstrated immunohistochemically to be in the periventricular nucleus (Alpert et al., 1976; Hokfelt et al., 1978), appears to be the the source of approximately two-thirds of the somatostatin in the median eminence and of approximately 50 percent of the somatostatin in the arcuate nucleus. These findings suggest that the decrease in SLI observed in the median eminence after large hypothalamic lesions or frontal deafferentation in earlier studies (Brownstein et al., 1977; Critchlow et al., 1978; Epelbaum et al., 1977b) may be attributable in part to destruction of this periventricular system. However, the use of the microdissection technique in the present study has also revealed that the somatostatin present in the adjacent ventromedial nucleus probably derives from other sources because neither periventricular nor amygdala lesions affected the SLI of this structure. However, it still cannot be excluded that more extensive damage to the anterior hypothalamus might decrease further the SLI in this area.

The present data also raise the possibility that these periventricular cells send projections in the anterior direction because their destruction significantly decreased SLI in the periventricular nucleus rostral to the lesion and in the medial preoptic nucleus. This is consistent with the results of previous studies (Epelbaum et al., 1977b), showing that ventromedial hypothalamic lesions or deafferentation of the medial-basal hypothalamus lower the somatostatin content of the preoptic area.

The present study is the first report on the possible projections of the immunohistochemically identified medial-basal amygdaloid somatostatin-positive cell group. It was originally hypothesized that lesions of this region would decrease SLI in the interstitial nucleus of the stria terminalis, medial preoptic and ventromedial nuclei, areas known to receive amygdaloid efferents from the stria terminalis (De Olmos and Ingram, 1972). However, no decreases of SLI were observed in these areas following amygdaloid or stria terminalis lesions, and identification of their sources of somatostatin requires further investigation. It was unexpected that bilateral destruction of the medial amygdala would decrease SLI in the median eminence and suprachiasmatic nucleus. To the author's knowledge, there are no reports of amygdaloid projections to these regions, and therefore, the present results should be interpreted with caution. However, similar depletions of SLI in these nuclei were observed following interruption of the stria terminalis, implicating this tract as the route for these putative somatostatinergic amygdalo-hypothalamic projections. It is suggested that the previously described (Brownstein et al. 1977; Critchlow et al., 1978; Epelbaum et al., 1977b) effects on somatostatin of rostral hypothalamic lesions and medial-basal hypothalamic deafferentation may be due in part to interruption of fibers of amygdaloid origin, as well as to periventricular hypothalamic damage.

It is interesting to note that besides somatostatin, there is evidence that the stria terminalis carries met-enkephalin, neurotensin (Uhl et al., 1978), and α-melanotropin-containing (O'Donohue et al., 1979) fibers. Thus, this tract may contain several peptidergic systems involved in limbic-hypothalamic function.

The present findings that both the amygdala and periventricular nucleus contribute somatostatin to the median eminence, where presumably it is released to affect GH secretion, is consistent with studies on the involvement of these brain regions in control of growth hormone (GH) secretion. For example, GH secretion is facilitated by medial preoptic lesions and is diminished by stimulation of this area (Epelbaum et at., 1977b; Martin et al., 1975). Basal GH release is also decreased by stimulation of the medial amygdala (Martin, et al., 1974b). In addition, the suppression of GH secretion by stress is prevented by either medial preoptic lesions (Rice et al., 1978) or by passive immunization with antisomatostatin (Arimura et al., 1976; Terry et al., 1976b). Therefore, it is possible that these experimental manipulations affect GH release by interacting with the somatostatinergic systems tentatively identified in the present study. Further experiments are in progress to test this possibility and to determine the origin and functional significance of the somatostatin present in other forebrain structures.

III. B. 2. THE EFFECT OF HYPOPHYSECTOMY ON SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN DISCRETE HYPOTHALAMIC AND EXTRAHYPOTHALAMIC NUCLEI*

ABSTRACT

Several hypothalamic and extrahypothalamic sites that have high concentrations of somatostatin-positive nerve terminals and/or cell bodies are important in the regulation of GH secretion. GH is capable of inhibiting its own secretion under certain prescribed conditions, and a short loop feedback regulatory mechanism may involve somatostatinergic pathways. The purpose of this investigation was to determine the effect of removal of GH by hypophysectomy on the content of somatostatin-like immunoreactivity (SLI) in discrete hypothalamic and extrahypothalamic nuclei.

Individual nuclei were removed from frozen brain sections of hypophysectomized and sham-operated male rats. The tissue content of somatostatin was determined by a specific RIA. The content of SLI in the median eminence of hypophysectomized animals was significantly reduced by 38 percent, compared to sham-operated controls (278 $\pm$ 53.2 vs 447.0 $\pm$ 57.4 pg/µg protein, respectively). Significant reductions of SLI in the medial preoptic (50 percent), arcuate (33 percent), and periventricular (30 percent) nuclei were also observed in hypophysectomized animals when compared to controls (10.2 $\pm$ 1.6 vs 20.0 $\pm$ 3.0; 60.2 $\pm$ 8.2 vs 89.8 $\pm$ 13.3; and 19.4 $\pm$ 1.8 vs 27.8 $\pm$ 3.1 pg/µg protein, respectively). No significant changes were detected in the ventromedial, suprachiasmatic, medial, central, or cortical amygdaloid nuclei nor in the nucleus interstitialis striae terminalis.

These data suggest that GH may exert a feedback effect on specific hypothalamic nuclei that involves somatostatin-containing systems.

*Terry and Crowley, 1980a.

INTRODUCTION

The secretion of GH is precisely regulated by both stimulatory and inhibitory neural influences. This control is achieved by at least two hypothalamic hormones: GH-releasing factor, the structure of which is still unknown, and the structurally identified tetradecapeptide GH-releasing inhibiting factor (somatostatin) (Martin et al., 1978a; Martin, 1976). Administration of specific antiserum to somatostatin to unanesthetized rats elevates basal GH secretion and partially reverses stress-induced GH inhibition (Arimura et al., 1976; Ferland et al., 1976; Terry et al., 1976b, 1977). These findings support a role of somatostatin in physiological GH secretion.

Evidence reviewed elsewhere (Martin, 1976) indicates that under certain prescribed conditions, GH also is capable of inhibiting its own secretion. Administration of GH, implantation of GH-secreting tumors, or direct hypothalamic placement of GH pellets reduce pituitary GH levels. These effects occur over a rather prolonged time course and may indicate only that the setpoint of the GH regulatory system is sensitive to circulating levels of GH. However, acute experiments have also shown that pharmacologically stimulated GH secretion can partially block the subsequent GH response to a second stimulus (Martin, 1976).

These inhibitory effects of GH may involve an interaction with somatostatinergic systems. Several hypothalamic and extrahypothalamic sites that are important for the regulation of GH secretion contain high

concentrations of somatostatin-positive cell bodies and/or nerve terminals (Martin, 1976; Martin et al., 1978a). An increase in hypothalamic somatostatin content occurs after GH administration to normal rats (Kanatsuka et al., 1979), and intracerebroventricular injections of GH in rats release somatostatin into the hypothalamic portal blood (Chihara, 1979). Furthermore, hypothalamic fragments incubated in vitro release somatostatin after exposure to GH (Sheppard et al., 1978).

If a short loop feedback regulation of GH involves an interaction with somatostatin, then removal of GH by hypophysectomy should affect the content of somatostatin in the brain. Hypophysectomy results in depletion of somatostatin in the hypothalamus, septum, and preoptic areas (Baker and Yen, 1976; Wakabayashi et al., 1976; Ferandaz-Durango et al., 1978; Berelowitz et al., 1978), an effect reversible by administration of GH (Hoffman and Baker, 1977). However, the localization of these effects to specific nuclei within these regions remains undefined. The purpose of the present study was to determine the effects of hypophysectomy on the concentration of somatostatin in individual nuclei of the hypothalamus and telencephalon.

MATERIALS AND METHODS

Hypophysectomized (n = 10) and sham-operated control (n = 11) male Sprague-Dawley rats (200 g) were purchased from Zivic-Miller Laboratories (Allison Park, PA). Animals were housed in groups of four and maintained under constant temperature (24°C) and a 14h light, 10h dark cycle with lights on at 0600h. All animals were given rat chow and a solution that contained 35 mM NaCl, 1.1 mM KCl, 0.32 mM CaCl, and 0.09 mM MgCl, ad libitum.

Dissection of the brain

Two weeks after surgery hypophysectomized and sham-operated rats were alternately sacrificed by decapitation, and their brains were immediately removed and snap frozen on powdered dry ice. In order to define precisely the localization of somatostatin within the various regions of the brain, a microdissection procedure was used. Serial coronal sections, 300 μm in thickness, were cut in a cryostat at -7C. Individual brain nuclear regions were removed from the frozen sections with hollow stainless steel needles under a stereomicroscope, according to the method of Palkovits (1975). Dissections of the regions have been described elsewhere (Crowley et al., 1978), and details of the microdissection are presented in Table VII. For determination of somatostatin, tissue areas punched from the frozen sections were expelled into 50 μl ice cold 1 N HCl and sonicated for approximately 3 sec with a Kontes Micro-Ultrasonic Cell Disruptor (Kontes Co., Vineland, N.J.). After sonication, 12-μl (arcuate and suprachiasmatic nuclei) or 5-μl (all other nuclear regions) aliquots of each sonicate were removed for determination of protein by the method of Lowry et al. (1951), using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as the standard. The sonicates were then centrifuged in a Beckman Microfuge (Houston, TX) for 2 min, and the supernatants were removed and stored at -90 C. Total hypophysectomy was confirmed by careful inspection of the sella turcica, failure to gain weight over the 2-week period, and measurement of serum GH with materials supplied by the NIAMDD.

Assay of somatostatin

The somatostatin content of tissue samples was determined using a highly specific and sensitive antiserum to somatostatin, as described elsewhere (Rorstad et al., 1979a,b). Synthetic cyclic somatostatin and [Tyr^{11}] somatostatin were purchased from Bachem, Inc. (Torrance, CA). The purity of each peptide was verified by high pressure liquid chromatography (courtesy of Dr. D. Desiderio) and amino acid analysis (courtesy of Dr. A. Kang). [Tyr^{11}]somatostatin was radioiodinated by a modification of the method of Greenwood et al. (1963) and stored in aliquots at -90 C. To 25 µl 0.5 M sodium phosphate buffer, pH 7.4, were added 50 µl 5.78 x 10^{-5} M (10 µg) [Tyr^{11}]somatostatin in 0.002 M ammonium acetate buffer, pH 4.6 Two mCi Na^{125}I (100 mCi/ml) were added, followed by 25 µl freshly prepared 6.59 x 10^{-4} M chloramine-T (0.15 mg/ml) dissolved in 0.05 M sodium phosphate buffer, pH 7.4. After a reaction time of 40 sec at room temperature, 50 µl 1.3. x 10^{-3} M sodium metabisulfite (0.25 mg/ml), dissolved in 0.05 M sodium phosphate buffer, pH 7.4, were added. This was followed, after an additional 10 sec, by 100 µl 0.1 percent (wt/vol) human serum albumin (Cohn fraction V; Sigma Chemical Co., St. Louis, MO), dissolved in 0.1 M sodium phosphate buffer, pH 7.4. The radioiodinated product was purified by ion exchange chromatography on a column (0.7 x 14 cm) of carboxymethyl cellulose (CM-52; Whatman, Inc., Clifton, NJ) by a previously described method (Arimura et al., 1975; Epelbaum et al., 1977a).

To compare the binding and displacement characteristics of ^{125}I-labeled [Tyr^{11}]somatostatin to ^{125}I-labeled [Tyr^{1}]somatostatin, as used in previous RIAs (Rorstad et al., 1979b; Terry et al., 1981a),

[Tyr^1]somatostatin was radioiodinated by methods described elsewhere (Rorstad et al., 1979b).

Supernatants of the sonicates were neutralized with 1 N sodium hydroxide and 0.1 M sodium phosphate buffer immediately before introduction into the assay. Median eminence extracts were diluted 1:15 in the assay buffer, and the other regions were measured undiluted. Samples of 10 µl were assayed for somatostatin-like immunoreactivity (SLI) in triplicate.

Aliquots of median eminence extracts were also assayed using the ^{125}I-labeled [Tyr^1]somatostatin and a 1:25,000 final dilution of sheep B antisomatostatin (Rorstad et al., 1979b). Individual samples from the same nuclear region of hypophysectomized and sham-operated animals were measured for SLI in the same RIA to eliminate between-assay variation. RIA was performed using a 1:150,000 final dilution of sheep antisomatostatin (Sheep B) with an assay buffer of 0.1 M sodium phosphate, pH 7.2, which contained 0.01 M EDTA, 0.05 M NaCl, sodium azide (0.1 percent wt/vol), and human serum albumin. Standards and unknown samples were incubated for 24 h at 4°C before the addition of approximately 12,000 cpm ^{125}I-labeled [Tyr^{11}]- or ^{125}I-labeled [Tyr^1]somatostatin in a final volume of 0.4 ml. The reaction mixture was incubated an additional 24 h before the addition of a 1:400 final dilution of nonimmune sheep serum and an appropriately diluted portion of burro antiserum raised against sheep gammaglobulin, at a dilution determined experimentally to yield an easily visualized pellet and a satisfactory precipitation of antisomatostatin. After an additional 18-24 h incubation at 4 C, the tubes were centrifuged and the radioactivity in the pellet was determined.

To assess if peptidase activity was eliminated after the extraction procedure, 100 pg of synthetic cyclic somatostatin was added to supernatants of tissue sonicates and assayed for somatostatin. In addition, median eminence extracts were reassayed for SLI 3 months after the initial determinations because the concentration of SLI in 0.1 M HCl extracts has been shown to decline with storage at -20 C over several weeks to months (Rorstad et al. 1979b).

The values were pooled by the treatment groups and means and SEs were calculated. The mean concentration of SLI in each brain area was expressed as picograms of SLI per μg protein ± SE. Statistical analysis of the data was performed by the unpaired Student's t-test.

RESULTS

The adequacy of hypophysectomy was demonstrated by total failure of weight gain over the 2-week period, absence of recognizable pituitary tissue in the pituitary fossa post mortem, and disappearance of GH from the serum.

Somatostatin RIA

Sheep B antisomatostatin bound 32.8 ± 2.1 percent of ^{125}I-labeled [Tyr^{11}]somatostatin at a final dilution of 1:150,000. The label was stable up to 4 weeks when stored in aliquots at -90 C. The minimal detectable concentration of the RIA, defined as the concentration of somatostatin that resulted in a binding of ^{125}I-labeled [Tyr^{11}]somatostatin to antiserum that was 2 SD below the mean binding achieved in the absence of somatostatin,

was 10.2 pg/tube (mean of 23 assays). The within-assay coefficients of variation for samples that contained means of 78 and 242 pg/tube somatostatin were, respectively, 8.1% (12 determinations) and 12.8% (12 determinations). The between-assay coefficients of variation of samples that contained means of 52, 106, and 194 pg/tube were 12.4%, 10.6%, and 11.8%, respectively (23 assays). The somatostatin RIA did not exhibit any cross-reactivities with a wide variety of hypothalamic peptides examined up to 10 µg/ml. Linear regression lines relating logit B/B_0 and the natural logarithms of the mass of synthetic somatostatin per tube and serial dilutions of the HCl extract of the median eminence using the ^{125}I-labeled [Tyr^{11}]somatostatin were parallel (somatostatin: $r = 0.992$; $y = 1.05x + 3.61$; median eminence extract: $r = 0.983$; $y = -1.1x + 4.15$), indicating immunological similarity. The standard curve and displacement curve of median eminence extracts using the ^{125}I-labeled [Tyr^{1}]somatostatin were also shown to be parallel (somatostatin: $r = -0.970$; $y = 1.07x + 4.21$; median eminence extract: $r = -0.999$; $y = 0.99x + 5.12$), in agreement with studies described elsewhere (Rorstad et al., 1977a,b). In addition, the standard curves for somatostatin using ^{125}I-labeled [Tyr^{11}]- or [Tyr^{1}]somatostatin were parallel.

The recovery of synthetic cyclic somatostatin from supernatants of tissue extracts was 98.6 percent $\pm$ 7.91 percent ($n = 10$), indicating that peptidase activity was eliminated after the extraction procedure. Moreover, there was no change in SLI in median eminence extracts reassayed 3 months after the initial determinations (sham operated controls: 462.8 ± 80.4 vs. 447.0 ± 57.4 pg/µg protein; hypophysectomized: 272.6 ± 52.6 vs. 278.8 ± 53.2; $n = 10$, $p > 0.2$).

Effects of hypophysectomy

The effect of hypophysectomy on SLI in selected hypothalamic and extrahypothalamic nuclear regions is shown in Table VIII. The content of SLI in the median eminence of hypophysectomized animals was significantly lowered by 38 percent, compared to sham-operated controls. A significant reduction of SLI in the medial preoptic (50 percent), arcuate (33 percent), and periventricular (30 percent) nuclei was also observed in hypophysectomized animals when compared to control rats. No significant changes were detected in the ventromedial, suprachiasmatic, medial amygdaloid, central amygdaloid, or cortical amygdaloid nuclei nor in the nucleus interstitialis striae terminalis.

To confirm these findings, SLI was measured in median eminence extracts from hypophysectomized and sham-operated animals using ^{125}I-labeled [Tyr^{1}]somatostatin, as described earlier (Rorstad et al., 1979b; Terry et al., 1980). The median eminence concentrations of SLI did not differ significantly ($n = 10$, $P > 0.1$) in the two RIAs comparing ^{125}I-labeled [Tyr^{1}]- to ^{125}I-labeled [Tyr^{11}]somatostatin (hypophysectomized: 243.2 $\pm$ 36.6 vs. 272.6 $\pm$ 52.6 pg/µg protein, respectively; sham-operated: 471.6 $\pm$ 93.1 vs. 462.8 $\pm$ 94.3 µg/pg protein, respectively). These data indicate that both RIAs measure the same amount of SLI in the same sample. It has been shown previously, using ^{125}I-labeled [Tyr^{1}]somatostatin and Sheep B antisomatostatin, that SLI from hypothalamic and extrahypothalamic extracts has biological activity (Rorstad et al. 1979b). Thus, these data suggest hypophysectomy results in a reduction in immunoreactive and bioactive somatostatin in discrete diencephalic and telencephalic sites.

Table VII. Microdissection of brain regions in hypophysectomized and sham-operated control rats

Brain region	Punches/brain	Cannula size (mm)	Approximate Coordinates (μm)[a]
Median eminence	3	0.5	A4620-A4110
N. arcuatus	6	0.3	A4620-A4110
N. periventricularis	4	0.5	A6360-A6060
N. preopticus medialis	2	0.75	A6860
N. ventromedialis	4	0.5	A4620
N. suprachiasmaticus	4	0.3	A6360-A6060
N. interstitialis striae terminalis	4	0.5	A6860
N. amygdaloideus medialis	4	0.5	A4620-A4380
N. amygdaloideus centralis	4	0.5	A4620-A4380
N. amygdaloideus corticalis	4	0.5	A4620-A4380

N, Nucleus.

[a]See (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974)

Table VIII. Effects of hypophysectomy on SLI in selected hypothalamic and extrahypothalamic nuclei of the male rat

Brain region	SLI (pg/μg protein ± SE)[a]	
	Hypophysectomized	Sham-operated control
Median eminence	278.8 ± 53.2[b]	447.0 ± 57.4
N. arcuatus	60.2 ± 8.2[c]	89.8 ± 13.3
N. periventricularis	19.4 ± 1.8[d]	27.8 ± 3.1
N. preopticus medialis	10.2 ± 1.6[d]	20.0 ± 3.0
N. ventromedialis	18.2 ± 2.2	20.5 ± 3.3
N. suprachiasmaticus	19.4 ± 2.2	20.9 ± 3.0
N. interstitialis striae terminalis	20.2 ± 2.4	19.6 ± 1.9
N. amygdaloideus medialis	20.0 ± 2.6	16.8 ± 1.5
N. amygdaloideus centralis	16.5 ± 1.2	13.9 ± 0.9
N. amygdaloideus corticalis	17.2 ± 1.9	15.6 ± 2.3

N, Nucleus.

[a] Means and standard errors of 10 and 11 separate determinations for hypophysectomized and sham-operated control animals, respectively.

[b] $P < 0.01$ vs. sham operated.

[c] $P < 0.05$ vs. sham operated.

[d] $P < 0.025$ vs. sham operated.

DISCUSSION

The present study indicates that hypophysectomy results in a significant decrease in the somatostatin content of four discrete regions of the rat brain. The regions affected were the median eminence, arcuate, hypothalamic periventricular, and medial preoptic nuclei. Thus, removal of the target gland, the pituitary, results in a decrease in the concentration of the inhibitory hormone for GH secretion. Similarly, Wakabayashi et al. (1976). Fernandez-Durango et al. (1978), and Kanatsuka et al (1979) also reported decreased tissue levels of radioimmunoassayable somatostatin in hypothalamic fragments after hypophysectomy, and Baker and Yen (1976) observed depletion of somatostatin, as measured immunocytochemically, in the median eminence after hypophysectomy. In contrast, Berelowitz et al. (1978) observed a depletion of immunoreactive somatostatin in the septum and preoptic area of the brain, but not in the hypothalamus, after hypophysectomy, and Epelbaum et al. (1977b) found no effect of hypophysectomy on somatostatin content in the mediobasal hypothalamus, preoptic area, amygdala, or cortex. It is possible that these discrepancies may be due to differences in the tissue dissection.

Reduction of hypothalamic somatostatin content after hypophysectomy could be the result of either decreased synthesis, increased release, a greater rate of secretion than synthesis, increased degradation, or degeneration and repair of the median eminence-pituitary stalk (Raisman, 1973). It is unknown at present which of these conditions results in the posthypophysectomy decrease in hypothalamic somatostatin observed in the present and in previous (Kanatsuka et al., 1979; Wakabayashi et al. 1976; Fernandez-Durango et al.,

1978) studies. However, it is unlikely that these changes are due to nonspecific effects. In addition to somatostatin, LHRH (Fernandez-Durango et al., 1978; Baker and Dermody, 1976) and TRH (Bassiri and Utiger, 1974) are also depleted by hypophysectomy, but there seem to be differences among these hormones in their pattern of change after surgery. For example, somatostatin was observed to progressively decrease up to 2.5 months after hypophysectomy, whereas LHRH did not decrease further after the 24th postoperative day (Fernandez-Durango et al., 1978). Moreover, although hypothalamic LHRH decreased after hypophysectomy, the LHRH in the organum vasculosum of the lamina terminalis increased in female rats (Wenger et al., 1978). Furthermore, TRH was found to decrease 2 weeks posthypophysectomy but was partially restored after 4 weeks (Bassiri and Utiger, 1974). Therefore, it seems that hypophysectomy has separate effects on somatostatin, LHRH, and TRH systems.

It is possible that hypophysectomy interrupts a short loop inhibitory feedback mechanism involving an interaction of GH with somatostatinergic systems. Using immunocytochemical techniques, Hoffman and Baker (1977) observed that GH treatment prevented the depletion of somatostatin in the median eminence if therapy was initiated immediately after hypophysectomy and partially restored somatostatin when treatment began after a prolonged postoperative interval. Similar results were obtained with RIA measurements (Kanatsuka et al., 1979). The question of whether removal of other pituitary hormones may also affect brain somatostatin remains unanswered. Although somatostatin has been implicated in the physiological regulation of TSH

secretion (Martin, 1976; Martin et al., 1978a), neither thyroidectomy nor T_4 treatment modify the hypothalamic content of somatostatin, LHRH, or TRH (Fernandez-Durango et al., 1978; Berelowitz et al., 1978).

Brownstein et al. (1975) have described the regional localization of somatostatin in several hypothalamic and extrahypothalamic sites of the rat brain, using the biochemical mapping approach. Immunocytochemicl studies (Elde et al., 1978; Elde and Hökfelt, 1978) have demonstrated somatostatin-postive cell bodies in several telencephalic and diencephalic nuclei, including the caudal periventricular hypothalamic and corticomedial amygdaloid nuclei (Brownstein et al., 1975; Elde et al., 1978). Evidence from this laboratory indicates that these two somatostatin cell groups project axons to the median eminence (Crowley and Terry, 1980a). In addition, the periventricular system also seems to innervate the medial preoptic, rostral periventricular, and arcuate nuclei, all of which showed changes in somatostatin content after hypophysectomy in the present study. Taken together, these findings raise the possibility that GH exerts an inhibitory feedback action on its own secretion by affecting somatostatin cell bodies in periventricular hypothalamus (and possibly the amygdala) that innervate the preoptic area and medial basal hypothalamus.

III. B. 3. THE EFFECTS OF EXERCISE STRESS ON SOMATOSTATIN CONCENTRATIONS IN DISCRETE BRAIN NUCLEI*

ABSTRACT

Plasma growth hormone levels fall and remain low for several hours after stress in the rat. This effect is partially reversed by administration of antiserum to somatostatin. The present study was undertaken to determine the role of central nervous system somatostatin in stress-induced growth hormone suppression. Adult male rats were forced to swim for 30 minutes in a tank filled with water at 37°C. They were sacrificed immediately afterwards by decapitation. Their brains were snap-frozen and serum was collected for growth hormone radioimmunoassay. Somatostatin was measured in 10 microdissected, individual brain nuclei by a specific and sensitive radioimmunoassay. Serum growth hormone was significantly lower in stressed rats compared to "nonstressed" controls. Swimming stress resulted in a significant reduction of somatostatin in the median eminence and medial portion of the caudate nucleus. There were no significant changes of somatostatin levels in the periventricular, arcuate, ventromedial, medial preoptic, suprachiasmatic, accumbens, central amygdaloid or interstitial stria terminalis nuclear regions. These results suggest (1) stress-induced suppression of growth hormone secretion in the rat is mediated by the release of somatostatin from nerve endings in the median eminence into the portal system to inhibit pituitary release and, (2) somatostatin-containing nerve fibers which innervate the caudate nucleus may influence extrapyramidal mechanisms associated with prolonged physical stress.

*Terry and Crowley, 1980b.

INTRODUCTION

Acute or chronic exposure to stressful stimuli consistently suppresses growth hormone (GH) secretion in the rat, as demonstrated by a reduction in mean plasma GH levels with abolition of the ultradian rhythm (Tache et al., 1978; Terry et al., 1976b, 1977d). These effects appear to involve the hypothalamus, rather than the pituitary gland directly, and may be mediated through altered release of the hypothalamic hormones that regulate pituitary GH secretion (Campbell et al., 1977; Tache et al., 1976b, 1977d). Thus, stress-induced GH suppression may be due to the diminished release of GH-releasing hormone, the structure of which is still unknown, or to the augmented release of the structurally identified tetradecapeptide GH-release inhibiting hormone (somatostatin). In order to test this latter hypothesis, the present experiment has investigated the effect of an acute stress on somatostatin-like immunoreactivity (SLI) in individual nuclei microdissected from hypothalamic and extrahypothalamic regions known to contain somatostatin-positive nerve terminals and/or cell bodies (Brownstein et al., 1975; Elde et al., 1978).

METHODS

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, Ind.) were housed in groups of 4, maintained under constant temperature (24°C) on a 14-10 h light-dark cycle with lights on at 0600 h, and ad libitum food and water. Animals were removed from their cages at 1000 h, placed in a large

water bath at 37°C and forced to swim for 30 min as previously described (Terry et al., 1976). The control group was left undisturbed in a separate room. After 30 min of forced swimming, stressed and control animals were sacrificed alternately by decapitation and their brains were rapidly removed and frozen with powdered dry ice. Samples of trunk blood were centrifuged and stored at -20°C for the subsequent measurement of serum GH with materials supplied by the NIAMDD. Serial coronal sections, 300 µl thick, were cut on a microtome cryostat at -7°C and frozen onto microscope slides. Individual brain nuclei were removed with stainless steel cannulae (Crowley et al., 1978). Table IX provides further details of the microdissection procedure. Tissue specimens were expelled into 50 µl of ice cold 1 N HCl and sonicated for approximately 3 sec. Aliquots of 5-10 µl were removed for analysis of protein content, and the remaining homogenate was stored at -90°C until the somatostatin content of tissue samples was determined by a sensitive and specific radioimmunoassay (RIA), as described elsewhere (Rorstad et al., 1979; Terry et al., 1980). Synthetic cyclic somatostatin and Tyr^{11}-somatostatin were purchased from Bachem, Inc. (Torrance, Calif.), and the purity of each was verified by high pressure liquid chromatography and amino acid analysis. Tyr^{11}-somatostatin was radioiodinated by a modification (Rorstad et al., 1979b; Terry et al., 1980a) of the method of Greenwood et al. (1963) and purified by a procedure described elsewhere (Arimura et al., 1975; Epelbaum et al., 1977). Sheep B antisomatostatin (Rorstad et al., 1979b; Terry et al., 1980a) bound 32.8 ± 2.1 percent of ^{125}I-labeled Tyr^{11}-somatostatin at a final dilution of 1:150,000, with a minimal detectable concentration of 10.2 pg/tube. Supernatants of the homogenates were neutralized with 1 M NaOH and

0.1 M sodium phosphate. Median eminence extracts were diluted 1:15 in the assay buffer, and the other regions were measured undiluted. Samples of 10 µl were assayed for SLI in triplicate.

RESULTS

Table X presents somatostatin concentrations in 12 brain nuclei from non-stressed and stressed animals. These data were analyzed by unpaired Student's t-tests. Swimming stress significantly decreased the levels of SLI in the median eminence (-47 percent) and the medial portion of the caudate nucleus (-17 percent). No significant changes in somatostatin concentration were noted in the other telencephalic, diencephalic or mesencephalic structures dissected. Serum GH was significantly ($P < 0.001$) lower in stressed animals compared to non-stressed controls (6.4 $\pm$ 1.0 vs. 49.5 $\pm$ 12.5 ng/ml, respectively).

Table IX. Microdissection of discrete brain regions

Brain region	No. punches/ brain	Cannula size (mm)	Approximate Coordinates (μm)*
N. caudatus (medial part)	2	0.75	A8920
N. accumbens	4	0.5	A8930
N. interstitialis striae terminalis	4	0.50	A6860
N. preopticus medialis	2	0.75	A6860
N. periventricularis	4	0.5	A6670, A6360
N. suprachiasmaticus	4	0.30	A6360, A6060
Median eminence	3	0.50	A4620, A4380
N. arcuatus	6	0.50	A4620, A4380
N. ventromedialis	4	0.50	A4620
N. amygdaloideus centralis	4	0.50	A4620, A4380
N. interpeduncularis	2	0.75	A1800, A1400
Periaqueductal gray	4	0.50	A1800, A1400

*Based on (Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974)

Table X. Effects of stress on somatostatin-like immunoreactivity (SLI) in selected brain nuclei in male rats

Brain region	SLI (pg/µg protein ± S.E.M.)	
	Control (n = 13)	Stress (n = 12)
N. caudatus (medial part)	11.6 ± 0.9	9.6 ± 0.5*
N. accumbens	24.8 ± 1.9	22.4 ± 2.9
N. interstitialis striae terminalis	31.8 ± 2.4	31.9 ± 3.1
N. preopticus medialis	11.9 ± 1.7	10.1 ± 1.6
N. periventricularis	56.8 ± 2.8	53.5 ± 5.5
N. suprachiasmaticus	24.2 ± 2.6	20.5 ± 2.2
Median eminence	679.1 ± 96.2	362.4 ±32.6**
N. arcuatus	67.5 ± 7.3	56.9 ± 4.8
N. ventromedialis	17.2 ± 1.5	14.4 ± 1.6
N. amygdaloideus centralis	20.1 ± 3.2	14.6 ± 1.6
N. interpeduncularis	4.2 ± 0.5	4.1 ± 0.5
Periaqueductal gray	13.6 ± 1.3	13.7 ± 1.5

* $P < 0.05$; ** $P < 0.001$ vs sham, based on unpaired Student's t-tests.

DISCUSSION

The present study indicates that acute physical stress in the rat results in a marked depletion of somatostatin in the median eminence, concomitant with suppression of mean serum GH. These results suggest that stress-induced GH suppression may be due to augmented release of somatostatin from the median eminence. Several recent reports provide compelling evidence in favor of this hypothesis. First, treatment with antiserum to somatostatin prevents stress-induced inhibition of GH secretion in the rat (Arimura et al., 1976; Terry et al., 1976b), suggesting that this effect is due to circulating somatostatin. Second, recent evidence from this laboratory indicates that the somatostatin-positive cell bodies that are localized to the anterior hypothalamic periventricular region (Elde et al., 1978) appear to be the source of at least two-thirds of the somatostatin present in the median eminence (Crowley and Terry, 1980, 1981a). Destruction of this area, or deafferentation of the medial-basal hypothalamus, greatly decrease median eminence somatostatin content (Brownstein et al., 1977; Epelbaum et al., 1977b) and also abolish the stress-induced suppression of GH release (Collu et al., 1973; Mitchell et al., 1973; Mitchell et al., 1973; Rice et al., 1978). Therefore, the decrease of somatostatin in the median eminence observed after stress could be the result of increased release of the peptide from this periventricular system into the adenohypophyseal portal blood. This in turn would inhibit pituitary GH secretion. Although increased somatostatin release

from the median eminence appears to be the most likely explanation for the reduced tissue levels, observed in the present study, increased degradation or decreased synthesis of the hormone cannot be ruled out.

It is interesting to note that a small, but significant, reduction of somatostatin occurred in the medial aspect of the caudate nucleus after stress. This region contains somatostatin-positive nerve fibers (Elde et al., 1978), but the functional significance of this system is not clear. The present findings raise the possibility that somatostatin-containing nerve fibers that innervate the caudate nucleus (Elde et al., 1978) may influence extrapyramidal mechanisms associated with prolonged physical stress.

4. CONCLUSIONS

The present data indicate that somatostatin-containing perikarya in the caudal hypothalamic periventricular and corticomedial amygdalar nuclei project axons to the median eminence. These two projections contribute 98 percent of hypothalamic somatostatin. The periventricular cells also project axons to the arcuate, medial preoptic, and rostral periventricular nuclei.

Hypophysectomy caused a significant reduction of somatostatin in the terminal projection areas of the periventricular somatostatinergic system, but did not lower somatostatin in the corticomedial amygdala. Swimming stress reduced median eminence somatostatin, suggesting increased release.

Thus, it is possible that the periventricular and amygdalar somatostatinergic systems function to regulate growth hormone secretion through their projections to the median eminence and by innervation of neurons at sites known to influence growth hormone release, i.e. arcuate nucleus.

Because the arcuate nucleus has a high somatostatin concentration and stimulation of the ventromedial-arcuate complex causes growth hormone release, attention was focused on a method to selectively lesion arcuate perikarya. Monosodium glutamate is known to rapidly destroy neurons in the arcuate and was employed as a "selective neurotoxin" to study the role of this nucleus in regulation of growth hormone secretion (section III,C).

III. C. EFFECTS OF THE NEUROTOXIN MONOSODIUM GLUTAMATE ON GROWTH HORMONE SECRETION AND BRAIN SOMATOSTATIN

1. MONOSODIUM GLUTAMATE: ACUTE AND CHRONIC EFFECTS ON RHYTHMIC GROWTH HORMONE AND PROLACTIN SECRETION, AND SOMATOSTATIN IN THE UNDISTURBED MALE RAT*

ABSTRACT

The present investigation was designed to determine the chronic effects of neonatal monosodium glutamate (MSG) administration (4 g/kg s.c.) and the acute effects of MSG (1 g/kg i.p.) on episodic growth hormone (GH) and prolactin (PRL) secretion and brain somatostatin (SRIF) in unanesthetized, chronically cannulated male rats.

Adult rats showed the typical physical characteristics that result from neonatal MSG administration. Analysis of episodic GH secretion showed a significant reduction in: (1) the amplitude of GH secretory peaks; and (2) the mean 5.5-h plasma level of GH. Bursts of plasma PRL were inhibited by MSG, but the mean 5.5.-h plasma levels were not affected. SRIF concentrations in the medial basal hypothalamus were reduced by 60 percent after neonatal MSG. Acute administration of MSG to adult rats caused an immediate, long-lasting suppression of rhythmic GH secretion and a rapid, transient release of PRL.

These results suggest: (1) neonatally administered MSG causes a marked disturbance in episodic GH and PRL secretion in adult rat; (2) MSG induces a

*Terry et al., 1981a.

decrease in hypothalamic SRIF and possibly GH-releasing factor; and (3) the acute effects of MSG on GH and PRL may be due to the inhibition and/or excitation of a complex neuronal network involving monoaminergic and peptidergic systems.

INTRODUCTION

Large doses of monosodium glutamate (MSG) administered neonatally cause a well-described syndrome in adult rats manifested by behavioral disturbances, tail automutilation, and neuroendocrine deficiencies characterized by stunted growth, obesity, hypothyroidism, hypogonadism and pituitary atrophy (Bakke et al., 1978; Cameron et al., 1976; Holzwarth-McBride et al., 1976; Lamperti et al., 1980; Millard et al., 1980; Nasagawa et al., 1974; Nemeroff et al., 1977a,b, 1978; Olney, 1969; Pizzi et al., 1977; Redding et al., 1976; Schubert et al., 1980; Terry et al., 1977c). The pituitary content and plasma levels of growth hormone (GH) are decreased after neonatal MSG administration (Bakke et al., 1978; Nasagawa et al., 1974; Nemeroff et al., 1977a,b; Redding et al., 1976; Terry et al. 1977c). The prolactin (PRL) response is less clear and there may be a difference between sexes. It is well established that MSG, administered either orally or subcutaneously, rapidly destroys neurons in the arcuate nucleus, circumventricular regions, and inner retina in a number of species (Olney, 1969a,b; Olney et al., 1976). Biochemical and immunohistochemical studies indicate that the neurotoxicity of neonatally administered MSG is associated with destruction of dopaminergic, cholinergic and probably GABAergic systems in the tuberoinfundibular tracts which

originate in the region of the arcuate nucleus (Clemens et al., 1978; Holzworth-McBride et al., 1976; Lamperti et al., 1980; Nemeroff et al., 1977a,b; Walaas and Fonnum, 1978). Since the tuberinfundibular tract contains peptidergic and several neurotransmitter elements (Hokfelt et al., 1978), destruction of these pathways may be the neurochemical basis for the neuroendocrine abnormalities that occur in adult rats after neonatal MSG treatment.

Monosodium glutamate administered to adult rats does not induce neurotoxic lesions in the brain, but it does alter the secretion of anterior pituitary hormones (Nemeroff et al., 1978). Intraventricular injections of MSG increased plasma luteinizing hormone levels in anesthetized male rats (Ondo et al., 1976). Acute treatment of adult rats with low doses of MSG was reported to cause a significant increase in LH and testosterone (Olney et al., 1976).

The purpose of the present investigation was to determine: (1) the chronic effects of neonatal MSG administration; and (2) the acute effects of MSG on rhythmic GH and PRL secretion, and brain levels of somatostatin (SRIF) in undisturbed, chronically cannulated adult male rats.

MATERIALS AND METHODS

Animals and experimental procedure

To study the chronic effects of MSG on pulsatile GH and PRL release, pregnant Charles River Sprague-Dawley rats were housed in air-conditioned quarters on a 12:12 h hight:dark schedule with lights on at 06:00 h and given laboratory chow and water ad libitum. Female and some male pups were removed

and sacrificed immediately after birth so that each litter contained 6 males. On alternate days for the first 10 days of life, the offspring were injected with either 5 doses of monosodium glutamate (4 g/kg body weight s.c.) (Sigma Chemicals, St. Louis, Mo.) or an equal volume of vehicle (0.9 percent NaCl, pH 7.4), according to methods described previously (Nemeroff et al., 1977). All animals were weaned after 21-22 days of age and placed in groups of 2/cage. Body weight, nasal-anal length, and the Lee index ($\sqrt[3]{\text{body weight}}$/nasal-anal length) (Lee, 1929) were recorded periodically. One group of MSG-treated and control animals, 4 months of age, were implanted with chronic indwelling silastic cannulae placed in the right atrium via the right external jugular vein and adapted to isolation test chambers, using methods described elsewhere (Tannenbaum and Martin, 1976; Terry et al., 1977d). After recovery of preoperative body weight (usually 2 weeks post-operatively) blood samples were withdrawn every 15 min and immediately centrifuged, the plasma frozen until assay, and red blood cells resuspended in normal saline were returned to animals at the time of the next sample. Each rat was sampled for 5.5. h beginning at 10:00 h. A second group of 110-day-old MSG- and vehicle-injected rats were sacrificed by decapitation, and samples of the medial basal hypothalamus, medial preoptic area, corticomedial and basolateral amygdala, and cortex were dissected, as described previously (Epelbaum et al., 1977b; Rorstad et al., 1979b; Schubert et al., 1980). After determination of wet weight, brain fragments were placed in 1 ml ice-cold 2 M acetic acid (tissue w/v, < 0.1). The fragments were homogenized using a glass Potter-Elvehjem homogenizer. The homogenate was frozen at -20°C overnight and, after thawing at room temperature, was centrifuged at 2000 X g a 4°C for 30 min.

The supernatant was collected and stored at -20°C. The pituitary gland was simultaneously removed, weighed, homogenized in 2 ml of 0.1 M phosphosaline-1 percent bovine serum albumin buffer pH 7.6, and centrifuged at 2000 X g for 30 min. The supernatant was diluted 1:100 in the RIA buffer and frozen until subsequent assay for GH and PRL. Representative animals from each group were anesthetized with ether and perfused with 10 percent formalin. Brains were removed and processed for conventional light microscopy, as described elsewhere (Terry and Martin, 1978a).

In a second experiment, designed to determine the acute effects of MSG on episodic GH and PRL secretion, normal male rats (400-500 g) with chronic indwelling cannulae were administered MSG, 1 g/kg i.p., or an equal volume of vehicle (0.9 percent NaCl, pH 7.4) at 10.50 h. Individual rats were usually used as their own control, receiving vehicle injections on one experimental day and MSG on a subsequent day. Each animal was sampled for 5.5 h beginning at 10.00 h.

Radioimmunoassay for somatostatin

Somatostatin was determined by methods described previously (Rorstad et al., 1979b). Results are expressed as somatostatin-like immunoreactivity (SLI) in pg/mg tissue.

GH and PRL radioimmunoassays

Growth hormone and PRL were determined in duplicate by a double antibody RIA using materials supplied by the National Pituitary Agency. Values are expressed in terms of the respective NIAMDD reference preparation. Minimum sensitivities of the assays are 3.2 ng/ml of GH and 1.9 ng/ml for PRL.

Analysis of data

Data was analyzed using the student's t-test to compare differences in GH, PRL and SRIF levels or growth parameters between treatment groups. Differences were considered significant at the $P < 0.05$ level.

RESULTS

Chronic effects of MSG on growth (Table XI

Animals treated neonatally with MSG showed stunted linear growth, obesity and tail automutilation, as described in previous studies (Bakke et al., 1978; Cameron et al., 1976; Holzworth-McBride et al., 1976; Lamperti et al., 1980; Millard et al., 1980: Nasagawa et al., 1974; Nemeroff et al., 1977a,b; Olney, 1969; Pizzi et al., 1977; Redding et al., 1976; Schubert et al., 1980; Terry et al., 1977c). The body weight and nasal-anal length of 110-day-old MSG-treated rats was reduced significantly when compared to the control group. The Lee index, a measure of body fat (Bernadakis and Patterson, 1968; Lee, 1929), was elevated in the MSG-treated group compared to controls. Tail automutilation was observed in 67 percent of MSG-treated rats at 24 days of age, and in virtually 100 percent at 110 days of age. A few animals afflicted with open skin lesions were removed from the study. Food and water consumption were not recorded; previous studies have shown no significant difference between MSG- or saline-injected male or female rats (Nemeroff et al., 1977a,b; Redding et al., 1976).

Chronic effects of MSG on pituitary GH and PRL (Table XII)

The size and weight of the pituitary glands of MSG-treated rats were reduced by approximately 62 percent ($P < 0.001$). There was a marked decrease in total GH content and concentration in pituitaries from MSG-treated animals. Total content of PRL was markedly reduced by MSG. However, the pituitary concentration of PRL was not decreased significantly.

Chronic effects of MSG on brain SRIF (Fig. 25)

The concentration of SRIF was reduced by 60 percent in the medial-basal hypothalamus. Although there was a slight decrease in SRIF in the basolateral amygdala, it was not significant.

Somatostatin levels in the medial preoptic area, corticomedial amygdala and cortex were not affected by MSG.

Chronic effects of MSG on rhythmic GH and PRL release

Individual control animals ($n = 9$) exhibited the typical ultradian GH rhythm (Fig. 26). Two major episodes of GH secretion were evident during the 5.5-sampling period, with most peak values greater than 400 ng/ml and trough values less than 6 ng/ml. Prominent GH secretory bursts were consistently observed at 10:30-11:30 and 13:30-14:30 h. Individual MSG-treated animals had markedly suppressed pulse amplitudes (usually < 100 ng/ml) of short duration (usually < 1 h) (Fig. 27). Mean plasma GH levels of animals administered MSG were reduced to baseline trough values except for 2 low amplitude peaks that occurred at 10:30 and 14:15 h (Fig. 28). The mean 5.5-h plasma GH level was reduced significantly by neonatal administration of MSG (Table XIII).

Mean PRL levels in control rats were generally low throughout with 2-4 random bursts that occurred most frequently in the afternoon (Fig. 29), as described elsewhere (Terry et al., 1977d). Neonatal administration of MSG suppressed random PRL bursts, but did not affect baseline levels (Fig. 29). The mean 5.5-h PRL was not affected significantly by MSG (Table XIII).

Acute effects of MSG on episodic GH and PRL secretion

The injection of vehicle at 10:50 h did not affect pulsatile GH secretion (Fig. 30). Acute administration of MSG (1 g/kg i.p.) to normal rats caused immediate suppression of episodic GH release which persisted up to 5h (Fig. 31). The mean 5.5-h GH level of MSG-administered rats was 44.4 $\pm$ 4.4 ng/ml (n = 11), significantly less ($P < 0.001$) than saline-injected animals, 126.9 $\pm$ 12.4 (n = 11).

The injection of MSG to normal animals caused a rapid, transient release of PRL when compared to the slight effect of normal saline injections (Fig. 32). Prolactin levels rose significantly ($P < 0.01$, n = 11) to 114.0 $\pm$ 24.0 ng/ml within 15 min after MSG administration and then decreased rapidly to baseline levels. MSG did not affect the random bursts of PRL observed in the afternoon. The minor elevation in PRL after saline injections was probably the result of a non-specific stress effect.

Histological verification of MSG-induced brain lesions

Examination of Nissl-stained hypothalamic sections from rats treated neonatally with MSG revealed a striking reduction in the number of arcuate neurons. No evidence of nerve cell destruction was observed in the

ventromedial, medial preoptic, anterior hypothalamic or suprachiasmatic nuclei. The optic nerves and chiasma were smaller than controls. There was no histological evidence of neuronal destruction in adult rats treated acutely with MSG.

Table XI. Effects of MSG on body weight, length and Lee index in rats at 110 days of age

Values given are the mean ± S.E.M.; the number of animals is given in parentheses.

Group	Body weight (g)	Nasal-anal length (cm)	Lee index*
Control (30)	506.8 ± 12.8	27.0 ± 0.3	0.295 ± 0.001
MSG-treated (18)	408.7 ± 10.2**	22.3 ± 0.2***	0.334 ± 0.002***

* $\sqrt[3]{\text{body weight (g)}}$/nasal-anal length (cm).

** P <0.02.

*** P <0.001.

Table XII. Effects of MSG on pituitary GH and PRL

Values given are the mean ± S.E.M.; the number of animals is given in parentheses.

Group	Pituitary wet weight (mg)	Pituitary GH		Pituitary PRL	
		µg/gland	µg/mg wet weight	µg/gland	µg/mg wet weight
Control (8)	11.98 ± 0.48	288.6 ± 2.3	24.1 ± 1.7	21.9 ± 1.9	1.8 ± 0.1
MSG-related (7)	4.26 ± 0.51*	43.1 ± 9.9*	8.2 ± 1.6*	7.7 ± 1.8*	1.5 ± 0.2**

* $P < 0.001$.

** Not significant.

Table XII. Mean 5.5-h GH and PRL levels in control and MSG-treated rats

There were 9 animals in each group. Values given are the mean ± S.E.M.

Experimantal group	Mean 5.5-h plasma GH level (ng/ml)	Mean 5.5-h plasma PRL levels (ng/ml)
Control	75.1 ± 11.1	5.8 ± 1.38
MSG-treated	12.5 ± 1.2*	5.3 ± 1.5**

* $P < 0.001$.

** Not significant.

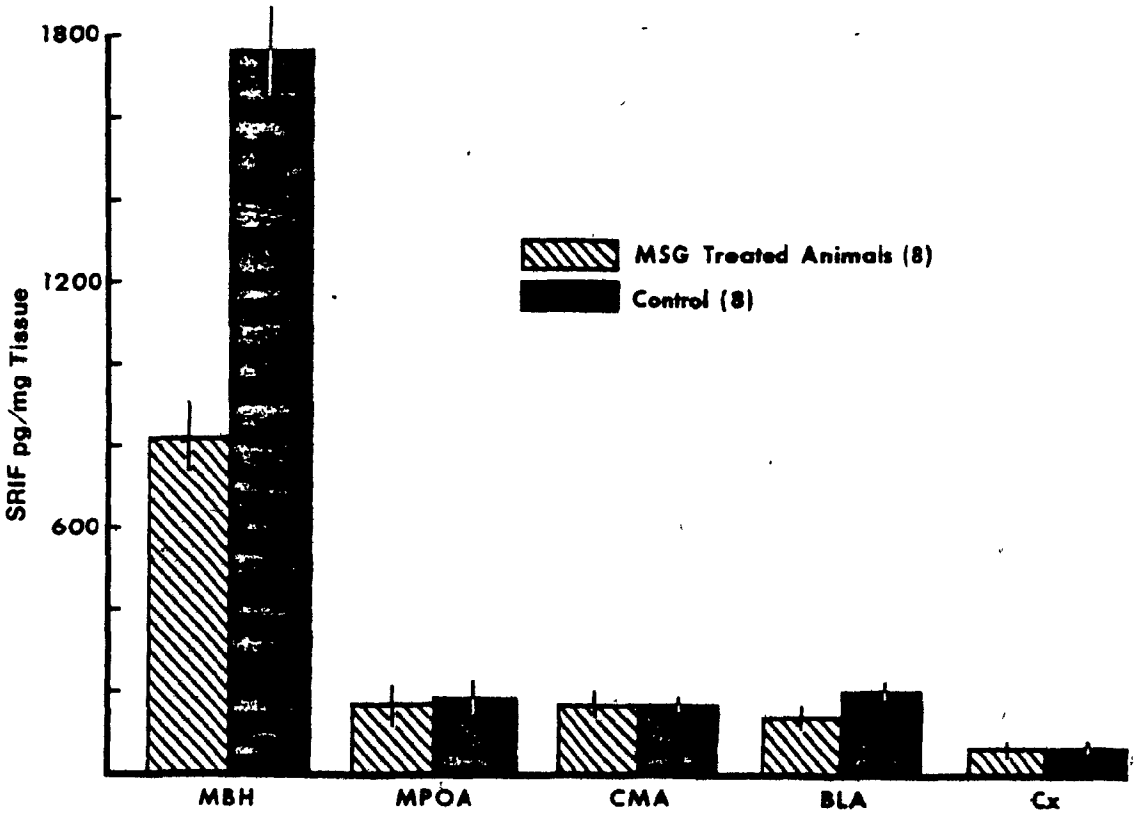

Fig. 25. Effects of neonatally administered MSG on SRIF concentrations in the brain of adult rats. MSG caused a significant reduction ($P < 0.001$) in MBH SRIF. In this and subsequent figures, number of animals in each group are shown in parentheses and vertical bars indicate SE of the mean. Abbreviations: MBH, medial basal hypothalamus; MPOA, medial preoptic area; CMA, corticomedial amygdala; BLA, basolateral amygdala; Cx, cortex.

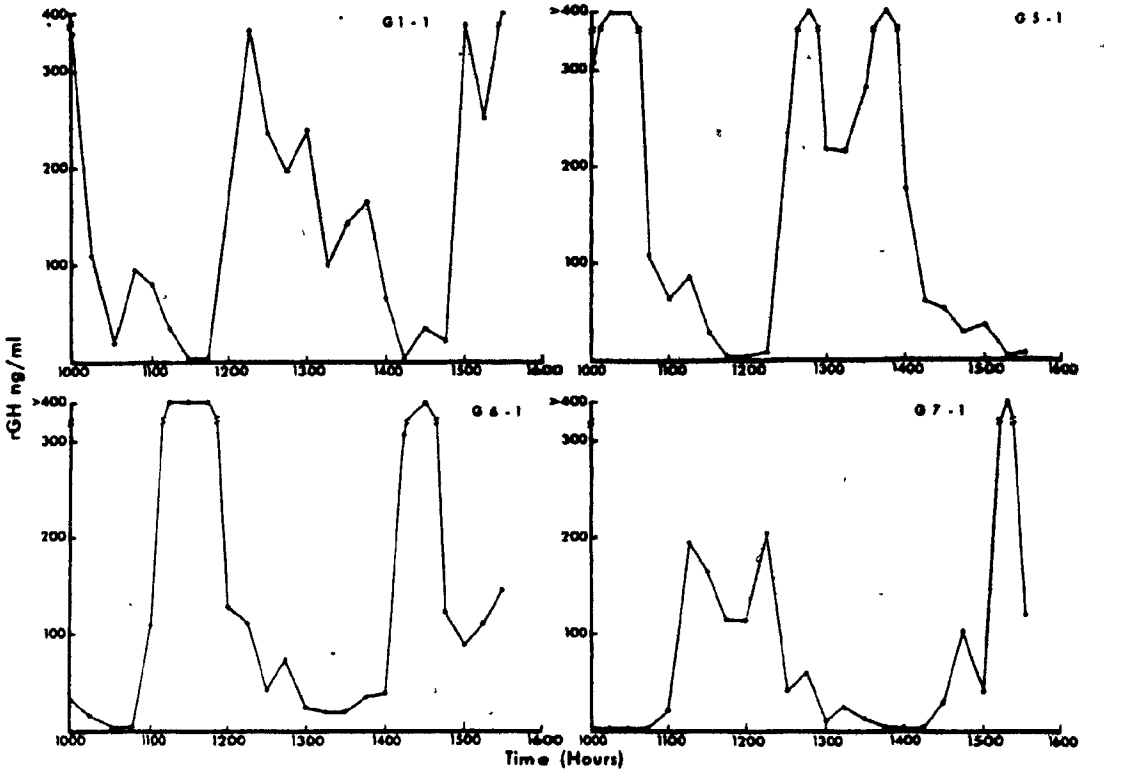

Fig. 26. Pulsatile GH secretion in individual adult control rats after neonatal administration of 0.9% NaCl s.c. GH secretion is characterized by high-amplitude secretory bursts that showed regular 3-4h interpeak intervals.

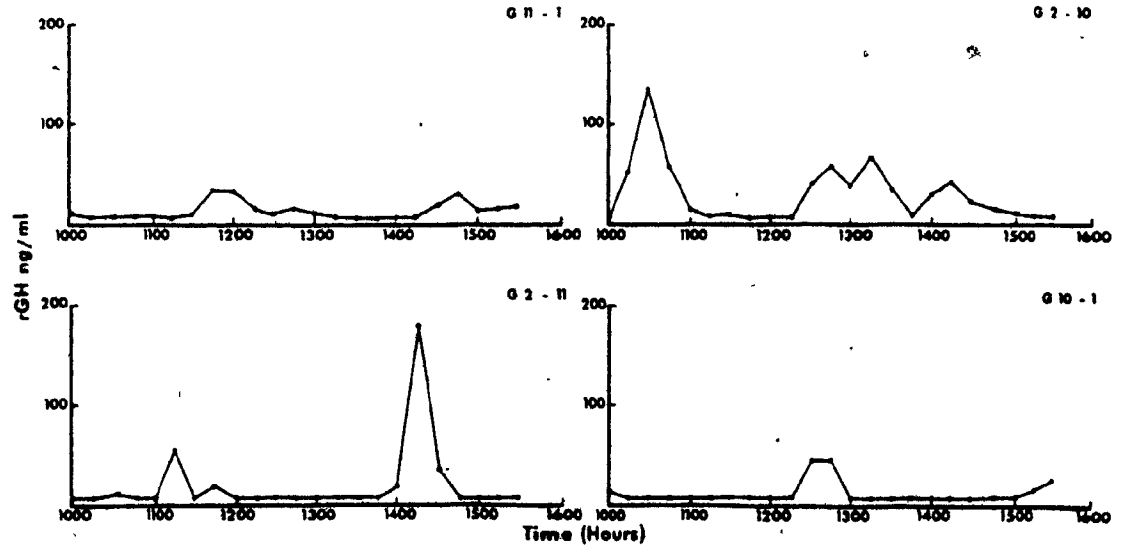

Fig. 27. Pulsatile GH secretion in individual adult rats after neonatal MSG administration (4g/kg s.c.). MSG-treated animals show evidence of pulsatile GH release with marked suppression of pulse amplitude (usually < 100 ng/ml) and pulse duration (usually < 1h).

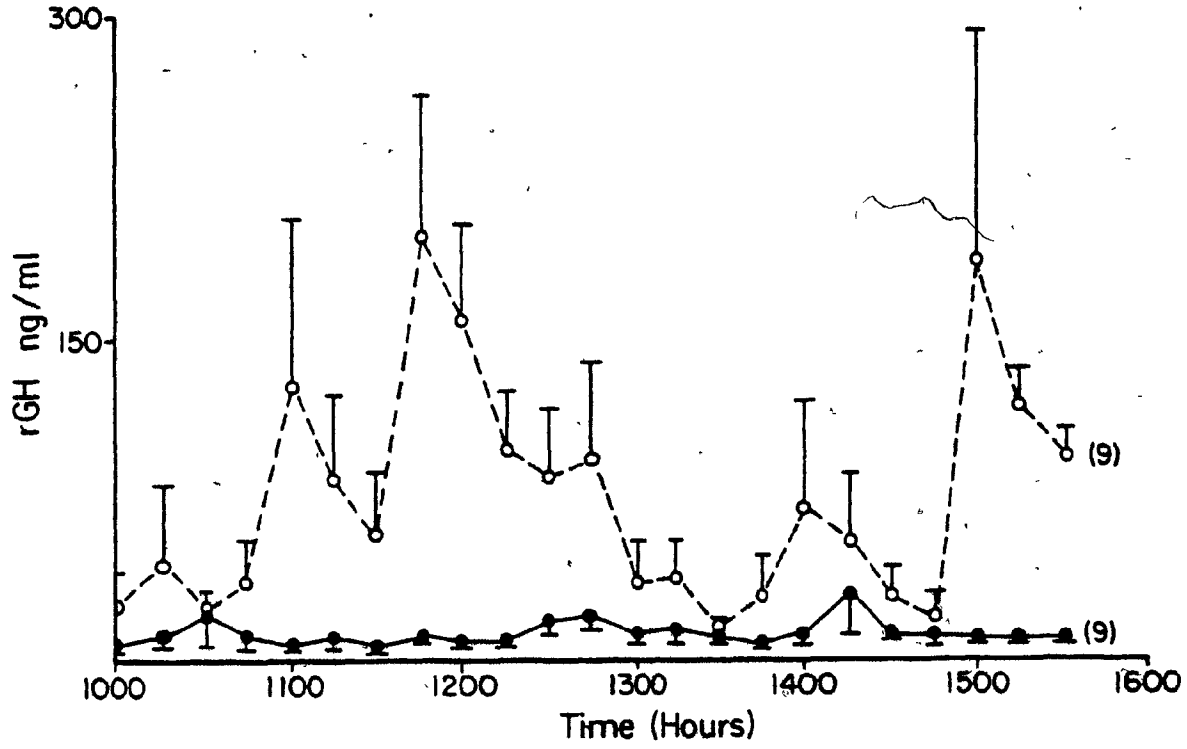

Fig. 28. Suppression of mean plasma GH levels in adult rats after neonatal MSG administration. Typical pulsatile GH secretion is seen in control animals (O—O). Mean plasma GH levels of MSG-treated rats are reduced to baseline levels with two low-amplitude short-duration peaks at 10.30h and 14.15h (●—●).

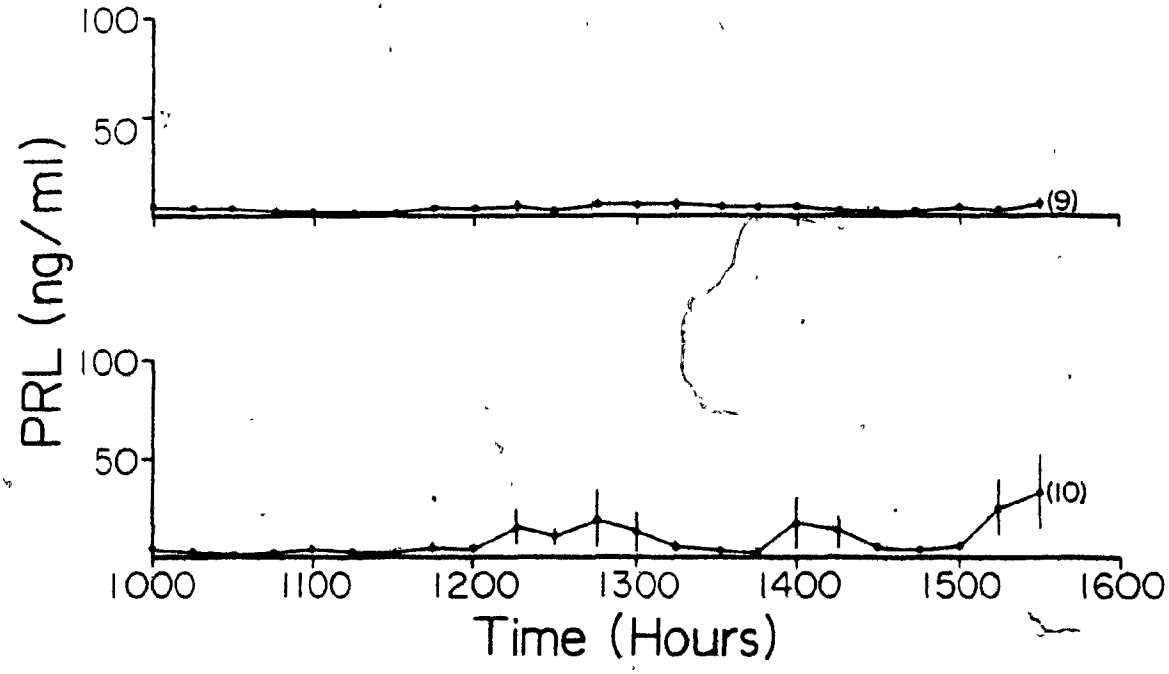

Fig. 29. Mean plasma PRL levels in adult rats after neonatal MSG administration (4 g/kg s.c., upper graph) and vehicle control (0.9% NaCl, lower graph). MSG suppressed random PRL bursts, but did not affect low basal levels.

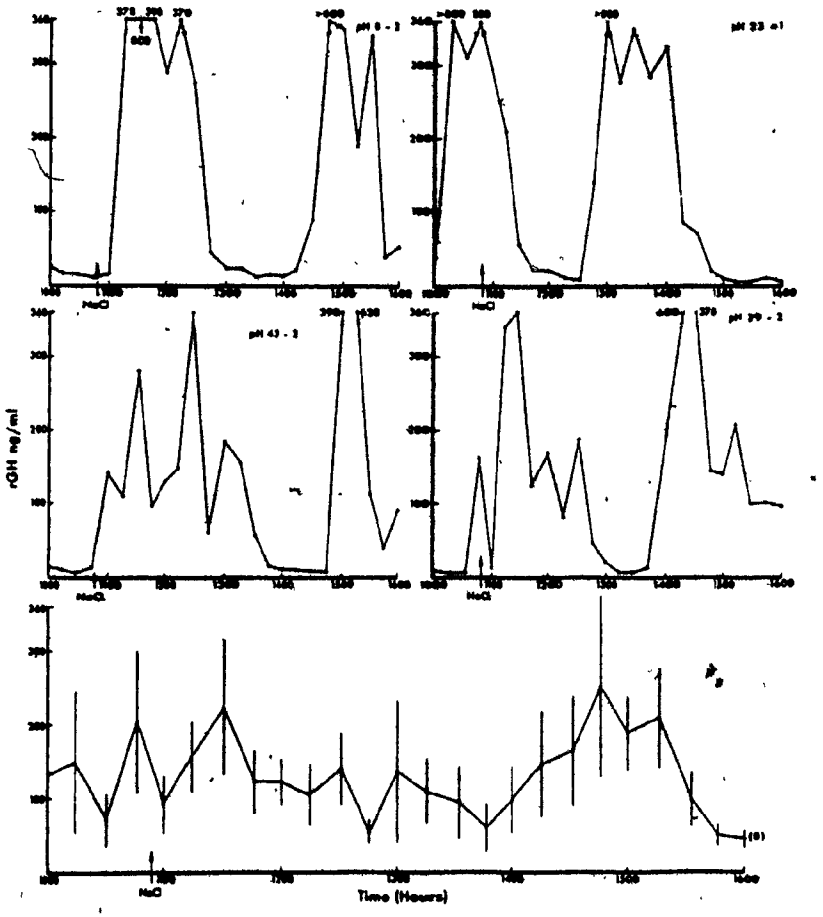

Fig. 30. Pulsatile GH secretion in individual control rats (upper and middle panels) and mean plasma GH (lower panel) after the injection of 0.9% NaCl i.p. at 10.50h (indicated by arrows). The injection of normal saline did not affect pulsatile GH secretion.

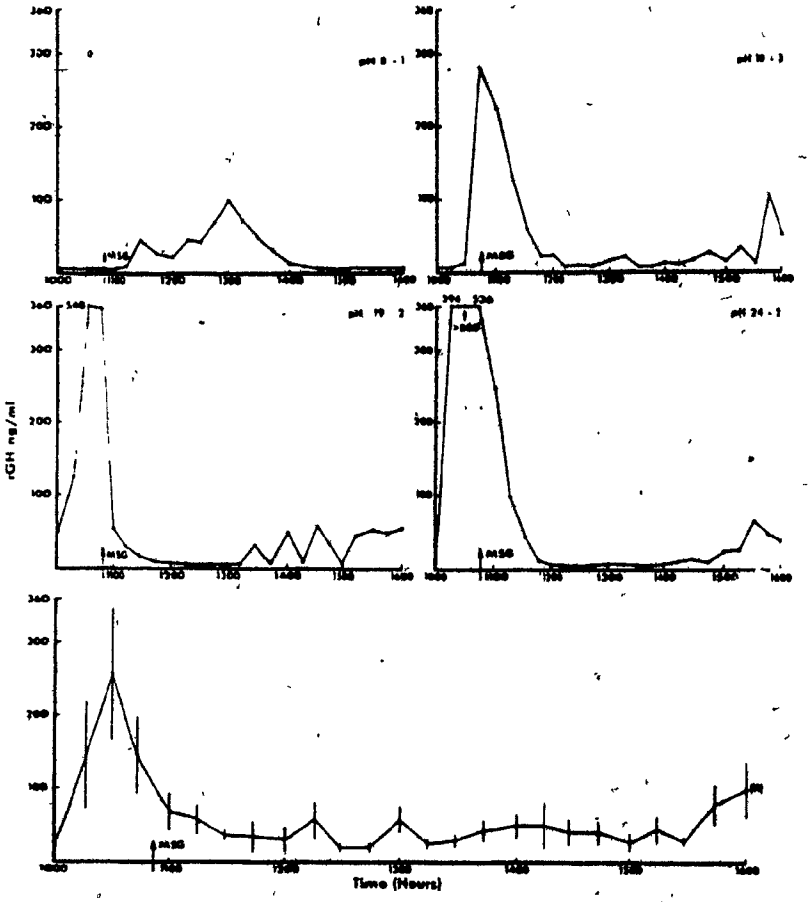

Fig. 31. Acute effects of MSG (1 g/kg i.p.) on pulsatile GH secretion in individual rats (upper and middle panels) and mean plasma GH (lower panel). MSG caused immediate and long-lasting suppression of GH release.

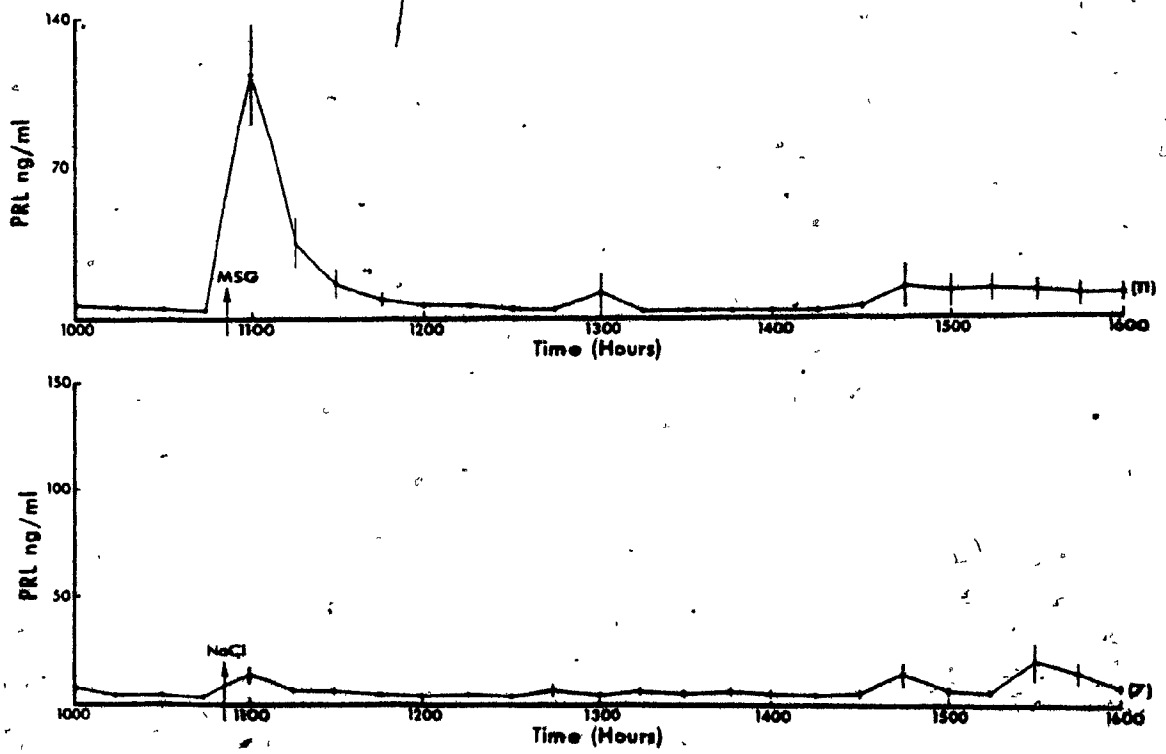

Fig. 32. Acute effects of MSG (1 g/kg i.p., upper graph) and vehicle control (0.9% NaCl, lower graph) on mean plasma PRL levels. MSG caused a rapid, transient release of PRL when compared to vehicle controls. The minor elevation in PRL after NaCl was probably a non-specific stress effect.

DISCUSSION

Growth hormone secretion in control rats was characterized by high-amplitude secretory bursts which reached levels greater than 400ng/ml. The bursts showed a striking acute profile with a rapid rise in plasma GH, abrupt termination of secretion, and decline in plasma GH to low or detectable levels. The surges of GH secretion showed regular 3-4-h interpeak intervals which were entrained to the light-dark cycle. These results are in agreement with studies described elsewhere (Martin et al., 1978a; Tannenbaum and Martin, 1976; Terry et al., 1977d) Neonatal MSG administration resulted in growth retardation, increased body fat, decreased pituitary mass and a significant reduction in the mean 5.5-h plasma GH levels and pituitary content of GH, confirming previous studies (Bakke et al., 1978; Cameron et al., 1976; Holzworth-McBride et al., 1976; Lamperti et al., 1980; Millard et al., 1980; Nasagawa et al., 1974; Nemeroff et al., 1977a,b; Olney, 1969; Pizzi et al., 1977; Redding et al., 1976; Schubert et al., 1980; Terry et al., 1977c). Growth hormone secretory profiles in MSG-treated rats were characterized by low amplitude bursts (usually < 100 ng/ml) of short duration (usually < 1 h); trough levels remained low or undetectable.

The pulsatile nature of GH secretion is thought to be regulated by stimulatory (GH-releasing factor, GRF) and inhibitory (GH-release inhibiting factor, somatostatin or SRIF) hypothalamic hormones (Martin, 1979; Martin et al., 1978a; Terry and Martin, 1981b). The structure of GRF is unknown whereas SRIF has been characterized as a tetradecapeptide (Brazeau et al., 1973). The release of these hormones is in turn regulated by monoamines (Martin, 1979; Martin et al., 1978a). The reduced amplitude and duration of GH pulses observed in the present study could result from decreased levels of stimulatory GRF or by increased secretion of SRIF. We favor the former hypothesis.

Although the precise localization of hypothalamic GRF-containing neurons is unknown, electrical stimulation of the ventromedial-arcuate region of the hypothalamus stimulates GH secretion in anesthetized rats (Martin, 1979). It is likely that destruction of arcuate neurons by MSG affects GRF neurons to result in defective pulsatile GH secretion. This idea is supported by recent studies of Millard et al., (1980) which show that GH release induced by morphine is decreased in MSG-treated rats. The alternate hypothesis is that MSG rats have an increased secretion of SRIF. Hypothalamic neurons containing SRIF are located primarily in the anterior hypothalamic-periventricular zone, which is unaffected by MSG. The decreased concentration of SRIF in the hypothalamus in MSG-treated rats is unlikely to be due to the direct effects of the neurotoxin, and may reflect altered GH feedback effects on SRIF levels. Hypophysectomy is reported to reduce SRIF levels in the hypothalamus (Baker and Yen, 1976; Fernandez-Durango et al., 1978; Terry and Crowley, 1980a).

Pharmacologic studies indicate that pulsatile GH secretion in the rat is dependent upon facilitatory noradrenergic and serotoninergic inputs to the medial basal hypothalamus (Martin, 1979; Martin et al., 1978a,b). Dopamine appears to have a minor role in GH regulation in the rat. The neurotoxicity of neonatally administered MSG is associated with destruction of dopaminergic and cholinergic systems in the arcuate nucleus-median eminence region (Clemens et al., 1978; Holzworth-McBride et al., 1976; Lamperti et al., 1980; Nemeroff et al., 1977a,b; Walaas and Fonnum, 1978) but norepinephrine and serotonin levels are unaffected. In conclusion, we favor the hypothesis that decreased GH secretion in MSG-treated is due to a defect in hypothalamic GRF resulting in decreased pituitary GH synthesis and failure of pulsatile release.

Neonatal administration of MSG suppressed random PRL bursts that usually occur in the afternoon in undisturbed, freely-behaving male rats (Terry et al., 1977d). However, mean 5.5-h plasma PRL levels and the pituitary concentration of PRL were not affected by MSG despite a significant reduction in the total pituitary PRL content. The results are in agreement with those of Clemens et al. (1978) and Nasagawa et al. (1974). Low plasma PRL levels were unexpected since it is known that dopamine has a direct inhibitory effect on pituitary prolactin release (MacLeod, 1976) and MSG decreases tuberoinfindibular dopamine. Nemeroff et al.(1977a,b) reported increased plasma PRL levels in adult male, but not female rats after neonatally administered MSG. The failure to demonstrate increased PRL levels in female rats may be related to decreased estrogen levels secondary to functional hypogonadism that occurs in MSG-treated animals. Clemens et al. (1978) found that 5-hydroxytryptophan, a serotonin precursor, caused marked enhancement of

PRL release in MSG-treated animals. These results suggest that MSG-induced lesions result in an impaired release mechanism for PRL, possibly due to the absence of a PRL releasing factor.

The acute administration of MSG to freely-behaving adult rats caused an immediate, long-lasting suppression of rhythmic GH secretion and rapid, transient release of PRL. Olney et al. (1976) suggested that a dose of MSG below that required to destroy arcuate neurons might nevertheless stimulate them to fire at increased rates and therby disturb endocrine systems regulated by these neurons. They reported that low doses of MSG caused elevations of luteinizing hormone and testosterone, presumably mediated by stimulation of the release of luteinizing hormone releasing hormone. These results have not been confirmed by other investigators (Nemeroff et al., 1978). Neither haloperidol nor atropine blocked the acute effects of MSG on serum PRL and GH, suggesting that postsynaptic dopaminergic and cholinergic receptors do not mediate these effects (Nemeroff et al., 1978). However, these results should be interpreted with caution since atropine also inhibits episodic GH secretion (Martin et al. 1978). Results of the present study do not support the hypothesis that MSG administration excites tuberoinfindibular dopaminergic neurons, since acute activation of this system alone would be expected to inhibit PRL release (MacLeod, 1976) and have little, if any, effect on episodic GH secretion (Willoughby et al., 1977).

A number of hypothalamic peptides, including substance P, β-endorphin, α-MSH and neurotensin, are reported to stimulate PRL secretion in the rat (Martin, 1979). Acute MSG administration may activate one of these peptidergic systems.

2. CONCLUSIONS

Neonatal administration of monosidium glutamate is associated with destruction of dopaminergic, cholinergic and GABAergic systems in the tuberoinfindibular tracts that originate in the arcuate region. It is also associated with cell loss in the retina and circumventricular organs and causes multiple neuroendocrine deficiencies including stunted growth, increased body fat, hypogonadism and hypothyroidism. Thus, it is not a "selective neurotoxin" and its effects on growth hormone secretion are difficult to interpret.

Despite these caveats, results of this study suggest that monododium glutamate caused a marked disturbance in rhythmic growth hormone secretion characterized by decreased amplitude and duration of growth hormone rises with low trough values. These findings were accompanied by a significant reduction of somatostatin in the medial basal hypothalamus. The reduced amplitude and duration of growth hormone pulses probably resulted from decreased levels of a growth hormone releasing factor, possibly due to destruction of arcuate neurons. Since hypophysectomy reduced somatostatin in the medial basal hypothalamus, decreased levels after monosodium glutamate were thought to reflect a growth hormone feedback effect and not a direct action of the neurotoxin.

Acute monosodium glutamate administration is thought to excite tuberoinfindibular neurons without causing cell death. If dopamine stimulates growth hormone release, then one would expect acute monosodium glutamate administration to increase plasma growth hormone levels. Such an effect was not observed in the present experiments. Nevertheless, other studies have shown that dopamine has a minor role in growth hormone regulation in the rat.

The next series of experiments were devised to ascertain the role of the two other major catecholaminergic neurotransmitters, norepinephrine and epinephrine, in growth hormone regulation. To this end, animals were administered selective inhibitors of enzymes responsible for the biosynthesis of norepinephrine and epinephrine, dopamine-B-hydroxylase and norepinephrine-N-methyltransferase, respectively (section IV,A,B,).

IV. ROLE OF CATECHOMAMINES IN REGULATION OF GROWTH HORMONE SECRETION

A. EVIDENCE FOR α-ADRENERGIC REGULATION OF EPISODIC GROWTH HORMONE AND PROLACTIN SECRETION IN THE UNDISTURBED MALE RAT.*

ABSTRACT

The present experiments were designed to study the effect of the centrally active α-adrenergic receptor agonist, clonidine, on episodic GH and PRL secretion in male rats after selective blockade of norepinephrine (NE) and epinephrine (EP) synthesis with the dopamine-β-hydroxylase inhibitor, FLA-63.

Freely behaving, chronically cannulated rats were maintained on a constant light-dark cycle in isolation test chambers. Beginning at 1000h, blood samples were removed every 20 min for 5-h periods without disturbing the animal. FLA-63 was administered (10 or 20 mg/kg ip) at 0845 h. Clonidine (15 or 150 μg/kg iv) was given at times that coincided with the spontaneous occurrence of episodic GH peaks or troughs observed in control animals.

Results of the present study are summarized as follows: 1) selective blockade of NE and EP synthesis with FLA-63 (20 mg/kg) caused complete suppression of episodic GH but had no significant effect on PRL release; 2) clonidine (150 μg/kg) restored the pulsatile pattern of GH secretion in FLA-63-treated rats, and 3) clonidine (15 and 150 μg/kg) stimulated PRL release in a dose-dependent manner.

These findings suggest a major stimulatory role of α-adrenergic receptors in episodic GH and PRL secretion.

*Terry and Martin, 1981a.

INTRODUCTION

GH is thought to be regulated by the release of both inhibitory (SRIF) and excitatory (GH-releasing factor) hypothalamic hypophysiotropic hormones that are, in turn, regulated by monoaminergic neurons. GH release induced by amygdalar or hippocampal stimulation is prevented by α-methyl-ρ-tyrosine (α-MT) and α-adrenergic blockade (Martin, 1974b; 1976), results that are consistent with the interpretation that catecholamines, particularly norepinephrine (NE), may function as neurotransmitters in the relay of these responses from higher neural centers to hypothalamic peptidergic neurons. Several investigators have provided evidence for a stimulatory role of the central noradrenergic system in primates operating through α-adrenergic receptors (Martin et al., 1978a; Krulich, 1979). Recent reports suggest a similar function of the NE system in rats (Durand et al., 1977; Martin et al., 1978b; Vijayan et al., 1978; Eden et al., 1979; Negro-Vilar et al., 1979).

The pattern of GH release has been documented in several species, including man (Martin, 1974; Martin et al., 1978a; Krulich, 1979). In the rat the surges of GH occur in a well defined ultradian rhythm that is entrained to the light-dark cycle (Tannenbaum and Martin, 1976). In contrast to primates, rats react to stress by an inhibition of GH secretion (Terry et al., 1977d). These factors make the study of the role of central neurotransmitters difficult, and stress-free experimental conditions are a prerequisite for meaningful results.

The role of the central noradrenergic system in the regulation of PRL secretion is still largely unknown, and the available information is contradictory. Systemic administration of clonidine induced elevation of PRL levels (Durand et al., 1977; Stevens and Lawson, 1977). Administration of phenoxybenzamine had a similar effect, whereas α-blockade with phentolamine or β-blockade with propranolol had no consistent influence (Martin et al., 1978a; Lawson and Gala, 1975).

The present experiments were designed to determine the effects of dopamine-β-hydroxylase inhibition and central α-adrenergic activation on episodic GH and PRL secretion in the undisturbed, freely behaving male rat.

Materials and Methods

Animals

Male Charles River Sprague-Dawley rats (300-350 g) were housed in temperature and humidity controlled laboratories with a 12 h light:12 h dark cycle (lights on at 0600 h). The animals were given free access to laboratory chow and water. Animals were implanted with chronic indwelling Silastic cannulae placed in the right atrium via the right external jugular vein and adapted to isolation test chambers, using methods previously described (Tannenbaum and Martin, 1976; Terry et al., 1977d). After recovery of preoperative body weight (usually 1 week postoperatively), blood samples were withdrawn every 20 min and immediately centrifuged, the plasma was frozen until assay, and red blood cells resuspended in normal saline were returned to animals at the time of the next sample. Individual rats were usually used as their own control, receiving control injections on one experimental day and the drug(s) to be tested on a subsequent day. Each animal was sampled for 5 h beginning at 1000 h.

Drug treatments

The dopamine-β-hydroxylase inhibitor, FLA-63 [bis(4-methyl-1-homopiperazinylthiocarbonyl)disulfide; Regis Chemical Co., Chicago, IL] was dissolved in normal saline containing 2 percent Tween 20 by treating with HCl and adjusting to pH 7.0 with NaOH. It was administered in doses of 10 or 20 mg/kg ip at 0845 h. The same animal received an equivalent volume of vehicle on another day. After demonstration that FLA-63 (20 mg/kg) suppressed episodic GH secretion, FLA-63-treated rats were subsequently administered the α-receptor agonist clonidine (15 and 150 μg/kg; Boehringer Laboratories, Montreal, Canada) or vehicle (normal saline) iv at 1035, 1210, or 1335 h without disturbing the animals. Plasma PRL was also measured in all animals.

Hormone assays

Growth hormone and PRL levels were determined in duplicate by a double antibody RIA using materials supplied by Dr. Parlow and the NIAMDD. Values are expressed in terms of the respective NIAMDD reference preparation. Minimum sensitivities of the assays are 3.2 ng/ml of GH and 1.9 ng/ml for PRL.

Analysis of data

The secretory profiles of GH and PRL were compared in drug-treated and control animals to determine 1) the effect of FLA-63 (10 and 20 mg/kg) on pulsatile GH and PRL release, and 2) the effect of clonidine (15 and 150

μg/kg) on GH and PRL secretion in FLA-63-pretreated animals. The student's t test was used to compare the overall mean plasma 5-h PRL levels in FLA-63-treated rats.

RESULTS

GH and PRL secretion in control rats

The 5-h GH secretory profile in 10 control rats is shown in Fig. 33. GH secretion in individual animals confirmed previous observations (Tannenbaum and Martin, 1976; Terry et al., 1977) that the hormone is secreted in an ultradian rhythm with 3- to 3.5-h intervals between bursts. Peak GH levels were greater than 400 ng/ml, and trough values were less than 7 ng/ml. GH levels were characterized by a broad surge at 1040-1140 h, a trough at 1200-1300 h, and an afternoon surge at 1300-1400 h. PRL levels were low throughout, measuring less than 10 ng/ml. Individual rats showed 2-4 random bursts of PRL, occurring most frequently in the afternoon, as described elsewhere (Terry et al., 1977d; results not shown).

Effects of FLA-63 on episodic GH and PRL secretion

Administration of FLA-63 resulted in a marked, dose-dependent suppression of pulsatile GH secretion (Fig. 33 and 34). Episodic GH release began to return within 4 h after 10 mg/kg FLA-63 but remained suppressed for 6 h after 20 mg/kg (Fig. 34). Therefore, the larger dose was used in all further experiments. In contrast, FLA-63 had no significant effect either on overall

PRL levels when compared to the control group (3.5 ± 0.5 vs. 3.7 ± 0.6 ng/ml, respectively; n = 10 animals per group) or on the PRL secretory pulses (data not shown).

Effects of clonidine on GH and PRL in FLA-63-treated rats

Clonidine (15 or 150 µg/kg) or saline were administered randomly at times that coincided with the anticipated occurrence of spontaneous bursts (1035 and 1335 h) or low trough levels (1210 h) of GH, as had been documented in the same animal on a previous control day. Previous studies in rats kept on a constant light-dark cycle have shown that GH bursts occur at approximately the same time (within 1h) between animals or in the same animal tested on separate occasions (Tannenbaum and Martin, 1976). In FLA-63-treated rats, the larger dose of clonidine elevated GH levels to values comparable to those seen during the spontaneous pulses of untreated controls (Fig. 35). The 15 µg/kg dose had a noticeable stimulatory effect, but it was not significant (Fig. 35). Clonidine also caused a significant, dose-dependent elevation in PRL secretion (Fig. 36). The stimulatory effect of clonidine on GH and PRL secretion in FLA-63-pretreated animals was not dependent on the time of administration.

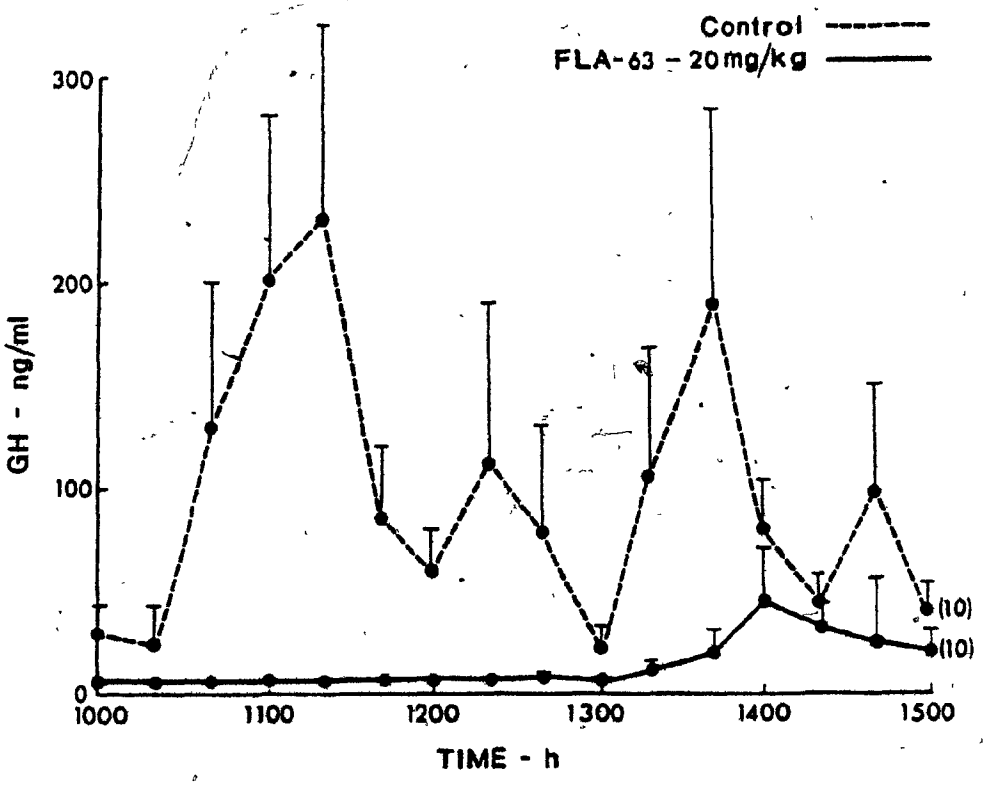

Fig. 33. Effects of an ip injection of FLA-63 (20 mg/kg) at 0845h on mean plasma GH in freely behaving male rats. Vehicle-injected control rats show typical episodic GH secretion with pulses at 1040–1140 and 1300–1400h. FLA-63 causes suppression of pulsatile GH release for 6 h. In this, and subsequent figures, number of animals in each group are shown in parentheses and vertical lines indicate SE of the mean.

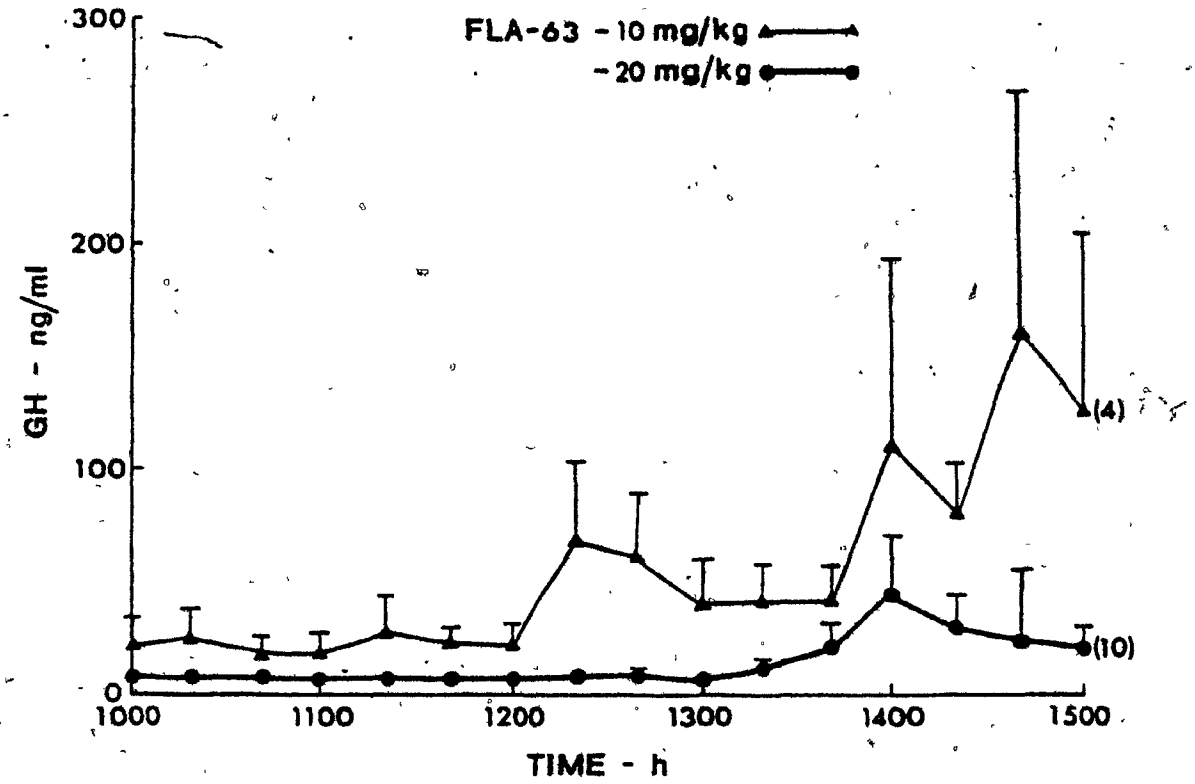

Fig. 34. Dose-dependent suppression of episodic GH secretion by FLA-63. The smaller dose of FLA-63 caused less suppression of GH release and had a shorter duration of action.

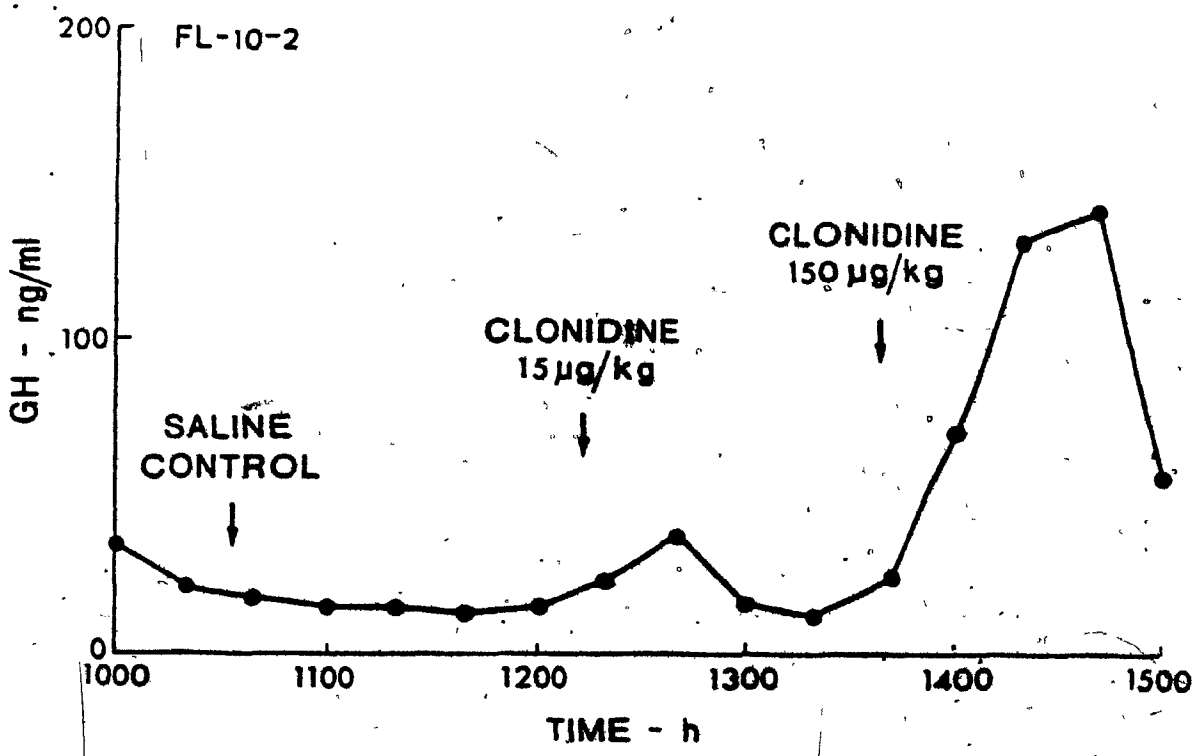

Fig. 35. Stimulation of GH secretion by clonidine (15 and 150 µg/kg) administered at 1210 and 1335 h, respectively, to a rat pretreated with FLA-63 (20 mg/kg) at 0845 h. The rise in GH after 150 µg/kg of clonidine is similar in magnitude to physiological spontaneous surges in GH.

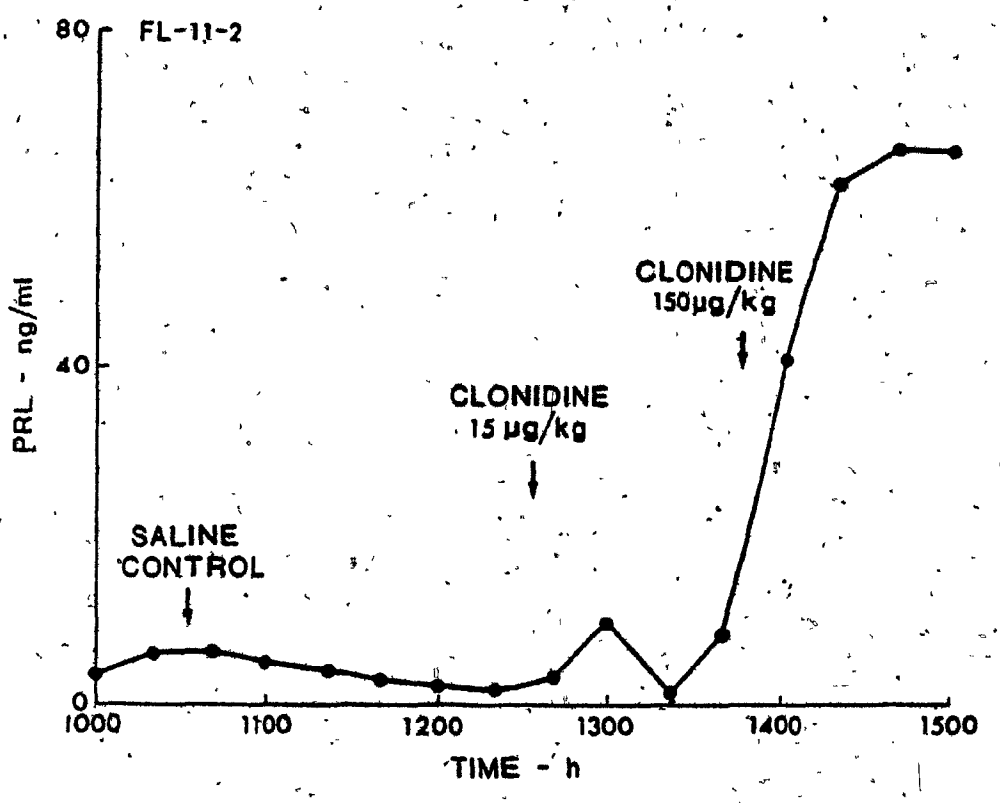

Fig. 36. Stimulation of PRL secretion by clonidine (15 and 150 µg/kg) administered at 1210 and 1335 h, respectively, to a FLA-63-treated rat.

Discussion

Results of this study indicate that the central α-adrenergic system is involved in the generation of episodic GH release in the undisturbed male rat. Treatment with the dopamine-β-hydroxylase inhibitor FLA-63 in a dose that is known to cause significant depletion of NE and epinephrine (EP) in the hypothalamus and other brain regions (Fuxe et al., 1979) resulted in total suppression of pulsatile GH release for 6 h. Administration of clonidine, a centrally active α-adrenergic receptor agonist, to FLA-63-pretreated animals caused an elevation of GH to levels that occur during physiological episodic release.

The results are in agreement with previously reported experiments that showed that inhibition of catecholamine synthesis with α-MT, blockade of α-receptors with phenoxybenzamine, or suppression of monoaminergic transmission with reserpine, abolished GH surges in chronically cannulated, unanesthetized male rats (Durand et al., 1977; Martin et al., 1978a; Eden et al., 1979). Blockade of β-receptors with propranolol had no effect on GH secretion (Martin et al., 1978b). GH release similar to natural secretory pulses could be induced with clonidine but not with the dopamine receptor agonist apomorphine (Durand et al., 1977). Similar results have been reported in the female rat. Selective blockade of NE and EP synthesis by diethyldithiocarbamate (DDC) completely abolished the episodic release of GH in unanesthetized, ovariectomized rats, and clonidine stimulated GH release to levels that occur during spontaneous release (Negro-Vilar et al., 1979).

These results are supported further by evidence of Ruch et al. (1976) that intraventricular administration of clonidine stimulates GH release in the male rat. Taken together, these findings suggest a major stimulatory role of α-adrenergic receptors in GH release.

Hypothalamic somatostatin cell bodies are present in the periventricular nucleus, an area richly innervated by NE nerve terminals and to some extent also by EP terminals (Fuxe, 1965; Lofstrom et al., 1976). Thus, the periventricular somatostatin-positive nerve cells that innervate the median eminence (Hokfelt et al., 1978) may be under the control of NE and possibly EP nerve cells. Andersson et al., (1977) found that treatment of hypophysectomized rats with rat GH reduced NE levels in the hypothalamic subependymal layer and decreased NE turnover in the posterior periventricular hypothalamic region. These results are significant because they provide further evidence of noradrenergic mechanisms in the control of GH secretion.

The present data indicate that selective blockade of NE and EP synthesis with FLA-63 had no significant effect on PRL secretion. In contrast, it was recently reported that inhibition of dopamine-β-hydroxylase by DDC in ovariectomized rats resulted in suppression of PRL release (Negro-Vilar et al., 1979). The reason(s) for this difference between sexes is not clear.

The effects of clonidine on PRL secretion support the findings of Durand et al., (1977) and Donoso et al., (1971) who showed that administration of either clonidine or DL-hydroxyphenylserine, respectively, increased PRL levels in α-MT-treated male rats. Stimulation of α-adrenergic receptors in ovariectomized rats pretreated with DDC resulted in a small rise in PRL (Negro-Vilar et al., 1979). The concept is well established that PRL

secretion is under the tonic inhibitory influence of the tuberoinfundibular dopaminergic system (MacLeod et al., 1976). Fuxe and Hokfelt (1970) have shown that activation of NE receptors by clonidine decreased activity in these tuberoinfundibular dopaminergic neurons. It is possible, therefore, that clonidine stimulated PRL secretion in the present experiments by the suppression of dopamine release from this neuronal system.

Clonidine has been classed as a preferential α_2-adrenergic agonist, and such receptors may be present on pre- and/or postsynaptic membranes (Starke, 1977). The present results demonstrating that clonidine stimulated GH secretion in animals pretreated with a dopamine-β-hydroxylase inhibitor suggest that clonidine activated postsynaptically located α-receptors. Recently, Fuxe et al. (1979) reported that clonidine also activates post- or presynaptic EP receptors in discrete brain regions. However, the predominant effect of clonidine was on NE, rather than EP, in the hypothalamus. In addition, clonidine may activate central histamine (H_2) receptors (Schwartz, 1979). However, there is no evidence that H_2-receptors are involved in the stimulation of GH or PRL secretion in the rat (Weiner and Ganong, 1978; Arakelian and Libertun, 1977; Rivier and Vale, 1977). Therefore, it is most likely that the stimulatory effect of clonidine on GH and PRL are due to activation of NE receptors.

IV. B. REGULATION OF EPISODIC GROWTH HORMONE SECRETION BY THE CENTRAL EPINEPHRINE SYSTEM - STUDIES IN THE CHRONICALLY CANNULATED RAT*

ABSTRACT

Catecholamines are postulated to regulate growth hormone (GH) secretion by their influence on the release of two hypothalamic substances, somatostatin (SRIF), which inhibits GH release, and GH-releasing factor, as yet unidentified. Extensive pharmacologic studies in man and animals indicate a stimulatory effect of central norepinephrine and dopamine on GH, but the function of epinephrine (EPI) is uncertain. Furthermore, many of the agents used to study the role of catecholamines in GH regulation are not selective in that they affect adrenergic as well as noradrenergic and/or dopaminergic, neurotransmission. In the present investigation, central nervous system (CNS) EPI biosynthesis was selectively interrupted with the specific norepinephrine N-methyltransferase inhibitors (NMT), SK and F 64139 and LY 78335, and the effects of central EPI depletion on episodic GH secretion in the chronically cannulated rat were determined. Inhibition of CNS EPI synthesis with SK and F 64139 caused complete suppression of episodic GH secretion, and concomitantly reduced the EPI level in the hypothalamus without affecting dopamine or norepinephrine. Administration of LY 78335 produced similar effects on pulsatile GH. Morphine-, but not clonidine-induced, GH release also was blocked by SK and F 64139. These results indicate that 1) the

*Terry et al., 1982

central EPI system has a major stimulatory function in episodic GH release, 2) morphine-induced GH release is mediated by the central EPI system, and 3) clonidine stimulates GH release by activation of postsynaptic alpha-adrenergic receptors. Drugs that affect CNS adrenergic systems have a potential role in the diagnosis and treatment of disorders of GH secretion.

INTRODUCTION

Growth hormone (GH) secretion by the pituitary gland appears to be regulated by two hypothalamic hormones, one stimulatory (GH-releasing factor), and the other inhibitory (somatostatin, SRIF). Although there is substantial physiological and biochemical evidence for the existence of GH-releasing factor, its structure has not been determined (Martin et al., 1978a) SRIF has been characterized as a tetradecapeptide (Brazeau et al., 1973). Extensive studies in man and experimental animals suggest that the release of SRIF and GH-releasing factor is regulated by catecholamine-containing neurons in the central nervous system (CNS) (Martin et al., 1978a; Martin, 1980).

Catecholamines were first implicated in GH regulation in man by the demonstration that insulin- and vasopressin-induced GH secretion were partially inhibited by the α-adrenergic blocker, phentolamine (Blackard and Heidingsfelder, 1968). It was shown subsequently that L-dopa stimulates GH secretion in humans (Boyd et al., 1970; Lal et al., 1975). Because L-dopa is a metabolic precursor of dopamine (DA), norepinephrine (NE), and epinephrine (EPI), it became essential to examine the effects of more selective dopaminergic and noradenergic drugs on GH release. For example, CNS

α-adrenergic receptor stimulation with clonidine elicits GH release in humans (Lal et al., 1975) and a number of lower species (Chambers and Brown, 1976; Lovinger et al., 1976; Durand et al., 1977; Terry et al., 1981a). Evidence concerning the role of brain DA in GH control is less consistent. In humans, CNS DA receptor stimulation with apomorphine causes GH release (Lal et al., 1973). However, activation or blockade of DA receptors in experimental animals has little effect on GH (Chambers and Brown, 1976; Lovinger et al., 1976; Durand et al., 1977; Willoughby et al., 1977), indicating that its effects may be specific to humans.

Serial measurements of plasma GH levels in humans and experimental animals show abrupt, spontaneous fluctuations (Martin et al., 1978a; Quabbe et al., 1976; Takahashi et al., 1968). For these oscillations to occur, the hypothalamic-pituitary connections must be intact (Martin, 1978). Because episodic GH secretion is particularly prominent in the male rat, this animal has provided a useful experimental model to study the adrenergic mechanisms involved in its regulation. In rats, a variety of noradrenergic synthesis inhibitors and receptor antagonists block the pulsatile secretion of GH (Martin, 1980). Moreover, noradrenergic antagonists prevent the increase in GH induced by morphine (Koenig et al., 1980).

In all the previous studies, the noradrenergic agonists and antagonists used may also have affected transmission in epinephrine (adrenergic) systems. Results of earlier experiments suggest that EPI stimulates GH in primates (Meyer and Knobil, 1967) and rodents (Muller et al., 1967; 1968), but not in man (Roth et al., 1963; Schalch, 1967; Massara and Strumina, 1970; Rabinowitz et al., 1968). The availability of several selective EPI synthesis

inhibitors, developed for possible use as antihypertensive agents in humans (Pendleton et al., 1979, 1980; Sauter et al., 1977; Fuller et al., 1977) have made it possible to alter the synthesis of EPI without affecting norepinephrine or dopamine. These compounds have been used in the present investigation to assess the involvement of brain EPI systems in regulation of episodic and morphine-induced GH secretion in the rat. Results of this study indicate that the CNS adrenergic system has a major function in the regulation of GH secretion.

METHODS

Animals

Male Sprague-Dawley rats (300-350 g, Harlan Industries Inc., Indianapolis, IN) were housed in temperature and humidity-controlled laboratories with a 12:12h light-dark cycle (lights on at 0600h). The animals were given free access to laboratory chow and water.

Drugs

The selective EPI synthesis inhibitors, SK and F 64139 and SK and F 29661 [7, 8-dichloro-1,2,3,4-tetrahydroisoquinoline and 1,2,3,4-tetrahydroisoquinoline-7-sulfonamide, respectively; Smith, Kline and French Laboratories, Philadelphia, PA], which act by inhibiting norepinephrine-N-methyltransferase (NMT, EC 2.1.1.28), were dissolved in 0.15 M NaCl (pH 7.0) to yield a final concentration of 25 mg/ml and administered in doses of 10, 25 and 50 mg/kg body weight ip. A third NMT inhibitor, LY 78335 (2,3-dichloro-α-methylbenzylamine; Eli Lilly and Company Research

Laboratories, Indianapolis, IN) was dissolved in the same vehicle and administered in doses of 25 and 50 mg/kg body weight ip. SK and F 64139 and LY 78335 cross the blood brain barrier and inhibit central and peripheral EPI synthesis (Pendleton et al., 1980; Fuller et al., 1977). SK and F 29661 only inhibits peripheral EPI synthesis because it does not cross the blood brain barrier (Pendleton et al., 1979). The α-agonist, clonidine (Boehringer Laboratories, Montreal, Quebec, Canada) was given in a dose of 150 μg/kg iv. Morphine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ) was diluted in 0.15 M NaCl to a final concentration of 0.5 mg/ml and administered in a dose of 43 mg/kg iv.

Experimental procedure

Experiment 1: Animals were implanted with chronic indwelling cannulae placed in the right atrium via the right external jugular vein and adapted to isolation test chambers, using methods described previously (Terry and Martin, 1978a). Sampling began after recovery of preoperative body weight (usually ~1 week postoperatively). Blood samples (4 x 50 μl) were withdrawn every 15 minutes and immediately centrifuged. The plasma was frozen at -90°C until radioimmunoassay of GH and prolactin (PRL), and red blood cells suspended in normal saline were returned to animals at the time of the next sample. Individual rats were usually used as their own control, receiving control injections on one experimental day and the drug(s) to be tested on a subsequent day. Animals (n=8/group) received either the centrally active EPI synthesis inhibitors, SK and F 64139, and LY 78335, the peripherally active,

SK and F 29661, or saline vehicle at 0930h and were sampled for 3h or 6h beginning at 1000h, in order to encompass one or two GH secretory episodes. The animal's behavior was monitored through one way observation ports in the cubicles.

In a parallel experiment, male rats (8/group) were sacrificed by cervical dislocation 1.5, 2.5, and 3.5h after receiving either SK and F 64139, 29661 or normal saline at 0930h. Their brains were removed immediately and hypothalami were dissected, weighed, snap frozen on dry ice, and stored at -90°C for subsequent extraction and determination of DA, NE, and EPI. The hypothalamic dissection was limited rostrally by the optic chiasm, caudally by the mammillary bodies, and laterally by the hypothalamic sulci, and the dorsal cut was approximately 2 mm from the base of the brain.

Experiment 2: Clonidine or vehicle was given to SK and F 64139-pretreated animals at 1105h, using methods described in Experiment 1.

Experiment 3: In order to determine if blockade of central EPI synthesis affected morphine-stimulated GH, morphine sulfate (3 mg/kg iv) or normal saline was administered at 1105h to animals pretreated with either SK and F 64139 or vehicle at 0930h. Samples were removed from 1000-1300h, as described above.

GH and PRL Radioimmunoassays

GH and PRL were measured in duplicate by double antibody radioimmunoassays using materials supplied by the National Pituitary Agency, as described previously (Terry and Martin, 1978a). Values were determined using the weighted Rodbard method (Rodbard, 1974) and expressed in terms of the NIAMDD

reference preparation, rGH-RP-1 and rPRL-RP-1. The minimum detectable amount of GH was 2.5 ng/ml and the intra- and interassay coefficients of variation were 3.8 percent and 9.6 percent, respectively (mean of 10 determinations). The minimum detectable amount of PRL was 1.0 ng/ml and the intra- and interassay coefficients of variation were 3.4 percent and 10.9 percent, respectively (means of 6 determinations).

Catecholamine assays

Concentrations of EPI, NE and DA in the hypothalamus were measured by a sensitive radioenzymatic assay. The catecholamines were extracted according to the method of Sole and Hussain (1977), and separated by thin layer chromatography, according to Peuler and Johnson (1977). The minimum detectable level of each catecholamine was 10 pg. All samples were measured in the same assay to avoid interassay variation.

Analysis of data

The secretory profiles of GH were compared in drug-treated and control animals to determine: 1) the effect of SK and F 64139, SK and F 29661, and LY 78335 on pulsatile GH and PRL release, and 2) the effects of clonidine, and morphine on GH secretion in vehicle- and/or SK and F 64139-pretreated animals. The effects of saline vehicle, SK and F 64139, SK and F 29661 and LY 78335 on GH and PRL levels over time were analyzed by two factor, repeated measures analyses of variance (drugs x time). In addition, single factor analyses of variance, followed by Newman-Keuls tests, were used to compare 1)

plasma GH and PRL levels in SK and F 64139-, SK and F 29661-, and LY 78335-treated rats, and 2) plasma GH levels from 1100-1300h in vehicle or SK and F 64139-pretreated animals that received drugs at 1105h. Single factor analyses of variance and Newman-Keuls tests (Weiner, 1971) also were used to compare tissue levels of catecholamines 1.5, 2.5, and 3.5 after receiving SK and F 64139, 29661 or vehicle. A P value less than .05 was defined as significant.

RESULTS

Effects of SK and 64139, SK and F 29661, and LY 78335 on Episodic GH and PRL Secretion

The pattern of GH secretion in individual animals confirmed previous observations (Tannenbaum and Martin, 1976) that male rats kept on a constant light-dark cycle have episodes of GH release that occur at approximately the same time (1100-1200 and 1300-1500h) (Fig. 37, left). The centrally active SK and F 64139 suppressed ($P < .001$) pulsatile GH for 6h (Fig. 37, right) in a dose-dependent manner (Fig. 38). Administration of the other centrally active EPI antagonist, LY 78335, also suppressed ($P < .001$) GH release, and this effect also was dose-related (Fig. 39). However, the peripheral antagonist, SK and F 29661 (50 mg/kg ip) had no effect on GH when compared to the normal saline control group (Fig. 40).

SK and F 64139 caused a significant, dose-related increase in mean 3h plasma levels of PRL (Table XIV). Administration of the peripheral EPI synthesis inhibitor, SK and F 29661, also elevated PRL levels (Table XIV). However, LY 78335 did not significantly affect plasma PRL when compared to the

normal saline control group (Table XIV). None of the drugs caused a discernible change in the animal's behavior and, specifically, there were no indications that the drug-treated animals were stressed.

Effects of SK and F 64139 and 29661 on Hypothalamic EPI

As demonstrated previously (Sauter et al., 1977; Crowley and Terry, 1981b), the centrally active EPI synthesis inhibitor, SK and F 64139 (50 mg/kg ip), caused a significant reduction ($P < .05$) in the hypothalamic concentration of EPI in male rats from 1.5 to 3.5h after administration, whereas, SK and F 29661, the peripheral EPI synthesis inhibitor, had no effect when compared to time-matched controls (Fig. 41). Neither of the drugs affected tissue levels of DA or NE (Table XV).

Effect of clonidine on GH in SK and F 64139-pretreated male rats

To test whether the inhibition of GH release by SK and F 64139 could be reversed by activation of central postsynaptic α-adrenergic receptors, clonidine (150 µg/kg) or vehicle were administered at a time (1105h) that coincided with the anticipated occurrence of a GH burst, as had been documented in the same animal on a previous control day. In SK and F 64139-pretreated rats, clonidine caused a significant elevation in mean GH levels above saline-injected controls ($P < .0001$, Fig. 42). Clonidine-stimulated mean GH levels were comparable to those seen during spontaneous pulses in untreated animals.

Effect of Morphine on GH in SK and F 64139-pretreated male rats

To determine if EPI was involved in morphine-induced GH release, morphine sulfate (3 mg/kg iv at 1105h) was administered to vehicle or SK and F 64139 pretreated male rats. In the vehicle-pretreated group, morphine had an immediate stimulatory effect on GH that resulted in mean plasma levels greater then 1000 ng/ml (Fig. 43), significantly higher than the ($P < .01$) group receiving saline vehicle (not shown). Previous studies have shown that morphine-stimulated GH release in the rat is inhibited by naloxone (Koenig et al., 1980), indicating that this is a specific effect due to activation of opiate receptors. In the present study, the stimulatory effect of morphine on GH was completely blocked by pretreatment with SK and F 64139 (Fig. 43).

TABLE XIV

EFFECTS OF SK&F 64139, SK&F 29661, AND LY 78335 ON MEAN 3h PLASMA PROLACTIN (PRL) LEVELS

	Plasma PRL (ng/ml) ± SE (n)
Normal Saline (ip)	4.7 ± 1.1 (10)
SK&F 29661 (50 mg/kg ip)	*20.2 ± 4.8 (10)
SK&F 64139 (ip)	
10 mg/kg	*14.4 ± 4.1 (6)
25 mg/kg	*25.8 ± 7.5 (6)
50 mg/kg	*39.7 ± 8.0 (9)
LY 78335 (ip)	
25 mg/kg	6.5 ± 1.5 (6)
50 mg/kg	9.7 ± 1.3 (6)

*P less than .05 compared to normal saline

TABLE XV

EFFECTS OF SKF 29661 AND SKF 64139 ON HYPOTHALAMIC CONCENTRATIONS OF DOPAMINE (DA) AND NOREPINEPHRINE (NE) IN MALE RATS 1.5, 2.5, AND 3.5 h AFTER INJECTION

	Normal Saline (ip)	SKF 29661 (50 mg/kg ip)	SKF 64139 (50 mg/kg ip)
DA (ng/g tissue)			
1.5 h	223.5 ± 17.8	213.4 ± 9.9	242.8 ± 6.5
2.5 h	221.8 ± 11.4	219.6 ± 10.3	250.1 ± 11.8
3.5 h	206.1 ± 16.6	190.9 ± 13.3	231.2 ± 15.0
NE (ng/g tissue)			
1.5 h	1498.5 ± 60.8	1384.0 ± 63.0	1313.0 ± 36.0
2.5 h	1503.0 ±118.0	1433.0 ±111.0	1327.0 ± 7.5
3.5 h	1275.2 ± 61.3	1300.0 ± 62.0	1300.0 ± 84.0

n = 8 animals in each group.

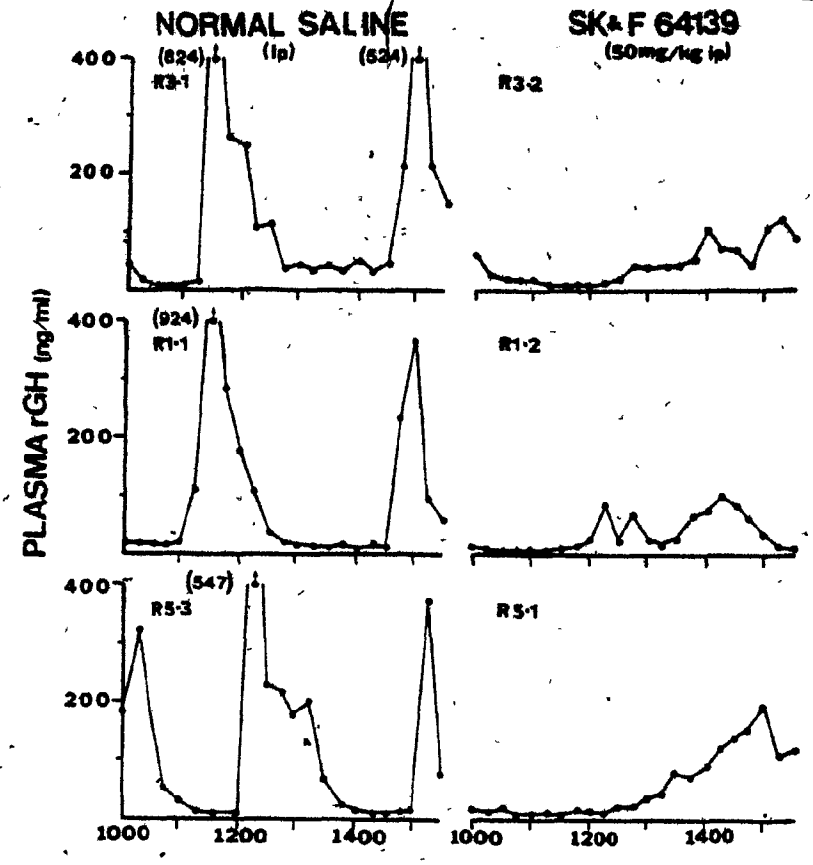

Fig. 37. Effects of SK&F 64139, a central EPI synthesis inhibitor, on episodic GH secretion in individual male rats sampled for 5-1/2h. Normal saline-treated control animals showed typical episodic GH release with pulses occurring between 1100-1200 and 1400-1500h. SK&F 64139 completely inhibited the first and partially suppressed the second pulse. Normal saline or SK&F 64139 were administered at 0930h. The letter R followed by a number in the upper left corner refers to individual rat identifications.

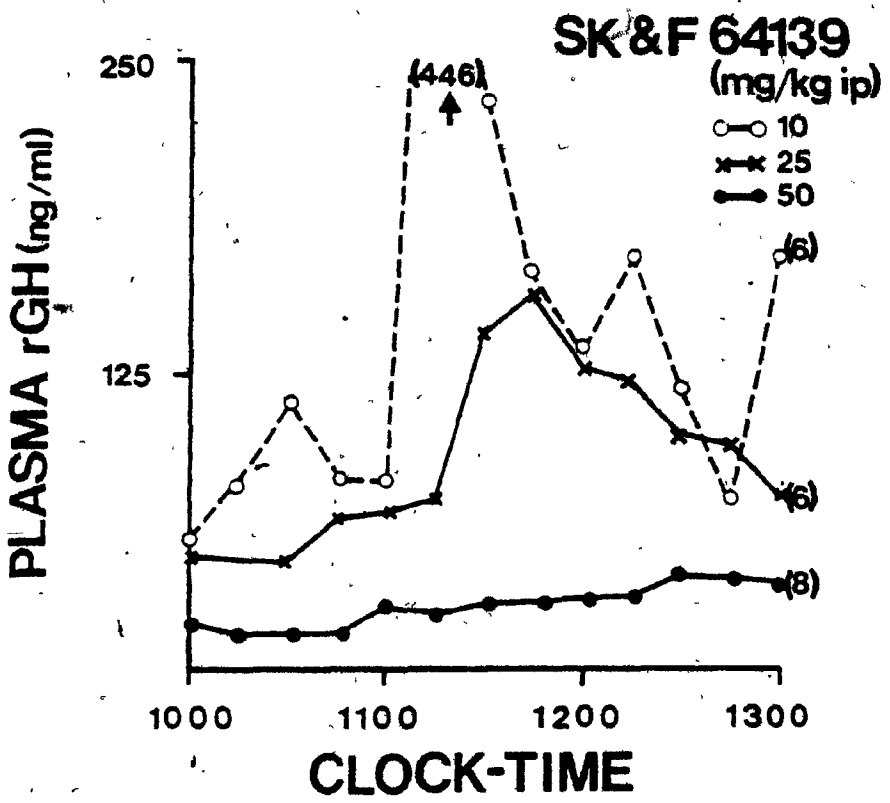

Fig. 38. Dose-related suppression of episodic GH secretion by SK&F 64139 administered at 0930h. SK&F 64139 inhibited ($P < .002$) GH release in doses of 25 and 50 mg/kg ip. The 10 mg/kg dose did not suppress GH significantly. Standard errors of the mean were omitted for clarity. In this, and subsequent figures, numbers in parentheses at 1300h refer to the number of animals in each treatment group.

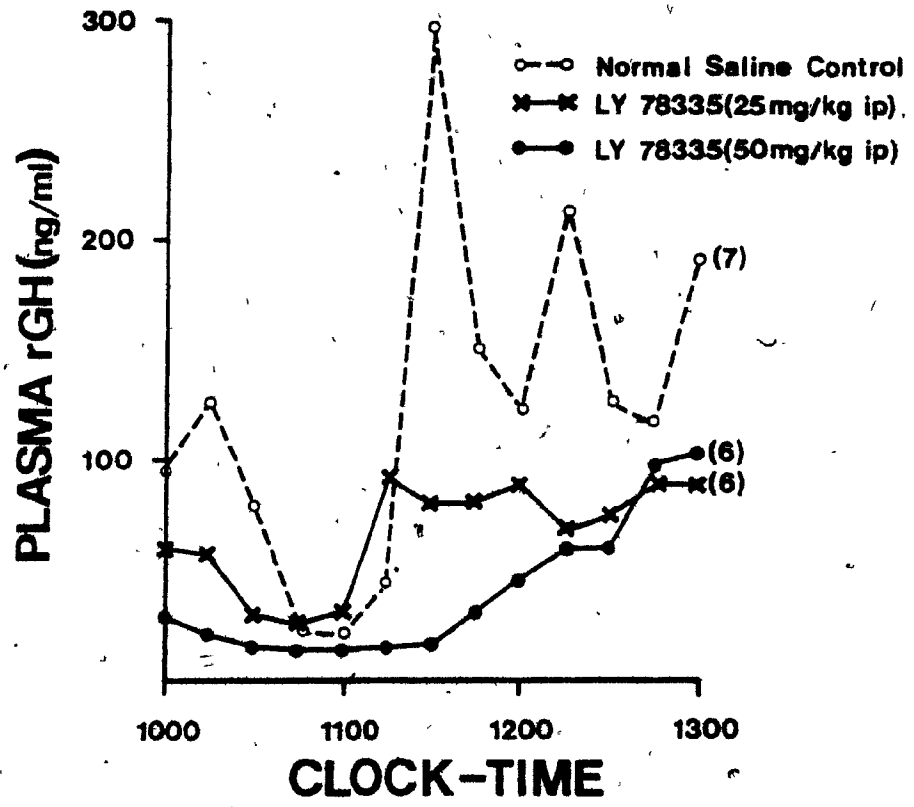

Fig. 39. Dose-related inhibition of pulsatile GH release by the central EPI synthesis inhibitor, LY 78335, administered at 0930h. Doses of 25 and 50 mg/kg inhibited ($P < .01$) GH. Standard errors were omitted for clarity.

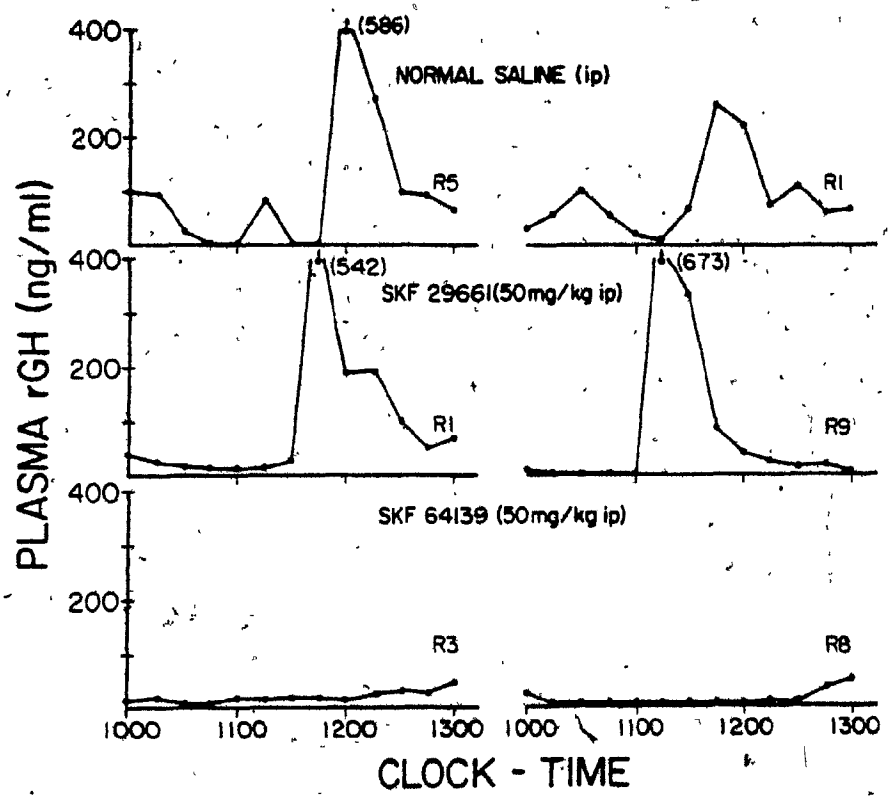

Fig. 40. Effects of SK&F 29661, a peripheral (EPI) synthesis inhibitor, and SK&F 64139 on episodic GH secretion in individual male rats. Normal saline- and SK&F 29661-treated animals showed pulsatile GH release (top and middle, respectively). SK&F 64139 completely suppressed episodic GH secretion (bottom). The letter R followed by a number in the lower right corner refers to individual rat identifications.

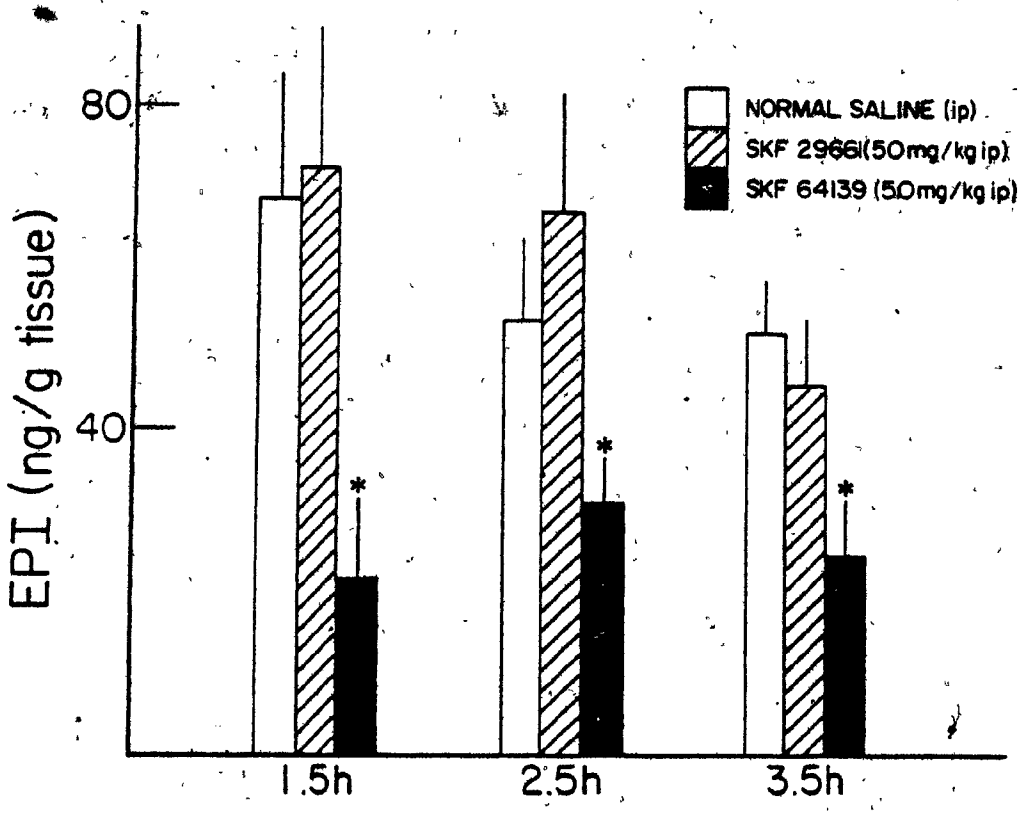

Fig. 41. Effects of SK&F 29661 and 64139 on the concentration of EPI in the hypothalamus 1.5, 2.5 and 3.5h after administration at 0930h. SK&F 64139 caused a significant reduction in EPI, but 29661 had no significant effect. *indicates P less than 0.05. There were 8 animals in each treatment group. Vertical lines represent SEM in this and subsequent figures.

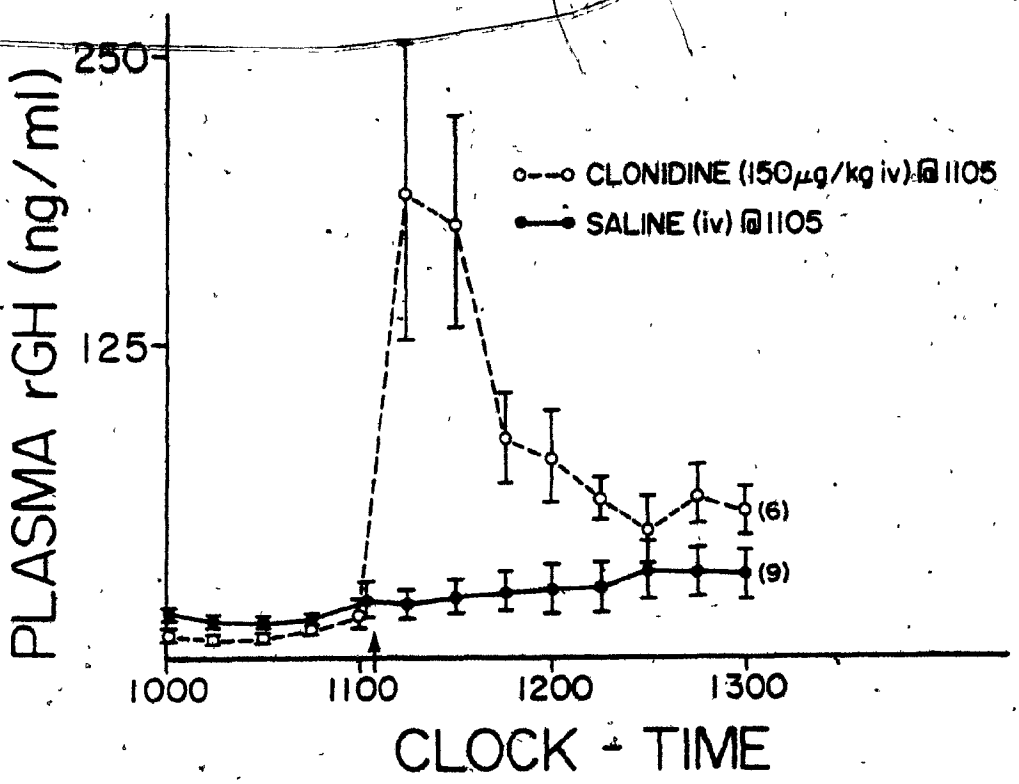

Fig. 42. Effect of clonidine, a centrally active α-adrenergic receptor agonist, on GH secretion in SK&F 64139- pretreated (0930h) male rats. Clonidine administration at 1105h (indicated by arrow) caused a significant elevation in GH levels.

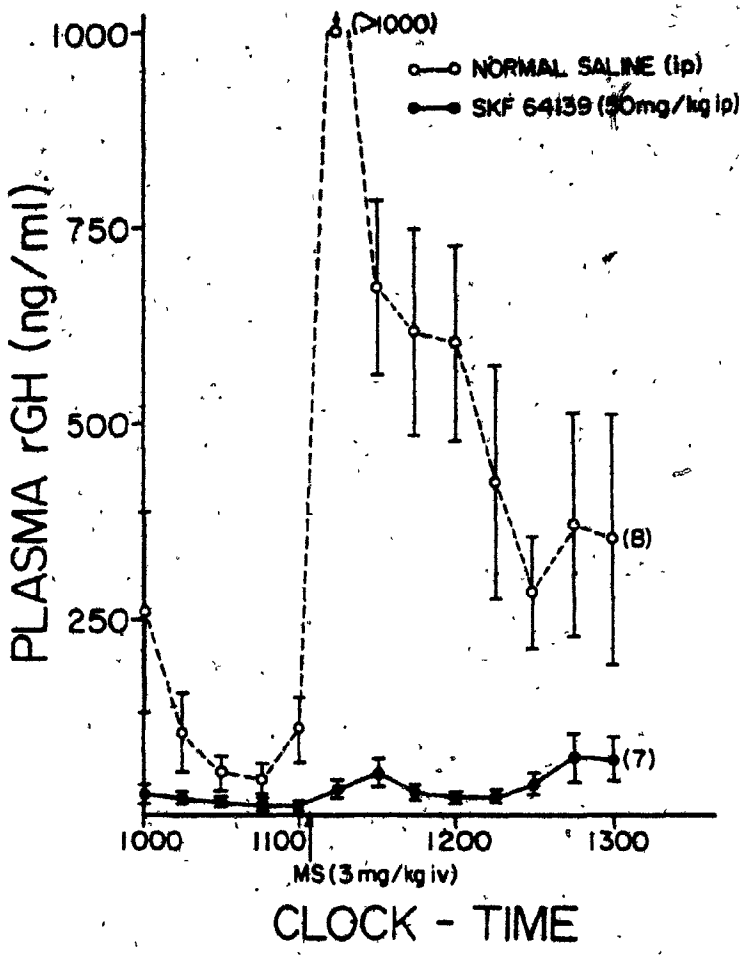

Fig. 43. Effects of morphine sulfate (MS) on GH secretion in SK&F 64139- and normal saline- pretreated (0930h) male rats. MS administration (1150h, indicated by arrow) to saline- pretreated rats caused a significant elevation in GH levels. This effect was blocked completely by pretreatment with SK&F 64139.

DISCUSSION

Results of the present study show that inhibition of CNS EPI synthesis with SK and F 64139 and LY 78335 completely suppressed physiologic, pulsatile GH release in male rats. In contrast, the peripheral EPI synthesis blocker, SK and F 29661, had no effect on GH secretion. This indicates that inhibition of brain, rather than adrenal, EPI synthesis is responsible. The inhibition of episodic GH secretion by SK and F 64139 was accompanied by a significant reduction in the hypothalamic concentration of EPI, while SK and F 29661 had no effect. Earlier studies have shown that LY 78335 inhibited hypothalamic EPI synthesis (Fuller and Perry, 1977). None of these drugs altered hypothalamic NE nor DA.

Previous attempts to investigate the function of EPI in GH regulation in man and experimental animals were limited by the poor penetrability of the blood brain barrier by EPI, and the unavailability of agents that directly affect brain EPI neurotransmission. In man, peripheral administration of EPI in doses sufficient to produce significant hyperglycemia did not induce GH secretion (Roth et al., 1963; Schalch, 1967; Massara and Strumina, 1970) nor inhibit arginine-stimulated GH release (Rabinowitz et al., 1968). However, large doses of EPI initiated a marked and prompt increase in plasma GH levels in female rhesus monkeys (Meyer and Knobil, 1967). Moreover, intracerebroventricular or intravenous EPI administration to rats caused release of bioassayable GH (Muller et al., 1967, 1968). Data from the present experiments, which employed selective EPI antagonists, indicate that the central EPI system has a major function in the generation of episodic GH secretion in the rat.

Morphine and the endogenous opioid peptides, met-enkephalin and β-endorphin, have a potent stimulatory effect on GH secretion in numerous experimental animals, an effect prevented by the specific opiate receptor blocker, naloxone (Martin et al., 1978a; Koenig et al., 1980; Van Vugt and Meites, 1980; Imura et al., 1981; Chihara et al., 1978). Furthermore, the synthetic enkephalin analogue, FK-33-824, causes GH release in man (von Graffenried et al., 1978). None of the opioids appear to act directly at the pituitary level and it is likely that they influence the release of GH either by modulating the secretion of releasing/inhibiting factors and/or by affecting neurotransmitters (Rivier et al., 1977; Dupont et al., 1977). Evidence to support the latter hypothesis was provided in a recent study demonstrating that α-adrenergic receptor blockade with phenoxybenzamine, or inhibition of NE and EPI synthesis with diethyldithiocarbamate, a dopamine-β-hydroxylase inhibitor, abolished the GH-releasing effect of morphine in the rat (Koenig et al., 1980). These effects were attributed to noradrenergic mechanisms. Data from the present study suggest that morphine enhances GH secretion by activation of CNS EPI systems because the selective EPI antagonist SK and F 64139 completely blocked morphine-induced GH release. These results do not necessarily exclude a similar role for NE.

Previous studies have shown that clonidine restores pulsatile GH release in rats after blockade of NE and EPI synthesis with FLA-63, a dopamine-β-hydroxylase inhibitor (Terry and Martin, 1981). In the present study, clonidine administration to SK and F 64139-pretreated animals elevated GH to levels that occur during episodic release. Clonidine also stimulates GH secretion in man and several species of experimental animals (Lal et al.,

1975; Chambers and Brown, 1976; Louinger et al., 1976; Durand et al., 1977; Terry and Martin, 1981a). The stimulatory action of clonidine is most likely due to activation of postsynaptic α-adrenergic receptors. Clonidine activates post-or presynaptic EPI receptors in discrete brain regions (Fuxe et al., 1979). It is not possible at present to determine whether the effect of clonidine is due to activation of receptors normally occupied by NE or EPI. However, the ability of clonidine to stimulate GH release in SK and F 64139-treated rats demonstrates that the EPI synthesis inhibitor does not compromise the ability of the pituitary gland to release GH.

In addition to its NMT-inhibiting actions, SK and F 64139 has been reported to have some adrenergic blocking effects mediated via α_2- and α_1-adrenoreceptors in vitro (Drew, 1981). Thus, caution should be exercised when interpreting the interaction between SK and F 64139, EPI, and GH, because NMT inhibition or α-receptor blockade could produce similar effects on GH release. It is unlikely that these α-blocking actions can account for the inhibitory effects of SK and F 64139 on pulsatile GH for several reasons. First, SK and F 64139 has little postsynaptic adrenergic blocking action, but does have presynaptic antagonistic properties (Drew, 1981). However, this would enhance NE and EPI release (Starke, 1977), and thus would not be expected to block GH release. Secondly, central EPI synthesis inhibition with LY 78335, which does not possess α-blocking properties (Fuller and Perry, 1977), also inhibited GH secretion. Finally, postsynaptic α-blockade by SK and F 64139 would be expected to inhibit clonidine-stimulated GH release (Schaub et al., 1980), but it did not.

The tetrahydroisoquinoline NMT inhibitors, SK and F 64139 and SK and F 29661, both elevated PRL levels. The mechanism underlying this effect is unknown at present but does not appear to be related to EPI synthesis inhibition because elevation of PRL was not obtained with the benzylamine NMT inhibitor, LY 78335. It is possible that the SK and F compounds exert a direct action on pituitary lactotrophs unrelated to their effects on EPI synthesis.

The dissociation between the effects of the SK and F agents and LY 78335 on PRL also argue against the possibility that the drug-induced suppression of GH release is due to stress. Stress suppresses pulsatile GH release and stimulates PRL release in rats (Terry et al., 1977d). Both SK and F 64139 and SK and F 29661 elevated plasma PRL, but only SK and F 64139 suppressed GH secretion. LY 78335 also inhibited pulsatile GH release but had no effect on PRL. These dissociations favor the interpretation that the inhibitory effects on GH are due to inhibition of central adrenergic neurotransmission.

Because octopamine can serve as an alternate substrate for NMT (Nagatsu, 1973), in addition to EPI, it is conceivable that the result of that interaction or others like it could have a role in CNS regulation of GH secretion. However, the distribution and role of octopamine in the mammalian CNS are unclear and there are no reports that other known metabolic products of alternative NMT substrates are active at α-receptor sites within the CNS.

In summary, results of the prsent experiments indicate 1) that central EPI systems exert a major stimulatory effect on episodic GH secretion and 2) that morphine-stimulated GH release is mediated by central EPI systems. 3) clonidine enhances GH release by activation of postsynaptic α-adrenergic

receptors. The selective EPI antagonist, SK and F 64139 is currently being investigated in humans as an antihypertensive agent (Dubb et al., 1979). This drug may also prove useful in the diagnosis and treatment of disorders of GH secretion.

C. CONCLUSIONS

Selective blockade of norepinephrine biosynthesis with FLA-63 resulted in complete suppression of rhythmic growth hormone secretion. Administration of clonidine to FLA-63-treated animals caused immediate release of growth hormone to levels observed during a spontaneous burst. Since norepinephrine is the biosynthetic precursor of epinephrine, one cannot conclude with certainty that the effects of FLA-63 on growth hormone resulted only from interruption of norepinephrine neurotransmission.

To circumvent this problem, epinephrine biosynthesis was blocked with agents that inhibited norepinephrine-N-methyltransferase. These drugs suppressed pulsatile and morphine-stimulated growth hormone secretion. In addition, clonidine released growth hormone after inhibition of epinephrine biosynthesis.

Thus, epinephrine and probably norepinephrine have an important function in the generation of rhythmic growth hormone secretion. The effect of clonidine on growth hormone most likely resulted from activation of postsynaptic alpha-adrenergic receptors that could be stimulated by either catecholamine.

There is clear evidence that somatostatin and the catecholamines, norepinephrine and epinephrine, are involved in regulation of rhythmic growth

hormone. It was hypothesized that catecholamines control growth hormone secretion by their influence on hypothalamic peptidergic neurons. To test this hypothesis, a perifusion system was designed wherein the effects of neurotransmitters on somatostatin release could be observed in vitro (section V).

V. EFFECT OF CENTRAL NEUROTRANSMITTERS ON SOMATOSTATIN RELEASE IN VITRO.

A. THE RELEASE OF BIOLOGICALLY AND IMMUNOLOGICALLY REACTIVE SOMATOSTATIN FROM PERIFUSED HYPOTHALAMIC FRAGMENTS*

ABSTRACT

The purpose of this investigation was to 1) develop a hypothalamic perifusion system which would allow measurement of spontaneous basal somatostatin (SRIF) release, 2) compare the immunological and biological activities of released SRIF, and 3) study the effect of membrane depolarization, extracellular calcium, and several neurotransmitters on SRIF release. Release was greater at the beginning of the perifusion and decayed with time for 90 min, after which it stabilized and remained constant for 3 h, the period used to determine the mean rates of release. Basal release was 20.2 pg/fragment · 10 min. Membrane depolarization with 55mM K^+ increased SRIF release 3- to 4-fold in a calcium-dependent manner.

The immunoreactivity and biological activity of SFIF concentrated by affinity chromatography of hypothalamic perifusates were compared to those of synthetic SRIF and rat hypothalamic extract. Biological activity was assessed by the inhibition of radioimmunoassayable rat GH released from cultured dispersed rat anterior pituitary cells. Hypothalamic fragments were exposed to several neurotransmitters as well as other substances known to influence rat GH secretion.

Our results may be summarized as follows 1) the perifused medial basal hypothalamus releases immunoactive and bioactive SRIF at a constant basal

*Terry et al., 1981b.

rate 2) membrane depolarization with high potassium stimulates a small releasable pool of SRIF in a calcium-dependent manner 3) affinity chromatography is an alternative technique to collect and concentrate SRIF released from tissues 4) common neurotransmitter agents did not modify SRIF release from the medial basal hypothalamus under the present conditions.

INTRODUCTION

The wide distribution of somatostatin (SRIF) together with its effects on neuronal activity and behavior place it in the same category as other neuropeptides with similar properties (Rorstad et al., 1980). Several of these peptides, such as substance P, LHRH, TRH, opioids, neurotensin, and vasoactive intestinal peptide, are concentrated in nerve endings and are postulated to comprise a new category of peptide neurotransmitters (Bennett and Edwardson, 1977; Duffy et al., 1975; Giachetti et al., 1977; Uhl and Snyder, 1976; Terry and Martin, 1978b; Elde and Hökfelt, 1978; Snyder, 1978; Iversen et al., 1978; Hokfelt, 1979).

The highest concentrations of SRIF are found in the median eminence (ME) and mediobasal hypothalamus (MBH). Brownstein et al. (1975) demonstrated that the ME, arcuate nucleus (ARCN), periventricular nucleus, ventral premammillary nucleus, and ventromedial nucleus (VMN) contain the highest SRIF concentrations of several hypothalamic nuclei. The localization of SRIF in nerve terminals, synaptosomes, and secretory granules (similar in size to those in nerve terminals) suggests a synaptic transmitter role for the peptide (Rorstad et al., 1980).

An important step in establishing a potential neurotransmitter role for the neuropeptides is the demonstration of their release from neuronal sites. Patel et al. (1977) first described calcium-dependent SRIF release from statically incubated rat neurohypophysis in the presence of depolarizing concentrations of potassium. Several investigators have demonstrated LHRH release from hypothalamic tissue and nerve endings incubated in vitro (Terry and Martin, 1978b; Rotsztejn et al., 1978; Gallardo and Ramirez, 1977; Kao and Weisz, 1977). Similar results have been demonstrated for TRH (Terry and Martin, 1978b; Schaeffer et al., 1977) and the enkephalins (Iverson et al., 1978; Henderson et al., 1978). Static incubation systems do not resolve the time course of events involved in a dynamic process such as neurosecretion; also, the hypothalamus is always subjected to the influence of its own metabolic and secretory products that accumulate during the incubation. These restrictions can be avoided by perifusion (superfusion) of isolated tissues or dispersed cells (Rorstad et al., 1980; Terry and Martin, 1978b; Gallardo and Ramirez, 1977; Kao and Weisz, 1977; Mulder and Smelik, 1977). In preliminary reports we (Terry and Martin, 1978c) determined the release of immunoreactive and bioreactive SRIF from perifused MBH. Similar results have been reported by others (Iversen et al., 1978; Wakabayashi et al., 1977; Patel et al., 1978; Negro-Vilar et al., 1978; Epelbaum et al., 1979a,b). In the present report we describe the release of immunoreactive and bioreactive SRIF from perifused MBH and the factors which affect it. The effect of several putative neurotransmitters and the use of immunoaffinity chromatography to remove and concentrate SRIF released from tissue were assessed.

MATERIALS AND METHODS

Animals

Adult male Charles River CD rats, weighing 200-250 g, were housed at constant room temperature (25 C) on a 12-h light, 12-h dark cycle (lights on, 0600h) for 1 week before sacrifice. They were given water and rat chow ad libitum

Preparation of hypothalamic fragments

All animals were decapitated between 0800-0900 h and their brains were rapidly removed. MBH fragments which contained a portion of the preoptic-anterior hypothalamic area were dissected with the following landmarks: anterior border of the optic chiasm, anterior border of the mammillary bodies, and 1 mm bilaterally and a depth of 1.0-1.2 mm from the lateral borders to include the ME, VMN, and portions of the ARCN, medial preoptic, and periventricular areas. Dimensions of representative fragments were verified by examination under a dissecting microscope. The tissue was immediately placed in Krebs-Ringer bicarbonate buffer (KRB; 119 mM NaCl, 1.2 mM KH_2PO_4, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 24.9 mM $NaHCO_3$) to which were added 11 mM glucose and 0.025 percent human serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO) with pH 7.4 when saturated with 5 percent CO_2-95 percent O_2 at 37.5C. Twenty-four MBHs were removed in 30 min and transferred to perifusion chambers. To ascertain which areas and nuclei were included in the MBH fragment, random samples were

removed immediately after dissection and 4.5 h of perifusion. Half of the fragments were snap frozen, cut at 50 μm on a cryotome, and stained with thionin-cresyl violet. The other half were immediately immersed in chilled 10 percent buffered formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined for tissue integrity by light microscopy.

The perifusion chambers were made of 1-ml disposable syringes fitted tightly with rubber gaskets into a Plexiglas outer chamber which enclosed the syringes. This system maintained the chambers at 37.5 C with a constant temperature circulator. The temperature inside each chamber was monitored with an electronic thermistor. Six MBH fragments were placed at the bottom of each chamber on top of a circular nylon screen to prevent tissue loss. We elected to use six fragments because individual fluctuations are not apparent when the average of several superfusions is calculated (Gallardo and Ramirez, 1977). The chambers were filled with KRB and sealed on top with a rubber plug which held the inlet tube. Each chamber was adjusted to contain 0.3 ml KRB in addition to the tissue volume. The inlet tube was connected to a Manostat Cassette pump (Manostat, NY) and medium was pumped from the reservoir at 3 ml/h. The pH of KRB was checked frequently. Ten-minute fractions (0.5 ml) were collected in 0.01 N HCl and immediately frozen at -20C. MBH fragments were perifused for 4.5 h. To compare the tissue contents of SRIF before and after perifusion, MBH fragments were extracted in 0.5 ml 0.1 N HCl and stored at -20C. The recovery of SRIF was studied, with and without the tissue present, by the addition of synthetic cyclic SRIF (100 pg/0.1 ml) to KRB and the measurement of SRIF-like immunoreactivity (SLI) in aliquots of media from

the inlet tube and collected fractions. Recoveries were determined as follows: percent recovery = 10^2 X SLI in collected fractions (picograms per 0.1 ml)/SLI in the inlet tube (picograms per 0.1 ml).

RIA for SRIF

SRIF was determined by methods previously described (Rorstad et al., 1979a). Fractions were brought to pH 7.2 with 0.1 N NaOH and 0.1 M Na Phosphate. The same amounts of neutralized HCl and KRB was added to the standard curve. Results were expressed as SLI.

Hypothalamic extracts

For bioassay studies, freshly dissected male rat hypothalami were extracted in 0.1 N HCl by methods previously described (Rorstad et al., 1979a). The extracts were frozen and stored at -20°C.

Recovery of released SLI by affinity chromatography (AFC)

Antiserum to SRIF was coupled to cyanogen-bromide-activated Sepharose 4B by a previously described method (Rorstad et al., 1979b). Small AFC columns were prepared from 1-ml syringes so that each contained 0.5 ml of the gel. The columns were connected in a series to collect the effluent from the perifusion chambers. To obtain sufficient SRIF for bioassay studies, 10 hypothalamic fragments were stimulated with K^+ for a period of 3 h after preincubation. The columns were eluted with 0.1 N HCl to remove SRIF, according to previously described methods (Rorstad et al., 1979a,b). Eluted fractions were frozen and stored at -20°C for RIA and bioassay.

The recovery of SLI from AFC was assessed by methods previously described (Rorstad et al., 1979a). Values for SRIF release after AFC were corrected for recoveries.

Dispersed rat anterior pituitary cell bioassay

Dispersed rat anterior pituitary cell cultures were prepared according to the method of Vale et al. (1976) with omission of the resuspension of cells in Viokase. After a preincubation period of 3 days in 35 X 10-mm dishes (Corning Glass Works, Corning, NY) containing 2 ml culture medium, the cells were washed once with culture medium. To 800 µl culture medium/dish were added 200 µl of either 1) a synthetic cyclic SRIF (Ayerst, Montreal, Canada) standard solution which contained 20-640 pg in 10 mM sodium phosphate buffer (pH 7.4)-0.1 M NaCl-0.1 percent human serum albumin (fraction V; Sigma), 2) the SLI eluted by acid from the AFC columns used to collect SLI released from perifused hypothalami, or 3) a HCl extract of rat hypothalamus.

The latter two experimental samples were neutralized with NaOH using phenol red as a pH indicator and were subsequently diluted in the same buffer as that used for synthetic SRIF. After incubation for 3 h, the culture medium was aspirated and stored frozen for subsequent assay of rat GH (rGH) by RIA using materials supplied by the NIAMDD (Bethesda, MD).

Preparation of experimental test substances

High potassium solutions (K^+) contained 55 mM K^+, and the NaCl concentration was reduced correspondingly (70 mM); low sodium (Na^+) solutions contained 70 mM NaCl and 5.6 mM K^+. Calcium-free (Ca^{+2}-free)

medium was prepared by omission of $CaCl_2$ from KRB. No EDTA or EGTA was added to Ca^{+2}-free solutions. The following substances were tested for releasing activity at concentrations of 10^{-4} -10^{-8} M in KRB: acetylcholine, apomorphine, aspartate, carbamylcholine, dopamine, L-dihydroxyphenylacetic acid, γ-aminobutyric acid, glutamate, glycine, L-isoproterenol, melatonin, morphine sulfate, neostigmine, norepinephrine, and prostaglandin E_2. Ascorbic acid was added (10^{-2}-10^{-6} M) to prevent oxidation of monoamines. All test materials were prepared in fresh, well-gassed KRB less than 5 min before introduction into perifusion chambers.

Analysis of data

Descriptions of the bioassay and RIA data for SRIF have been previously reported (Rorstad et al., 1979a,b). RIA data are expressed as: ln B/Bo vs. ln dilution of hypothalamic extract, perfusate, or ln pg SRIF. Slopes of the regression lines calculated by use of the least squares method were compared for parallelism. Bioassay data are presented as micrograms per ml rGH released vs. picograms of SRIF and dilution of hypothalamic perfusates or extract. Paired and unpaired t tests were used to compare data where appropriate.

RESULTS

Histology of the MBH fragment

Serial sections of paraffin-embedded and frozen MBH fragments contained the ME, VMN, and portions of the ARCN, medial preoptic, and periventricular areas at different levels (Fig. 44). There was good replication of MBH

dissections as assessed by histological examination. Histological examination of tissue perifused for 4.5 h showed variable perineuronal edema, but the nuclei were of normal appearance and none showed pyknotic changes that would be associated with cell degeneration.

Basal and K^+-stimulated SRIF release

The large quantity of SRIF released at the initiation of perifusion decreased with time for 60 min, after which it stabilized and remained constant for 3h, the period used to determine the mean rates of release for all subsequent experiments (Fig. 45). Fragments released 21.6 ± 1.9 pg SRIF/fragment·10 min over a 3 h basal period, which was less than 1.5 percent of the total tissue content. There was no significant change in the weight of the MBH fragments before and after perifusion (14.8 ± 0.5 vs. 14.6 ± 0.6 mg, respectively). Total MBH SRIF content was 22.2 ± 2.1 ng/fragment before perifusion, not significantly different from that after perifusion (22.8 ± 3.6).

The recovery of exogenous synthetic cyclic SRIF from the perifusion system was 91.2 ± 3.8 percent (n = 6) without and 100 ± 10.2 percent (n = 6) with MBH fragments present. The high recovery of SRIF in the presence of tissue represents endogenously released and exogenous material.

Stimulation with high K^+/low Na^+ in the presence of 2.5 mM Ca^{+2} significantly ($P < 0.001$) increased SRIF release to 83.9 ± 6.94 (n = 8) and 55.8 ± 10.6 (n = 8) pg/fragment·10 min (Figs. 46 and 47 respectively), which represented 0.37 percent and 0.25 percent of the total tissue content, respectively. High K^+/low Na^+ stimulation in Ca^{+2}-free KRB and low

Na^+/normal K^+ in the presence of 2.5 mM Ca^{+2}-KRB had no effect (Fig. 46). The first depolarizing pulse of high K^+/low Na^+ resulted in a significantly greater ($P < 0.025$, by paired t test; $n = 8$) SRIF release than the second (52.9 ± 8.6 vs. 41.0 ± 6.6 pg/fragment·10 min, respectively; Fig. 47).

RIA of affinity-recovered SRIF

Serial dilutions of SRIF recovered from affinity columns inhibited the binding of [^{125}I]Tyr^1-SRIF to antiserum in a parallel fashion to synthetic SRIF and a HCl extract of rat hypothalamus, indicating immunological similarity (Fig. 48). The recovery of SLI from AFC columns was 90.2 ± 0.98 percent. There was no SLI in effluents collected from AFC columns during K^+ exposure, which indicated that SRIF was completely subtracted from perifusates.

Biological activity of released SRIF

Synthetic SRIF, immunoaffinity-purified hypothalamic perifusates, and a HCl extract of rat hypothalamus inhibited the release of GH from cultured rat anterior pituitary cells in a dose-related manner (Fig. 49). The concentration of biologically active SRIF in the perifusates was determined by comparison of the amount of rGH released in the presence of the hypothalamic perifusates to a standard curve, relating rGH released and the concentration of synthetic SRIF. The perifusates shown in Fig. 49 contained in total 2.18 and 1.70 ng bioassayable SRIF, respectively. The hypothalamic extract contained 2.15 ng bioassayable SRIF/mg tissue wet wt. The amount of

bioassayable SRIF recovered from seven perifusion chambers (10 MBH fragments/chamber) was 1.64 ± 0.14 ng and represented 0.74 percent of the tissue content released. These results were consistent with the release of immunologically reactive SRIF (< 1.5 percent).

Effect of several putative neurotransmitters

None of the following substances had a significantly reproducible stimulatory effect on SRIF release at concentrations of 10^{-4}-10^{-8} M: acetylcholine, apomorphine, aspartate, carbamylcholine, dopamine, L-dihydroxyphenylacetic acid, γ-aminobutyric acid, glutamate, glycine, L-isoproterenol, melatonin, morphine sulfate, neostigmine, norepinephrine, or prostaglandin E_2. Fragments were exposed to K^+ to determine their responsivities and viabilities at the end of each experiment. Those that did not respond were excluded.

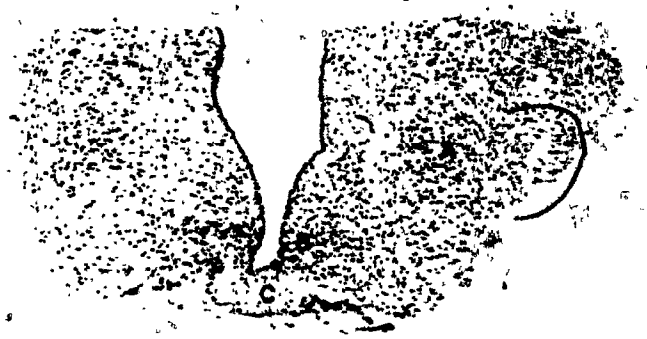

Fig. 44. Light microscopy of a coronal section of the rat MBH fragment. The MBH shown was perifused for 4.5h. The tissue was fixed in chilled 10% buffered formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and photographed at a magnification of 50 times its actual size. A, VMN; B, ARCN; C, ME. The third ventricle is not labeled.

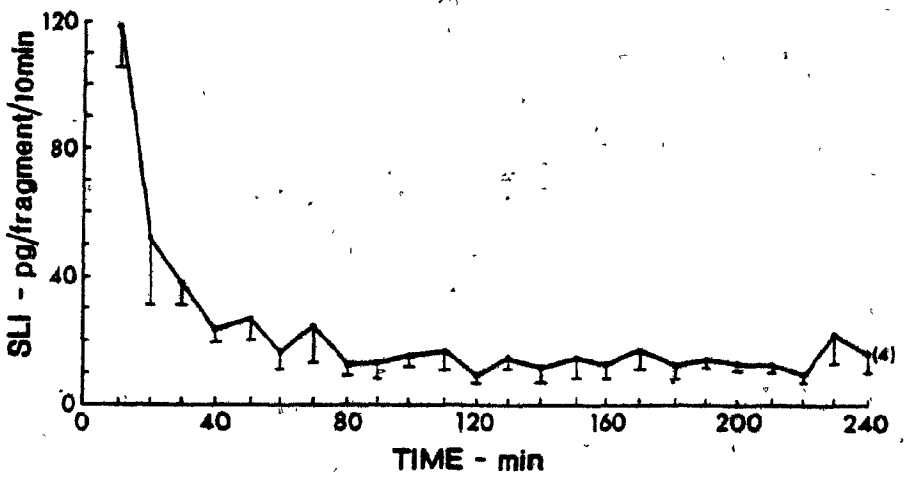

Fig. 45. Release of SLI from rat MBH perifused with KRB. Each point represents the mean ± SE of four chambers, each of which contained six MBH fragments.

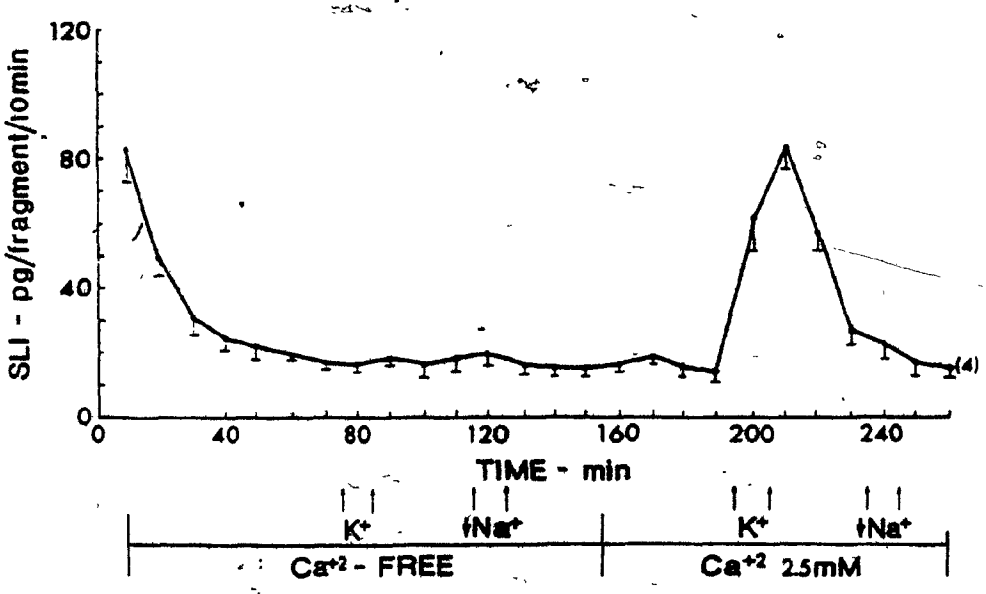

Fig. 46. Release of SLI from rat MBH perifused with Ca^{+2}-free KRB or standard KRB with 2.5 mM Ca^{+2}. K^+ refers to perifusion with 55 mM K^+ where the Na^+ was lowered correspondingly (70 mM) to maintain isotonicity. Na^+ refers to KRB with 70 mM Na^+ and 5.5 mM K.

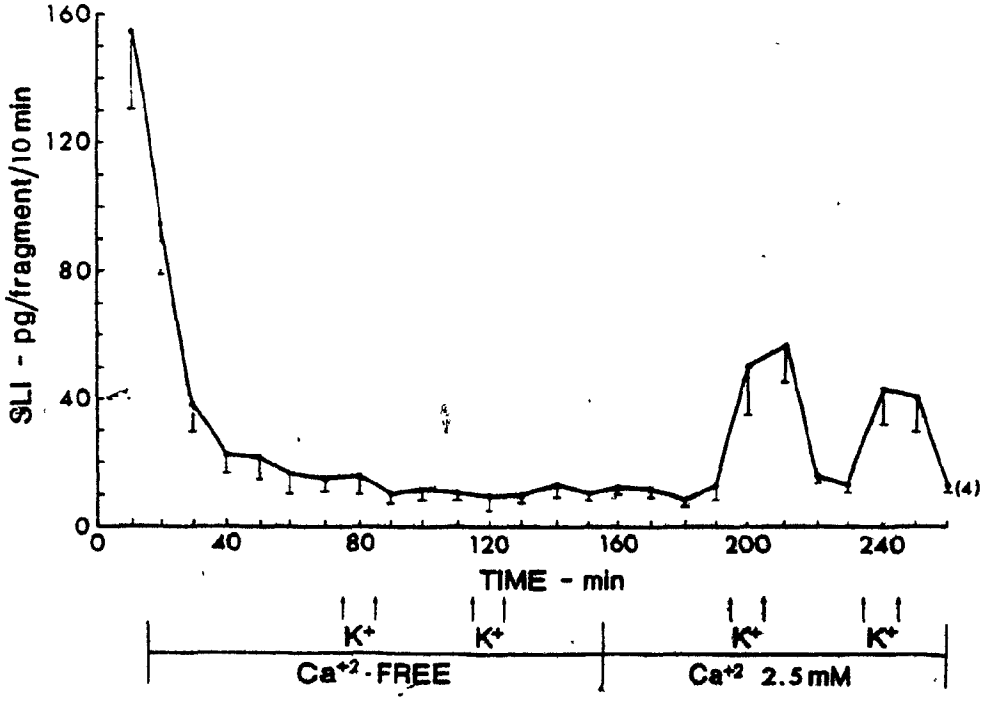

Fig. 47. Release of SLI from rat MBH perifused with Ca^{+2}-free KRB or standard KRB with 2.5 mM Ca^{+2}. K^+ refers to 55 mM K^+ and 70 mM Na^+.

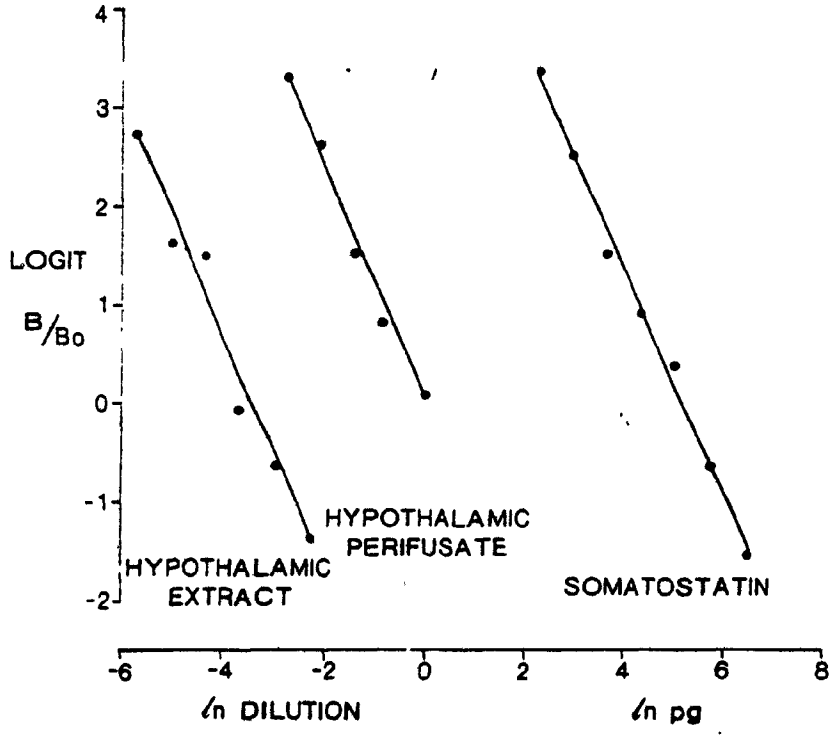

Fig. 48. Linear regression lines relating logit B/Bo and the natural logarithms of serial dilutions of a HCl extract of rat hypothalamus, serial dilutions of immunoaffinity-concentrated perifusates of rat hypothalami, and the mass of synthetic SRIF per RIA tube. (hypothalamic extract: $r = 0.9780$; $y = 1.186x + 4.114$; hypothalamic perifusate: $r = 0.9938$; $y = -1.189x + 0.1076$; SRIF: $r = 0.9923$; $y = -1.151x + 5.998$). B/Bo is the ratio of $[^{125}I]Tyr^{1}$-SRIF bound to antiserum in the presence of added SRIF or an experimental smaple to that bound in the absence of SRIF. Each point represents the mean of duplicate determinations.

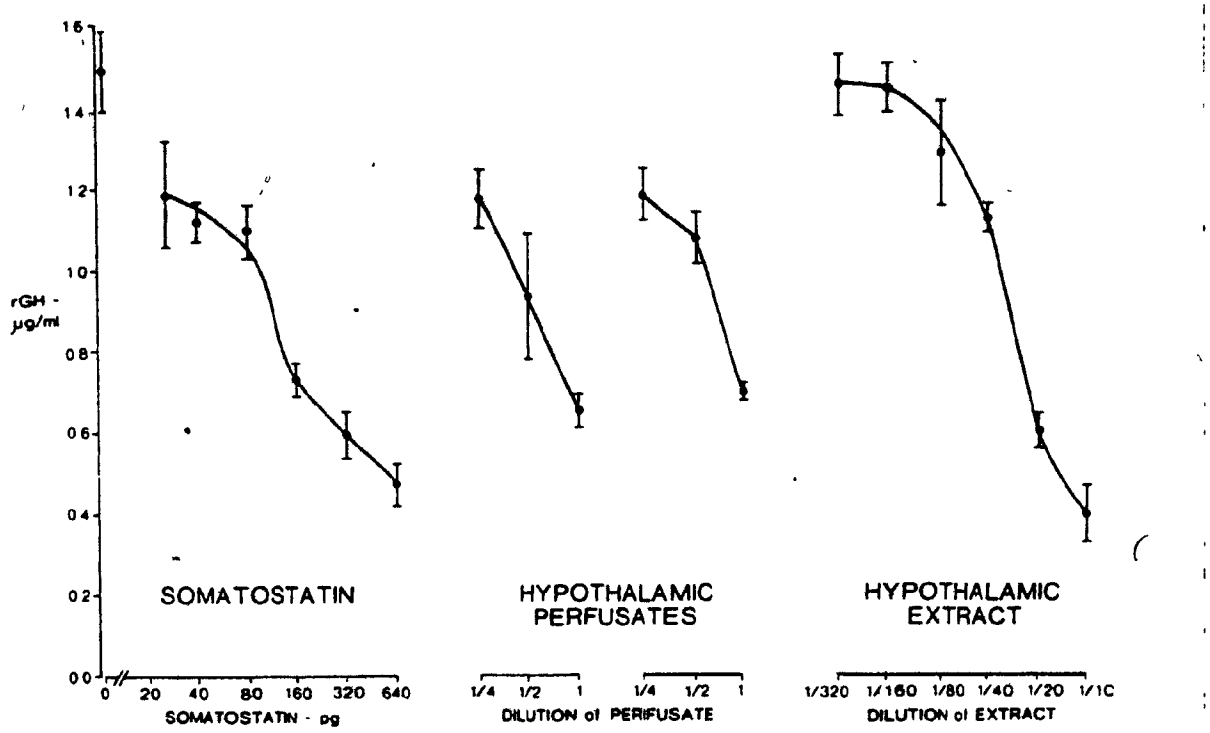

Fig. 49. Inhibition curves relating rGH released into the incubation medium in 3 h from cultured rat anterior pituitary cells and 1) the mass of synthetic SRIF per culture dish, 2) serial dilutions of immunoaffinity-purified hypothalamic perifusates, or 3) serial dilutions of a HCl extract of rat hypothalamus. Each point represents the mean ± SE of six determinations in the case of synthetic SRIF or three determinations for the hypothalamic perifusates or extracts.

DISCUSSION

To investigate the physiological significance of membrane depolarization on peptide release, certain criteria have been established (Terry and Martin, 1978). 1) Basal and depolarization-induced peptide release must be temperature and calcium dependent. 2) Membrane depolarization produced by excess potassium, electrical stimulation, or other depolarizing agents should be effective in releasing the peptide. 3) Degradation of the peptide must be inhibited in order to yield an accurate evaluation of the substances released. 4) Identification and quantification of the peptide released should be verified by more than one method, i.e. immunological and biological activities. Our results show that after an initial equilibration or washout period of 90 min, SRIF is released from perifused MBH at a constant basal rate which can be maintained for at least 4 h. Exposure to 55mM K^+, which causes cell depolarization, results in a 3- to 4-fold increase in SRIF release, representing approximately 0.3 percent of the total tissue content. This would suggest that membrane depolarization with K^+ stimulates a small releasable pool of the peptide.

Calcium has long been known to be an essential link in the process of neurotransmission. When its concentration in extracellular fluid is decreased, the release of synaptic transmitters is reduced and eventually abolished. The importance of calcium in the release of synaptic transmitters has been established at all chemical synapses tested regardless of the nature of the transmitter. Its role is further generalized to other secretory

processes, for example the liberation of hormones by cells in the posterior pituitary gland, adrenal medulla, and salivary glands (Douglas, 1968). In our studies, high concentrations of K^+ did not cause SRIF release in Ca^{+2}-free media; this provides direct evidence for a Ca^{+2}-dependent release mechanism. These results are in agreement with several other investigators who have described K^+-stimulated, Ca^{+2}-dependent SRIF release from the hypothalamus and amygdala as well as from hypothalamic and cortical synaptosomes (Iversen et al., 1978; Wakabayashi et al., 1977; Patel et al., 1978; Berelowitz et al., 1978). Peptidase inhibitors were not added to the perifusion system because the recovery of exogenous SRIF was greater than 90 percent.

The validity of the perifusion system was demonstrated by good preservation of the fine structure of incubated MBH fragments and no change in the tissue content of SRIF after perifusion. There was satisfactory replication of MBH dissections, as assessed by histological examination and reproducibility of SRIF content. The histological examination of the MBH fragment together with the constant SRIF content and preservation of calcium-dependent stimulated release suggest that, although there is variable perineuronal edema, the tissue is viable at the end of the 4.5-h perifusion.

Immunoaffinity chromatography was an alternative technique to remove and concentrate SRIF released from perifused tissues. The immunoreactivity and biological activity of SRIF concentrated by AFC were compared to those of synthetic cyclic SRIF and an acid extract of rat hypothalamus. The hypothalamic perifusate demonstrated a dose-response regression line parallel to synthetic SRIF and the hypothalamic extract, consistent with immunological

similarity. When tested for biological activity, MBH perifusates suppressed rGH release in a dose-related manner, similar to synthetic SRIF and the hypothalamic extract. Therefore, SRIF released from MBH fragments was immunologically and biologically similar to synthetic cyclic and endogenous hypothalamic SRIF.

The secretion of hypothalamic peptides is thought to be modified by the actions of central nervous system neurotransmitters. Dopamine, norepinephrine, and acetylcholine were reported to increase the concentration of SRIF in the pituitary portal blood of urethane-anesthetized rats (Chihara et al., 1978). It has also been observed that dopamine releases SRIF from incubated hypothalamic tissue (Negro-Vilar et al., 1978) and synaptosomal preparations (Wakabayashi et al., 1977) and that acetylcholine and serotonin inhibit SRIF release (Richardson et al., 1979). We were unable to demonstrate a significantly reproducible increase increase in SRIF release after exposure of the MBH to physiological concentrations of several common neurotransmitters. Recently, Epelbaum et al. (1979a,c) reported that SRIF release was unaffected by substance P, dopamine, γ-aminobutyric acid, or serotonin but was inhibited by vasoactive intestinal peptide. Because basal SRIF release was very low and approached the sensitivity of our assay, we could not conclusively determine inhibition of release below the basal level. One explanation for the discrepancy in reported neurotransmitter effects is the type of tissue preparation used to study release <u>in vitro</u>. The isolated ME is composed predominantly of nerve endings and, thus, could have a response different from the MBH which contains cell bodies and axons. Specific neurotransmitters may act at axoaxonic, axosomatic, or axodendritic sites to

exert an inhibitory and/or excitatory effect. It is even more difficult to compare in vivo with in vitro data because of the complex neuronal circuitry of hypothalamic-extrahypothalamic connections and accessability to specific sites which depends on the method of administration.

The presence of SRIF in synaptosomes of the ME is consistent with a hypophysiotropic role for SRIF in GH secretion. Its function in other parts of the nervous system remains to be established. Evidence that SRIF could function as a neurotransmitter includes: 1) its selective distribution in several anatomic pathways, 2) its localization in nerve terminals, synaptosomes, and secretory granules, suggesting its presence in nerve terminals; 3) its influences on the electrophysiological properties of neurons; and 4) its potassium-stimulated, calcium-dependent release from central nervous system tissue.

B. CONCLUSIONS

Although somatostatin was released from the medial basal hypothalamus in a calcium-dependent manner, exposure to several neurotransmitters did not cause a reproducible increase in somatostatin release. Because basal somatostatin release approached the lower sensitivity of the radioimmunoassay, it was impossible to accurately determine inhibition of release. These results emphasize the difficulties encountered when attempting to compare in vitro and in vivo data from systems with complex neuronal circuitry.

CONCLUSIONS

Growth hormone secretion in undisturbed, freely-behaving male rats was exemplified by spontaneous rises that occurred approximately every 3 1/2 hours, confirming results described elsewhere (Tannenbaum and Martin, 1976). Nadirs in plasma levels were low or undetectable. Peaks were frequently greater than 800 ng/ml and they appeared to be entrained to the light-dark cycle. There was very little intra- or interanimal variation in the amplitude, frequency or timing of this intrinsic rhythm.

In the female rat, episodic growth hormone secretion occurred approximately once each hour during all phases of the estrus cycle with a mean amplitude of 74 ng/ml. However, some animals had secretory bursts every two hours. Light entrainment of these pulses was not evident. Growth hormone rose during pregnancy and remained elevated for several hours after parturition. Suckling caused an immediate and brief rise in plasma growth hormone. Because of these multiformities, the female rat was not used in subsequent studies.

Neutralization of circulating somatostatin with specific antisera caused an elevation in the nadir levels of plasma growth hormone, but had no significant effect on summit nor mean levels. Thus, episodic rises in plasma growth hormone are not due to intermittent bursts of somatostatin release followed by postinhibitory rebound of growth hormone. These data favor the concept of a growth hormone releasing factor that is released episodically.

Further studies provided evidence to support the hypothesis that growth hormone is regulated by episodic secretion of at least two hypothalamic hormones. Electrical stimulation of the lateral hypothalamic-medial forebrain area had both excitatory and inhibitory effects on growth hormone secretion that were dependent on preexisting plasma levels. Stimulation during spontaneous growth hormone bursts caused an immediate decline in plasma levels, whereas, excitation when levels were low caused release of growth hormone. Passive immunization against somatostatin prevented stimulation-induced growth hormone suppression, but did not augment stimulation-induced growth hormone release, suggesting the former response was mediated by somatostatin, and the latter by a growth hormone releasing factor. The possibility also exists that circulating growth hormone has feedback effects that could influence responses to stimulation.

Although the precise localization of growth hormone releasing factor neurons is unknown, it has been proposed that they reside in the arcuate-ventromedial nuclear region. Neonatal administration of monosodium caused destruction of hypothalamic arcuate neurons and a marked reduction in the amplitude of growth hormone peaks. Even though the somatostatin concentration in the medial basal hypothalamus was reduced significantly, nadir levels of growth hormone remained low. Despite the fact that monosodium glutamate was not a selective neurotoxin, it is likely that destruction of arcuate neurons by this agent caused a deficiency of growth hormone releasing factor and resulted in absent episodic growth hormone secretion. The reduction in hypothalamic somatostatin was probably due to altered growth hormone feedback effects on this peptide.

Swimming stress in the rat caused prolonged suppression of rhythmic growth hormone secretion. Intracranial self-stimulation through electrodes in the lateral hypothalamic-medial forebrain region also suppressed episodic growth hormone release. There was no discernible difference between growth hormone responses to stimulation in reinforcing versus non-reinforcing sites. Moreover, the growth hormone responses to lateral hypothalamic stimulation could not be distinguished clearly from the response to swimming stress. These effects could be mediated by decreased growth hormone releasing factor or increased somatostatin release. Data from further studies favor the latter hypothesis. Passive immunization with somatostatin antiserum partially prevented or restored stress- and lateral hypothalamic stimulation-induced growth hormone inhibition. In addition, swimming stress decreased the concentration of somatostatin in the median eminence, suggesting increased release.

Somatostatin-containing cell bodies are concentrated in the periventricular nucleus of the hypothalamus and in the medial-basal amygdala. Data from the present experiments indicate that the periventricular cell group projects axons to the median eminence, arcuate, medial preoptic and rostral periventricular nuclei, and the medial-basal amygdalar group innervates the median eminence and suprachiasmatic nucleus via the stria terminalis. This somatostatinergic amygdalofugal pathway was confirmed recently by Sanaka et al. (1981). These results suggest that both the periventricular and amygdalar somatostatinergic systems may participate in the regulation of growth hormone secretion via their projections to the median eminence and other medial hypothalamic nuclei.

Further studies provided evidence to support this hypothesis. Removal of the target gland, the pituitary, caused a decrease in the somatostatin content of the median eminence, arcuate, periventricular and medial preoptic nuclei. These findings suggest that growth hormone exerts a short-loop inhibitory feedback action on its own secretion by affecting somatostatinergic cell bodies in the periventricular hypothalamus (and possibly the amygdala) that innervate the preoptic area and medial basal hypothalamus. Recent studies have shown that the central nervous system is involved in growth hormone autoregulation (Tannenbaum, 1980) and that growth hormone stimulates somatostatin release (Patel, 1979; Sheppard et al., 1978; Chihara et al., 1979).

Central adrenergic (and probably noradrenergic) systems have a major role in the generation of episodic growth hormone secretion. Initial studies with the dopamine-B-hydroxylase inhibitor, FLA-63, led to the conclusion that norepinephrine was the major catecholamine involved in pulsatile growth hormone secretion. However, FLA-63 also blocked epinephrine synthesis and it was impossible to be certain if one or both catecholamines were involved. Stimulation of growth hormone release by clonidine did not resolve this issue because it could have stimulated alpha-adrenergic receptors normally occupied by norepinephrine or epinephrine.

To determine the function of epinephrine in growth hormone regulation, epinephrine biosynthesis was blocked by selective norepinephrine-N-methyltransferase inhibitors, SK and F 29661, 64139 and LY 78335. SK and F 29661 does not cross the blood brain barrier and, although it inhibits adrenal epinephrine synthesis, had no effect on growth hormone.

Conversely, the other two agents blocked central epinephrine synthesis, evidenced by decreased hypothalamic levels, and inhibited episodic growth secretion in a dose-dependent manner. Levels of norepinephrine and dopamine were unaffected. These two drugs also blocked morphine-induced growth hormone release. Thus, adrenergic systems must be intact to maintain spontaneous rhythmic growth hormone secretion. Furthermore, growth hormone release induced by morphine is dependent upon adrenergic neurotransmission.

Epinephrine could generate growth hormone pulses by inhibition of somatostatin or stimulation of growth hormone releasing factor. Administration of somatostatin antiserum to SK and F-treated animals caused growth hormone release (Terry et al., 1981), favoring the former hypothesis. However, similar effects were observed in non-drug-treated control groups (data not shown). Other studies concerning thyrotropin secretion, indicate that epinephrine stimulates thyrotropin releasing hormone and does not inhibit somatostatin (Terry, 1981).

To determine the site of action of neurotransmitters that regulate growth hormone secretion, hypothalamic fragments were perifused in vitro and somatostatin release was measured. Somatostatin was released by membrane depolarization in a calcium-dependent manner. However, several different neurotransmitters did not stimulate somatostatin release. It could not be determined if somatostatin release was inhibited because basal secretion approached the lower limits of detection of the radioimmunoassay.

Results from earlier studies suggest that the isolated medial basal hypothalamus contains neural substrates necessary for episodic growth hormone secretion (Willoughby et al., 1977). Hypothalamic deafferentation causes a

decrease in the activity of norepinephrine-N-methyltransferase, indicating the extrahypothalamic origin of hypothalamic epinephrine (Brownstein et al., 1976). So far, norepinephrine-N-methyltransferase-positive cell bodies have been identified only in the brainstem. Since epinephrine is essential for spontaneous episodic growth hormone secretion, it may reach hypothalamic peptidergic neurons by an alternate route. Peptidergic neurons within hypothalamic islands may be hyperresponsive to smaller amounts of epinephrine as a result of denervation supersensitivity. Alternatively, there may be undiscovered epinephrine perikarya within the hypothalamic island, since complete deafferentation only reduces levels by 60 percent (Brownstein et al., 1976).

In conclusion, rhythmic growth hormone secretion in the rat is generated by excitatory and inhibitory neural elements. Peptidergic neurons in the medial hypothalamus are thought to generate this rhythm by releasing somatostatin and growth hormone releasing factor into the hypothalamic-adenohypophyseal portal system. Afferent inputs (i.e. epinephrine) to these neurons regulate their activity and therefore control the amplitude, frequency and entrainment of growth hormone rises and ebbs.

STATEMENT OF ORIGINALITY

With the exception of spontaneous pulsatile growth hormone secretion in the male rat (Section II,A), all of the observations described in Sections II through V are original. Other investigators have shown that stress suppresses plasma growth hormone in the rat, but the effect of swimming stress on pulsatile growth hormone secretion has not been reported previously.

Studies on the effects of somatostatin antiserum on growth hormone and stress-induced suppression of this hormone were reported by others about the same time the present data was published; some did not examine pulsatile secretion. The chronic effects of monosodium glutamate on growth hormone and somatostatin were studied simultaneously by other investigators, but the effects of this neurotoxin on the dynamics of rhythmic growth hormone secretion were not examined. Also, two studies describing somatostatin release in vitro were published while the present studies were in progress.

There have been reports describing the effects of hypothalamic deafferentation and medial preoptic lesions on hypothalamic somatostatin, though none described specific projections of periventricular somatostatinergic neurons to the medial preoptic, arcuate and rostral periventricular nuclei. Although it had been shown previously that hypophysectomy reduced somatostatin levels in the hypothalamic region, the present study was the first to localize this effect to discrete hypothalamic nuclei.

Using other less specific pharmacological agents, several investigators suggested that noradrenergic systems have a major role in the regulation of growth hormone secretion. However, it has not been shown that selective inhibition of dopamine-B-hydroxylase blocks rhythmic rises in growth hormone.

The remainder of the observations in this dissertation are described for the first time.

The entire contents of this thesis have been reported in the following publications:

Section II:

Saunders, A., L.C. Terry, J. Audet, P. Brazeau, and J.B. Martin (1976). Dynamic Studies of Growth Hormone and Prolactin Secretion in the Female Rat. Neuroendocrinology, 21:193-203.

Terry, L.C., J. Audet, P. Brazeau, and J.B. Martin (1976). Neuroendocrine Concomitants of Intracranial Self-Stimulation: Effects on Growth Hormone, Prolactin, and Corticosterone. Proc. Sco. Neurosciences, Toronto, Ontario, p. 660.

Terry, L.C., A. Saunders, J. Audet, J.O. Willoughby, P. Brazeau, and J.B. Martin (1977). Physiologic Secretion of Growth Hormone and Prolactin in Male and Female Rats. Clin. Endocrinology, 6:19S-28S.

Terry, L.C., P. Brazeau, and J.B. Martin (1977). Intracranial Self-Stimulation: Effects on Hypothalamic-Pituitary Regulation. Neurology, 27:405.

Terry, L.C., and J.B. Martin (1978). Hypothalamic-Pituitary Responses to Intracranial Self-Stimulation. Brain Res., 157:89-104.

Section III

Terry, L.C., J.O. Willoughby, P. Brazeau, J.B. Martin, and Y. Patel (1976). Antiserum to Somatostatin Prevents Stress-Induced Inhibition of Growth Hormone in the Rat. Science, 192:565-567.

Terry, L.C., J.B. Martin, J.O. Willoughby, and P. Brazeau (1976). Antiserum to Somatostatin Prevents Stress-Induced Inhibition of Growth Hormone in the rat. Fed. Proc. 2:35, 782.

Terry, L.C., J. Epelbaum, P. Brazeau, and J.B. Martin (1977). Antiserum to Somatostatin: Effects on the Dynamics of Growth Hormone, Prolactin, and Thyroid Stimulating Hormone Secretion in Cannulated Rats. 7th Annual Society for Neuroscience Meeting, ANAHEIM, A 1156.

Terry, L.C., J. Epelbaum, P. Brazeau, and J.B. Martin (1977). Monosodium Glutamate: Acute and Chronic Effects on Growth Hormone, Prolactin, and Somatostatin in the Rat. Fed. Proc., 36:364.

Terry, L.C., and W.R. Crowley (1979). Effect of Stress on the Concentration of Somatostatin (SRIF) in Discrete Hypothalamic and Extrahypothalamic Regions of the Rat. Soc. for Neuroscience Meeting, Atlanta.

Terry, L.C., and W.R. Crowley (1980). The Effect of Stress on Immunoreactive Somatostatin in Discrete Hypothalamic and Extrahypothalamic Nuclei of the Rat Brain. Brain Res., 197:543-546.

Crowley, W.R., and L.C. Terry (1980). The Effect of Anterior Periventricular Lesions on the Concentration of Somatostatin (SRIF) in Discrete Hypothalamic and Extrahypothalamic Regions of the Rat. Endocrine Soc. Abstracts, 62nd Annual Meeting, Washington, D.C.

Crowley, W.R., and L.C. Terry (1980). Biochemical Mapping of Somatostatinergic Systems in Rat Brain: Effects of Periventricular and Hypothalamic and Medial Basal Amygdaloid Lesions on Somatostatin-like Immunoreactivity in Discrete Brain Nuclei. Brain Res., 200:283-291.

Terry, L.C., and W.E. Crowley (1980). The Effect of Hypophysectomy on Immunoreactive Somatostatin in Discrete Nuclei of Hypothalamic and Extra-Hypothalamic Regions of the Rat Brain. Endocrinology, 107:1771-1775.

Terry, L.C., and W.R. Crowley (1980). Selective Depletions of Somatostatin in Discrete Nuclei by Hypophysectomy, Periventricular Hypothalamic, and Medial Basal Amygdaloid Lesions. Society for Neuroscience, 6:30(14.7), Cincinnati.

Terry, L.C., J. Epelbaum, and J.B. Martin (1981). Monosodium Glutamate: Acute and Chronic Effects on Rhythmic Growth Hormone and Prolactin Secretion, and Somatostatin in Undisturbed Rats. Brain Res., 217:129-142.

Terry, L.C., and J.B. Martin (1981). The Effects of Lateral Hypothalamic-Medial Forebrain Stimulation and Somatostatin Antiserum on Pulsatile GH Secretion in Freely-Behaving Rats: Evidence for a Dual Regulatory Mechanism. Endocrinology, 109:622-627.

Terry, L.C., W.R. Crowley, C. Lynch, C. Longserre, and M.D. Johnson (1982). Role of Central Adrenergic Neurons in the Regulation of Anterior Pituitary Hormone Secretion by Hypothalamic Peptides (Winter Neuropeptide Meeting, Breckenridge, Colorado).

Section IV:

Terry, L.C., and J.B. Martin (1978). Role of a-Adrenergic Mechanisms in the Generation of Episodic Growth Hormone (GH) and Prolactin (PRL) Secretion. Neurology, 28:366 (NC40).

Terry, L.C., and J.B. Martin (1981). Evidence for a-Adrenergic Regulation of Episodic Growth Hormone and Prolactin Secretion in the Undisturbed Male Rat. Endocrinology, 108:1869-1873.

Terry, L. (1981). Regulation of Episodic Growth Hormone and Thyrotropin Stimulating Hormone Secretion in the Rat by the Central Epinephrine System. Psychoneuroendocrinology Symposium.

Terry, L.C., W.R. Crowley, C. Longserre, C. Lynch, and M. Johnson (1981). Role of Epinephrine in Pulsatile Growth Hormone Secretion in the Rat. Endocrine Soc. Abstracts, 63rd Annual Meeting, Cincinnati, Ohio.

Terry, L.C. (1981). Regulation of Growth Hormone and Thyrotropin Stimulating Hormone Secretion by the Central Adrenergic System. American Neurological Association, San Francisco, Abstract No. 37.

Terry, L.C. (1981). Regulation of Growth Hormone and Thyrotropin Secretion by the Central Adrenergic System. Trans. Am. Neurol. Asc. (in press).

Terry, L.C., W.R. Crowley, and M.D. Johnson (in press, January 1982). Regulation of Episodic Growth Hormone Secretion by the Central Epinephrine System - Studies in the Chronically Cannulated Rat, J. Clin. Investigation.

Section V:

Terry, L.C., and J.B. Martin (1978). Release of Somatostatin (SRIF) from Perifused Rat Hypothalamic Fragments. Fed. Proc., 37:665.

Terry, L.C., O. Rorstad, and J.B. Martin (1978). Release of Somatostatin-like Immunoreactivity (SLI) from Perifused Mediobasal Hypothalami (MBE). Endocrine Society, 60th Annual Meeting, p. 86 (no. 24), Miami.

Terry, L.C., and J.B. Martin (1978). Release of Endogenous Somatostatin (SRIF) from Isolated, Perifused Medial-Basal Hypothalami. Neurology, 28:367 (NC5).

Terry, L.C., O. Rorstad, and J.B. Martin (1981). The Release of Immunologically and Biologically Active Somatostatin from Perifused Hypothalamic Fragments. Endocrinology, 107:794-800.

Terry, L.C., and J.B. Martin (1978). Hypothalamic Hormones: Subcellular Distribution and Mechanisms of Release. Ann. Rev. Pharmacol. and Toxicol., 18:111-123.

BIBLIOGRAPHY

Abrahms, R.L., M.L. Parker, S. Blanco, S. Reichlin and W.H. Daughaday (1966). Hypothalamic regulation of growth hormone secretion. Endocrinology 78:605-613.

Adey, W.R. and M. Meyer (1952). Hippocampal and hypothalamic connections of the temporal lobe in the monkey. Brain 75:358-384.

Ajika, K. and T. Hokfelt (1973). Ultrastructural identification of catecholamine neurones in the hypothalamic periventricular-arcuate nucleus-median eminence complex with special reference to quantitative aspects. Brain Res. 57:97-117.

Ajika, K. and T. Hokfelt (1975). Projections to the median eminence and the arcuate nucleus with special reference to monoamine systems: Effects of lesions. Cell Tiss. Res. 158:15-35.

Allen, J.P., C.F. Allen, M.A. Greer and J.J. Jacobs (1973). Stress-induced secretion of ACTH. In A. Brodish and E.S. Redgate (Eds.), Brain-Pituitary-Adrenal Interrelationships, Karger, Basel, pp.99-127.

Alpert, L.C., J.R. Brawer, Y.C. Patel, and S. Reichlin (1976). Somatostatinergic neurons in anterior hypothalamus: immunohistochemical localization. Endocrinology 98:255-258.

Amenori, Y., C.L. Chen and J. Meites (1970). Serum prolactin levels in rats during the different reproductive states. Endocrinology 86:506-510.

Abrahms, R.L., M.L. Parker, S. Blanco, S. Reichlin and W.H. Daughaday (1966). Hypothalamic regulation of growth hormone secretion. Endocrinology 78:605-613.

Adey, W.R. and M. Meyer (1952). Hippocampal and hypothalamic connections of the temporal lobe in the monkey. Brain 75:358-384.

Ajika, K. and T. Hokfelt (1973). Ultrastructural identification of catecholamine neurones in the hypothalamic periventricular-arcuate nucleus-median eminence complex with special reference to quantitative aspects. Brain Res. 57:97-117.

Ajika, K. and T. Hokfelt (1975). Projections to the median eminence and the arcuate nucleus with special reference to monoamine systems: Effects of lesions. Cell Tiss. Res. 158:15-35.

Allen, J.P., C.F. Allen, M.A. Greer and J.J. Jacobs (1973). Stress-induced secretion of ACTH. In A. Brodish and E.S. Redgate (Eds.), Brain-Pituitary-Adrenal Interrelationships, Karger, Basel, pp.99-127.

Alpert, L.C., J.R. Brawer, Y.C. Patel, and S. Reichlin (1976). Somatostatinergic neurons in anterior hypothalamus: immunohistochemical localization. Endocrinology 98:255-258.

Amenori, Y., C.L. Chen and J. Meites (1970). Serum prolactin levels in rats during the different reproductive states. Endocrinology 86:506-510.

Andersson K, K. Fuxe, P. Eneroth, J.A. Gustafsson, and P. Skett (1977). On the catecholamine control of growth hormone regulation. Evidence for discrete changes in dopamine and noradrenaline turnover following growth hormone administration. - Neurosci. Let. 5:83-89.

Antelman, S.M. and A. Caggiula (1977). Norepinephrine-dopamine interactions and behavior. Science, 195:646-653.

Arakelian MC and C. Libertun (1977). H1 and H2 histamine receptor participation in the brain control of prolactin secretion in lactating rats. Endocrinology 100:890-895.

Arimura, A., H. Sato, D.H. Coy and A.V. Schally (1975). Radioimmunoassay for GH-release inhibiting hormone. Proc. Soc. Exp. Biol. (N.Y.), 148:784-789.

Arimura, A., H. Sato, A. Dupont, N. Nishi and A.V. Schally (1975). Somatostatin: abundance of immunoreactive hormone in rat stomach and pancreas. Science 189:1007-1009.

Arimura A. and A.V. Schally (1976). Increase in basal and thyrotropin-releasing hormone (TRH)-stimulated secretion of thyrotropin (TSH) by passive immunization with antiserum to somatostatin in rats. Endocrinology 98:1069-1072.

Arimura, A., W.D. Smith and A.V. Schally (1976). Blockade of the stress-induced decrease in blood GH by anti-somatostatin serum in rats. Endocrinology 98:540-543.

Arnold, M.A. and J.D. Fernstrom (1980). Administration of antisomatostatin serum to rats reverses the inhibition of pulsatile growth hormone secretion produced by injection of methergoline but not yohimbine. Neuroendocrinology. 31:194-199.

Baker, B.L. and W.E. Dermody (1976). Effect of hypophysectomy on immunocytochemically demonstrated gonadotropin-releasing hormone in rat brain. Endocrinology 98:1116-1122.

Baker, B.L. and Y.Y. Yen (1976). The influence of hypophysectomy on stores of somatostatin in the hypothalamus and pituitary stem. Proc. Soc. Exp. Biol. Med. 151:599-602.

Baker, B.L. and Y.-K. Yu (1976). Distribution of growth hormone-release inhibiting hormone (somatostatin) in the rat brain as observed with immunocytochemistry, Anat. Rec., 186:343-356.

Bakke, J.L., N. Lawrence, J. Bennett, S. Robinson and C.Y. Bowers (1978). Late endocrine effects of administering monosodium glutamate to neonatal rats. Neuroendocrinology 26:220-228.

Ban, T. and F. Omukai (1959). Experimental studies on the fiber connections of the amygdaloid nuclei in the rabbit. J. Comp. Neurol. 113:245-280.

Bard, P., D. McK. Rioch (1937). A study of four cats deprived of neocortex and additional portions of the forebrain. Bull. Johns Hopkins Hosp. 60:73-147.

Bassiri, R.M. and R.D. Utiger (1974). Thyrotropin-releasing hormone in the hypothalamus of the rat. Endocrinology 94:188-197.

Beaulac-Baillargeon, L., T. DiPaolo, V. Raymond, V. Ginguere and F. Labrie (1980). Alpha-adrenergic sites in bovine pituitary cells in culture. Soc. Neurosci. Abstr. 6:29.

Bennett, G.W. and J.A. Edwardson (1977). Synaptic interactions between thyrotrophin releasing hormone and putative amino acid neurotransmitters in the central nervous system. J. Endocrinol. 73:27P.

Berelowitz, M., S. Kronheim, B. Pimstone and M. Sheppard (1978). Potassium stimulated calcium dependent release of immunoreactive somatostatin from incubated rat hypothalamus. J. Neurochem 31:1537-1539.

Berlowitz, M., B. Pimstone, B. Shapiro, S. Kronheim and D. DeWit (1978). Tissue growth hormone release inhibiting hormone-like immunoreactivity in experimental hypothyroidism and hypopituitarism. Clin. Endocrinol. 9:185-191.

Bernadakis, L.L. and B.D. Patterson (1968). Correlation between 'Lee Index' and caracass fat content in weaning and adult female rats with hypothalamic lesions. J. Endocr., 40:526-528.

Besser, G.M., C.H. Mortimer, A.A. McNeilly, M.D. Thorner, G.A. Bastitoni, S.R. Bloom, K.W. Kastrup, R.F. Hanson, R. Hall, D.H. Coy, A.J. Kastin and A.V. Schally (1974). Long-term infusion of growth hormone release inhibiting hormone in acromegaly: Effects on pituitary and pancreatic hormones. Br. Med. J. 4:622-627.

Bjorklund, A., B. Falck, F. Hromek, C. Owman and K.A. West (1970). Identification and terminal distribution of the tubero-hypophyseal monoamine fiber system in the rat by means of stereotaxic and mircrospectrofluorimetric techniques. Brain Res. 17:1-23.

Bjorklund, A., B. Falck, A. Mobin and U. Stenevi (1974). Organization of the dopamine and noradrenaline innervations of the median eminence-pituitary region in the rat. In Neurosecretion - The Final Endocrine Pathway, F. Knowles and L. Vollrath (eds). Springer, Berlin, pp.209-222.

Bjorklund, A., O. Lindvall and A. Mobin (1975). Evidence of an incertohypothalamic dopamine neurone system in the rat. Brain Res. 89:29-42.

Bjorklund, A., R.Y. Moore, A. Nobin and U. Steineri (1973). The organization of tuberohypophyseal and reticulo-infindibular catecholamine neuron systems in the rat brain. Brain Res. 51:171-191.

Bjorklund, A. and A. Nobin (1973). Fluorescence histochemical and microspectrofluorometric mapping of dopamine and noradrenaline cell groups in the rat diencephalon. Brain Res. 51:193-205.

Blackard, W.G. and S.A. Heidingsfelder (1968). Adrenergic receptor control mechanism for growth hormone secretion. J. Clin. Invest. 47:1407-1414.

Bloom, S.R., C.H. Mortimer, M.O. Thorner, G. M. Besser, R. Hall, A. Gomez-Pan, V.M. Roy, R.C.G. Russell, D.H. Coy, A.J. Kastin and A.V. Schally (1974). Inhibition of gastrin and gastric-acid secretion by growth-hormone release-inhibiting hormone. Lancet II:1106-1108.

Bohnet, H.G. and H.G. Friesen (1976). Control of prolactin secretion in man. In F. Labrie, J. Meites and G. Pelletier (Eds.), Hypothalamus and Endocrine Functions, Plenum Press, (N.Y.), pp.257-281.

Boyar, R.M. (1978). Sleep-related endocrine rhythms. In The Hypothalamus. S. Reichlin, R.J. Baldessarini, and T.B. Martin, (Eds.), Raven Press, (N.Y.), 56:373-386.

Boyd, A., H. Lebovitz and J.B. Pfeiffer (1970). Stimulation of human-growth hormone secretion by L-dopa. N. Engl. J. Med. 283:1425-1429.

Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemin (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77-79.

Brazeau, P., J. Rivier, W. and Vale R. Guillemin (1974). Inhibition of growth hormone secretion in the rat by synthetic somatostatin. Endocrinology 94:184-187.

Brown, G.M. and J.B. Martin (1974). Corticosterone, prolactin and growth hormone responses to handling and new environment in the rat, Psychosom. Med. 36:241-247.

Brown, G.M. and S. Reichlin (1972). Psychological and neural regulation of growth hormone secretion. Psychosom. Med. 34:45-51.

Brown, G.M., D.S. Schalch and S. Reichlin (1971). Hypothalamic mediation of growth hormone and adrenal stress response in the squirrel monkey. Endocrinology 89:694-703.

Brown, M. and W. Vale (1975). Central nervous system effects of hypothalamic peptides. Endocrinology 96:1333-1336.

Brown, G.M., I.V. Uhlir, J. Seggie, A.V. Schally and A.J. Kastin (1973). Effect of septal lesions on plasma levels of MSH, corticosterone, GH and prolactin before and after exposure to novel environment:role of MSH in the septal syndrome. Endocrinology 94:583-587.

Brownstein, M.J., A. Arimura, R. Fernandez-Durango, A.V. Schally, M. Palkovits and J.S. Kizer (1977). The effect of hypothalamic deafferentation on somatostatin-like activity in the rat brain. Endocrinology 100:246-248.

Brownstein, M.J., A. Arimura, H. Sato, A.V. Schally and J.S. Kizer (1975). The regional distribution of somatostatin in the rat brain. Endocrinology 96:1456-1461.

Brownstein, M., M. Palkovits, M.L. Tappaz, J.M. Saavedra and J.S. Kizer (1976). Effect of surgical isolation of the hypothalamus on its neurotransmitter content. Brain Res. 117:287-295.

Cameron, D.P., T.K.Y. Poon and G.C. Smith (1976). Effects of monosodium glutamate administration in the neonatal period on the diabetic syndrome in KK mice. Diabetologia 12:621-626.

Campbell, G.A., M. Kurcz, S. Marshall and J. Meites (1977). Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone, and prolactin; response to LH-releasing hormone and thyrotropin releasing hormone. Endocrinology 100:580-587.

Carlson, H.E., I.K. Mariz and W.H. Daughaday (1974). Thyrotropin-releasing hormone stimulation and somatostatin inhibition of growth hormone secretion. Endocrinology 94:1709-1713.

Carter, D.A. and A.G. Phillips (1975). Intracranial self-stimulation at sites in the dorsal medulla oblongata. Brain Research 94:155-160.

Chambers, J.W. and G.M. Brown (1976). Neurotransmitter regulation of growth hormone and ACTH in the rhesus monkey: effects of biogenic amines. Endocrinology 98:420-428.

Chen, H.J., G.P. Mueller and J. Meites (1974). Effects of L-dopa and somatostatin on suckling-induced release of prolactin and GH. Endocrine Research Communications 1:283-291.

Cheng, K.W., J.B. Martin and H.G. Friesen (1972). Studies on neurophysin release. Endocrinology 91:177-184.

Chihara K. (1979). Stimulation by rat growth hormone of somatostatin release into hypophyseal portal blood in the rat. Program of the 61st Annual Meeting of The Endocrine Society, Anaheim, CA., p.145 (Abstr).

Chihara K, A. Arimura, M. Chihara and A.V. Schally (1978). Studies on the mechanism of growth hormone and thyrotropin responses to somatostatin antiserum in anesthetized rats. Endocrinology 103:1916-1923.

Chihara, K., A. Arimura, D.H. Coy and A.V. Schally (1978). Studies on the interaction of endorphins, substance P and endogenous somatostatin in growth hormone and prolactin release in rats. Endocrinology 102:281-290.

Chihara K., A. Arimura and A.V. Schally (1979). Effect of intraventricular injection of dopamine, norepinephrine, acetylcholine, and 5-hydroxytryptamine on immunoreactive somatostatin release into rat hypophyseal portal blood. Endocrinology 104:1656-1662.

Clemens, J.A., M.E. Roush, R.W. Fuller and C.J. Shaar (1978). Changes in luteinizing hormone and prolactin control mechanisms produced by glutamate lesions of the arcuate nucleus. Endocrinology 103:1304-1312.

Collu, R., J.C. Jequier, J. Letarte, G. Leboeuf and J.R. Ducharme (1973). Effects of stress and hypothalamic deafferentation on the secretion of growth hormone in the rat. Neuroendocrinology 11:183-190.

Conrad, L.C.A. and D.W. Pfaff (1976a). Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J. Comp. Neurol. 169:185-226.

Conrad, L.C.A. and D.W. Pfaff (1976b). Efferents from medial basal forebrain and hypothalamus in rat. II. An autoradiographic study of the anterior hypothalamus. J. Comp. Neurol. 169:221-262.

Conrad, L.C.A. and D.W. Pfaff (1976c). Autoradiographic tracing of nucleus accumbens efferents in the rat. Brain Res. 113:589-596.

Conrad, C.D. and W.E. Stumpf (1975). Endocrine-optic pathways to the hypothalamus. In Anatomical Neuroendocrinology, W.E. Stumpf and L.D. Grant (Eds.), Karger, Basel, pp.15-29.

Cooper, J.R., F.E. Bloom and R.H. Roth (1978). The Biochemical Basis of Neuropharmacology, Oxford, New York, pp.326.

Critchlow, V., K. Abe, S. Urman and W. Vale (1981). Effects of lesions in the periventricular nucleus of the preoptic-anterior hypothalamus on growth hormone and thyrotropin secretion and brain somatostatin. Brain Res. 222:267-276.

Critchlow, V., R.W. Rice, K. Abe and W. Vale (1978). Somatostatin content of median eminence in female rats with lesion induced disruption of the inhibitory control of growth hormone secretion. Endocrinology 103:817-825.

Crowley, W.R., T.L. O'Donohue and D.M. Jacobowitz (1978). Changes in catecholamine content in discrete brain nuclei during the estrous cycle of the rat. Brain Res. 147:315-326.

Crowley, W.R. and L.C. Terry (1980). The effect of anterior periventricular lesions on the concentration of somatostatin (SRIF) in discrete hypothalamic and extrahypothalamic regions of the rat. Proc. 62nd Ann. Meet. Endocr. Soc. Washington, D.C., p.401.

Crowley, W.R. and L.C. Terry (1981a). Biochemical mapping of somatostatinergic systems in rat brain: effects of periventricular hypothalamic and medial basal amygdaloid lesions on somatostatin-like immunoreactivity in discrete brain nuclei. Brain Res.200:283-291

Crowley, W.R. and L.C. Terry (1981b). Effects of an epinephrine synthesis inhibitor, SK and F 64139, on the secretion of luteinizing hormone in ovariectomized female rats. Brain Res. 204:231-235.

Dahlstrom, A. and K. Fuxe (1964). Evidence for the existence of monoamine neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol. Scand. 64, Suppl. 232, p.1-55.

DeGroot, J. (1959). The Rat Forebrain in Stereotoxic Coordinates. North-Holland Publ., Amsterdam.

De Olmos, J.S. (1972). The amygdaloid projection field in the rat as studied with the cupric-silver method. In The Neurobiology of the Amygdala, B.E. Eleftheriou (Ed.), Plenum, New York, pp.145-204.

De Olmos, J.S. and W.R. Ingram (1972). The projection field of the stria terminalis in the rat brain. An experimental study. J. Comp. Neurol. 146:303-334.

De Wied, D. and W.H. Gispen (1977). Behavioral effects of peptides. In Peptides in Neurobiology, H. Gainer (Ed.), New York, Plenum Press, pp.397-448.

Donoso, A., W. Bishop, C.P. Fawcett, L. Krulich and S.M. McCann (1971). Effects of drugs that modify brain monoamine concentrations on plasma gonadotropin and plasma prolactin levels in the rat. Endocrinology 89:774-784.

Douglas, W.W. (1968). Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. Br. J. Pharmacol. 34:451-474.

Drew, G.M. (1981). α_2-adrenoreceptor-blocking action of the phenylethanolamine-N-methyl-transferase inhibitor SK and F 64139. J. Pharm. Pharmacol. 33:188-189.

Dubb, J.W., R.M. Stote, F. Alexander, A.P. Intoccia, M. Geczy and R.G. Pendleton (1979). Studies with a PNMT inhibitor. Clin. Pharmacol. Ther. 25:837-843.

Dube, D., R. LeClerc, G. Pelletier, A. Arimura and A.V. Schally (1975). Immunohistochemical detection of growth hormone-release inhibiting hormone (somatostatin) in the guinea pig brain. Cell Tiss. Res. 161:385-392.

Duffy M.J., D. Malhall and D. Powell (1975). Subcellular distribution of substance P in bovine hypothalamus and substantia nigra. Neurochem. 25:305-307.

Dupont, A., L. Cusan, M. Garon, F. Labrie and C.H. Li (1977). β-Endorphin: stimulation of growth hormone release in vivo. Proc. Natl. Acad. Sci. USA 74:358-359.

Durand, D., J.B. Martin and P. Brazeau (1977). Evidence for a role of α-adrenergic mechanisms in regulation of episodic growth hormone secretion in the rat. Endocrinology 100:722-728.

Dyer, R.C. and B.A. Cross (1972). Antidromic identification of units in the preoptic and anterior hypothalamic areas projecting to the ventromedial and arcuate nuclei. Brain Res. 43:254-258.

Eager, R.P., C.C. Chi and G. Wolf (1971). Lateral hypothalamic projections to the hypothalamic ventromedial nucleus in the albino rat. Demonstration by means of a simplified ammonical silver degeneration method. Brain Res. 29:128-132.

Eden, S., P. Bolle and K. Modigh (1979). Monoaminergic control of episodic growth hormone secretion in the rat: effects of reserpine, α-methyl-p-tyrosine, p-chlorophenylalamine, and haloperidol. Endocrinology 105:523-529.

Eden, S., E. Eriksson, J.B. Martin and K. Modigh (1981). Evidence for a growth hormone releasing factor mediating alpha-adrenergic influence on growth hormone secretion in the rat. Neuroendocrinology 33:24-27.

Ehle, AL, J.W. Mason and L.L. Pennington (1977). Plasma growth hormone and cortisol changes following limbic stimulation in conscious monkeys. Neuroendocrinology 23:52-60.

Elde, R. and T. Hokfelt (1978). Distribution of hypothalamic hormones and other peptides in the brain. In: Ganong WF, Martini L (Eds), Frontiers in Neuroendocrinology. Raven Press, New York, vol 5:1-33.

Elde, R., T. Hokfelt, O. Johansson, A. Ljungdahl, G. Nilsson and S.L. Jeffcoate (1978). Immunohistochemical localization of peptides in the nervous system. In J. Hughes (Ed.), Centrally Acting Peptides, University Park Press, Baltimore, pp.17-35.

Elde, R.P. and J.A. Parsons (1975). Immunocytochemical localization of somatostatin in cell bodies of the rat hypothalamus. Amer. J. Anat. 144:541-548.

Eleftheriou, B.E., C. Desjardins, M.L. Pattison, R.L. Norman and A.J. Zolovick (1969). Effects of amygdaloid lesions on hypothalamic-hypophyseal growth-hormone activity. Neuroendocrinology 5:132-139.

Endroczi, E., G. Hartmann and K. Lissak (1967). Meso-diencephalic activatory and inhibitory mechanisms: Their relations with conditioning, self-stimulation behavior and control of the pituitary-adrenocortical function. Acta. Physiol. Acad. Sci. Hung. 31:115-126.

Enjalbert, A., M. Ruberg, S. Arancibia, L. Fiore, M. Priam and C. Kordon (1979). Independent inhibition of prolactin secretion by dopamine and γ-aminobutyric acid in vitro. Endocrinology 105:823-826.

Epelbaum, J. P. Brazeau, D. Tsang, J. Brawer and J.B. Martin (1977a). Subcellular distribution of radioimmunoassayable somatostatin in rat brain. Brain Res. 126:309-323.

Epelbaum J., L. Tapia-Arancibia, J. Besson, W. Rotsztejn and C. Kordon (1979a). Vasoactive intestinal peptide inhibits release of somatostatin from hypothalamus in vitro. Eur. J. Pharmacol. 58:493-495.

Epelbaum, J., L. Tapia-Arancibia, C. Kordon, O.P. Ottersen and Y. Ben-Ari (1979b). Regional distribution of somatostatin within the amygdaloid complex of the rat brain. Brain Res. 174:172-174.

Epelbaum J., L. Tapia-Arancibia, W. Besson and C. Kordon (1979c). Inhibition by vasoactive intestinal peptide of the in vitro release of somatostatin from rat mediobasal hypothalamic slices. 61st Annual Meeting of The Endocrine Society, Anaheim, CA, p.145 (Abstr 289).

Epelbaum, J., J.O. Willoughby, P. Brazeau and I.B. Martin (1977b). Effects of brain lesions and hypothalamic deafferentation on somatostatin distribution in the rat brain. Endocrinology 101:1495-1502.

Eriksson, E., S. Eden and K. Modigh (1981). Importance of norepinephrine α_2-receptor activation for morphine-induced rat GH secretion. Neuroendocrinology 33:91-96.

Feldman, S., N. Conforti and I. Chowers (1971). The role of the medial forebrain bundle in mediating adrenocortical responses to neurogenic stimuli. J. Endocrinol. 51:745-749.

Feldman, S., N. Conforti and I. Chowers (1972). Effects of partial hypothalamic deafferentations on adrenocortical responses, Acta Endocr. (Kbh.) 69:526-530.

Feldman, S., N. Conforti and I. Chowers (1972). Neural pathways mediating adrenocortical responses to photic and acoustic stimuli. Neuroendocrinology 10:316-323.

Ferland, L., L. Cusan, A. Dupont, M. Garon and F. Labrie (1977). Role of somatostatin in the basal and stimulated release of GH and TSH. Endocrinology 100:230 (Abstr).

Ferland, L., F. Labrie, M. Jobin, A. Arimura and A.V. Schally (1976). Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. Biochem. Biophys. Res. Commun. 78:149-155.

Fernandez-Durango, R., A. Arimura, J. Fisback and A.V. Schally (1978). Hypothalamic somatostatin and LH-RH after hypophysectomy, in hyper- or hypothyroidism, and during anesthesia in rats. Proc. Soc. Exp. Biol. Med. 157:235-240.

Field, P.M. (1972). A quantitative ultrastructural analysis of the distribution of amygdaloid fibers in the preoptic area and ventromedial hypothalamic nucleus. Exp. Brain Res. 14:369-374.

Finklestein, J.W., H.P. Roffwarg, R.M. Boyar, J. Kream and L. Hellman (1972). Age-related changes in the twenty-four hour spontaneous secretion of growth hormone. J. Clin. Endocrinol. Metab. 35:665-670.

Frohman, L.A. and L.L. Bernardis (1968). Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Endocrinology 82:1125-1132.

Frohman, L.A., L.D. Bernardis, L. Burck, J.W. Maran and A.P.S. Dhariwal (1972). Hypothalamic control of growth hormone secretion in the rat. In Growth and Growth Hormone, A. Pecile and E.E. Muller (Eds.), Excerpta Medica, Amsterdam, pp.271-282.

Frohman, L.A., L.L. Bernardis and K. Kant (1962). Hypothalamic stimulation of growth hormone secretion. Science 162:580-582.

Frohman, L.A., J.W. Maran and A.P.S. Dhariwal (1971). Plasma growth hormone responses to intrapituitary injections of GH RF in the rat. Endocrinology 88:1483-1488.

Fuller, R.W. and K.W. Perry (1977). Lowering of epinephrine concentration in rat brain by 2,3-dichloro-α-methylbenzylamine, an inhibitor of norepinephrine N-methyltransferase. Biochem. Pharmacol. 26:2087-2090.

Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. (Suppl. 64) 247:37-85.

Fuxe, K. and T. Hokfelt (1966). Further evidence for the existence of tuberoinfindibular dopamine neurons. Acta Physiol. Scand. 66:245-246.

Fuxe K and T. Hokfelt (1970). Central monoaminergic systems and hypothalamic function. In: Hypothalamus, Martini L, Motta M, and F. Fraschini (Eds.), Academic Press, New York, p.136.

Fuxe, K., T. Hokfelt, G. Jonsson, S. Levine, P. Lidbrink and A. Lofstrom (1973). Brain and pituitary-adrenal interactions; studies on central monoamine neurons. In Brain-Pituitary-Adrenal Interrelationships, A. Brodish and E.S. Redgate (Eds.), Karger, Basel, pp.239-269.

Fuxe, K., G. Jonsson, P. Bolme, K. Anderson, L.F. Agnati, M. Goldstein and T. Hokfelt (1979). Reduction in adrenalin turnover in cardiovascular areas of rat medulla oblongata by clonidine. Acta Physiol. Scand. 107:177-179.

Gallardo E. and V.D. Ramirez (1977). A method for the superfusion of rat hypothalami: secretion of luteinizing hormone-releasing hormone (LH-RH). Proc. Soc. Exp. Biol. Med. 155:79-84.

Gerich, J.E., M. Lorenzi, W. Schneider, J.H. Karam, J. Rivier, R. Guillemin and P.H. Forsham (1974). Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. N. Engl. J. Med. 291:544-547.

Giachetti A, S.I. Said, R.C. Reynolds and F.C. Koniges (1977). Vasoactive intestinal polypeptide in brain: localization in and release from isolated nerve terminals. Proc. Natl. Acad. Sci. USA 74:3424-3428.

Gloor, P. (1955). Electrophysiological studies on the connections of the amygdaloid nucleus in the cat. Part I. The neuronal organization of the amygdaloid projection system. Electroenceph. Clin. Neurophysiol. 7:223-242.

Gloor, P. (1960). Amygdala. In Handbook of Physiology, Section I. Neurophysiology, Vol. II, I.H. Field, W. Magoun and V.E. Hall (Eds.), American Physiological Society, Washington, D.C., pp.1395-1420.

Gloor, P., I.T. Murphy and I.I. Dreifuss (1969). Electrophysiological studies of amygdalo-hypothalamic connections. Ann. N.Y. Acad. Sci. 157:629-635.

Gordin A, A. Arimura and A.V. Schally (1976). Effect of thyroid hormone excess and deficiency on serum thyrotropin in rats immunized passively with antiserum to somatostatin. Proc. Soc. Exp. Biol. Med. 153:319-323.

Grandison, L. and A. Guidotti (1979). γ-aminobutryric acid receptor function in the rat anterior pituitary: evidence for control of prolactin release. Endocrinology 105:754-759.

Greenwood, F.C., W.M. Hunter and J.S. Glover (1963). The preparation of I^{131}-labelled human growth hormone of high specific radioactivity. Biochem. J. 89:114-123.

Grosvenor, C.E. and C.W. Turner (1960). Pituitary lactogenic hormone concentration during pregnancy in the rat. Endocrinology 66:96-99.

Guillery, R.W. (1957). Degeneration in the hypothalamic connections of the albino rat. J. Anat. (London) 91:91-114.

Hall, E. (1963). Efferent connections of the basal and lateral nuclei of the amygdala in the cat. Am. J. Anat. 113:139-151.

Hall, R., G.M. Besser, A.V. Schally, D.H. Coy, D. Evered, D.J. Goldie, A.J. Kastin, A.S. McNeilly, C.H. Mortimer, C. Phenekos, W.M.G. Turnbridge and D. Weightman (1973). Action of growth-hormone-release inhibitory hormone in healthy men and acromegaly. Lancet 2:581-586.

Hall, R.D., F.E. Bloom and J. Olds (1977). Neuronal and neurochemical substrates of reinforcement. Neurosci. Res. Progr. Bull. 15:133-314.

Halasz, B., L. Pupp and S. Uhlarik (1962). Hypophysiotropic area in the hypothalamus. J. Endocrinol. (London) 25:147-154.

Halasz, B., K. Koves, M. Rethelyi, M. Bodiky and S. Koritsanszky (1975). Recent data on neuronal connections between nervous structures involved in the control of the adenohypophysis. In Anatomical Neuroendocrinology, W.E. Stumpf and L.D. Grant (Eds.), Karger, Basel, pp.9-14.

Harris, M.C. and M. Sanghera (1974). Projection of medial basal hypothalamic neurons to the preoptic anterior hypothalamic areas and the paraventricular nucleus in the rat. Brain Res. 81:401-411.

Hayhow, W.H. (1959). An experimental study of the accessory optic system in the cat. J. Comp. Neurol. 113:281-313.

Hayhow, W.R., C. Webb and A. Jeirue (1960). The accessory optic fiber system in the rat. J. Comp. Neurol. 115:187-215.

Heimer, L. (1975). Olfactory projections to the diencephalon. In Anatomical Neuroendocrinology, W.E. Stumpf and L.D. Grant (Eds.), Karger, Basel, pp.30-39.

Heimer, L. and W.J.H. Nauta (1969). The hypothalamic distribution of the stria terminalis in the rat. Brain Res. 13:284-297.

Henderson G., J. Hughes and H.W. Kosterlitz (1978). In vitro release of leu- and met-enkephalin from the corpus striatum. Nature 271:677-679.

Hökfelt, T. (1979). Polypeptides: localization. Neurosci. Res. Program Bull. 17:424-443.

Hökfelt, T., S. Efendic, C. Hellerstrom, O. Johanssen, R. Luft and A. Arimura (1975). Cellular localization of somatostatin endocrine-like cells and neurons of the rat with special references to the A_1 cells of the pancreatic islets and to the hypothalamus. Acta endocr. (Kbh.), Suppl. 200:5-41.

Hökfelt, T., R. Elde, K. Fuxe, O. Johanssen, A. Ljungdahl, M. Goldstein, R. Luft, S. Efendic, G. Nilsson, L. Terenius, D. Ganten, S.L. Jeffcoate, J. Rehfeld, S. Said, M. Perez de la Mora, L. Posaani, R. Rapia, L. Teran and R. Palacios (1978). Aminergic and peptidergic pathways in the nervous system with special reference to the hypothalamus. In The Hypothalamus, S. Reichlin, R.J. Baldessarini and J.B. Martin (Eds.), Raven Press, New York, pp.69-135.

Hoffman, D.L. and B.L. Baker (1977). Effect of treatment with growth hormone on somatostatin in the median eminence of hypophysectomized rats. Proc. Soc. Exp. Biol. Med. 156:265-271.

Holzwarth-McBride, M.A., E.M. Hurst and K.M. Knigge (1976). Monosodium glutamate-induced lesions of the arcuate nucleus. I. Endocrine deficiencies and ultra-structure of the median eminence. Anat. Rec. 186:185-196.

Imura, H., Y. Kato, H. Katakami and N. Matsushita (1981). Effect of CNS peptides on hypothalamic regulation of pituitary secretion. In Neurosecretion and Brain Peptides, J.B. Martin, S. Reichlin, and K.L. Bick, (Eds.), Raven Press, New York, pp.557-570.

Ishikawa, L., S. Kawamura and O. Tanaka (1969). An experimental study on the efferent connections of the amygdaloid complex in the cat. Acta Med. Okayama 23:519-539.

Iversen L.L., S.D. Iversen, F. Bloom, C. Douglas, M. Brown and W. Vale (1978). Calcium-dependent release of somatostatin and neurotensin from rat brain in vitro. Nature 273:161-163.

Iversen L.L., R.A. Nicoll and W. Vale (1978). VIII. The Neuropeptides: a new generation of neurotransmitters. Neurosci. Res. Program Bull. 16:336-340.

Jacobowitz, D.M. (1975). Fluorescence microscopic mapping of CNS norepinephrine systems in the rat forebrain. In Anatomical Neuroendocrinology, W.E. Stumpf and L.D. Grant (Eds.), Karger, Basel, pp.368-380.

Jacobowitz, D.M. and M. Palkovits (1974). Topographic atlas of catecholamines and acetylcholinesterase-containing neurons in the brain. I. Forebrain (telencephalon, diencephalon), J. Comp. Neurol. 157:13-28.

Johnson, T.N. and C.D. Clemente (1959). An experimental study of fiber connections between the putamen, globus pallidus, ventral thalamus, and midbrain tegmentum in the cat. J. Comp. Neurol. 113:83-101.

Jones, M.T., E. Hillhouse and J. Burden (1975). Secretion of corticotropin-releasing hormone in vitro. In Frontiers in Neuroendocrinology, Vol. 4, L. Martini and W.F. Ganong (Eds.), Raven Press, New York, pp.195-226.

Jonsson, G., K. Fuxe and T. Hokfelt (1972). On the catecholamine innervation of the hypothalamus, with special reference to the median eminence. Brain Res. 40:271-281.

Kanatsuka, A., H. Makino, Y. Matsushima, M. Osegawa, M. Yamamoto and A. Kumagai (1979). Effect of hypophysectomy and growth hormone administration on somatostatin content in the rat hypothalamus. Neuroendocrinology 29:186-190.

Kao, L.W.L. and J. Weisz (1977). Release of gonadotropin-releasing hormone (Gn-RH) from isolated, perifused medial-basal hypothalamus by melatonin. Endocrinology 100:1723-1726.

Kato, Y., K. Chihara, S. Ohgo and H. Imura (1974). Effects of hypothalamic surgery and somatostatin on chlopromazine-induced growth hormone release in rats. Endocrinology 95:1608-1613.

Kizer, J.S., M. Palkovits and M.J. Brownstein (1976). The projections of the A8, A9, and A10 dopaminergic cell bodies: Evidence for a nigral-hypothalamic-median eminence dopaminergic pathway. Brain Res. 108:363-370.

Knook, L.H. (1965). The Fibre Connections of the Forebrain. Davis, Philadelphia.

Kobayashi, R.M., M. Palkovits, I.J. Kopin and D.M. Jacobowitz (1974). Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Res. 77:269-279.

Koenig, J., M.A. Mayfield, R.J. Coppings, S.M. McCann and L. Krulich (1980). Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone - and prolactin - secretion in the rat. Brain Res. 197:453-468.

Kao, L.W.L. and J. Weisz (1977). Release of gonadotropin-releasing hormone (Gn-RH) from isolated, perifused medial-basal hypothalamus by melatonin. Endocrinology 100:1723-1726.

Kato, Y., K. Chihara, S. Ohgo and H. Imura (1974). Effects of hypothalamic surgery and somatostatin on chlorpomazine-induced growth hormone release in rats. Endocrinology 95:1608-1613.

Kizer, J.S., M. Palkovits and M.J. Brownstein (1976). The projections of the A8, A9, and A10 dopaminergic cell bodies: Evidence for a nigral-hypothalamic-median eminence dopaminergic pathway. Brain Res. 108:363-370.

Knook, L.H. (1965). The Fibre Connections of the Forebrain. Davis, Philadelphia.

Kobayashi, R.M., M. Palkovits, I.J. Kopin and D.M. Jacobowitz (1974). Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Res. 77:269-279.

Koenig, J., M.A. Mayfield, R.J. Coppings, S.M. McCann and L. Krulich (1980). Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone - and prolactin - secretion in the rat. Brain Res. 197:453-468.

Kokka, N., J.F. Garcia, R. George and H.W. Elliott (1972). Growth hormone and ACTH secretion: evidence for an inverse relationship in rats. Endocrinology 90:735-743.

Kokka, N., R.M. Eisenberg, J. Garcia and R. George (1972b). Blood glucose, growth hormone, and cortisol levels after hypothalamic stimulation. Am. J. Physiol. 222:236-301.

Koritsansky, S. and K. Koves (1976). Data on the absence of axon terminals of medial preoptic area neurons in the surface zone of the median eminence. J. Neurol. Transm. 38:159-167.

Koves, K. and M. Rethelyi (1976). Direct neural connection from the medial preoptic area to the hypothalamic arcuate nucleus of the rat. Exp. Brain Res. 25:529-539.

Krey, L.C., K.H. Lu, W.R. Butler, J. Hotchkiss, R. Piva and E. Knobil (1975). Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. Endocrinology 96:1088-1093.

Krieg, W.J.S. (1932). The hypothalamus of the albino rat. J. Comp. Neurol. 84:211-276.

Krieger, D.T. and Glick, S.M. (1974). Sleep EEG stages and plasma growth hormone concentration in states of endogenous and exogenous hypercortisolemia of ACTH elevation. J. Clin. Endocrinol. Metab. 39:986-1000.

Krulich, L. (1979). Central neurotransmitters and the secretion of prolactin, GH, LH and TSH. Ann Rev. Physiol. 41:603-615.

Krulich, L., E. Hefco and J.E. Aschenbrenner (1975). Mechanism of the effects of hypothalamic deafferentation on prolactin secretion in the rat. Endocrinology 96:107-118.

Krulich, L., P. Illner, C.P. Fawcett, M. Quijada and S. McCann (1972). Dual hypothalamic regulation of growth hormone secretion. In Growth and Growth Hormone, A. Pecile and E.E. Muller (Eds.), Excerpta Medica, Amsterdam, pp.306-316.

Lal, S., C.E. De La Vega, T.L. Sourkes and H.G. Friesen (1973). Effect of apomorphine on growth hormone and follicle-stimulating hormone levels in human serum. J. Clin. Endocrinol. Metab. 37:719-724.

Lal, S., J.B. Martin, C. De La Vega and H.G. Friesen (1975). Comparison of the effect of apomorphine and L-dopa on serum growth hormone levels in man. Clin. Endocrinol. 4:277-285.

Lal, S. and J.B. Martin (1980). Neuroanatomy and neuropharymacological regulation of neuroendocrine function. In Handbook of Biological Psychiatry Part III. Brain Mechanisms and Abnormal Behavior - Genetics and Neuroendocrinology, H.M. van Praag, M.H. Lader, O.J. Rafaelson and E. Sachar (Eds.), Marcel Dekker, New York, pp.101-167.

Lal, S., G. Tolis, J.B. Martin, G.M. Brown and H. Guyda (1975). Effect of clonidine on growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone in the serum of normal man. J. Clin. Endocrinol. Metab. 41:827-832.

Lamperti, A., L. Pupa and T. Tafelski (1980). Time-related effects of monosodium glutamate on the reproductive neuroendocrine axis of the hamster. Endocrinology 106:553-558.

Laursen, A.M. (1955). An experimental study of pathways from the basal ganglia. J. Comp. Neurol. 102:1-26.

Lawson D.M. and R.R. Gala (1975). The influence of adrenergic, dopaminergic, cholinergic and serotoninergic drugs on plasma prolactin levels in ovariectomized, estrogen-treated rats. Endocrinology 96:313-318.

Lee, M.O. (1929). Determination of the surface area of the white rat with its application to the expression of metabolic results. Amer. J. Physiol. 89:24-31.

Leonard, C.M. and J.W. Scott (1971). Origin and distribution of the amygdalofugal pathways in the rat. An experimental-neuroanatomical study. J. Comp. Neurol. 141:313-330.

Leranth, Cs., L. Zaborszky, J. Marton and M. Palkovits (1975). Quantitative studies on the supraoptic nucleus in the rat. I. Synaptic organization. Exp. Brain Res. 22:509-523.

Lofstrom A, G. Jonsson and K. Fuxe (1976). Microfluorometric quantitation of catecholamine fluorescence in rat median eminence. I. Aspects on the distribution of dopamine and noradrenaline nerve terminals. J. Histochem Cytochem 24:415-429.

Lovinger, R., A.T. Boryczka, R. Shackelford, S.L. Kaplan, W.F. Ganong, and M.M. Grumbach (1974). Effect of synthetic somatotropin release inhibiting factor on the increase in plasma growth hormone elicited by L-dopa in the dog. Endocrinology 95:943-946.

Lovinger, R., J. Holland, S. Kaplan, M. Grumbach, A.J. Boryczka, R. Shackelford, J. Salmon, I.A. Reid and W.F. Ganong (1976). Pharmacological evidence for stimulation of growth hormone secretion by a central noradrenergic system in dogs. Neuroscience 1:443-450.

Lowry D, M. Rosebrough, A. Farr and R. Randall (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Lundberg, P.O. (1960). Cortico-hypothalamic connections in the rabbit. An experimental neuroanatomical study. Acta Physiol. Scand. 49 (Suppl. 171):1-84.

Machlin, L.J., L.S. Jacobs, N. Arulis, R. Kimes and R. Miller (1974). An assay for growth hormone and prolactin-releasing activities using a bovine pituitary cell culture system. Endocrinology 95:1350-1358.

MacLeod R.M. (1976). Regulation of prolactin secretion. In: Frontiers in Neuroendocrinology, L. Martini and W.F. Ganong (Eds.), Raven Press, New York, vol. 4:169-194.

Makara, G.B., M.C. Harris and K.M. Spyer (1972). Identification and distribution of tubero-infindibular neurons. Brain Res. 40:283-290.

Makara, G.B. and L. Hodacs (1975). Rostral projections from the hypothalamic arcuate nucleus. Brain Res. 84:23-29.

Malacara, J.M., R. Valverde and S. Reichlin (1972). Elevation of plasma radioimmunoassayable growth hormone in the rat induced by porcine hypothalamic extract. Endocrinology 91:1189-1198.

Martin, J.B. (1972). Plasma growth hormone (GH) response to hypothalamic or extrahypothalamic electrical stimulation. Endocrinology 91:107-115.

Martin, J.B. (1974a). Inhibitory effect of somatostatin (SRIF) on the release of growth hormone (GH) induced in the rat by electrical stimulation. Endocrinology 94:497-503.

Martin J.B. (1974b). The role of hypothalamic and extrahypothalamic structures in the control of GH secretion. In: Proceedings of the NIH Symposium on Growth Hormone, S. Raiti (Ed.), NIH Publication no. 74-612. NIH, Bethesda MD, p.223.

Martin, J.B. (1976). Brain regulation of growth hormone secretion. In: Frontiers in Neuroendocrinology, Vol. 4, L. Martini and W.F. Ganong (Eds.), Raven Press, New York, pp.129-168.

Martin, J.B. (1978). Bowditch Lecture: Brain mechanisms for integration of growth hormone secretion. Physiologist 22:23-29.

Martin, J.B. (1980). Functions of central nervous system neurotransmitters in regulation of growth hormone secretion. Fed. Proc. 39:2902-2906.

Martin, J.B. and I. Jackson (1975). Neural regulation of TSH and GH secretion. In Anatomical Neuroendocrinology, W. Stumpf and L. Grant (Eds.), Karger, New York, pp.343-353.

Martin, J.B., J. Audet and A. Saunders (1975). Effect of somatostatin and hypothalamic ventromedial lesions on GH release induced by morphine. Endocrinology 96:839-847.

Martin, J.B., P. Brazeau, G.S. Tannenbaum, J.O. Willoughby, J. Epelbaum, L.C. Terry and D. Durand (1978a). Neuroendocrine organization of growth hormone regulation. In: The Hypothalamus, S. Reichlin, R.J. Baldessarini, and J.B. Martin, (Eds.), Raven Press, New York 56:329-357.

Martin, J.B., D. Durand, W. Gurd, G. Faille, J. Audet and P. Brazeau (1978b). Neuropharmacologic regulation of episodic growth hormone and prolactin secretion in the rat. Endocrinology 102:106-113.

Martin, J.B., S. Reichlin and G. Brown (1977). Clinical Neuroendocrinology, Vol. 14, Davis, Philadelphia.

Martin, J.B., L.P. Renaud and P. Brazeau (1974). Pulsatile growth hormone secretion: Suppression by hypothalamic ventromedial lesions and by long acting somatostatin. Science 186:538-540.

Martin, J.B., G. Tannenbaum, J.O. Willoughby, L.P. Renaud and P. Brazeau (1975). Functions of the central nervous system in regulation of pituitary GH secretion. In Hypothalamic Hormones: Chemistry, Physiology, Pharmacology and Clinical Uses, M. Motta, P.G. Crosignani and L. Martini (Eds.), Academic Press, New York, pp.217-236.

Massara, F. and E. Strumina (1970). Increase in plasma growth hormone concentration in man after infusion of adrenaline-propranolol. J. Endocrinol. 47:95-100.

McIntyre, H.B. and W.D. Odell (1974). Physiological control of growth hormone in the rabbit. Neuroendocrinology 16:8-21.

McHugh, P.R., W.C. Black and J.W. Mason (1966). Some hormonal responses to electrical self-stimulation in the Macaca mulatta. Amer. J. Physiol. 210:109-113.

Meibach, R.C. and A. Siegel (1977). Efferent connections of the septal area in the rat: An analysis utilizing retrograde and anterograde transport methods. Brain Res. 119:1-20.

Merimee, T.J. (1979). Growth Hormone: Secretion and Action. In Endocrinology Vol. 1, L.J. DeGroot et al. (Eds.), Grune and Stratton, New York, pp.123-132.

Meszaros, T., I.J. Csuri, J. Hazas and M. Palkovits (1969). Esterase activity in the hypothalamus. Acta Morphol. Acada. Sci. Hung. 17:201-215.

Mettler, F.A. (1945). Fiber connections of the corpus striatum of the monkey and baboon. J. Comp. Neurol. 82:169-204.

Meyer, V. and E. Knobil (1967). Growth hormone secretion in the unanesthetized rhesus monkey in response to noxious stimuli. Endocrinology 80:163-171.

Millard, W.J., K. Gordon, J.B. Martin, Jr., J. Audet, S. Sagar and J.B. Martin (1980). Effects of morphine (MS), α-melanocyte stimulating hormone (α-MSH) and pentobarbital on plasma growth hormone (GH) and prolactin (PRL) in monosodium glutamate (MSG)-treated rats. Fed. Proc. 39:980.

Millhouse, O.E. (1969). A Golgi study of the descending medial forebrain bundle. Brain Res. 15:341-363.

Millhouse, O.E. (1973). Certain ventromedial hypothalamic afferents. Brain Res. 55:89-105.

Mitchell, J.A., M. Hutchins, W.J. Schindler and V. Critchlow (1973). Increases in plasma growth hormone concentration and naso-anal length in rats following isolation of the medial basal hypothalamus. Neuroendocrinology 12:161-173.

Moore, R.Y. (1978). Central neural control of circadian rhythms. In Frontiers in Neuroendocrinology, Vol. 5, W.F. Ganong and L. Martini (Eds.), Raven Press, New York, pp.185-206.

Moore, R.Y. and F.E. Bloom (1978). Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. Ann. Rev. Neurosci. 1:129-169.

Moore, R.Y. and F.E. Bloom (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Ann. Rev. Neurosci. 2:113-168.

Morgan, M.V. (1968). Some efferent fiber projections of the amygdala in the cat. Ph.D. Thesis, Duke University, North Carolina.

Morin, F. (1950). An experimental study of hypothalamic connections in the guinea pig. J. Comp. Neurol. 92:193-213.

Moss, R.L., M. Kelly and P. Riskind (1975). Tubero-infindibular neurons: Dopaminergic and norepinephrinergic sensitivity. Brain Res. 89:265-277.

Mulder, G.H. and P.G. Smelik (1977). A superfusion system technique for the study of the sites of action of glucocorticoids in the rat hypothalamus-pituitary-adrenal system in vitro. I. Pituitary cell superfusion. Endocrinology 100:1143-1152.

Müller, E.E. (1973). Nervous control of growth hormone secretion. Neuroendocrinology 11:338-369.

Müller, E.E., P. Dal Pra and A. Pecile (1968). Influence of brain neurohumors injected into the lateral ventricle of the rat on growth hormone release. Endocrinology 83:893-896.

Müller, E.E., G. Nistico and U. Scapagnini (1977). Neurotransmitters and Anterior Pituitary Function. Academic Press, New York.

Müller, E.E., T. Saito, A. Arimura and A.V. Schally (1967). Blockade of release of growth hormone by brain norepinephrine depletors. Endocrinology 80:471-476.

Murphy, B.E. (1967). Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J. Clin. Endocr. 27:973-990.

Nagatsu, T. (1973). Biochemistry of Catecholamines. University Park Press, Baltimore.

Nasagawa, H., R. Yanai and S. Kikuyama (1974). Irreversible inhibition of pituitary prolactin and growth hormone secretion and of mammary gland development in mice by monosodium glutamate administered neonatally. Acta Endocrinol. (Dbh.), 75:249-252.

Natelson, B.H., G.P. Smith, P.E. Stokes and A.W. Root (1974). Changes of 17-hydroxycorticosteroids and growth hormone during self-stimulation in monkeys. Physiol. Behav. 12:121-126.

Nauta, W.J.H. (1956). An experimental study of the fornix system in the rat. J. Comp. Neurol. 104:247-272.

Nauta, W.J.H. (1961). Fibre degeneration following lesions of the amygdaloid complex in the monkey. J. Anat. (London) 95:515-531.

Nauta, W.J.H. and W.R. Mehler (1966). Projections of the lentiform nucleus in the monkey. Brain Res. 1:3-42.

Negro-Vilar A., S.R. Ojeda, J.P. Advis and S.M. McCann (1979). Evidence for noradrenergic involvement in episodic prolactin and growth hormone release in ovariectomized rats. Endocrinology 105:86-91.

Negro-Vilar A., S.R. Ojeda, A. Arimura and S.M. McCann (1978). Dopamine and norepinephrine stimulate somatostatin release by median eminence fragments in vitro. Life Sci. 23:1493-1497.

Nemeroff, C.B., G. Bissett, G.H. Greeley, R.B. Mailman, J.B. Martin, P. Brazeau and J.S. Kizer (1978). Effects of acute administration of monosodium-L-glutamate (MSG), atropine or haloperidol on anterior pituitary hormone secretion in the rat. Brain Research 156:198-201.

Nemeroff, C.B., L.D. Grant, G. Bissett, G.N. Ervin, L.E. Harrell and A.J. Prange (1977). Growth, endocrinological and behavioral deficits after monosodium-L-glutamate in the neonatal rat: possible involvement of arcuate dopamine neuron damage. Psychoneuroendocrinology 2:179-196.

Nemeroff, C. B., R.J. Konkel, G. Bissett, W.W. Youngblood, J.B. Martin, P. Brazeau, M.S. Rone, A.J. Prange, Jr., G.R. Breese and J.S. Kizer (1977). Analysis of the disruption in hypothalamic-pituitary regulation in rats treated neonatally with monosodium-L-glutamate (MSG): evidence for the involvement of tuberoinfindibular cholinergic and dopaminergic systems in neuroendocrine regulation. Endocrinology 101:613-622.

Newman, G., W. Roberts, L.A. Frohman and L.L. Bernardis (1967). Plasma GH levels in rats following amygdala pyriform cortex lesions. Proc. Soc. Can. Fed. Biol. Soc. 10:41.

O'Donohue, T.L., W.R. Crowley and D.M. Jacobowitz (1979). Biochemical mapping of the noradrenergic ventral bundle projection sites: evidence for a noradrenergic-dopaminergic interaction. Brain Res. 172:87-100.

O'Donohue, T.L., R.L. Miller and D.M. Jacobowitz (1979). Identification, characterization, and stereotaxic mapping of intraneuronal α-melanocyte stimulating hormone-like immunoreactive peptides in discrete regions of the rat brain. Brain Res. 176:101-123.

Olds, J. (1977). Drives and Reinforcements: Behavioral Studies of Hypothalamic Functions. Raven Press, New York.

Olds, J. and P. Milner (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol., 47:419-427.

Olds, M.E. and A. Yuwiler (1972). Effect of brain stimulation in positive and negative reinforcing regions in the rat on content of catecholamines in hypothalamus and brain. Brain Res. 36:385-398.

Olney, J.W. (1969). Brain lesions, obesity and other distrubances in mice treated with monosodium glutamate. Science 164:719-721.

Olney, J.W. (1969). Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. J. Neuropath. Exp. Neurol. 28:455-474.

Olney, J.W., T.J. Circero, E.R. Meyer and T. de Gubareff (1976). Acute glutamate-induced elevations in serum testosterone and luteinizing hormone. Brain Res. 112:420-424.

Olney, J.W., B. Schainker and W. Rhee (1976). Chemical lesioning of the hypothalamus as a means of studying neuroendocrine function. In Hormones, Behavior and Psychopathology, E. Sachar (Ed.), Raven Press, New York, pp.153-158.

Ondo, J.G., K.A. Pass and R. Baldwin (1976). The effects of neurally active amino acids on pituitary gonadotropin secretion. Neuroendocrinology 21:79-87.

O'Steen, W.K. and G.H. Waughm (1968). Radioactivity in the optic pathway and hypothalamus of the rat after intraocular injection of tritiated 5-hydroxytryptophan. Brain Res. 8:209-212.

Palkovits M. (1975). Isolated removal of hypothalamic nuclei for neuroendocrinological and neurochemical studies. In Anatomical Neuroendocrinology, W.E. Stumpf, L.D. Grant (Eds.), Karger, Basel, p.72.

Palkovits, M. (1977a). Transmitters of the suprainfundibular system. In Advances in Biochemical Psychopharmacology, Vol. 6, E. Costa and G.L. Gessa, (Eds.), Raven Press, New York, pp.71-78.

Palkovits, M. (1977b). Biochemical neuroanatomy (review and considerations). Proceedings Fifth International Congress of Endocrinology, International Congress Series, Excerpta Medica, Amsterdam, pp.105-110.

Palkovits, M. (1981). Catecholamines in the hypothalamus. Neuroendocrinology 33:123-128.

Palkovits, M., M.J. Brownstein, A. Arimura, H. Sato, A.V. Schally and J.S. Kizer, (1976). Somatostatin content of the hypothalamic ventromedial and arcuate nuclei and the circumventricular organs in the rat. Brain Research 109:430-434.

Palkovits, M., M. Brownstein, J.M. Saavedra and J. Axelrod (1974). Norepinephrine and dopamine content of hypothalamic nuclei of rat. Brain Res. 77:137-149.

Palkovits, M., M. Fekete, G.B. Makara and J.P. Herman (1977a). Total and partial hypothalamic deafferentations for topographical identification of catecholaminergic innervations of certain preoptic and hypothalamic nuclei. Brain Res. 127:127-136.

Palkovits, M. and D.M. Jacobowitz (1974). Topographic atlas of catecholamine and acetylcholinesterase containing neurons in the rat brain, II. Hindbrain (mesencephalon, rhombencephalon). J. Comp. Neurol. 157:29-42.

Palkovits, M., Cs. Leranth, L. Zaborszky and M. J. Brownstein (1977b). Electron microscopic evidence of direct neuronal connections from the lower brain stem to the median eminence. Brain Res. 136:339-344.

Palkovits, M. and L. Zaborszky (1979). Neural connections of the hypothalamus. In Anatomy of the Hypothalamus, P.J. Morgane and J. Panskepp (Eds.), Vol. 1, Marcel Dekker, Inc., New York, 1979, pp.379-501.

Papez, J.W. (1938). Reciprocal connections of the striatum and pallidum in the brain of Pithecus (Macacus) rhesus. J. Comp. Neurol. 69:329-349.

Papez, J.W. (1942). A summary of fiber connections of the basal ganglia with each other and with other protions of the brain. Res. Publ. Assoc. Neur. Ment. Dis. 21:21-58.

Parker, D.C., L.G. Rossman, T.M. Siler, J. Rivier, S.S.C. Yen and R. Guillemin (1974). Inhibition of the sleep-related peak in physiologic human growth hormone release by somatostatin. J. Clin. Endocrinol. Metab. 38:496-499.

Parsons, J.A., S.L. Erlandsen, O.D. Hegre, R.C. McEvoy and R.P. Elde (1976). Central and peripheral localization of somatostatin. Immunoenzyme immunocytochemical studies. J. Histochem. Cytochem. 24:872-882.

Patel, Y.C. (1979). Growth hormone stimulates hypothalamic somatostatin. Life Sci. 24:1589-1594.

Patel, Y., G.C. Weir, and S. Reichlin (1975). Anatomic distribution of somatostatin in brain and pancreatic islets as studied by radioimmunoassay. Endocrinology 96:1347 (Abstract A154).

Patel, Y.C., H.H. Zingg and J.J. Dreifus (1977). Calcium-dependent somatostatin secretion from rat neurohypophysis in vitro. Nature 267:852-853.

Patel, Y.C., H.H. Zingg and J.J. Dreifuss (1978). Somatostatin secretion from the rat neurohypophysis and stalk median eminence (SME) in vitro: calcium-dependent release by high potassium and electrical stimulation. Metabolism (Suppl 1) 27:1243-1245.

Penke, G.T., M. Wilson, A.L. Steiner and A.P.S. Dhariwal (1973). Effects of a purified growth hormone releasing factor on growth hormone secretion and pituitary cyclic nucleotide content. Metabolism 22:769-772.

Pendleton, R.G., G. Gessner, G. Weiner, B. Jenkins, J. Sawyer, W. Bondinell and A. Intoccia (1979). Studies on SK and F 29661, an organ specific inhibitor of phenylethanolamine-N-methyl-transferase. J. Pharmacol. Exp. Ther. 208:24-30.

Pendleton, R.G., J.P. McCafferty and J.M. Roesler (1980). Effects of PNMT inhibitors upon cardiovascular changes induced by hemorrhage in the rat. Eur. J. Pharmacol. 66:1-10.

Peuler, J.B. and G.A. Johnson (1977). Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci. 21:625-636.

Pfizzi, W.J., J.E. Barnhart and D.J. Fanslow (1977). Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. Science 196:452-454.

Powell, E.W. and Leman, R.B. (1976). Connections of the nucleus accumbens. Brain Res. 105:389-404.

Printz, R.H. and J.L. Hall (1974). Evidence for a retinohypothalamic pathway in the golden hamster. Anat. Rec. 179:57-66.

Quabbe, H-J., E. Schilling and H. Helge (1966). Pattern of growth hormone secretion during a 24-hour fast in normal adults. J. Clin. Endocrinol. Metab. 26:1173-1177.

Rabinowitz, D., T.J. Merimee, J.K. Nelson, R.B. Schultz and J.A. Burgess (1968). The influence of proteins and amino acids on growth hormone release in man. In Growth Hormone, A. Pecile and E.E. Muller (Eds.). Proc. Int. Symp. Growth Horm., 1st, 1967 Excerpta Medica Found. Int. Congress Ser. No. 158, pp105-115.

Raisman, G. (1970). An evaluation of the basic pattern of connections between the limbic system and the hypothalamus. Am. J. Anat. 129:197-202.

Raisman, G. (1973). Electron microscopic studies of the development of new neurohemal contacts in the median eminence of the rat after hypophysectomy. Brain Res. 55:245-261.

Ranson, S.W. and M. Ranson (1939). Pallidofugal fibers in the monkey. Arch. Neurol. Psychiatry 42:1059-1067.

Ranson, S.W. and S.W. Ranson., Jr. (1941). Efferent fibers of the corpus striatum. Res. Publ. Assoc. Neur. Ment. Dis. 21:69-76.

Ranson, S.W., S.W. Ranson, Jr. and M. Ranson (1941). Fiber connections of corpus striatum as seen in Marchi preparations. Arch. Neurol. Psychiatry 46:230-249.

Redding, T.W., A.V. Schally, A. Arimura and I. Wakaboyashi (1976). Effect of monosodium glutamate on some endocrine functions. Neuroendocrinology 8:245-250.

Reichlin, S. (1974a). Regulation of somatotropic hormone secretion. In Handbook of Physiology, Sect. VII, Vol. 4, R.O. Greep and W.H. Sawyer (Eds.), American Physiological Society, Washington, pp.405-447.

Reichlin, S. (1974b). Regulation of somatotrophic hormone secretion. In The Pituitary Gland, G.W. Harris and B.T. Donovan, (Eds.), Butterworths, London, pp.270-298.

Renaud, L.P. (1976a). An electrophysiological study of the amygdalo-hypothalamic projections to the ventromedial nucleus of the rat. Brain Res. 105:45-58.

Renaud, L.P. (1976b). Tuberoinfundibular neurones in the basomedial hypothalamus of the rat: Electrophysiological evidence for axon collaterals to hypothalamic areas. Brain Res. 105:59-72.

Renaud, L.P., J.B. Martin and P. Brazeau (1975). Depressant action of TRH, LH-RH and somatostatin on activity of central neurons. Nature (Lond.) 255:233-235.

Rethelyi, M. and B. Halasz (1970). Origin of the nerve endings in the surface of the median eminence of the rat hypothalamus. Exp. Brain Res. 11:145-158.

Rice, R.W., K. Abe and V. Critchlow (1978). Abolition of plasma growth hormone response to stress and of the circadian rhythm in pituitary-adrenal function in female rats with preoptic-anterior hypothalamic lesions. Brain Research 148:129-141.

Rice, R.W. and V. Critchlow (1976). Extrahypothalamic control of stress-induced inhibition of growth hormone secretion in the rat. Endocrinology 99:970-976.

Rice, R.W., J. Kroning and V. Critchlow (1975). Effects of stress on plasma corticosterone and growth hormone levels in rats with median eminence-pituitary islands. Neuroendocrinology 19:339-351.

Rice, R.W., J. Kroning and V. Critchlow (1976). Sex differences in the effects of surgical isolation of the medial basal hypothalamus on linear growth and plasma growth hormone levels in the rat. Endocrinology 98:982-990.

Richardson, S.B., P. Greenleaf and I. Starker (1979). Serotonin and acetylcholine inhibit somatostatin release from rat hypothalami in organ culture. 61st Annual Meeting of The Endocrine Society, Anaheim CA, (Abst 284) p.143.

Rivier, J.E.F. (1974). Somatostatin: Total solid phase synthesis. J. Am. Chem. Soc. 96:2986-2992.

Rivier, C. and W. Vale (1977). Effects of γ-amino-butyric acid and histamine on prolactin secretion in the rat. Endocrinology 101:506-511.

Rivier, C., W. Vale, N. Ling, M. Brown and R. Guillemin (1977). Stimulation in vivo of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology 100:238-241.

Rodbard, D. (1974). Statistical quality control and routine data processing for radioimmunoassays (RIA) and immunoradiometric assays (IRMA). Clin. Chem. 20:1255-1270.

Rorstad, O.P., M.J. Brownstein and J.B. Martin (1979a). Demonstration of immunoreactive and biologically active somatostatin-like material in the rat retina. Proc. Nat. Acad. Sci. (Wash.), 76:3019-3023.

Rorstad, O.P., J. Epelbaum, P. Brazeau and J.B. Martin (1979b). Chromatographic and biological properties of immunoreactive somatostatin in hypothalamic and extrahypothalamic brain regions of the rat. Endocrinology 105:1083-1092.

Rorstad, O.P., J.B. Martin and L.C. Terry (1980). Somatostatin and the nervous system. In The Role of Peptides in Neuronal Function, J.L. Barker and T.G. Smith (Eds.), Marcel Dekker, New York, pp.573-614.

Roth, J., M. Glick, R.S. Yalow S.A. Berson (1963). Hypoglycemia: a point stimulus to secretion of growth hormone. Science 140:987-988.

Rotsztejn, W.H., S.V. Drouva, E. Pattou and C. Kordon (1978). Met-enkephalin inhibits in vitro dopamine-induced LHRH release from mediobasal hypothalamus of male rats. Nature 274:281-282.

Ruch, W., D.J. Koerker, M. Carino, S.D. Johnson, B.R. Webster, J.W. Ensinck, C.J. Goodner and C.C. Gak (1974). Studies on somatostatin (somatotropin release inhibiting factor) in conscious baboons. In Advances in Human Growth Research, S. Raiti (Ed.), Publication No. (NIH) 74-612, Dept. Health, Education and Welfare, Washington, D.C., pp.271-293.

Ruch, W., A.L. Jaton, B. Bucher, P. Marbach and W. Doepfner (1976). Alpha-adrenergic control of growth hormone secretion. Experientia 32:529-531.

Saavedra, J.M., M. Palkovits, M.J. Brownstein and J. Axelrod (1974). Serotonin distribution in the nuclei of the rat hypothalamus, and preoptic region. Brain Res. 77:157-165.

Sadowski, B. (1972). Intracranial self-stimulation patterns in dogs, Physiol. Behav. 8:189-193.

Sadowski, B. (1976). Physiological correlates of self-stimulation. 5. Endocrine responses. In Brain-Stimulation Reward, A. Wauquier and E.T. Rolls (Eds.), Elsevier, Amsterdam, pp.450-454.

Sanaka, M., S. Shiosaka, K. Takatsuki, S. Inagaki, H. Takagi, E. Senba, Y. Kawai, T. Matsuzaki and M. Tohyama (1981). Experimental immunohistochemical studies on the amygdalofugal peptidergic (substance P and somatostatin) fibers in the stria terminalis of the rat. Brain Res. 221:231-242.

Sandou, J., A. Arimura and A.V. Schally (1973). Stimulation of growth hormone release by anterior pituitary perifusion in the rat. Endocrinology 90:1315-1319.

Saper, C.B., L.W. Swanson and W.M. Cowan (1976). The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J. Comp. Neurol. 169:409-442.

Saavedra, J.M., M. Palkovits, M.J. Brownstein and J. Axelrod (1974). Serotonin distribution in the nuclei of the rat hypothalamus, and preoptic region. Brain Res. 77:157-165.

Sadowski, B. (1972). Intracranial self-stimulation patterns in dogs. Physiol. Behav. 8:189-193.

Sadowski, B. (1976). Physiological correlates of self-stimulation. 5. Endocrine responses. In Brain-Stimulation Reward, A. Wauquier and E.T. Rolls (Eds.), Elsevier, Amsterdam, pp.450-454.

Sanaka, M., S. Shiosaka, K. Takatsuki, S. Inagaki, H. Takagi, E. Senba, Y. Kawai, T. Matsuzaki and M. Tohyama (1981). Experimental immunohistochemical studies on the amygdalofugal peptidergic (substance P and somatostatin) fibers in the stria terminalis of the rat. Brain Res. 221:231-242.

Sandow, J., A. Arimura and A.V. Schally (1973). Stimulation of growth hormone release by anterior pituitary perifusion in the rat. Endocrinology 90:1315-1319.

Saper, C.B., L.W. Swanson and W.M. Cowan (1976). The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J. Comp. Neurol. 169:409-442.

Sassin, J.B., A.G. Frantz, E.D. Weitzman and S. Kapen (1972). Human prolactin: 24-hour pattern with increased release during sleep. Science 177:1205-1207.

Saunders, A., L.C. Terry, J. Audet, P. Bruneau and J.B. Martin (1976). Dynamic studies of growth hormone and prolactin secretion in the female rat. Neuroendocrinology 21:193-203.

Sauter, A.M., J.Y. Lew, Y. Baba and M. Goldstein (1977). Effect of phenylethanolamine N-methyltransferase and dopamine-β-hydroxylase inhibition on epinephrine levels in the brain. Life Sci. 21:261-266.

Schaeffer J.M., J. Axelrod and M.J. Brownstein (1977). Regional differences in dopamine-mediated release of TRH-like material from synaptosomes. Brain Res. 138:571-574.

Schaeffer, J.M. and A.J.W. Hsueh (1980). Acetylcholine receptors in the rat anterior pituitary gland. Endocrinology 106:1377-1381.

Schalch, D.S. (1967). The influence of physical stress and exercise on growth hormone and insulin secretion in man. J. Lab. Clin. Med. 69:256-269.

Schalch, D.S. and S. Reichlin (1966). Plasma growth hormone concentration in the rat determined by radioimmunoassay: influence of sex, pregnancy, lactation, anesthesia, hypophysectomy and extrasellar pituitary transplants. Endocrinology 79:275-280.

Schally A.V., D.H. Coy and C.A. Meyers (1978). Hypothalamic regulatory hormones. Annu. Rev. Biochem. 47:89-128.

Schally, A.V., T.W. Redding, A. Arimura, A. Dupont and G.L. Linthicum (1977). Isolation of gamma-aminobutyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) activity *in vivo* and *in vitro*. Endocrinology 100:681-691.

Schaub, C., M.T. Bluet-Pajot, F. Mounier, A. Segalen and J. Duhoult (1980). Effects of noradrenergic agonists and antagonists on growth hormone secretion under gamma-hydroxybutyrate narco-analgesia in the rat. Psychoneuroendocrinol. 5:139-146.

Schubert, F., J.N. George and M. Bhaskar Rao Bhashar (1980). Monosodium glutamate (MSG) alters protein, vasopressin and oxytocin in microdissected hypothalamic areas in newborn and adult rats. Life Sci. 26:651-656.

Schusdziarra V., D. Rouiller, A. Arimura and R.H. Unger (1978). Antisomatostatin serum increases levels of hormones from the pituitary and gut, but not from the pancreas. Endocrinology 102:1956-1959.

Schwartz, J.C. (1979). Histamine receptors in brain. Life Sci. 25:895911.

Setalo, G., S. Vigh, A.V. Schally, A. Arimura and B. Flerko (1975). GH-RIH-containing neural elements in the rat hypothalamus. Brain Research 90:352-356.

Sheppard M., S. Kronheim and B.L. Pimstone (1978). Stimulation by growth hormone of somatostatin release from the rat hypothalamus in vitro. Clin. Endocrinol. 9:583-586.

Siler, T.M., G. Vanderberg and S.S.C. Yen (1973). Inhibition of growth hormone release in humans by somatostatin. J. Clin. Endocrinol. Metab. 37:632-634.

Siler, T.M., S.S.C. Yen, W. Vale and R. Guillemin (1974). Inhibition by somatostatin on the release of TSH induced in man by thyrotropin-releasing factor. J. Clin. Endocrinol. Metab. 38:742-745.

Smith, G. and G. Fink (1972). Experimental studies on the origin of monoamine-containing fibers in the hypothalamo-hypophysial complex of the rat. Brain Res. 43:37-51.

Snyder, S.H. (1978). Peptide neurotransmitter candidates in the brain: focus on enkephalin, angiotensin II, and neurotensin. In The Hypothalamus, S. Reichlin, R.J. Baldessarini and J.B. Martin (Eds.), Raven Press, New York, vol. 56:233-243.

Sole, M.J. and M.N. Hussain (1977). A simple, specific radio-enzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine, and dopamine in plasma and tissues. Biochem. Med. 18:301-307.

Sonntag, W., L.J. Fonnam, N. Miki, R.W. Steger, T. Ramos, A. Arimura and J. Meites (1981). Effects of CNS active drugs and somatostatin antiserum on growth hormone release in young and old rats. Neuroendocrinology 33:73-78.

Sousa-Pinto, A. and J. Castro-Correia (1970). Light microscopic observations on the possible retinohypothalamic projection in the rat. Exp. Brain Res. 11:515-527.

Stachura, M.E. (1976). Influence of synthetic somatostatin upon growth hormone release from perifused rat pituitaries. Endocrinology 99:678-683.

Starke, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77:1-124.

Steiner, S.S., B. Beer and M.M. Shaffer (1969). Escape from self-produced rates of brain stimulation. Science 163:90-91.

Steiner R.A., J.K. Stewart, J. Barber, D. Koerker, C.J. Goodner, A. Brown, P. Illner and C.C. Gale (1978). Somatostatin: a physiological role in the regulation of growth hormone secretion in the adolescent male baboon. Endocrinology 102:1587-1594.

Stevens, R.W. and D.M. Lawson (1977). The influence of estrogen on plasma prolactin levels induced by thyrotropin releasing hormone (TRH), clonidine and serotonin in ovariectomized rats. Life Sci. 20:261-265.

Stewart, J.K., R.A. Steiner, J. Barber, D.J. Koerker, C.J. Goodner and G.C. Gale (1977). Effects of active immunization against somatostatin on secretion of growth hormone and insulin in adolescent male baboons. Progr. Endocr. Soc. 59th Ann. Meet. 340:230.

Swanson, L.W. (1976). An autoradiographic study of the efferent connections of the preoptic region in the rat. J. Comp. Neurol. 167:227-256.

Swanson, L.W. and W.M. Cowan (1975). The efferent connections of the suprachiasmatic nucleus of the hypothalamus. J. Comp. Neurol. 160:303-304.

Swanson, L.W. and B.K. Hartman (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. J. Comp. Neurol. 163:467-506.

Szabo, M. and L.A. Frohman (1975). Effects of porcine stalk median eminence and prostaglandin E_2 on rat growth hormone secretion in vivo and their inhibition by somatostatin. Endocrinology 96:955-961.

Szentagothai, J. (1964). The parvicellular neurosecretory system. Prog. Brain Res. 5:135-146.

Szentagothai, J., B. Flerko, B. Mess and B. Halasz (1968). Hypothalamic Control of the Anterior Pituitary. Akademiai Kiado, Budapest.

Tache, Y., P. DuRuisseau, G. Charpenet and R. Collu (1977). Pituitary responsiveness to LH-RH in female rats submitted to chronic immobilization stress. In Proc. 59th Ann. Meet. Endocr. Soc., Chicago, p.304.

Tache, Y., P. DuRuisseau, J.R. Ducharme and R. Collu (1978). Pattern of adenohypophyseal hormone changes in male rats following chronic stress. Neuroendocrinology 26:208-219.

Tache, Y., P. DuRuisseau, J. Tache, H. Seyle and R. Collu (1976). Shift in adenohypophyseal activity during chronic intermittent immobilization of rats. Neuroendocrinology 22:325-336.

Takahashi, K., W.H. Daughaday and D.M. Kipnis (1971). Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology 88:909-917.

Takahashi, Y., D.M. Kipnis and W.H. Daughaday (1968). Growth hormone secretion during sleep. J. Clin. Invest. 47:2079-2090.

Tannenbaum, G.S. (1980). Evidence for autoregulation of growth hormone secretion via the central nervous system. Endocrinology 107:2117-2120.

Tannenbaum, G.S. and J.B. Martin (1976). Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98:562-570.

Tannenbaum, G.S., J. Epelbaum, E. Cole, P. Brazeau and J.B. Martin (1978). Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. Endocrinology 102:1909-1914.

Tannenbaum, G.S. J.B. Martin and E. Cole (1976). Ultradian growth hormone rhythm in the rat: effects of feeding, hyperglycemia, and insulin-induced hypoglycemia. Endocrinology 99:720-727.

Terry, L.C. (1981). Regulation of growth hormone and thyrotropin secretion by the central adrenergic system. Trans. Amer. Neurol. Asc. (in press).

Terry, L.C. (1982). Catecholamines, Growth Hormone, Thyrotropin and Disorders of Mood, (in press).

Terry, L.C. and W.R. Crowley (1980a). The effect of hypophysectomy on immunoreactive somatostatin in discrete nuclei of hypothalamic and extra-hypothalamic regions of the rat brain. Endocrinology 107:1771-1775.

Terry, L.C. and W.R. Crowley (1980b). The effect of stress on immunoreactive somatostatin in discrete hypothalamic and extrahypothalamic nuclei of the rat brain. Brain Res. 197:543-546.

Terry, L.C. and J.B. Martin (1978a). Hypothalamic-pituitary responses to intracranial self-stimulation in the rat. Brain Res. 157:89-104.

Terry, L.C., and J.B. Martin (1978b). Hypothalamic hormones: subcellular distribution and mechanisms of release. Ann. Rev. Pharmacol, Toxicol. 18:111-123.

Terry, L.C. and J.B. Martin (1978c). Release of somatostatin (SRIF) from perifused rat hypothalamic fragments. Fed. Proc. 37:665.

Terry, L.C. and J.B. Martin (1981a). Evidence for α-adrenergic regulation of episodic growth hormone and prolactin secretion in the undisturbed male rat. Endocrinology 108:1869-1873.

Terry, L.C. and J.B. Martin (1981b). The effects of lateral hypothalamic-medial forebrain stimulation and somatostatin antiserum on pulsatile GH secretion in freely-behaving rats: evidence for a dual regulatory mechanism. Endocrinology 109:622-627.

Terry, L.C., J. Audet, P. Brazeau and J.B. Martin (1976a). Neuroendocrine concomitants of intracranial self-stimulation: effects on growth hormone prolactin and corticosterone. Proc. Soc. Neurosci., Toronto, Ontario 2:660.

Terry, L.C., P. Brazeau and J.B. Martin (1977a). Intracranial self-stimulation: effects on hypothalamic-pituitary regulation. Neurology 27:405.

Terry, L.C., M.E. Crowley and M.D. Johnson (1982). Regulation of episodic growth hormone secretion by the central epinephrine system - studies in the chronically cannulated rat. J. Clin. Invest. 69:(in press).

Terry, L.C., J. Epelbaum, P. Brazeau and J.B. Martin (1977b). Passive immunization with antiserum to somatostatin (AS-SS): effects on the dynamics of growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone (TSH) secretion in cannulated rats. Program of the Seventh Annual Meeting of the Society for Neuroscience, Anaheim, Vol.3:359 (Abstr. 1156).

Terry, L.C., J. Epelbaum, P. Brazeau and J.B. Martin (1977c). Monosodium glutamate: acute and chronic effects on growth hormone, prolactin and somatostatin in the rat. Fed. Proc. 36:364.

Terry, L.C., J. Epelbaum, P. Brazeau and J.B. Martin (1981a). Monosodium glutamate: acute and chronic effects on rhythmic growth hormone, and prolactin secretion, and somatostatin in undisturbed rats. Brain Res. 217:129-142.

Terry, L.C., O.P. Rorstad and J.B. Martin (1981b). The release of immunologically and biologically active somatostatin from perifused hypothalamic fragments. Endocrinology 107:794-800.

Terry, L.C., A. Saunders, J. Audet, J.O. Willoughby, P. Brazeau and J.B. Martin (1977d). Physiologic secretion of growth hormone and prolactin in male and female rats. Clin. Endocrinol. 6:19s-28s.

Terry, L.C., J.D. Willoughby, P. Brazeau, J.B. Martin and Y. Patel (1976b). Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. Science 192:565-567.

Tolliver, J.M., R.L. Taylor and D.R. Burt (1981). Muscarinic receptors in the posterior pituitary gland. Neuroendocrinology 32:33-37.

Turner, B.H. (1974). Projections of the nucleus and tracts of the rat stria terminalis. Anat. Rec. 178:479.

Uhl, G.R., R.R. Goodman, M.J. Kuhar and S.H. Snyder (1978). Enkephalin and neurotensin: Immunohistochemical localization and identification of an amygdalofugal pathway. Advanc. Biochem. Psychopharmacol. 18:71-87.

Uhl, G.R. and S.H. Snyder (1976). Regional and subcellular distributions of brain neurotensin. Life Sci. 19:1827-1832.

Unger, R.H. and L. Orci (1975). The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet I:14-16.

Uretsky, E., A. Kling and J. Orbach (1966). Plasma 17-hydroxycorticosteroid levels following intracranial self-stimulation in rats. Psychol. Rep. 19:891-901.

Vale, W., C. Rivier and M. Brown (1977). Regulatory peptides of the hypothalamus. Ann. Rev. Physiol. 39:473-527.

Vale, W., C. Rivier, M. Brown, L. Chan, N. Ling and J. Rivier (1976). Applications of adenohypophyseal cell culture to neuroendocrine studies. In Hypothalamus and Endocrine Functions, F. Labrie, J. Meites, and G. Pelletier (Eds.), Plenum Press, New York, p.397.

Valverde, F. (1963). Studies on the forebrain of the mouse. Golgi observations. J. Anat (London) 97:157-180.

Valverde, F. (1965). Studies on the Piriform Lobe. Harvard University Press, Cambridge, Mass.

Vander Gugten, J., M. Palkovits, H.L.J.M. Wijen and D.H.G. Versteeg (1976). Regional distribution of adrenaline in rat brain. Brain Res. 107:171-175.

Van Loon, G.R. (1973). Brain Catecholamines and ACTH secretion. In Frontiers in Neuroendocrinology, W.F. Ganong and L. Martini (Eds.), Oxford University Press, New York, pp.209-247.

Van Vugt, D.A. and J. Meites (1980). Influence of endogenous opiates on anterior pituitary function. Fed. Proc. 39:2533-2538.

Varner M.A., S.L. Darvis and J.J. Reeves (1980). Temporal serum cholesterol concentrations of growth hormone, thyrotropin, insulin and glucagon in sheep immunized against somatostatin. Endocrinology 106:1027-1032.

Versteeg, D.H.G., J. Vander Gugten, W. de Jong and M. Palkovits (1976). Regional concentrations of noradrenaline and dopamine in rat brain. Brain Res. 113:563-574.

Vidal, F. (1940). Pallidohypothalamic tract or x-bundle of Meynert in the rhesus monkey. A.M.A. Arch. Neurol. Psychiatry 44:1219-1223.

Vijayan E., L. Krulich and S.M. McCann (1978). Catecholaminergic regulation of TSH and growth hormone release in ovariectomized and ovariectomized, steroid-primed rats. Neuroendocrinology 26:174-185.

von Graffenried, B., E. Del Pozo, J. Roubicek, E. Krebs, W. Poldinger, P. Burmeister and L. Kerp (1978). Effects of the synthetic enkephalin analogue FK 33-824 in man. Nature 272:729-730.

Wakabayashi, I., R. Demura, M. Kanda, H. Demura and K. Shizume (1976). Effect of hypophysectomy on hypothalamic somatostatin content in rats. Endocrinol. Jpn. 23:439-442.

Wakabayashi, I., Y. Miyazawawa, M. Kanda, N. Miki, R. Demura, H. Demura and K. Shizume (1977). Stimulation of immunoreactive somatostatin release from hypothalamic synaptosomes by high (K^+) and dopamine. Endocrinol. Jpn. 24:601-604.

Walaas, I. and F. Fonnum (1978). The effect of parenteral glutamate treatment on the localization of neurotransmitters in the mediobasal hypothalamus. Brain Research 153:546-562.

Wauquier, A., and E.T. Rolls (1976). Brain-Stimulation Reward. Elsevier, New York, p.615.

Websters New Collegiate Dictionary, (1977) G. and C. Merriman Company, Massachusetts.

Weiner, R.I. and W.F. Ganong (1978). Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol. Rev. 58:905-976.

Wenger, T., I. Gerendai and B. Halasz (1978). Effect of hypophysectomy on the luteinizing hormone-releasing hormone content of the organum vasculosum of the lamina terminalis in female rat. Brain Res. 157:157-160.

Weiner, R.I., J.E. Shryne, R.A. Gorski and C.H. Sawyer (1972). Changes in the catecholamine content of the rat hypothalamus following deafferentation. Endocrinology 90:867-873.

Wilber, J.F., T. Nagel and W.F. White (1971). Hypothalamic growth hormone releasing activity (GRA): Characterization by the in vitro rat pituitary and radioimmunoassay. Endocrinology 89:1419-1424.

Willoughby, J.O., P. Brazeau and J.B. Martin (1977). Pulsatile growth hormone and prolactin: effects of (+) butaclamol, a dopamine receptor blocking agent. Endocrinology 101:1298-1303.

Willoughby, J.O. and T.A. Day (1981). Central catecholamine depletion: effects on physiological growth hormone and prolactin secretion. Neuroendocrinology 32:65-69.

Willoughby, J.O. and J.B. Martin (1977). Proceedings of the Congress on Hypothalamic Releasing Hormones, F. Labrie (Ed.), Plenum Press, New York.

Willoughby, J.O. and J.B. Martin (1977). Proceedings of the Congress on Hypothalamic Releasing Hormones, F. Labrie (Ed.), Plenum Press, New York.

Willoughby, J.O. and J.B. Martin (1978). The role of the limbic system in neuroendocrine regulation. In Continuing Evolution of the Limbic System Concept, K. Livingston and O. Hornykiewicz (Eds.), Plenum Press, New York.

Willoughby, J.O., M. Menadue, P. Zeegers, P.H. Wise and J.R. Oliver (1980). Effects of human growth hormone on the secretion of rat growth hormone. J. Endocr. 86:165-169.

Willoughby, J.O., L.C. Terry, P. Brazeau and J.B. Martin (1977). Pulsatile growth hormone, prolactin and thyrotropin secretion in rats with hypothalamic deafferentation. Brain Research 127:137-152.

Woodburne, R.T., E.C. Crosby and R.E. McCotler (1946). The mammalian isthmus and midbrain region. Part II. The fiber connections. A. The relation of the tegmentum of the midbrain with the basal ganglia in Macaca mulatta. J. Comp. Neurol. 85:67-92.

Yagi, K. and Y. Sawaki (1970). On the localization of neurosecretory cells controlling adenohypophyseal function. Jap. J. Physiol. 32:621-622.

Yen, S.S.C., T.M. Siler and G.W. DeVane (1974). Effect of somatostatin in patients with acromegaly: Suppression of growth hormone, prolactin, insulin and glucose levels. N. Engl. J. Med. 290:935-938.

Zaborszky, L. and M. Palkovits (1978). Ascending brain stem pathways to the diencephalon and limbic regions. A light and electron microscopic study in the rat. Acta Morphol. Acad. Sci. Hung. 26:49-71.

Zaborszky, L. and G.B. Makara (1979). Intrahypothalamic connections: An electron microscopic study in the rat. Exp. Brain Res. 34:201-215.

Zaborszky, L. Cs. Leranth, G.B. Makara and M. Palkovits (1975). Quantitative studies on the supraoptic nucleus in the rat. II. Afferent fiber connections. Exp. Brain Res. 22:525-540.

Zimmermann, E. and V. Critchlow (1967). Effects of diurnal variation in plasma corticosterone levels on adrenocortical response to stress. Proc. Soc. Exp. Biol., (New York), 125:658-663.