EFFECTS OF INTRACELLULAR GLUCOPENIA ON PULSATILE GROWTH HORMONE SECRETION: MEDIATION IN PART BY SOMATOSTATIN

by

© Jean-Claude Painson

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Neurology and Neurosurgery
McGill University, Montreal

August 15, 1985 ©

Running title:

MECHANISMS OF GROWTH HORMONE RESPONSE TO GLUCOPENIA

"Le génie scientifique, c'est d'inventer
le monde tel qu'il est."

A mes parents

TABLE OF CONTENTS

RESULTS

LIST OF FIGURES AND TABLES

Figures

Tables

ACKNOWLEDGMENTS

Though only my name appears as author, many people contributed in making this thesis a reality. I particularly acknowledge Dr. Gloria Tannenbaum for providing expert advice, counsel and support in organizing the manuscript. I also acknowledge Dr. Robert Dykes for his continuous encouragement.

I'm very grateful to Mrs. Wendy Gurd for her patience in teaching me the surgical and experimental techniques and to Mrs. Sylvie Barrett for performing many of the hormone assays. I would like to address a special thanks to Mrs. Dorothy Carruthers and Ms. Diane Lamy for their secretarial expertise and help in proofreading my thesis. I also thank the Montreal Children's Hospital Audio-Visual department for providing the illustrations of the manuscript.

I wish to express my appreciation to Dr. Nicholas Ling for generous supplies of the hGRF peptide used in my study, to the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases for providing the rat GH radioimmunoassay materials, and finally to Dr. R. Chance for the gift of rat insulin preparation for the insulin radioimmunoassay.

Last but not least, I'm deeply grateful to my family and friends for their continuous support and encouragement during the past two years.

I was the recipient of a McGill University-Montreal Children's Hospital Research Institute Graduate Studentship for the academic period between June 83 - July 85. A manuscript reporting a portion of these data is now in press in Endocrinology volume 117 September, 1985.

ABSTRACT

The present investigation examined the effects of 2-deoxy-D-glucose (2DG) [illegible] intracellular glucopenia on growth hormone (GH), [illegible] dynamics in [illegible] male rat [illegible] con-tra[illegible] [illegible] administration of 2DG (40[illegible] [illegible] lacking [illegible] of [illegible] pulses with marked hyperglycemia. Intracerebroventricular adminis-tration of low doses of 2DG ([illegible]) [illegible] also markedly attenuated GH pulse amplitude and raised plasma glucose levels, but these low doses were ineffective when injected iv, suggesting a central site of action.

To elucidate the mechanism(s) mediating the GH suppression response to glucoprivation, I assessed the involvement of the two hypothalamic GH-regulatory peptides, somatostatin (SRIF) and GH-releasing factor (GRF). Passive immunization of 2DG-treated rats with a specific SRIF antiserum caused an initial surge of GH release and significant elevation of both trough and mean 6-h plasma GH levels. However, SRIF AS failed to restore the amplitude of GH pulses to normal levels. Administration of 3 iv boluses of hGRF (10 μg), at 90 min intervals, to 2DG-treated rats resulted in GH release which was variable and time-dependent; the magnitude of the first response was significantly less than that of the other two. Immunoneutralization with SRIF antiserum eliminated this difference and significantly enhanced hGRF-induced GH release.

These results demonstrate that intracellular glucopenia is a potent inhibitor of pulsatile GH secretion in the rat, and that this response is mediated, at least in part, by an increase in SRIF release. The longer lasting suppression of GH pulses observed after glucose deprivation may be due to decreased output of GRF from the hypothalamus.

RÉSUMÉ

L'étude suivante examine les effets de la glucopénie intracellulaire provoquée par le [illegible]xy-[illegible]-glucose (2DG) sur la sécrétion [illegible] l'hormone [illegible] (GH), de l'insuline et du [illegible] chez [illegible] intracérébroventric[illegible] par voie [illegible] (4[illegible] mg[illegible] et [illegible] durée des [illegible] de [illegible] considérablement [illegible], et [illegible] particulièrement [illegible] pendant [illegible] 5 h. [illegible] niveau [illegible] insuline demeure normal. [illegible] de [illegible] (4 [illegible] 8 mg/[illegible] administrées dans [illegible] système [illegible] produisent le même effet [illegible] les [illegible] inefficaces lorsqu'administrées par voie intraveineuse.

Afin de clarifier le [illegible] les mécanisme[illegible] d'action du 2DG, les rôles de la somatostatine (SRIF) et de la somatocrinine (GRF) ont été évalués. L'immunisation de rats traités au 2DG avec un antisérum (A-) spécifique à SRIF provoque une brève sécrétion de GH suivie d'une augmentation du niveau moyen des creux de GH sans toutefois rétablir la concentration normale de GH dans le plasma. Par contre, l'administration de trois doses massives de hGRF (10 µg/0.3 ml) à 90 min d'intervalle entraîne une décharge variable de GH, la première (1 h après l'administration de 2DG) étant systématiquement moins importante que les deux autres. L'administration concomitante de SRIF A- et de hGRF parvient cependant à éliminer cette différence relative au temps d'injection. Ces résultats indiquent donc que chez le rat, la glucopénie intracellulaire telle que déclanchée par le 2DG, diminue la sécrétion de GH et qu'une augmentation de sécrétion de SRIF semble, du moins en partie, responsable de cette réponse. Cette hypothèse n'exclut toutefois pas la possibilité que la sécrétion de GRF en provenance de l'hypothalamus soit diminuée par l'administration du 2DG.

INTRODUCTION

Growth hormone (GH) is a trophic hormone of the anterior pituitary gland with a large molecular weight which, as opposed to most other hormones of the pituitary, does not have a specific target organ. Growth hormone has a more generalized action, in that it promotes somatic growth in both soft and skeletal tissues, and its action on these metabolically active tissues may vary depending on the tissue (Cheek and Hill, 1974). Growth hormone is also implicated in the metabolic control of intermediary metabolites such as carbohydrates, lipids and proteins (Reichlin, 1974). These observations have suggested to many earlier investigators that GH release may be regulated by metabolism and that adequate plasma concentration of this hormone was important for normal metabolic functions. It is therefore of interest to understand the mechanisms whereby this control is achieved and its importance in day-to-day metabolism.

Metabolic Regulation of GH Secretion

GH and carbohydrate metabolism. The role and action of GH in modulating carbohydrate metabolism was evidenced by the finding that administration of GH to animals produced alterations in carbohydrate metabolism. Altszuler and his co-workers in 1959 (Altszuler, Steele, Wall, Dunn and De

Bodo, 1959) presented the first report showing that GH was capable of increasing both glucose production and utilization in normal as well as in hypophysectomized animals (dogs). Thereafter, it was found that the effect of GH was biphasic, with an early insulin-like effect resulting in a fall in plasma glucose level, followed by an anti-insulin-like action (Altszuler, Steele, Rathgeb and De Bodo, 1968; Altszuler, 1974; Merimee, 1979). These earlier findings raised the possibility that GH release might be regulated by carbohydrate metabolism and perhaps be involved in maintaining glucose homeostasis, as well as regulating the metabolic adjustments to fasting and other situations of substrate deficiency (Cahill, Herrera, Morgan, Soeldner, Steinke, Levy, Reichard and Kipnis, 1966).

Roth and his co-workers, in 1963, were the first to report that hypoglycemia induced by insulin was a potent stimulator of GH secretion (Roth, Glick, Yalow and Berson, 1963(a)). They demonstrated that hypoglycemia stimulated GH release in fasted subjects and that this response was blocked by simultaneous infusion of glucose and insulin. These findings were at that time interpreted to indicate a direct relationship between plasma glucose and GH level. Furthermore, other stimuli such as moderate exercise, fasting and inhibition of intracellular glucose utilization were all found to cause a significant elevation of plasma GH level in

normal human subjects, (Faiman, 1965; Marks, Howorth and Greenwood, 1965; Hunter, Rigal and Sukkar, 1968), while administration of glucose produced an abrupt fall in plasma GH (Roth, Glick, Yalow and Berson, 1963(b), and 1964). In the light of these observations it was hypothesized that GH was directly involved in glucose homeostasis (Luft and Cerasi, 1968). However, the insulin-induced hypoglycemia test used in these studies does not simulate a normal physiological condition and the GH response observed might therefore, not be part of daily glucoregulatory mechanisms. Several experiments questioned the role of GH in glucose homeostasis. Induction of a small decrease in plasma glucose level by administration of low doses of insulin did not result in significant GH release (Koh, Kohn, Catt and Burger, 1968). In addition, this study also demonstrated that spontaneous fluctuations in plasma GH occurred with no apparent relation to the changes in plasma glucose levels. In 1970, Glick (Glick, 1970) presented a study in which he showed that the threshold fall in blood glucose that was necessary to induce significant GH release was beyond that observed under normal circumstances. The use of sequential blood sampling in normal adults demonstrated that changes in plasma concentration of GH were independent of plasma glucose during a 24-hour fast (Quabbe, Schilling and Helge, 1966) as well as independent of feeding regimens. Glick and Goldsmith

(Glick and Goldsmith, 1968) reported that rhythmic fluctuations of plasma GH occurred in both fasting and feeding conditions in normal male and female subjects. Thereafter, it was shown that neither glucose nor proteins are directly involved in modulation of GH secretion, and that the periodic rhythmic secretion of GH was independent of normal daily metabolic processes (Goldsmith and Glick, 1970). More recent investigations in man were not able to establish a direct correlation between plasma glucose level and GH release (Cryer, 1981; Tse, Clutter, Shah, Miller and Cryer, 1983) ruling out the possibility of a specific role for GH in glucose homeostasis under normal physiological conditions in healthy adult human subjects.

Similar findings were observed in other animals species although there appeared to be a wide degree of species specificity (Machlin, Takahashi, Horino, Hertelendy, Gordon and Kipnis, 1968). While the monkey was found to have a pattern of GH release that was similar to human (Meyer and Knobil, 1967), the pig exhibited only a slight elevation in plasma GH in response to an hypoglycemic challenge (Machlin, Horino, Hertelendy and Kipnis, 1968). Moreover, the rabbit (McIntyre and Odell, 1974), the cat (Kokka, Garcia, Morgan and George, 1971) and the mouse (Müller, Miedico, Giustina and Cocchi, 1971) were unresponsive to such a stimulus. Conflicting data have been reported in the rat. While earlier investigators reported a decrease (Takahashi, Daughaday and Kipnis, 1971)

in plasma GH level in response to cellular glucopenia, as well as decreased GH content in the anterior pituitary gland of starved rats (Friedman and Reichlin, 1965), more recent investigators have demonstrated both a decrease (Tannenbaum, Martin and Colle, 1976) and an increase (Bluet-Pajot and Schaub, 1977 and 1978; Okajima, Motomastu, Kato and Ibayashi, 1980) in plasma GH in response to hypoglycemia. These conflicting results further complicated the problem and thus this issue remains unclear and warrants further investigation.

GH and protein metabolism. Growth hormone stimulates protein synthesis (Kostyo and Nutting, 1974; Merimee, 1979) thus creating a positive nitrogen balance. In the light of this action of GH, the effects of protein and various amino acids on the secretion of GH were investigated. Even though infusion of arginine was followed by significant release of GH in the plasma of normal male and female subjects (Knopf, Conn, Fajans, Floyd, Guntsche and Rull, 1965; Merimee, Burgess and Rabinowitz, 1966), Best and his collaborators (Best, Catt and Burger, 1968) presented data suggesting that the GH response to an arginine infusion test was not a specific response since GH fluctuations were also observed in the plasma of control subjects only receiving a saline injection. Therefore, it cannot be assumed that in humans, amino acids, and consequently proteins, have a major role in GH regulation.

GH and fat metabolism. Growth hormone is also known to play an important role in the control of fat metabolism, since it shifts general metabolism towards fat mobilization from adipose tissue (Goodman and Knobil, 1959; Goodman and Schwartz, 1974; Heubi, Burstein, Sperling, Gregg, Subbiah and Matthews, 1983). More specifically, GH causes inhibition of lipogenesis while it accelerates fat catabolism (Goodman, 1963). However, beside the ruminants (Bertelendy and Kipnis, 1973) in which plasma free fatty acids were shown to play an important role in GH homeostasis, it was not possible to obtain such proof for other species; only supraphysiological levels of fat in the plasma of monkeys were able to induce a GH response (Blackard, Boylen, Hinson and Nelson, 1969). Moreover, the time lapse between the administration of the fat surplus and the GH response was often too great to establish a direct correlation (Glick, 1969). It was thence concluded that free fatty acids only play a minor role in GH regulation under normal circumstances.

Central Nervous System Regulation of GH Secretion

Hypothalamic control of GH secretion. Evidence for central nervous system (CNS) regulation of GH secretion arises from several clinical and experimental observations. From a clinical point of view, it was observed that patients with pituitary stalk section for non-pituitary disorders had

reduced plasma GH concentration with partial or complete loss of GH response to insulin-induced hypoglycemia (Glick, Roth, Yalow and Berson, 1965). These observations suggested that the integrity of the CNS-pituitary axis was essential for the reflex GH response to an hypoglycemic challenge. Direct experimental evidence for brain control of GH release was obtained from experimental investigations whereby discrete hypothalamic areas were either lesioned or stimulated.

Lesion experiments. Reichlin (Reichlin, 1960 and 1961) was the first to demonstrate that, in rats, extensive lesions located at the base of the hypothalamus, including the median eminence, caused a decrease in somatic growth, depleted pituitary GH content and decreased plasma GH levels as determined by the tibial epiphyseal assay. Moreover, Abrams and his coworkers (Abrams, Parker, Blanco, Reichlin and Daughaday, 1966) showed, using radioimmuoassay (RIA), that in the squirrel monkey hypothalamic lesions restricted to the median eminence resulted in failure of the GH response to insulin-induced hypoglycemia. More specifically, it was observed that lesions limited to the ventromedial hypothalamic (VMH) area which spared the median eminence, but infringed on the arcuate nucleus, caused growth retardation associated with depletion of pituitary GH content and decreased plasma GH concentration in weanling rats (Frohman and Bernardis, 1968). It was also shown that the extent of the

deficit was proportional to the lesion size (Bernardis and Frohman, 1970).

More recent investigations using the neurotoxic agent monosodium glutamate, which selectively destroys the arcuate nucleus (Terry, Epelbaum and Martin, 1981; Millard, Martin jr., Audet, Sagar and Martin, 1982), or electrolytic lesion of the VMH-arcuate complex (Martin, Renaud and Brazeau, 1974; Eikelboom and Tannenbaum, 1983) demonstrated that the medio-basal hypothalamus was stimulatory to GH secretion since its destruction was followed by suppressed plasma GH concentration. Furthermore, animals in which all the neural connections between the anterior and medio-basal hypothalamus were interrupted were found to have normal and often increased GH secretion (Collu, Jéquier, Letarte, Leboeuf and Ducharme, 1973; Mitchell, Hutchins, Schindler and Critchlow, 1973; Willoughby, Terry, Brazeau and Martin, 1977; Critchlow, Rice, Abe and Vale, 1978; Antoni, Makara and Rappay, 1981) and similarly, lesioning the medial preoptic anterior hypothalamic area resulted in elevated plasma GH concentration (Epelbaum, Willoughby, Brazeau and Martin, 1977; Willoughby and Martin, 1978; Critchlow, Abe, Urman and Vale, 1981). Taken together, these data provided support for the view that the medio-basal hypothalamus, more specifically the VMH-arcuate region, contained the putative GH-releasing factor (GRF) neurons, with the anterior-medial preoptic hypothalamic

region as the GH-inhibitory regulatory center.

Stimulation experiments. Data from experiments whereby selective brain regions were stimulated provided additional support to the idea that GH secretion was under hypothalamic control. Electrical stimulation of the VMH-arcuate complex was shown to be capable of inducing a prompt rise in plasma GH concentration within five minutes after the termination of the stimulus with the peak GH level reached by 10-15 minutes (Frohman, Bernardis and Kant, 1968; Bernardis and Frohman, 1971). Furthermore, positive GH responses were also obtained from regions located outside the hypothalamus (Martin, 1972); both dorsal and ventral hippocampus, and the basolateral amygdala were stimulatory loci to GH secretion while the corticomedial amygdala was inhibitory to the secretion of the hormone. In addition, electrical stimulation of the anterior hypothalamus caused a decrease in plasma GH levels further implicating this region as an inhibitory locus for GH control (Martin, Tannenbaum, Willoughby, Renaud and Brazeau, 1975).

Neurohormonal Control of GH Secretion

In parallel to the early lesion and stimulation experiments mentioned above, and in view of the existence of a chemical connection between the hypothalamus and the pituitary gland via the portal circulation, other investigators were interested in studying the effects of administration of hypo-

thalamic extracts on GH release to possibly identify the hypothalamic GH-regulatory factors. Deuben and Meites (Deuben and Meites, 1964) were the first to demonstrate the existence of a GH-releasing factor in acid extracts of rat hypothalamus. This factor was able to cause a four to six fold increase in the amount of bioassayable GH released by cultured rat anterior pituitaries. Furthermore, administration of extracts derived from other brain regions were ineffective in inducing GH release. With the advent of a specific RIA to measure GH in the plasma, it became possible to evaluate more precisely the effects of hypothalamic extracts on GH release from the anterior pituitary. In the rhesus monkey, in vivo administration of sheep hypothalamic extracts caused a significant increase in plasma GH level with a time course resembling that observed following an hypoglycemic challenge (Garcia and Geschwind, 1966). Similar observations were made in other species as well. Intracarotid administration of extracts from bovine stalk median eminence fragments induced an increase in the basal plasma GH level of sheep (Machlin, Horino, Kipnis, Phillips and Gordon, 1967) and intravenous administration of porcine hypothalamic extracts was very effective in inducing a GH response in rats (Malacara and Reichlin, 1971). Later, it was observed that other brain peptides were also effective in stimulating GH release from the pituitary gland although none of them was

specific to GH (Martin, Brazeau, Tannenbaum, Willoughby, Epelbaum, Terry and Durand, 1978). Other experiments, however, have shown that GH release was also under the control of inhibitory hypothalamic influences. Krulich and his co-workers (Krulich, Dhariwal and McCann, 1968; Krulich, Illner, Fawcett, Quijada and McCann, 1971) presented data indicating that bovine hypothalamic extracts also possessed the capacity to inhibit both synthesis and release of GH *in vitro* suggesting a dual control of GH secretion. However, it was not until later that the true nature of this GH-inhibitory factor was elucidated.

Somatostatin. Somatostatin (SRIF) is a tetradecapeptide which was discovered on the basis of its ability to inhibit GH release from pituitary cells in culture (Brazeau, Vale, Burgus, Ling, Butcher, Rivier and Guillemin, 1973). It was first isolated and characterized from sheep hypothalamic extract. The discovery of this peptide stimulated research and it soon became evident that SRIF was a potent inhibitor of GH release to virtually all known stimuli, both *in vivo* and *in vitro*, and in a wide variety of species including man (Martin, Tannenbaum, Willoughby, Renaud and Brazeau, 1975). Moreover, it was shown to have a widespread inhibitory effect on hormone release in general. Immunocytochemical studies have localized SRIF in various brain regions with the primary

somatostatinergic neurons in the anterior-preoptic hypothalamus (Brownstein, Arimura, Sato, Schally and Kizer, 1975; Alpert, Brawer, Patel and Reichlin, 1976; Filby and Gross, 1983) which is consistent with the earlier physiologic studies. In addition, SRIF was found in several peripheral organs such as the pancreatic islets (Luft, Efendic, Hökfelt, Johansson and Arimura, 1974; Pelletier, Leclerc, Arimura and Schally, 1975) and the gastrointestinal tract (Arimura, Sato, Coy and Schally, 1975; Patel and Reichlin, 1978). These later findings fitted well with the observations that SRIF was also effective in suppressing insulin (Alberti, Christensen, Christensen, Prange Hansen, Iversen, Lundbaek, Seyer-Hansen and Ørskov, 1973; Koerker, Ruch, Chideckel, Palmer, Ensinck, Goodner and Gale, 1974), glucagon (Gerich, Lorenzi, Schneider, Karam, Rivier, Guillemin and Forsham, 1974; Dobbs, Sakurai, Sasaki, Faloona, Valverde, Baetens, Orci and Unger, 1975), gastrin (Bloom, Mortimer, Thorner, Besser, Hall, Gomez-Pan, Roy, Russell, Coy, Kastin and Schally, 1974) and secretin release (Boden, Sivitz, Owen, Essa-Koumar and Landor, 1975).

Finally, experiments using a specific antiserum to SRIF have shown that SRIF has a physiological role in the regulation of GH secretion under both normal and pathological conditions. Administration of SRIF antiserum elevated basal GH levels in normal animals (Ferland, Labrie, Jobin, Arimura

and Schally, 1976; Steiner, Stewart, Barber, Koerker, Goodner, Brown, Illner and Gale, 1978; Terry and Martin, 1981; Eikelboom and Tannenbaum, 1983), blocked stress-induced GH suppression (Arimura, Smith and Schally, 1976; Terry, Willoughby, Brazeau, Martin and Patel, 1976), and restored high amplitude GH levels in both starved (Tannenbaum, Epelbaum, Colle, Brazeau and Martin, 1978) and streptozotocin diabetic rats (Tannenbaum, 1981) exhibiting suppressed plasma GH concentration. Studies of animal models of both diabetes (Patel, Cameron, Bankier, Malaisse-Lagae, Ravazzola, Studer and Orci, 1978; Tannenbaum, Colle, Wanamaker, Gurd, Goldman and Seemayer, 1981) and starvation (Tannenbaum, Rorstad and Brazeau, 1979) have confirmed marked alterations in tissue somatostatin content suggesting a role for SRIF in these conditions.

Growth hormone-releasing factor (GRF). Despite compelling evidence for GRF activity this peptide has only recently been isolated and characterized by two groups of investigators (Guillemin, Brazeau, Böhlen, Esch, Ling and Wehrenberg, 1982; Rivier, Spiess, Thorner and Vale, 1982). Three peptides with 37, 40 and 44 amino acids, respectively, were isolated from two carcinoid pancreatic tumors of patients exhibiting severe symptoms of acromegaly. All three peptides exhibit potent GH-releasing activity, both *in vitro* (Brazeau, Ling, Böhlen, Esch, Ying and Guillemin, 1982; Vale, Vaughan, Yamamoto,

Spiess and Rivier, 1983) and in vivo in a variety of species (Wehrenberg, Ling, Brazeau, Esch, Böhlen, Baird, Ying and Guillemin, 1982; Chihara, Minamitani, Kaji, Kodama, Kita and Fjuita, 1983; Gelato, Pescovitz, Cassorla, Loriaux and Merriam, 1983; Szabo, Dudlak, Thominet and Frohman, 1983; Harvey, Scanes and Marsh, 1984; Scanes, Garsia, Lauterio, Huybrechts, Rivier and Vale, 1984; Thorner, Rivier, Spiess, Borges, Vance, Bloom, Rogol, Cronin, Kaiser, Evans, Webster, MacLeod and Vale, 1983; Tannenbaum and Ling, 1984). The 44 amino acid form of the peptide was later found to be identical in structure and biological potency to the native peptide isolated from human hypothalamus (Ling, Esch, Böhlen, Brazeau, Wehrenberg and Guillemin, 1984). In addition, this peptide has been found to interact in a non-competitive manner with SRIF to control GH release both in vitro (Brazeau, Ling, Böhlen, Esch, Ying and Guillemin, 1982) and in vivo (Tannenbaum and Ling, 1984). More recently, Spiess and his collaborators (Spiess, Rivier and Vale, 1983) have reported the isolation and characterization of a 43 amino acid peptide which they proposed to be the rat hypothalamic GRF. Immunocytochemical studies soon thereafter localized GRF primarily within the arcuate nucleus of the hypothalamus (Bloch, Brazeau, Ling, Böhlen, Esch, Wehrenberg, Benoit, Bloom and Guillemin, 1983; Lin, Bollinger, Ling and Reichlin, 1984) as well as within some cell bodies within the VMH nucleus (Merchenthaler, Vigh, Schally, and Petrusz, 1984),

confirming the stimulatory role of these brain regions in GH control.

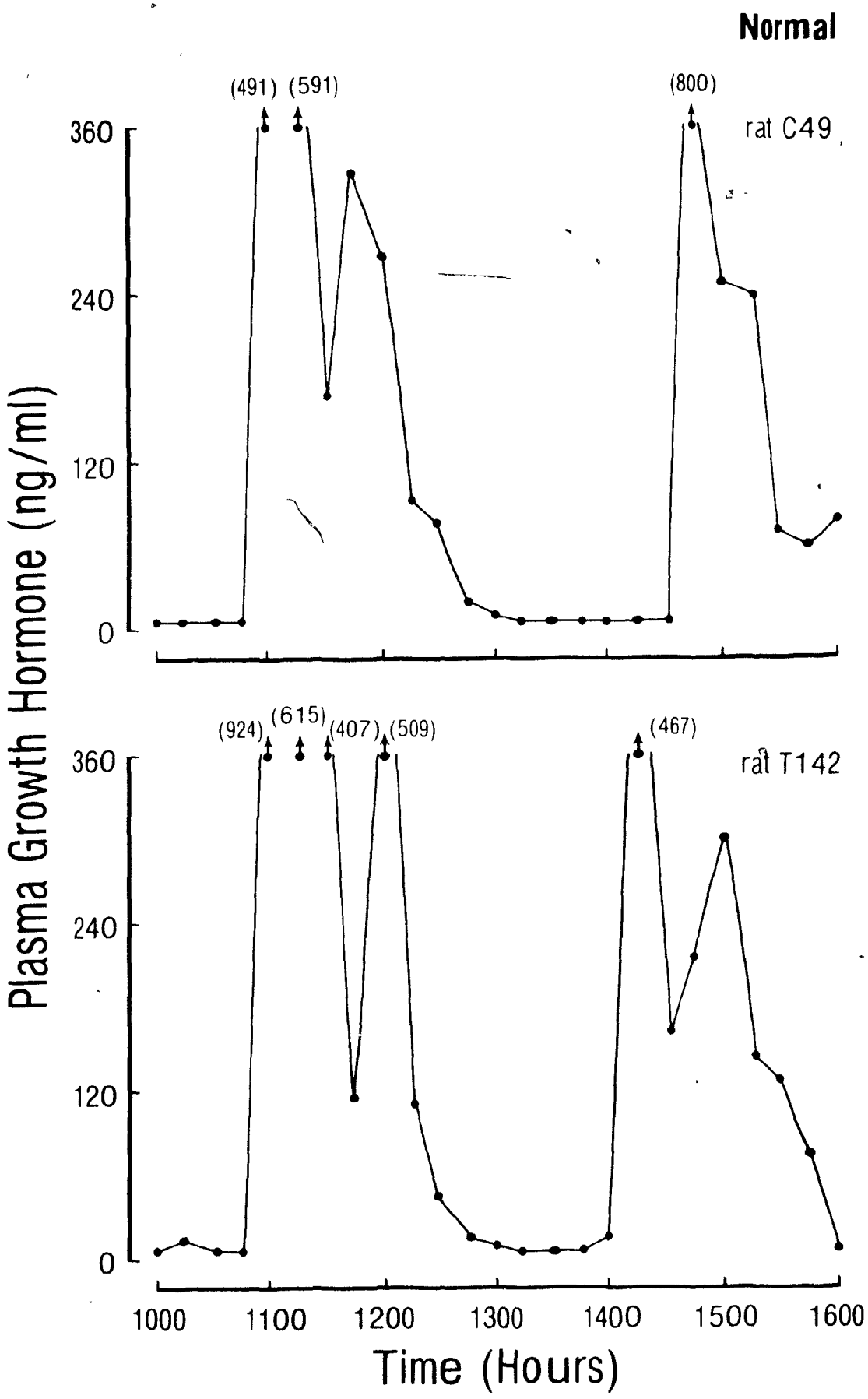
Growth Hormone Secretory Pattern

In all species investigated experimentally thus far (Meyer and Knobil, 1967; Machlin, Horino, Hertelendy and Kipnis, 1968; Takahashi, Daughaday and Kipnis, 1971; Tsushima, Irie and Sakuma, 1971), including humans (Quabbe, Schilling and Helge, 1966; Goldsmith and Glick, 1970), GH secretion is characterized by wide variations. Although factors such as sleep, exercise and stress are implicated in the physiologic variations observed in GH secretion, many of the surges are spontaneous. Typically, in healthy adult men, plasma GH levels may vary from basal levels of less than 5 ng/ml to levels as high as 60 ng/ml (Martin, Tannenbaum, Willoughby, Renaud and Brazeau, 1975). The highest levels are often observed during night sleep (Takahashi, Kipnis and Daughaday, 1968; Mendelson, Jacob, Gillin and Wyatt, 1979), and also during the stages of adolescence (Miller, Tannenbaum, Colle and Guyda, 1982). In the rat, GH secretion is also characterized by spontaneous bursts of GH release. In particular, in this species, GH secretion is governed by a striking ultradian rhythm (Tannenbaum and Martin, 1976) with a periodicity of approximately 3-4 hours, which in the male rat, is entrained to the light-dark cycle of the day

(Tannenbaum and Martin, 1976). The GH rhythm continues to function independent of environmental lighting and feeding and is not correlated to changes in plasma glucose or insulin, indicating that it is a true endogenous biological rhythm, likely controlled by CNS mechanisms (Tannenbaum, Martin and Colle, 1976). In male rats, the GH secretory episodes are often multiphasic in nature with two or even three spikes of GH released within each large GH secretory episode. This pattern of GH secretion persists unchanged throughout the 24-hour day (Tannenbaum and Martin, 1976). Figure 1 shows two individual, representative GH secretory profiles of rats sampled for periods of 6 hours which the author has obtained in this laboratory.

In summary, it is now generally believed that the regulation of GH release from the pituitary gland is mediated via the CNS by at least two hypothalamic factors which are released into the hypothalamic hypophyseal portal circulation. These factors which are both inhibitory (SRIF) and stimulatory (GRF) have been demonstrated to interact in a non-competitive manner and likely generate the episodic rhythmic secretory pattern which characterizes GH release in many species (for review, see Tannenbaum, 1985).

FIGURE 1 Individual rhythmic GH secretory patterns in two animals sampled over a 6-h period. Pulsatile GH secretion in the male rat monitored over this time interval (1000 - 1600 h) is characterized by two major episodes of GH secretion occurring at approximately 3 h intervals and separated by a trough period of mostly undetectable GH levels. A uniformity in the timing of the GH pulses is evident.


Normal
(491)
(591)
(800)
rat C49
360
240
120
0
(924)
(615)
(407)
(509)
(467)
rat T142
360
240
120
0
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

Aim of the Present Investigation

As was pointed out in the above discussion, GH is actively involved in the regulation of various metabolic processes, and its secretion is, in turn, controlled by metabolic as well as neuroendocrine factors. The possibility that GH might play a role in the regulation of glucose homeostasis after hypoglycemic stimulation, or prolonged periods of fasting, has been considered by many investigators in earlier studies. While there is general agreement that GH secretion in man is increased in response to insufficient glucose metabolism provoked by either insulin (Roth, Glick, Yalow and Berson, 1963(a); Luft and Cerasi, 1968), or the non-metabolizable glucose analog, 2DG (Roth, Glick, Yalow and Berson, 1963(b); Wegienka, Grodsky, Karam, Grasso and Forsham, 1967; Brodows, Pi-Sunyer and Campbell, 1973; Woolf, Lee, Leebaw, Thompson, Lilavivathana, Brodows and Campbell, 1977; Wakabayashi, Demura, Miki, Ohmura, Miyoshi and Shizume, 1980), conflicting results have been obtained in rats. Rat GH secretion has been shown to be either inhibited (Takahashi, Daughaday and Kipnis, 1971; Tannenbaum, Martin and Colle, 1976) or stimulated (Bluet-Pajot and Schaub, 1978) in response to insulin-induced hypoglycemia, and both a decrease (Takahashi, Daughaday and Kipnis, 1971) and increase (Okajima, Motomatsu, Kato and Ibayashi, 1980) in plasma GH

have been reported after 2DG treatment. Since spontaneous GH secretion in the rat exhibits a striking ultradian rhythm (Tannenbaum and Martin, 1976) it is possible that this disagreement stems from either the use of inappropriate blood sampling techniques, stressful anesthesia and/or differences in the route of administration of the glucopenic stimulus. Thus, the first series of experiments in the present investigation was undertaken to clarify the GH response to intracellular glucoprivation by examining the effects of both systemic and central administration of 2DG on GH secretory dynamics in conscious freely-behaving rats. The 2DG stimulus has the advantage of providing a more controlled stimulus and avoiding the superimposed systemic effects of insulin (See Appendix I).

The mechanism mediating the GH response to insufficient glucose remains to be elucidated. Participation of the CNS is indicated by reports of the existence of glucose-sensitive cells within the hypothalamus which modulate the GH response to hypoglycemia (Blanco, Schalch and Reichlin, 1966; Himsworth, Carmel and Frantz, 1972). It is now well-recognized that brain control of the rhythmic secretion of GH is achieved by way of the complex interaction of two hypothalamic hormones, GRF and SRIF (Tannenbaum and Ling,

1984). Yet, to date, the in vivo involvement of the two hypothalamic GH-regulatory peptides in glucose regulation of GH secretion has not been assessed. The recent isolation and characterization of GRF peptides from both human pancreas tumors and hypothalamus, together with the availability of a specific antiserum to SRIF, now provide powerful tools to address this question. In the second series of experiments reported here I investigated the involvement of SRIF and GRF in mediating the GH response to 2DG-induced intracellular glucopenia.

MATERIAL AND METHODS

Animals and Surgery

Adult male Sprague Dawley rats weighing 280-310 grams at the start of each experiment were obtained from Charles River Canada (St. Constant, Québec). All rats were implanted with chronic intracardiac venous cannulae under sodium pentobarbital (50 mg/kg, ip) anesthesia using a method previously described (Tannenbaum and Martin, 1976). The cannula which consisted of sterile silastic tubing (ID 0.025 in., OD 0.047 in.; 10 cm) was inserted into the right external jugular vein, 40 mm from the right atrium of the heart. The free end was brought subcutaneously to the skull surface, fastened to hypodermic tubing and affixed to the skull with dental cement. In some instances, a chronic intracerebroventricular (icv) cannula, constructed of aluminum, was simultaneously inserted into the left lateral ventricle of the brain (Tannenbaum, 1980). With the incisor bar at +5 mm, stereotaxic coordinates were: 1 mm posterior to bregma, 1 mm lateral to the mid-line and 4.5 mm below the skull surface (coordinates were taken from the Pellegrino and Cushman Atlas, 1967). After surgery, each rat was injected with 0.3 cc Penicillin G (300,000 IU/ml). They were immediately placed in isolation test chambers (lights on between 0600-1800 hours (h)) in a temperature (22±2°C)- and humidity

controlled room with Purina rat chow and tap water available ad libitum, until body weight returned to preoperative levels (usually 5-7 days). During this period, the rats were weighed and handled daily to habituate them to the experimental procedure to be used on the test day and thus minimize any stress associated with handling. The venous cannula was maintained patent by rinsing with a solution of 20% heparin (Heparin sodium 10,000 U.S.P. Units/ml, Hepalean) every 4-5 days.

Experimental Procedures

On the day prior to the test day, the animals were prepared with polyethylene (PE 100: void volume, 0.3 ml) tubing filled with 20% heparinized saline which extended from the hypodermic tubing on their head to the outside of the isolation cubicle via a stainless steel spring attached to a roller bearing at the top of the cage. This assembly permitted the animal to be freely-moving during all experimental manipulations. The tubing was connected to a 1.0 cc syringe and the animals were left so prepared overnight. On the test day, in most experiments, food was removed from the cage 1.5-2 h before the start of the experiment, at 1000 h, and returned at the end at 1600 h. Blood samples (0.45 ml) were withdrawn every 15 minutes (min) for periods of 6 h (1000 - 1600 h). All blood samples were immediately centrifuged and

the plasma was separated in six aliquots and stored at -20°C for subsequent assays of GH, insulin and glucose. The red blood cells were then resuspended in 0.25 ml normal saline (0.9% NaCl) and returned to the animal following removal of the next blood sample. This sampling technique allowed longitudinal sampling without hemodynamic depletion. At the termination of the experiments the accuracy of the icv cannula placement was determined by injection of 10 µl methylene blue dye via the cannula and macroscopic observance of the dye in the ventricles of the brain.

Study 1. In the first series of experiments, 8 groups of animals were used to investigate the effects of systemic and central administration of 2DG on GH, insulin and glucose secretory dynamics. Three groups were administered 2DG (Sigma Chemical Co., St. Louis, MO), iv, at three different doses, (400 mg/kg, 8 mg/kg and 4 mg/kg) whereas a fourth group received 2DG iv in a dose of 400 mg/kg and was allowed to feed ad libitum during the test. A known amount of rat chow pellets was given to these animals at 1000 h, at the start of the experiment. At the end of the test (1600 h), the remaining food was weighed and the amount eaten determined. Two other groups received 2DG icv in doses of 4 mg/10 µl and 8 mg/10 µl. In all experiments the 2DG was diluted in normal saline just before use and was injected after removal of the third blood sample (1030 h). Two additional groups

served as controls and received normal saline iv (0.6 ml) or icv (10 µl) at the same time point.

Study 2. The second series of experiments was undertaken to assess the involvement of the two hypothalamic GH-regulatory peptides, SRIF and GRF, in the 2DG-induced GH suppression response. To examine the role of endogenous SRIF, one group of 2DG-treated rats (400 mg/kg, iv) was administered 1 ml of a specific SRIF antiserum, iv, 15 min after administration of the 2DG (i.e. at 1045 h). This antiserum which was raised in sheep is specific for SRIF and does not bind other brain or gut peptides (Tannenbaum, Epelbaum, Colle, Brazeau and Martin, 1978). At a final dilution of 1/20,000 this antiserum binds 35% of (^{125}I-Tyr$_1$)-SRIF and can detect 10 pg/assay tube of synthetic SRIF. A second 2DG-treated group (400 mg/kg, iv) served as control and received 1 ml of normal sheep serum at the same time point.

To evaluate the possible involvement of GRF in mediating the GH response to the glucopenic stimulus, the ability of 2DG-treated animals to respond to exogenously administered GRF was assessed. For these experiments, two other groups of rats, treated with the same dose of 2DG (400 mg/kg, iv), were given three iv bolus injections, 90 min apart, (at 1130, 1300 and 1430 h) of either the 44-amino acid peptide, human (h)GRF (kindly provided by Dr. N. Ling, Salk Institute, La Jolla, CA) or normal saline as a control. The GRF peptide, which

was a synthetic replicate of the original peptide, was synthesized by solid phase techniques using a Beckman 990 peptide synthesizer (Ling, Esch, Davis, Mercado, Regno, Böhlen, Brazeau and Guillemin, 1980). The peptide was diluted in normal saline just before use to attain a concentration of 10 μg/0.3 ml. In order to document the rapidity of the response to hGRF, an additional blood sample was obtained 5 min after each injection.

Finally, to examine the interaction of GRF with endogenous circulating SRIF in glucopenic animals, I administered 1 ml SRIF antiserum, iv, at 1045 h followed by three iv bolus injections of hGRF (10 μg/0.3 ml), as described above, to 2DG (400 mg/kg, iv)-treated animals.

Hormone Assays

Plasma GH concentrations were determined in duplicate by a double antibody RIA using materials supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK, Bethesda, MD). This assay allowed accurate estimation of plasma GH in a small sample volume (25 μl). The averaged plasma GH values are reported in terms of the rat GH reference preparation (rGH-RP-1) which was used in the standard curve of the assay. The rGH purified antigen (NIADDK-rGH-I-5) was iodinated with ^{125}I using a modification of the chloramine T method (Greenwood, Hunter and

Glover, 1963). Monkey anti-rat serum (anti-rGH-S-4) served as the first antibody and was used at a final dilution of 1:30,000 to obtain approximately 40% binding in the assay. Goat anti-monkey serum (gamma-globulin, G-6-4, P4. Antibodies Inc., CA), at a dilution ranging from 1:9 to 1:7, was used as the precipitating antibody after 72 h of incubation at room temperature. Normal monkey serum (Flow Lab. Ltd., Ont.) was used as a carrier protein for the goat anti-monkey serum. The minimum and maximum detection limits of the assay were 6.2 and 800 ng/ml, respectively. When it was possible, plasma samples with concentrations of GH greater than 800 ng/ml were reassayed at dilutions of 1:5 or 1:10, otherwise such values were expressed as >800 ng/ml and considered to be 800 ng/ml in statistical analyses. Similarly, plasma samples with concentrations of GH less than 6.2 ng/ml were expresed as <6.2 ng/ml and considered to be 6.2 ng/ml in statistical analyses. The inter- and intra-assay coefficients of variation were 12.6% and 11.8%, respectively, for duplicate samples from pooled plasma containing a mean GH concentration of 28.3 ng/ml.

Plasma immunoreactive insulin (IRI) was measured by a dextran-coated charcoal method (Herbert, Lau, Gottlieb and Bleicher, 1965) using guinea pig antiporcine insulin serum at a dilution of 1:60,000 to attain a binding of approximately 60%. Purified crystalline rat insulin (lot No. 615-244-260,

courtesy of Dr. R. Chance, E. Lilly Co., Indianapolis, IN) served as a reference standard. Purified porcine insulin was iodinated with ^{125}I using the Chloramine T method and was repurified on the day of the assay. Separation of free and bound hormone was accomplished by addition of a 2.5% charcoal solution after 48 h of incubation at 4°C. The minimum and maximum detection limits of the assay were 0.32 and 20 ng/ml respectively and all samples (50 µl) were determined in duplicate. Plasma smaples with IRI concentrations less than 0.32 ng/ml were expressed as <0.32 and considered to be 0.32 in statistical analyses.

Plasma glucose, 10 µl aliquots, was measured by an automated glucose oxidase method (Glucose analyzer 2, Beckman Instruments, Palo Alto, CA). The apparatus determines the rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample.

SRIF Binding Capacity of Plasma of Rats after Administration of SRIF Antiserum

The SRIF binding capacity of plasma samples of rats treated with SRIF antiserum was assessed by determining the ability of aliquots of rat plasma (100 µl) obtained 45 min before, and 15, 165 and 315 min after administration of SRIF antiserum to bind (^{125}I-Tyr_1)-SRIF. Plasma samples from each rat were diluted 1:100 in the SRIF assay buffer (0.5 M. phosphate buffer, pH 7.2), and binding to labeled SRIF was

determined under standard conditions used for RIA of SRIF in this laboratory (Tannenbaum, Rorstad and Brazeau, 1979) using a double antibody RIA. In order to document that the normal sheep serum administered to the control group did not possess any SRIF binding capacity, plasma samples from control animals were also tested in the SRIF RIA.

Statistical Analyses

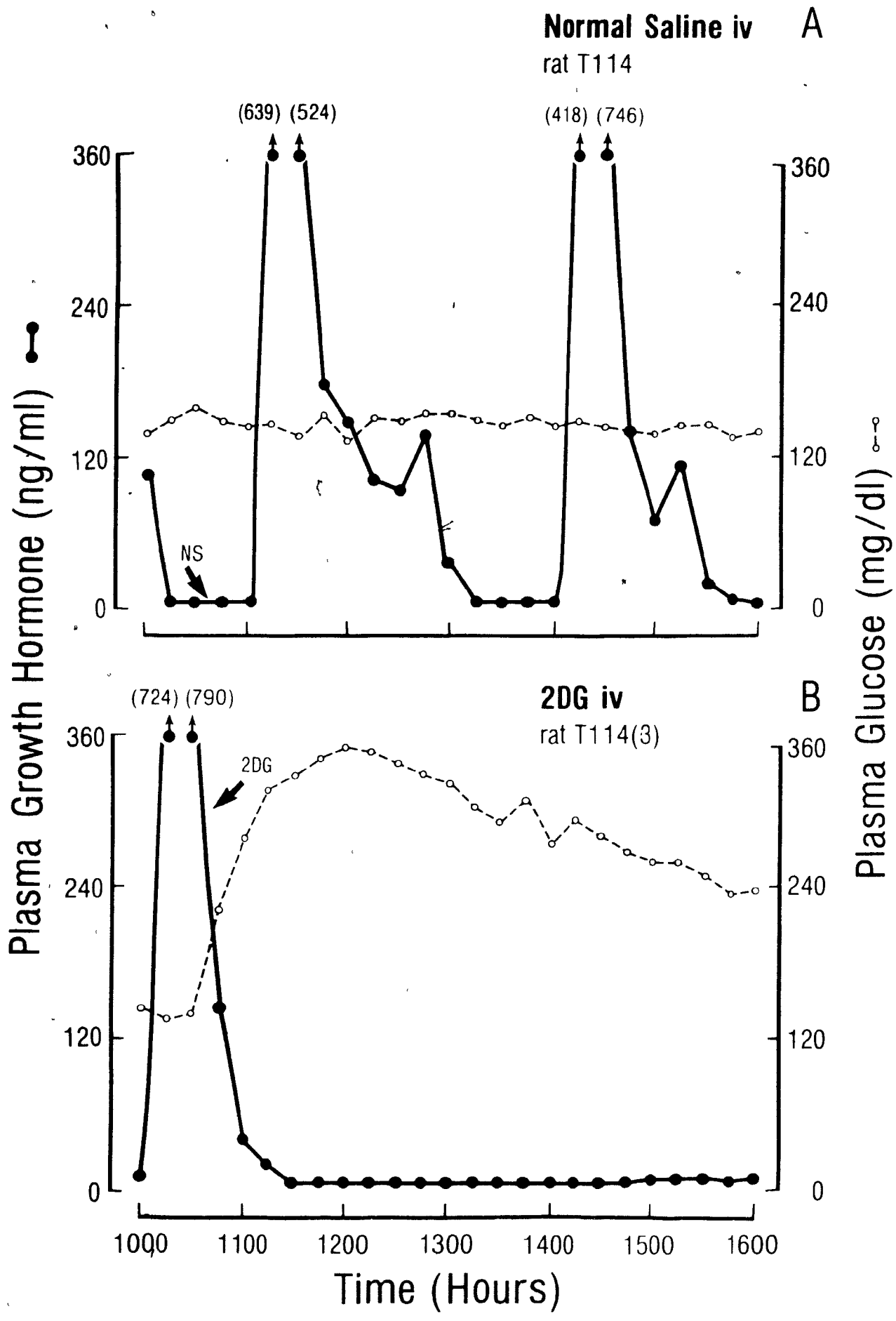
Analysis of variance for repeated measures and Student's t test were used for statistical comparisons between experimental groups (Winer, 1971). Student's paired t test was used to evaluate significant treatment effects within groups.

RESULTS

Effects of Intravenous Administration of 2DG on GH, Glucose, and Insulin Secretory Dynamics

Saline-injected control animals exhibited the typical pulsatile pattern of GH secretion previously documented in normal freely-moving rats (Tannenbaum and Martin, 1976) with two major episodes of GH secretion evident during the 6-h sampling period (Fig. 2A). As was the case for normal animals (See Fig. 1), the GH surges had a rapid onset and were mostly multiphasic in nature. The peak GH values often exceeded 500 ng/ml and the mean peak GH level in this group was 703.6±58.2 ng/ml. In all rats the secretory episodes were separated by trough periods characterized by mostly undetectable (<6.2 ng/ml) plasma GH levels. In addition, there was a synchrony in the timing of the GH pulses indicating entrainment to the light-dark cycle regimen. Typically, the first GH secretory episode occurred between 1100-1230 h with a second surge evident between 1400 and 1530 h. The duration of the episodes was somewhat variable with the mean duration being 111.6±4.5 min. During trough periods, the plasma GH levels remained low for 65.0±4.6 min and the mean plasma GH value for this period was 8.0±0.9 .ng/ml (range 6.2-14.2 ng/ml).

FIGURE 2 Individual, representative 6-h plasma GH and glucose profiles in a rat treated with normal saline (A) in comparison to those in the same rat after 2DG treatment (B). The intravenous injection of 400 mg/kg 2DG caused a significant suppression in amplitude and duration of spontaneous GH pulses. Plasma GH levels remained low for the entire sampling period and this response was accompanied by a pronounced hyperglycemia. Arrows indicate time of injection.


Normal Saline iv
A
rat T114
(639) (524)
(418) (746)
360
240
120
0
NS
Plasma Growth Hormone (ng/ml)
Plasma Glucose (mg/dl)
(724) (790)
2DG iv
B
rat T114(3)
2DG
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

In striking contrast, the 6-h plasma GH profiles of rats administered 400 mg/kg 2DG exhibited a marked suppression in both amplitude and duration of GH secretory episodes which was evident 30 min after the injection of 2DG. Typically, in these rats, the ultradian GH rhythm was lost and GH secretory peaks were no longer discernable (Fig. 2B); plasma GH levels seldom exceeded 90 ng/ml and a large proportion of the GH values were near or below the detectable limit of the GH RIA. In all rats, this suppresion in amplitude and duration of spontaneous GH pulses was evident throughout the 6-h sampling period and mean 6-h plasma GH levels were significantly ($P<0.001$) depressed compared to those of the normal saline-treated control group (mean 6-h plasma GH level: 28.7 ± 4.8 vs 146.9 ± 12.2 ng/ml) (Fig. 3 and Table 1). In some instances, recovery of GH secretion began to occur at the end of the sampling period.

Concomitant with the striking GH suppression, the iv administration of 2DG caused a pronounced hyperglycemia (Fig. 2B) with a characteristic time course. This hyperglycemia was evident within 15 min after injection and the mean plasma glucose level at this time point, 256.0 ± 9.5 mg/dl was significantly ($P<0.001$) higher than the preinjection value of 142.1 ± 6.2 mg/dl. Plasma glucose levels reached a peak 97.5 ± 9.2 min after administration of 2DG and gradually declined thereafter, although remaining significantly

FIGURE 3 Effect of iv administration of either normal saline (top graph) or 400 mg/kg 2DG in non-feeding (middle graph) and feeding (bottom graph) animals on mean 6-h plasma GH levels. Intravenous injection of a high dose of 2DG consistently caused a significant suppression of GH secretory pulses and plasma GH levels remained severely depressed for up to 5 h after the injection. Allowing the animals to eat did not prevent the suppression of GH and mean plasma GH levels in this group were similar to those observed in non-feeding 2DG-treated rats. Arrows indicate time of injection. Vertical bars represent the standard error of the mean (SEM). The number of animals in each group is shown in parentheses.

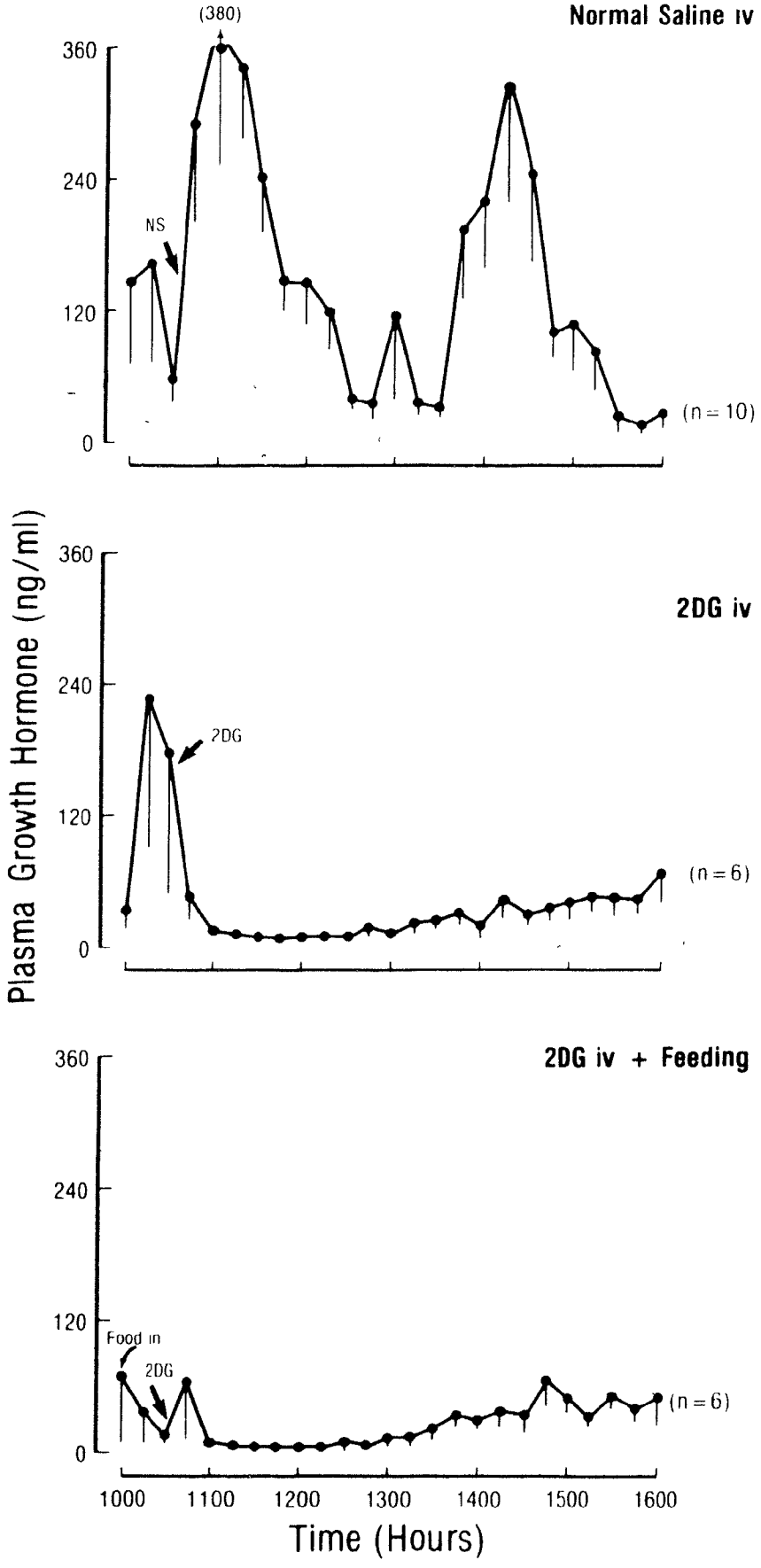

Normal Saline iv
(380)
360
240
120
0
NS
(n = 10)
2DG iv
360
240
120
0
2DG
(n = 6)
2DG iv + Feeding
360
240
120
0
Food in
2DG
(n = 6)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)
Plasma Growth Hormone (ng/ml)

TABLE 1

Summary of the effects of intravenous administration of 2DG on mean plasma growth hormone, glucose and insulin levels

Experimental groups	No. of animals per group	Mean 6-h plasma GH levels (ng/ml)	Mean 6-h plasma glucose levels (mg/dl)	Mean 6-h plasma insulin levels (ng/ml)
Normal Saline iv	10	146.9 ± 12.2	145.9 ± 2.8	0.77 ± 0.15
2DG (400 mg/kg) iv	6	28.7 ± 4.8[a]	307.1 ± 13.4[a]	0.59 ± 0.08
2DG (400 mg/kg) iv & Feeding	6	29.8 ± 8.0[a]	277.5 ± 10.6[a]	6.94 ± 1.12[a]
2DG (8 mg/kg) iv	6	113.3 ± 15.3	141.6 ± 1.3	0.70 ± 0.14
2DG (4mg/kg) iv	6	161.1 ± 24.4	141.2 ± 2.3	0.88 ± 0.17

Numbers represent mean ± standard error of the mean (S.E.M.)
a $P<0.001$ compared to normal saline-treated control group.

($P<0.001$) elevated until the last sample at 1600 h (221.0± 13.6 mg/dl) when compared to normal saline controls whose plasma glucose levels fluctuated minimally throughout the 6-h sampling period (Fig. 2A). Mean 6-h plasma glucose levels of 2DG-treated rats were significantly greater than those of the normal saline-treated group (Table 1).

Despite the marked hyperglycemia, there was no significant difference in mean 6-h plasma IRI levels of 2DG-treated animals compared to saline controls (Table 1).

When the animals were allowed to free feed during the sampling period, they ate 9.0±0.5 g of the provided food. However, feeding did not modify the hormonal effects induced by the iv administration of 2DG (400 mg/kg); the mean 6-h plasma GH level in this group (29.8±8.0 ng/ml) was not significantly different from that of the non-feeding 2DG-treated group (28.7±4.8 ng/ml) (Fig. 3 and Table 1). Similarly, these rats exhibited the typical hyperglycemic profile observed in all animals receiving 400 mg/kg of 2DG iv (Table 1). Nevertheless, feeding resulted in the typical marked fluctuations in plasma IRI levels usually observed in response to food intake and therefore significantly ($P<0.001$) elevated mean 6-h plasma IRI levels in this group (6.94±1.12 ng/ml) compared to the normal saline treated group (0.77±0.15 ng/ml) (Table 1).

Intravenous administration of fifty- to hundred-fold smaller doses of 2DG failed to induce any of the hormonal changes observed following the iv administration of the larger dose. The 6-h plasma GH profiles of these animals exhibited the typical rhythmic GH secretory pattern with two major episodes of GH secretion evident during the 6-h sampling period and many peak values greater than 400 ng/ml (Fig. 4). Mean 6-h plasma GH levels in these two groups of animals were not significantly different from those of the control group administered normal saline iv (Table 1). Similarly, plasma glucose and insulin levels fluctuated minimally and remained within their normal physiologic range throughout the 6-h sampling period (Table 1).

Effects of Intracerebroventricular Administration of 2DG on GH, Glucose and Insulin Secretory Profiles

Central administration of 10 µl normal saline did not alter the typical GH, glucose and insulin secretory patterns previously observed in the normal saline iv-injected control group. All animals in this control group exhibited two episodes of GH secretion (Fig. 5A) with peak GH values > 400 ng/ml. Plasma glucose and insulin levels fluctuated minimally during the sampling period (Table 2).

Intracerebroventricular administration of 2DG in doses of 4 mg and 8 mg/10 µl resulted in a significant dose-dependent suppression of pulsatile GH secretion. Administra-

FIGURE 4 Individual representative 6-h plasma GH profiles in two rats administered either 4 mg/kg or 8 mg/kg 2DG iv. The iv injection of fifty- to hundred-fold smaller doses of 2DG did not alter the typical rhythmic GH secretory pattern evident in normal rats under standard unstressed conditions. Arrows indicate time of injection.

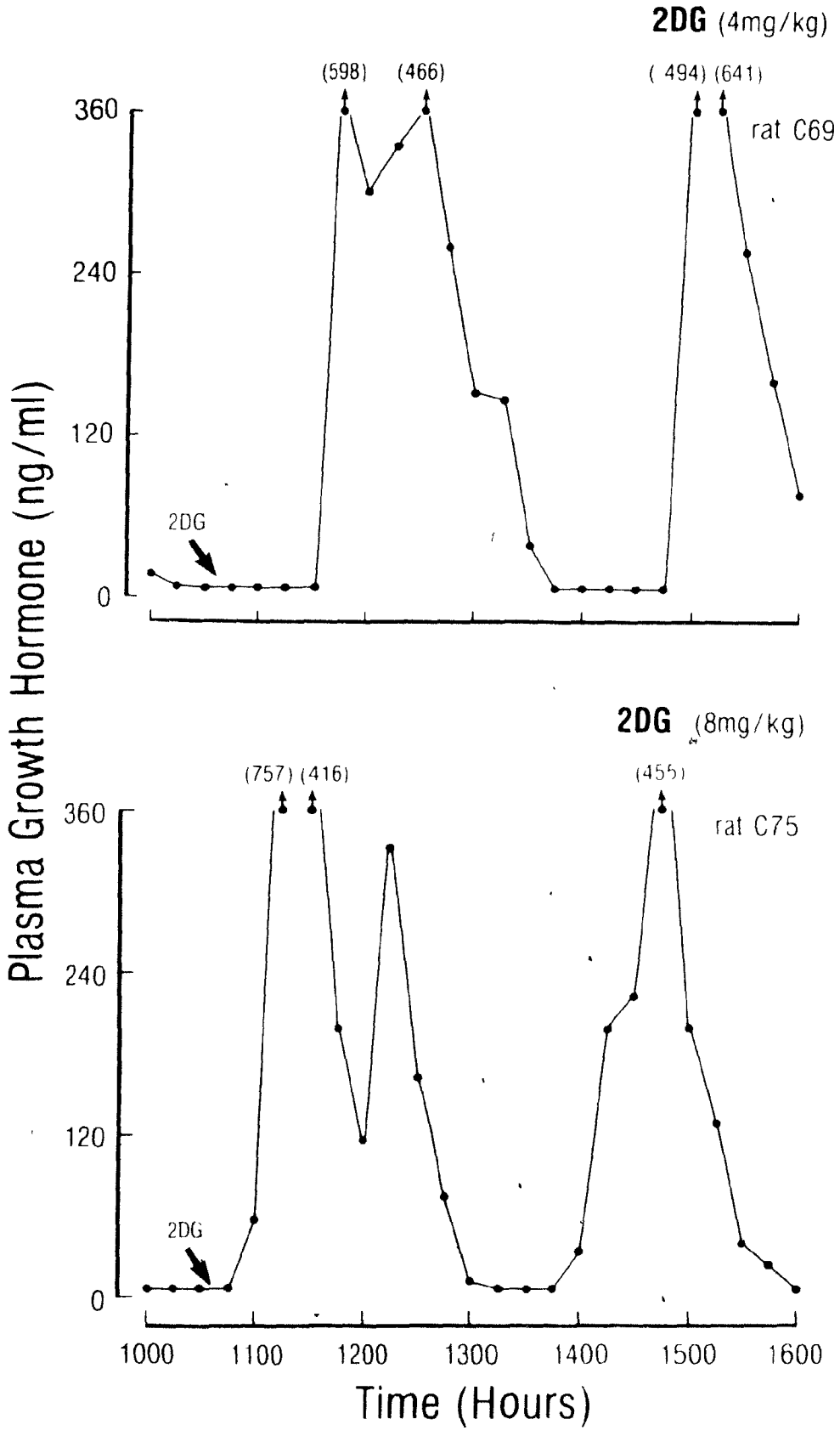

2DG (4mg/kg)
(598)
(466)
(494)
(641)
rat C69
360
240
120
0
2DG
2DG (8mg/kg)
(757)
(416)
(455)
rat C75
360
240
120
0
2DG
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

FIGURE 5 Effects of icv administration of either normal saline (A) or 8 mg/10 µl 2DG (B) on mean 6-h plasma GH levels. Central injection of the small dose of 2DG caused a significant suppression of pulsatile GH secretion within 30 minutes after injection. Plasma GH levels remained severely depressed for the entire duration of the sampling period. Arrows indicate the time of injection. Vertical lines represent SEM. The number of animals in each group is shown in parentheses.

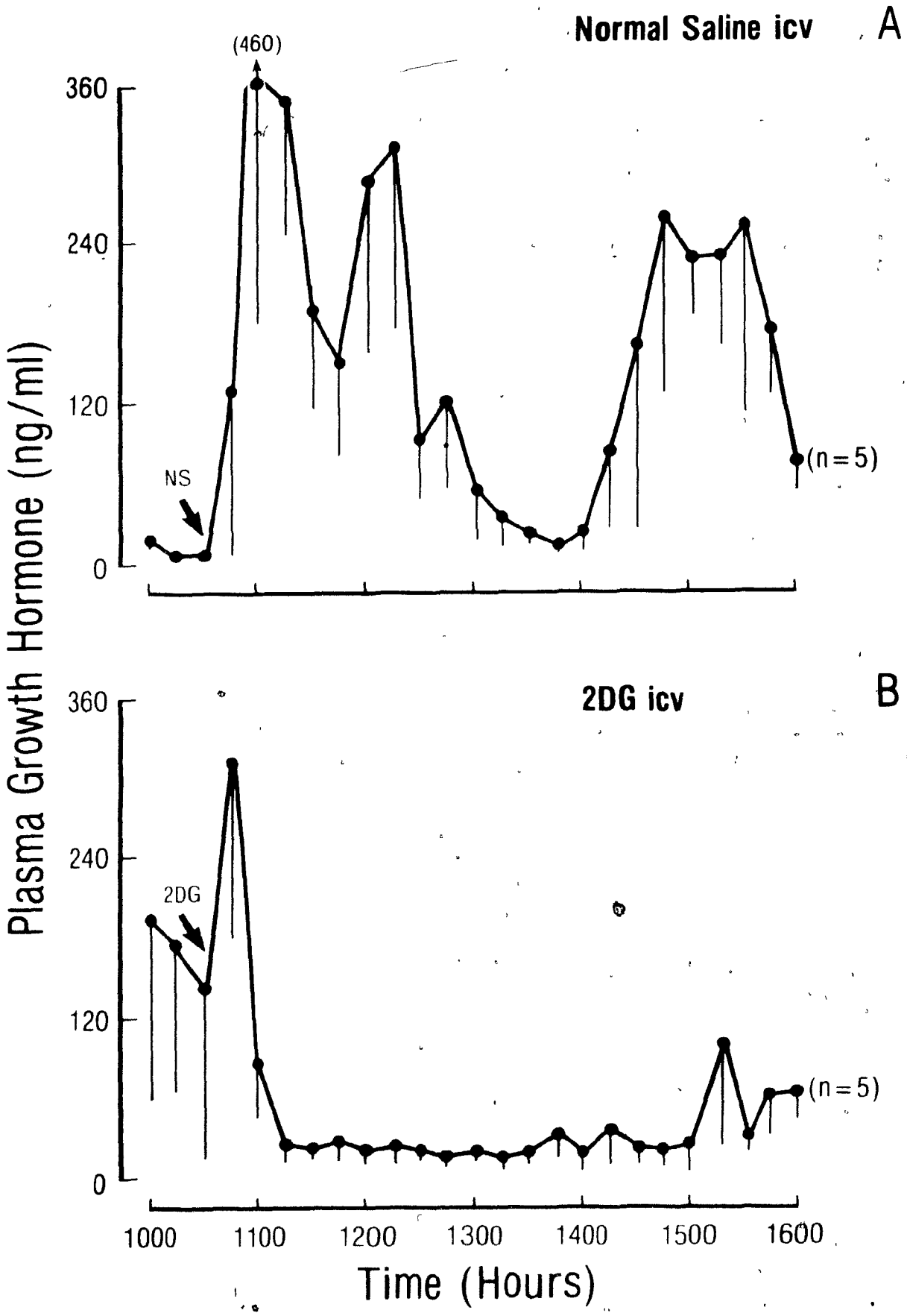

Normal Saline icv
A
(460)
360
240
120
0
NS
(n=5)
2DG icv
B
360
240
120
0
2DG
(n=5)
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

TABLE 2

Summary of the effects of intracerebroventricular administration of 2DG on mean plasma growth hormone, glucose and insulin levels.

Experimental groups	No. of animals per group	Mean 6-h plasma GH levels (ng/ml)	Mean 6-h plasma glucose levels (mg/dl)	Mean 6-h plasma insulin levels (ng/ml)
Normal Saline icv	5	170.3 ± 31.7	129.6 ± 7.0	1.43 ± 0.75
2DG (4 mg/10 µl) icv	5	80.1 ± 8.4[a]	176.6 ± 8.4[b]	0.83* (n=1)
2DG (8 mg/10 µl) icv	5	48.6 ± 14.2[b]	217.1 ± 7.4[c]	0.98 ± 0.13

a P<0.05
b P<0.01
c P<0.001
} compared to saline-treated control group.

* Because of a technical problem with the insulin RIA it is not possible to present the mean data for this group. Only the samples from one animal were assayed.

tion of the smaller dose caused a partial but significant ($P<0.05$) decrease in mean 6-h plasma GH concentration compared to normal saline icv-injected controls (Table 2). Although GH pulses were still evident in these animals, they were markedly attenuated. Concomitant with this GH suppression response there was a significant increase in plasma glucose level 45 min after the injection of 2DG and plasma glucose values remained significantly elevated for 255 min after injection. The mean 6-h plasma glucose level in this group was significantly greater than that of the saline icv-injected control group (Table 2).

Intracerebroventricular administration of 8 mg/10 µl 2DG caused a greater degree of GH suppression. Plasma GH levels in these rats were severely reduced with no major GH secretory episode evident during the 6 h of sampling (Fig. 5B). This suppression was similar in magnitude to that previously observed following the iv administration of the larger dose of 2DG (400 mg/kg). The depression in GH pulse amplitude was evident within 30 min after injection of the 2DG (Fig. 5B) and plasma GH levels remained significantly reduced for the 6-h period compared to normal saline controls (48.6 ± 14.2 vs 170.3 ± 31.7 ng/ml; $P<0.01$). It is interesting to note that in some instances, whether 2DG was administered iv or icv, some recovery of GH secretion was observed during the last 45 min of the sampling period (Fig. 5B).

This GH suppression response was accompanied by marked hyperglycemia (Table 2) with a time course similar to that observed following iv administration of the large dose of 2DG. In this group, significant elevation of plasma glucose levels occurred within 15 min and lasted until the last sample at 1600 h.

Effects of Somatostatin Antiserum on GH Secretory Profiles in 2DG-Treated Animals

Individual GH secretory profiles of 2DG (400 mg/kg), iv-treated rats administered normal sheep serum (NSS) were comparable to those of rats which had received 2DG alone. They continued to show a marked suppression in both GH pulse amplitude (Fig. 6, left panel) and mean 6-h plasma GH level (Fig. 7) associated with the hyperglycemia which usually accompanies iv administration of 400 mg/kg 2DG (mean 6-h plasma glucose level: 291.1±14.3 mg/dl). Administration of SRIF antiserum (AS) caused an initial increase in plasma GH level in most animals (Fig. 6, right panel) and mean plasma GH levels 15 min after injection were significantly elevated compared to those observed in 2DG-NSS treated controls (115.9±42.2 vs 13.6±5.7 ng/ml; $P<0.02$). In addition, passive immunization with SRIF AS caused a significant elevation of subsequent GH trough levels (mean GH trough level: 17.0±3.2 vs 6.2±0 ng/ml; $P<0.01$)(Table 3). However, immunoneutralization of 2DG-treated rats with SRIF AS, although signifi-

FIGURE 6 Effect of passive immunization with somatostatin antiserum (SRIF AS) on individual, representative 6-h plasma GH secretory profiles in rats administered 400 mg/kg 2DG, iv (right panel) compared to 2DG-treated rats given normal sheep serum (NSS) (left panel). Administration of SRIF AS caused an initial surge of GH secretion and significant elevation of GH trough levels. Nevertheless, it failed to restore the high amplitude of GH pulses. Arrows indicate the times of injection.

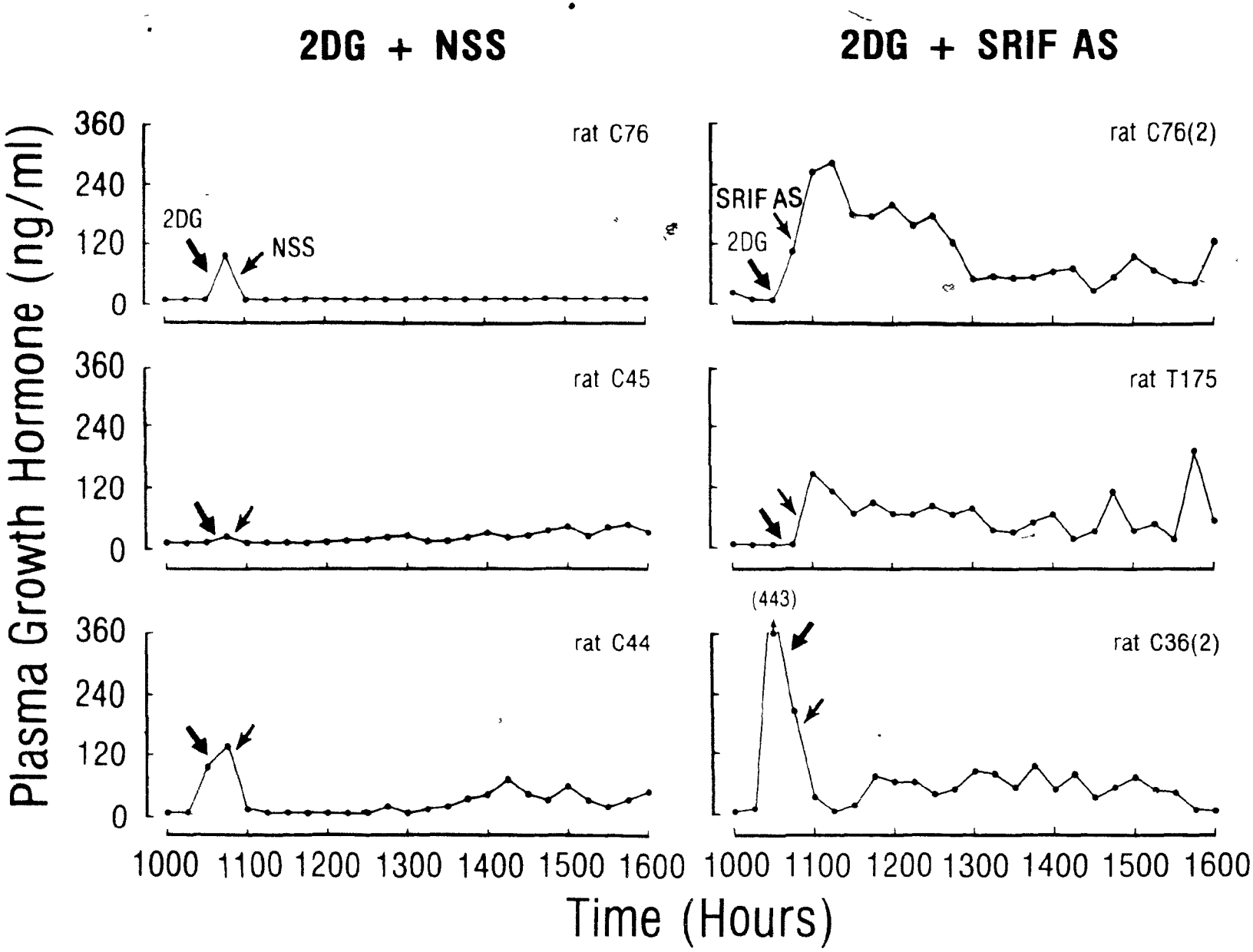
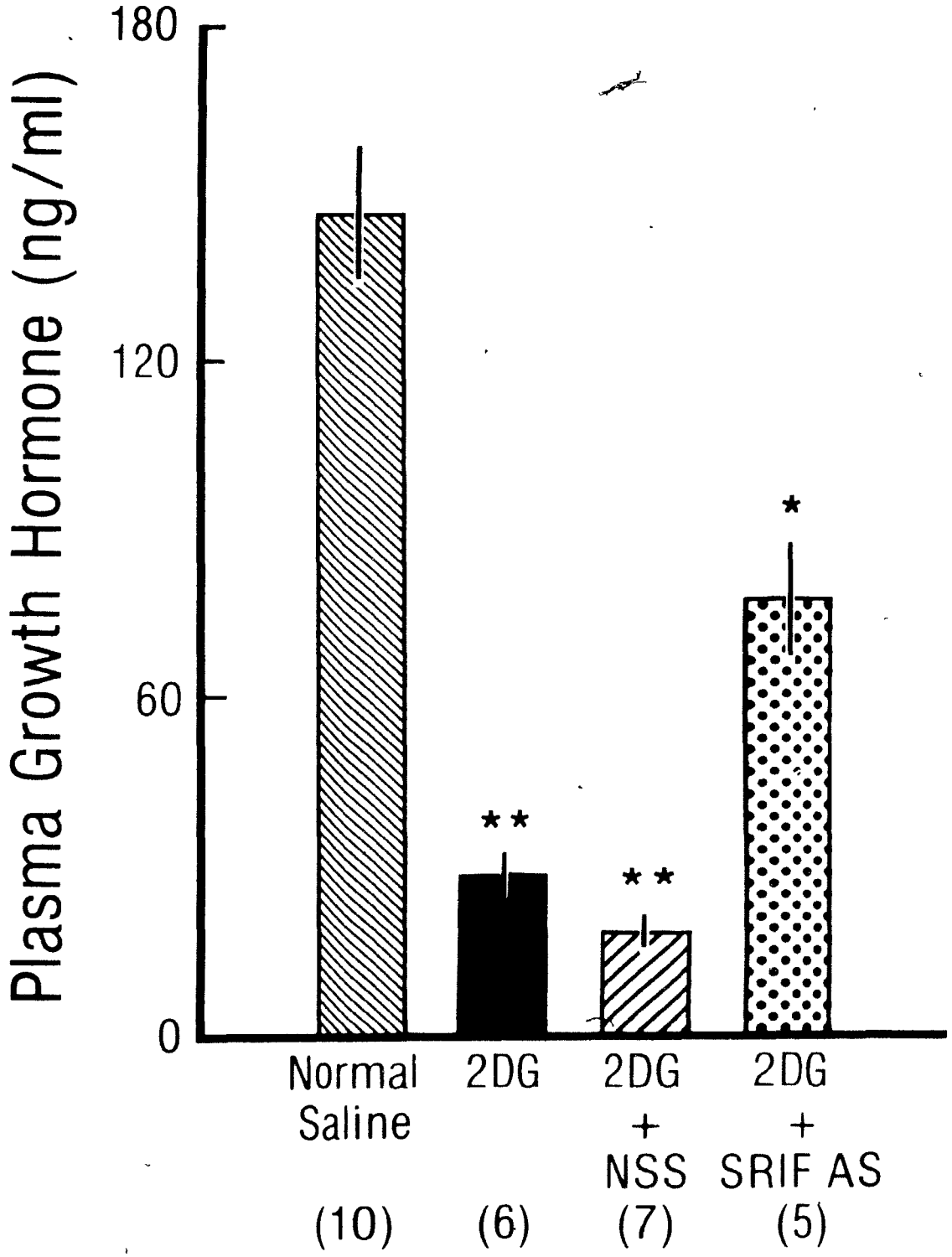

2DG + NSS
2DG + SRIF AS
Plasma Growth Hormone (ng/ml)
360
240
120
0
rat C76
2DG
NSS
rat C76(2)
SRIF AS
2DG
rat C45
rat T175
(443)
rat C44
rat C36(2)
1000 1100 1200 1300 1400 1500 1600
Time (Hours)

FIGURE 7 Mean 6-h plasma GH levels in the 4 groups of rats. Administration of SRIF AS to 2DG-treated rats caused a significant elevation of mean 6-h plasma GH levels compared to 2DG-NSS-treated controls, however it failed to restore the mean 6-h GH level to normal values. Each bar represents the mean $\pm$ SEM and the number of animals in each group is shown in parentheses. *$P<0.01$ compared to normal saline and 2DG + NSS groups. ** $P<0.001$ compared to normal saline group.


Plasma Growth Hormone (ng/ml)
180
120
60
0
**
**
*
Normal Saline
2DG
2DG + NSS
2DG + SRIF AS
(10)
(6)
(7)
(5)

cantly ($P<0.02$) elevating mean GH peak levels compared to 2DG-NSS controls, failed to restore the high amplitude of GH pulses previously observed in normal saline-treated control animals (Table 3). The highest GH peak level observed in this group ranged from 92.2 to 284.5 ng/ml which was significantly ($P<0.001$) lower than that observed under control conditions (range: 372.0 to 1049.9 ng/ml). Nevertheless, the mean 6-h plasma GH level of the 2DG-SRIF AS treated group (77.7 ± 10.7 ng/ml) was significantly ($P<0.01$) greater than that of the 2DG-NSS control group (18.8 ± 3.0 ng/ml) although, significantly ($P<0.01$) less than that of the normal saline-treated control group (Fig. 7), suggesting only a partial restoration of plasma GH concentration. Treatment with SRIF AS did not alter the typical effect of 2DG on plasma glucose and IRI levels; these parameters were not significantly different from those observed in 2DG-NSS treated animals (mean 6-h plasma glucose level: 305.4 ± 27.6 vs 291.1 ± 14.3 mg/dl and mean 6-h plasma IRI level: 0.69 ± 0.09 vs 0.59 ± 0.08 ng/ml).

Effects of hGRF on GH Release in 2DG-Treated Animals

Intravenous administration of normal saline to 2DG-treated animals did not modify the depressed plasma GH profiles and mean 6-h plasma GH levels were similar to those of rats treated with 2DG alone (Fig. 8). Administration of 3 iv boluses of 10 µg hGRF, at 90 min intervals, to 2DG-treated

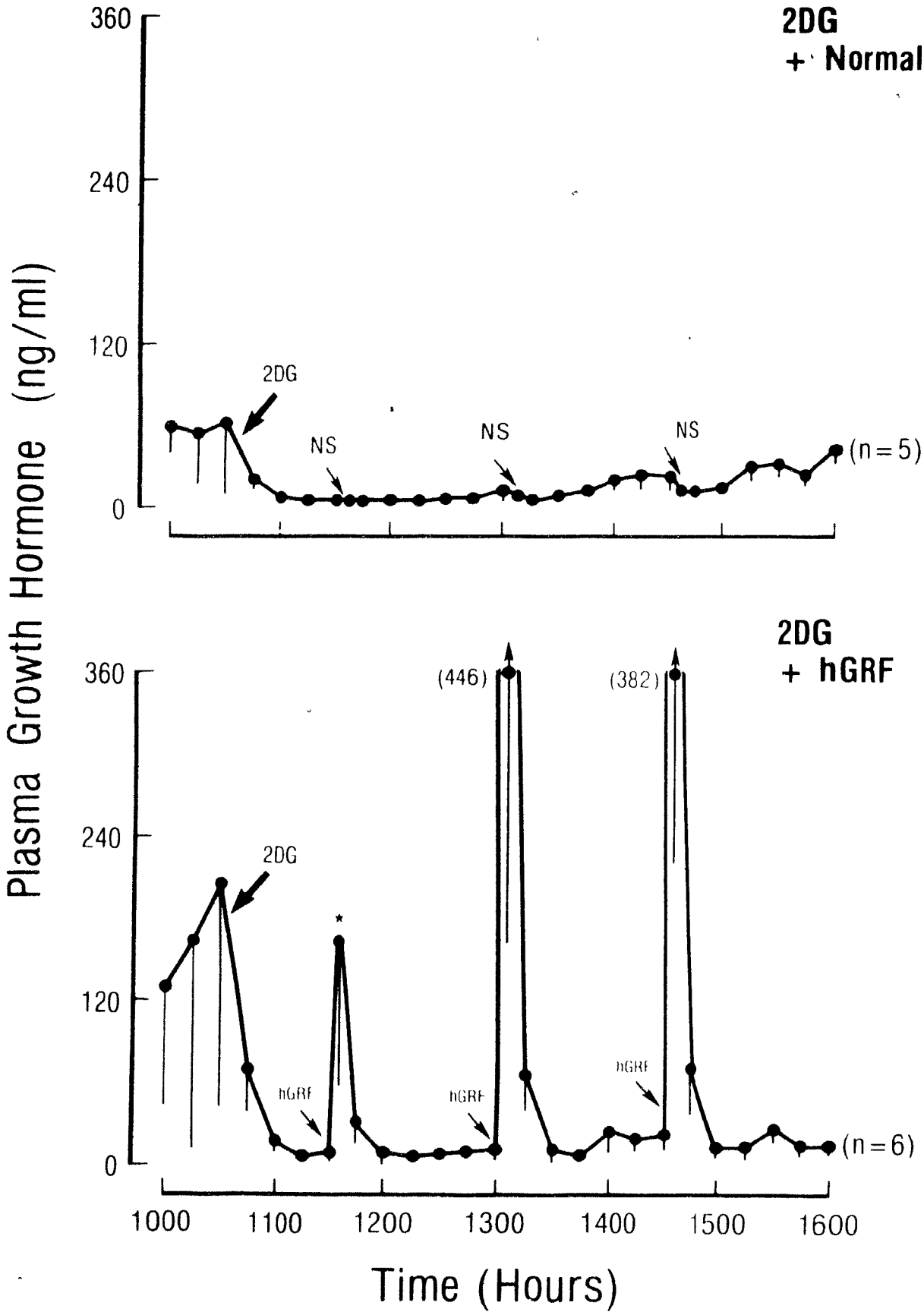
TABLE 3

Effects of passive immunization with SRIF AS on mean peak and trough GH levels in 2DG-treated rats

Experimental groups	No. of animals per group	Mean 6-h plasma GH peak levels (ng/ml)	Mean 6-h plasma GH trough levels (ng/ml)
Normal Saline iv	10	703.6 ± 58.2	8.0 ± 0.9
2DG (400 mg/kg) iv	6	101.6 ± 20.7[b]	7.0 ± 0.7
2DG + NSS	7	83.9 ± 15.1[b]	6.2 ± 0.0
2DG + SRIF AS	5	178. ± 34.7[b,c]	17.0 ± 3.2[a]

a $P < 0.01$ compared to the normal saline-, 2DG-, and 2DG + NSS treated groups.
b $P < 0.001$ compared to normal saline control group.
c $P < 0.02$ compared to 2DG + NSS control group.

FIGURE 8 Comparison of the effects of iv administration of either normal saline or 10 μg hGRF on mean 6-h plasma GH levels in 2DG (400 mg/kg, iv)-treated rats. The GRF peptide caused a surge of GH release after each injection which was variable and time-dependent. Arrows indicate times of injection. Vertical lines represent the SEM. The number of animals in each group is shown in parentheses. * $P<0.05$ compared to hGRF-induced GH release at 1430 h.


2DG
+ Normal Saline
360
240
120
0
2DG
NS
NS
NS
(n=5)
2DG
+ hGRF
(446)
(382)
2DG
hGRF
hGRF
hGRF
(n=6)
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

animals caused release of GH within 5 min after injection. However, this release was highly variable and dependent on the time of injection, the first response being consistently the weakest in all the animals investigated (Figs. 8 and 9). Peak GH responses in individual animals ranged from nil to values as high as 1246.9 ng/ml (Fig. 9) and the mean GH release observed 5 min after the first hGRF bolus (163.2±106.6 ng/ml) was significantly ($P < 0.05$) lower than that observed after the administration of the third bolus (382.8±133.0 ng/ml) (Fig. 8). Despite the fact that the second response had a higher mean plasma GH value, (446.9±198.4 ng/ml) this difference failed to reach significance because of the wide range of variation in response to hGRF at this particular time point (range: 22.5-1246.9 ng/ml).

Effects of Immunoneutralization with SRIF AS on hGRF-Induced GH Release in 2DG-Treated Animals

Figure 10 illustrates the mean plasma GH response to hGRF in 2DG-treated rats administered SRIF AS. Neutralization of endogenous circulating SRIF significantly enhanced the weak GH response to hGRF previously observed at 1130 h and also augmented GH release in response to exogenous administration of hGRF at 1300 h and 1430 h. Of interest, is the finding that pretreatment with SRIF AS eliminated the time-dependent difference in the magnitude of the hGRF-induced GH

FIGURE 9 Individual, representative 6-h plasma GH profiles of 2DG-treated rats administered 3 iv boluses of hGRF (10 μg/0.3 ml). The GH responses to the peptide were highly variable with the first response being consistently the weakest. Arrows indicate the times of injection.

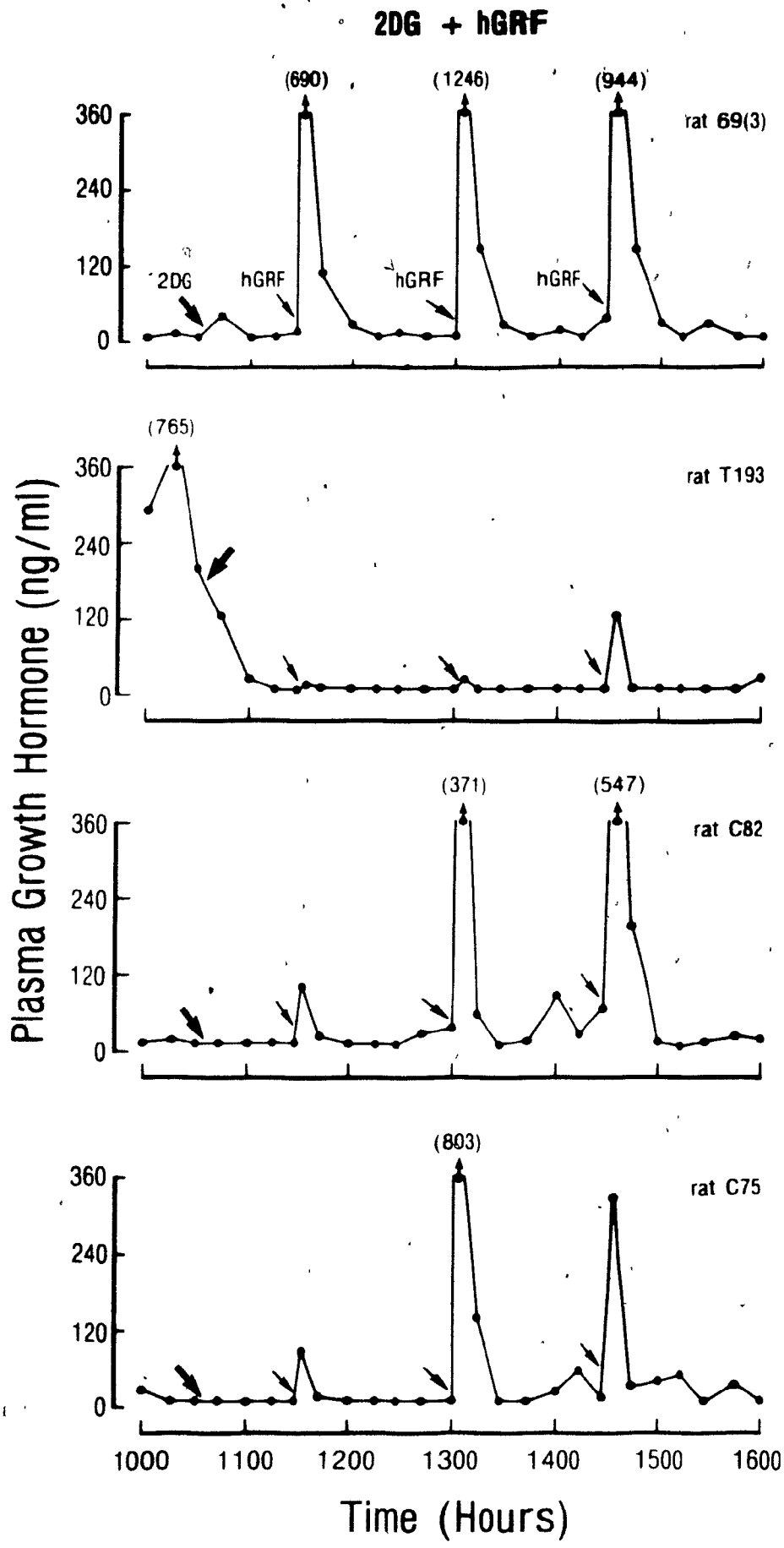
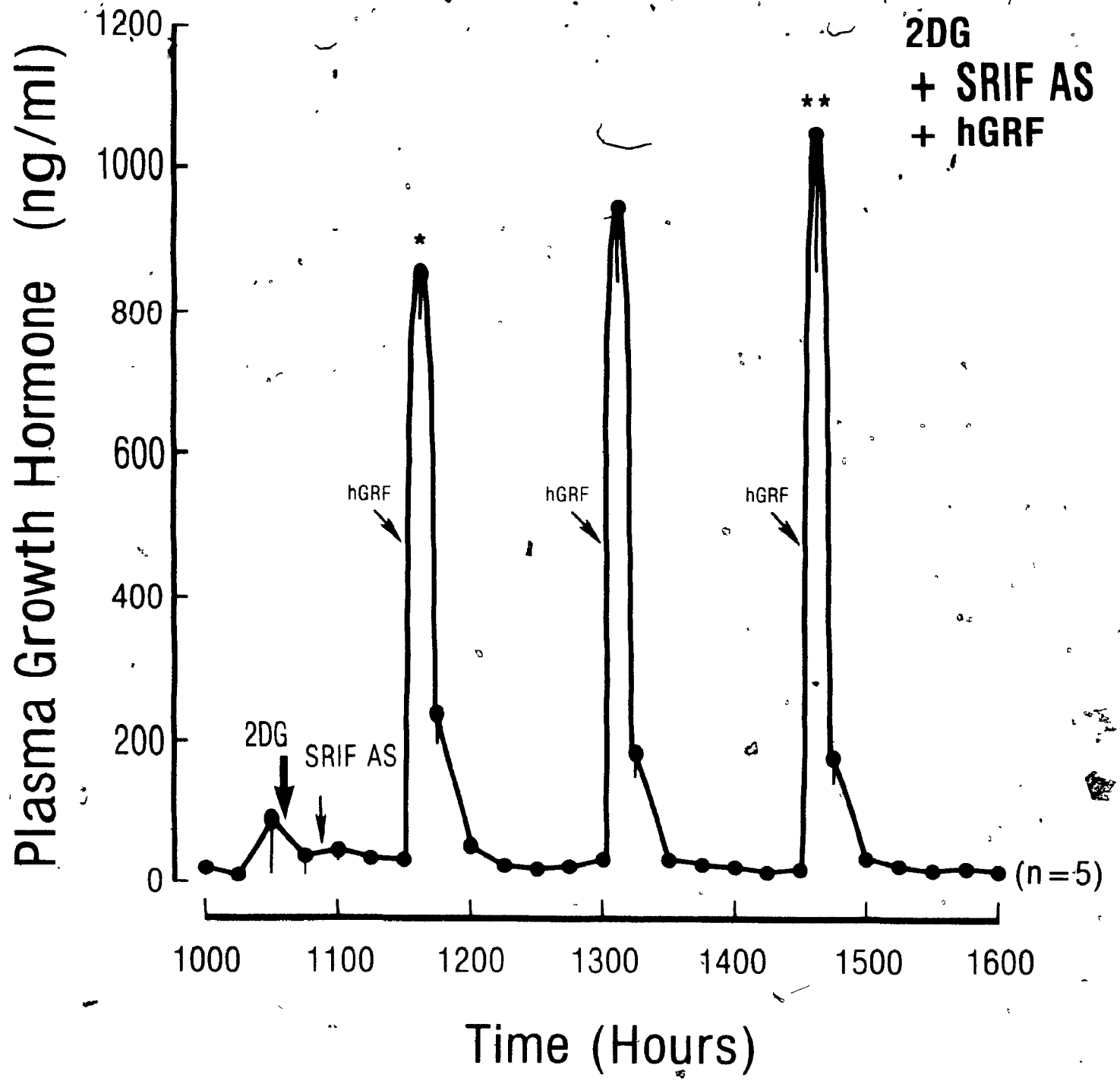

2DG + hGRF
(690)
(1246)
(944)
rat 69(3)
360
240
120
0
2DG
hGRF
hGRF
hGRF
(765)
rat T193
(371)
(547)
rat C82
(803)
rat C75
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

FIGURE 10 Effects of immunoneutralization with SRIF AS on hGRF-induced GH release in 2DG-treated rats. Administration of SRIF AS eliminated the time-dependent difference and significantly augmented the GH responses at 1130 h (*$P<0.001$) and 1430 (**$P<0.02$) h compared to those observed after hGRF alone (see Fig. 8). Arrows indicate times of injections. The number of animals is shown in parentheses. Vertical bars represent the SEM.


2DG
+ SRIF AS
+ hGRF
Plasma Growth Hormone (ng/ml)
1200
1000
800
600
400
200
0
hGRF
hGRF
hGRF
2DG
SRIF AS
*
**
(n=5)
1000
1100
1200
1300
1400
1500
1600
Time (Hours)

release in 2DG-treated animals. Thus, there was no longer a statistically significant difference between the first and the third response to the peptide. Plasma GH levels in this group of animals reached values which were comparable to those seen in normal animals during spontaneous GH secretory episodes (range: 506.0 - 1654.0 ng/ml).

SRIF Binding of Rat Plasma after SRIF AS Administration

The mean binding of a 1:100 dilution of plasma 45 min before and 15 min, 165 min and 315 min after SRIF AS treatment was found to be 0.5±0.2%, 42.3±3.8%, 43.0±3.3% and 38.0±1.1% respectively. No significant binding was observed in the plasma of rats treated with NSS.

DISCUSSION

Maintenance of adequate plasma glucose concentration and its metabolism are important in daily body function and critical for survival. Both human and animal species have evolved efficient mechanisms, either physiological or behavioral, to prevent excessive hypoglycemia or glucopenia. Among the physiological responses involved in glucoregulation, hormonal responses are of primary importance. Several hormones are known to be involved either directly or indirectly in glucoregulation. Of importance are insulin, which is the primary glucoregulatory hormone, glucagon, adrenaline, cortisol and to a lesser degree GH and thyroid stimulating hormone (Bolli, Gottesman, Cryer and Gerich, 1984). Each of these hormones has some specific function in carbohydrate metabolism, and understanding their mechanisms of control under both normal and pathological conditions is important in assessing their role in day-to-day metabolism. The experiments described in this thesis were undertaken to provide some insight into the mechanism(s) controlling the GH response to insufficient glucose metabolism as induced by the administration of 2DG in the rat.

The findings of the present study confirm that both systemic and central administration of 2DG cause a significant hyperglycemia without the expected rise in plasma insulin levels. These observations have been reported by several investigators in the past (Brown and Bachrach, 1959; Brown, 1962; Frohman, Müller and Cocchi, 1973; Müller, Frohman and Cocchi, 1973). The hyperglycemia is known to result from CNS-mediated activation of mechanisms involved in the defense of substrate availability. This response involves multisynaptic descending catecholamine pathways of the sympathetic limb of the autonomic nervous system, (Brown and Bachrach, 1959) and this whole activation system can be shut off by simple adrenal demedullation and denervation (Frohman, Müller and Cocchi, 1973) or spinal cord transsection (Brodows, Pi-Sunyer and Campbell, 1973). These pathways in turn stimulate the output of adrenaline (Hökfelt and Bydgeman, 1961) from the adrenal medulla which finally results in release of glucose from the liver via an increase in gluconeogenesis (Brown and Bachrach, 1959). The greater hyperglycemia we observed following systemic versus central administration of 2DG is likely due to the combination of the CNS effects superimposed upon the peripheral competition of 2DG and glucose (see Appendix I). The importance of the CNS in mediating 2DG-induced hyperglycemia is further demonstrated by the present finding that low doses of 2DG (4 mg and 8 mg),

which were capable of inducing marked hyperglycemia when given centrally, were ineffective when administered via the peripheral route.

The absence of an increase in insulin release, despite the hyperglycemia following central administration of 2DG, suggested to earlier investigators (Frohman, Müller and Cocchi, 1973) that the insulin response to cellular glucopenia was mediated via the CNS, probably via glucoreceptors located within the VMH area (Desiraju, Banerjee and Anand, 1968; Mayer and Arees, 1968; Oomura, Ono, Ooyama and Wayner, 1969) from which originate the sympathoadrenal fibers controlling adrenaline release. Furthermore, the finding that this insulin response was blocked by adrenal demedullation, suggested to these investigators that 2DG-induced insulin suppression was the result of adrenaline action on the B-cells of the pancreas (Brodows, Pi-Sunyer and Campbell, 1973; Frohman, Müller and Cocchi, 1973; Müller, Frohman and Cocchi, 1973).

Deoxyglucose is also a potent stimulator of appetite (Balagura and Kanner, 1971; Müller, Cocchi and Mantegazza, 1972; Smith, Gibbs, Strohmayer and Stokes, 1972; Thompson and Campbell, 1977; Ritter, Slusser and Stone, 1981). However, allowing the animals to eat after 2DG treatment in the present study did not alter the hyperglycemia, although it did cause significant elevation of plasma insulin levels.

These data suggest that the feeding response to 2DG-induced cellular glucopenia may be involved in reestablishing a normal level of glucose utilization by increasing insulin release directly via stimulation of the pancreas.

The ultradian GH rhythm was dramatically altered following 2DG-induced cellular glucopenia. The plasma longitudinal GH profiles of rats administered a large dose of 2DG intravenously exhibited a severe reduction in both amplitude and duration of spontaneous GH secretory episodes and plasma GH concentrations remained significantly suppressed for up to 5 h after the injection. The minimal GH release usually observed during the last 45 minutes of the sampling period may reflect some recovery of GH secretion which may be accounted for by the dissipation of 2DG from tissue. Allowing the animals to eat during the test did not modify the GH response induced by the administration of 2DG, and plasma GH levels were still markedly depressed suggesting that, feeding although stimulated by 2DG, was not a counterregulatory response to the depressed plasma GH levels.

The 2DG-induced suppression response we observed is in agreement with an earlier report (Takahashi, Daughaday and Kipnis, 1971) based on single blood sampling in the rat, indicating that systemic administration of a large dose of 2DG was capable of suppressing plasma GH levels. However, our results are in disagreement with a previous study

demonstrating that central administration of 2DG caused an increase in plasma GH in urethane anesthetized rats (Okajima, Motomatsu, Kato and Ibayashi, 1980). However, urethane itself has been reported to affect both plasma GH levels and blood glucose (Krzemien and Lipowska-Zabawska, 1976; Saito, Ogawa, Ishimaru, Oshima and Saito, 1979); and thus, the anesthesia, per se, may be a confounding variable in that study.

The finding that small doses of 2DG were ineffective in inducing GH suppression when given systemically, yet were potent inhibitors of GH release when administered centrally, provides strong support for the hypothesis of a central site of action for 2DG-induced GH suppression. In 1966, Blanco and his collaborators (Blanco, Schalch and Reichlin, 1966) reported that hyperglycemia induced locally in the median eminence of squirrel monkeys caused a suppression in plasma GH levels suggesting the existence of CNS glucoreceptors involved in GH regulation. Other investigators were able to localize glucose-sensitive cells to the ventromedial and lateral hypothalamic areas of the brain. They showed that the activity of these neurons was selectively altered by variations in the surrounding glucose concentration and by cellular glucopenia (Desiraju, Banerjee and Anand, 1968; Oomura, Ono, Ooyama and Wayner, 1969). These earlier findings were soon confirmed by the work of Himsworth and his

co-workers which showed that direct administration of 2DG to these brain regions (i.e. the ventromedial and lateral hypothalamic areas) of the monkey had marked effects on GH secretion (Himsworth, Carmel and Frantz, 1972). Of interest, is the fact that these glucose-sensitive brain sites encompass the GH hypothalamic regulatory centers. Thus we hypothesized that 2DG was having its effects on GH secretion via an action on the hypothalamic peptidergic neuronal system, both inhibitory (SRIF) and stimulatory (GRF), involved in GH regulation.

One potential explanation for the changes in the plasma GH levels observed after the administration of 2DG may be related to a direct effect of the compound on the brain somatostatinergic system. That is, the 2DG-induced GH suppression could be due to increased release of SRIF into the hypophyseal portal circulation. Evidence for direct stimulation of hypothalamic SRIF in response to insufficient glucose metabolism comes from recent <u>in vitro</u> studies which demonstrated that SRIF release from incubated rat hypothalamic fragments increased after addition of 2DG to the culture medium (Berelowitz, Dudlak and Frohman, 1982; Lengyel, Nieuwenhuyzen-Kruseman, Grossman, Rees and Besser, 1984). The specificity of this effect is indicated in that neither SRIF release from other brain regions such as the cerebral cortex (Berelowitz, Dudlak and Frohman, 1982) nor

the release of other hypothalamic releasing factors (i.e. LHRH) (Lengyel, Grossman, Nieuwenhuyzen-Kruseman, Ackland and Rees, 1984) was altered by changes in glucose metabolism. To assess the involvement of SRIF in vivo, 2DG-treated rats were passively immunized against SRIF with a specific SRIF antiserum. This antiserum was identical to that used in several previous studies and has been shown to reverse GH suppression in both starved (Tannenbaum, Epelbaum, Colle, Brazeau and Martin, 1978) and diabetic rats (Tannenbaum, 1981). Immunoneutralization with SRIF antiserum caused an initial surge of GH release and significant elevation of both trough and mean 6-h plasma GH levels compared to 2DG-treated rats administered normal sheep serum. However, the antiserum failed to restore either the amplitude of the GH pulses or the mean 6-h plasma GH levels to normal values. It is unlikely that inadequate immunoneutralization can account for the incomplete restoration of the GH pulses since significant SRIF antibody binding was measured in the plasma of SRIF antiserum-treated animals for the entire sampling period. These data therefore suggest that increase SRIF release is not the sole mechanism mediating the 2DG-induced GH suppression response and provide support for only a partial role of endogenous SRIF in this response. The results also suggest that additional mechanism(s) may be involved in glucose modulation of GH secretion; one such mechanism may be decreased release of the GH-stimulatory peptide, GRF.

In several situations in which GH secretion is decreased, in both animal species (Tannenbaum, Eikelboom and Ling, 1983; Harvey, Scanes and Marsh, 1984) and in man (Takano, Hizuka, Shizume, Asakawa, Miyakawa, Hirose, Shibasaki and Ling, 1984), it has been demonstrated that exogenous administration of GRF successfully released GH from the pituitary, indicating that the pituitary is still capable of responding to GRF. To assess the capacity of GRF to release GH from the pituitary of 2DG-treated rats we administered hGRF at a dose which has previously been shown to be a potent GH secretagogue in the rat in vivo (Tannenbaum, Eikelboom and Ling, 1983). In these experiments, exogenous administration of three intravenous boluses of hGRF caused a surge of GH release after each injection. However, the amount of GH released was variable and time-dependent. In particular, the hGRF-induced GH release observed 1 h after 2DG treatment was consistently less than that observed at the other two time points. Possible explanations for the weak GH response at this time point may be related to the following. First, this low responsiveness may result from a depletion of pituitary GH content as a consequence of decreased GH synthesis secondary to the blockade of glucose metabolism in the somatotrophs. This is unlikely to occur since it was reported that, in vitro, a decrease in GH synthesis in the pituitary somatotrophs was only observed after 5 h of incuba-

tion in the presence of 2DG (Betteridge and Wallis, 1974). Another possibility is that it is due to interference or antagonism by endogenous SRIF at the level of the pituitary as a result of increased SRIF release. The finding that immunoneutralization with SRIF antiserum eliminated the time-dependent difference in hGRF-induced GH release at 1130 h provides support for the view that endogenous circulating SRIF is interfering with the ability of hGRF to release GH at this time point (1130 h). The observation that passive immunization with SRIF antiserum also augmented the GH responsiveness at the other time points (although to a lesser degree) is also consistent with earlier reports demonstrating antagonism between SRIF and GRF both in vitro (Brazeau, Ling, Böhlen, Esch, Ying and Guillemin, 1982) and in vivo (Tannenbaum and Ling, 1984). While these results provide additional (in vivo) support for the view that SRIF release is increased in response to glucose deprivation, the data indicate that the time course of this response is of limited duration. This in vivo finding concords well with an in vitro observation of Dr. Michael Berelowitz (University of Cincinnati, Cincinnati, Ohio) that 2DG induced a short-lived, delayed stimulation of hypothalamic SRIF release with the peak response occurring at 20-25 min and a duration of 60 min (Personal Communication).

In summary, these *in vivo* results demonstrate that intracellular glucopenia is a potent inhibitor of pulsatile GH secretion in the rat and that this response is mediated, at least in part, by increased SRIF release. While the present findings are compatible with the hypothesis that glucoprivation induces an acute SRIF release in the rat, further experimentation is necessary to determine whether GRF release is also sensitive to changes in glucose utilization. A longer lasting decrease in GRF release could potentially account for the marked suppression in GH secretroy pulses observed several hours after 2DG administration. The ability to directly measure GRF levels in response to altered glucose metabolism may shed light on this question.

APPENDIX I

Actions of 2-deoxy-D-glucose

Deoxy-D-glucose has been used extensively both *in vitro* and *in vivo* in studies on carbohydrate metabolism and on the effects of intracellular glucopenia (See Table 4). Deoxy-D-glucose is a non-metabolizable glucose analog which is transported into the cells under insulin stimulation via the same carrier as glucose and is phosphorylated to form 2DG-phosphate which acts as an inhibitor of the enzymes phosphoisomerase and glucose-6-phosphate dehydrogenase (Brown and Bachrach, 1959). Therefore, 2DG and glucose are mutual competitive inhibitors. In the presence of 2DG, less glucose is metabolized and 2DG accumulates intracellularly as 2DG-6-phosphate which cannot be converted to fructose-6-phosphate and therefore induces a state of generalized intracellular glucopenia. Administration of 2DG in both humans and rats will induce a host of metabolic, hormonal and behavioral modification which are summarized in the following Table.

TABLE 4

Summary of Metabolic, Hormonal and Behavioral Effects induced by 2DG in Humans and Rats

	Humans	Rats
Metabolic	intracellular glucopenia (3,25,27) hyperglycemia (25,26) ↑free fatty acids (13) ↑lactate (13) ↑sympathetic activity (3)	intracellular glucopenia (4,5) hyperglycemia (4,16,22) ↑free fatty acids (6) ↑triglycerides (10) ↑sympathetic activity (8) ↑gastric acid secretion (7)
Hormonal	↑adrenaline (13) ↑cortisol (13) ↑glucagon (3) ↑growth hormone (26,27) ↑prolactin (27)	↑adrenaline (11,12) ↑corticosterone (23) ↓insulin (6,9,17) ↓↑growth hormone (19,24) ↑thyroid stimulating hormone (15) ↑brain somatostatin (2,14) ↓prolactin (19)
Behavioral	↑feeding (23) warmth, sweating, flushing and drowsiness, ↓oral temperature (25)	↑feeding (1,18,20,22) ↑drinking (21) stupor and ataxia (21) ↓rectal temperature (15)

- REFERENCES FOR APPENDIX -

1. Balagura, S., and Kanner, M., 1971. Hypothalamic sensitivity to 2-deoxy-D-glucose and glucose: Effects on feeding behavior. Physiology and Behavior, 7: 251-255.

2. Berelowitz, M., Dudlak, D., and Frohman, L.A., 1982. Release of somatostatin-like immunoreactivity from incubated rat hypothalamus and cerebral cortex. Journal of Clinical Investigation, 69: 1293-1301.

3. Brodows, R.G., Pi-Sunyer, F.X., and Campbell, R.G., 1973. Neural control of counter-regulatory events during glucopenia in man. Journal of Clinical Investigation, 52: 1841-1844.

4. Brown, J., and Bachrach, H.L., 1959. Effects of 2-Deoxyglucose on blood glucose levels in the rat. Proceedings of the Society for Experimental Biology and Medicine, 100: 641-643.

5. Brown, J., 1962. Effects of 2-Deoxyglucose on carbohydrate metabolism: Review of the literature and studies in the rat. Metabolism, 11: 1098-1112.

6. Coimbra, C.C., Gross, J.L., and Migliorini, R.H., 1979. Intraventricular 2-Deoxyglucose, glucose, insulin and free fatty acid mobilization. American Journal of Physiology, 236: E317-E327.

7. Colin-Jones, D.G., and Himsworth, R.L., 1970. The location of the chemoreceptor controlling gastric acid secretion during hypoglycemia. Journal of Physiology (London), 206: 397-410.

8. DiRocco, R.J., and Grill, H.J., 1979. The forebrain is not essential for sympathoadrenal hyperglycemic response to glucoprivation. Science, 204: 1112-1114.

9. Frohman, L.A., Müller, E.E., and Cocchi, D., 1973. Central nervous system mediated inhibition of insulin due to 2-deoxyglucose. Hormone and Metabolic Research, 5: 21-26

10. Haïto, K., Kagawa, S., Takeda, A., Shimizu, S., Mimura, K., and Matsuoka, A., 1984. Elevation of plasma triglyceride levels due to 2-deoxy-D-glucose in conscious rats. Life Sciences, 35: 1821-1827.

11. Himsworth, R.L., 1970. Hypothalamic control of adrenaline secretion in response to insufficient glucose. Journal of Physiology, (London), 206: 411-417.

12. Hökfelt, B., and Bydgeman, 1961. Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat. Proceedings of the Society for Experimental Biology and Medicine, 106: 537-539.

13. Kerr, D.S., Hansen, I.L., and Levy, M.M., 1983. Metabolic and hormonal responses of children and adolescents to fasting and 2-deoxyglucose. Metabolism, 32: 951-959.

14. Lengyel, A.M.J., Grossman, A., Nieuwenhuzen-Kruseman, A.C., Akland, J., and Rees, L.H., 1984. Glucose modulation of somatostatin and LHRH release from rat hypothalamic fragments in vitro. Neuroendocrinology, 39: 31-38.

15. Martino, E., Bambini, G., Aghini-Lombardi, F., Breccia, M., and Baschieri, L., 1984. Effects of 2-deoxy-D-glucose on hypothalamic-pituitary-thyroid axis in rats. Life Sciences, 35: 1569-1574.

16. Müller, E.E., Cocchi, D., and Forni, A., 1971. A central site for the hyperglycemic action of 2-deoxy-D-glucose in mouse and rat. Life Sciences, 10: 1057-1067.

17. Müller, E.E., Frohman, L.A., and Cocchi, D., 1973. Drug control of hyperglycemia and inhibition of insulin due to centrally administered 2-deoxy-D-glucose. American Journal of Physiology, 224: 1210-1217.

18. Müller, E.E., Cocchi, D., and Mantegazza, P., 1972. Brain adrenergic system in the feeding response induced by 2-deoxy-D-glucose. American Journal of Physiology, 223: 945-950.

19. Okajima, T., Motomatsu, T., Kato, K.I., and Ibayashi, H. 1980. Naloxone inhibits prolactin and growth hormone release induced by intracellular glucopenia in the rats. Life Sciences, 27: 755-760.

20. Ritter, B.C., Slusser, P.G., and Stone, S., 1981. Glucoreceptors controlling feeding and blood glucose; location in the hindbrain. Science, 213: 451-454.

21. Russell, P.J.D., and Mogenson, G.J., 1975. Drinking and feeding induced by jugular and portal infusion of 2-deoxy-D-glucose. American Journal of Physiology, 229: 1014-1018.

22. Smith, G.P., Gibbs, J., Strohmayer, A., and Stokes, P. E., 1972. Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys. American Journal of Physiology, 222: 77-81.

23. Sun, C.L., Thoa, N.B., Kopin, J., 1979. Comparison of the effects of 2-deoxyglucose and immobilization on plasma levels of catecholamines and corticosterone in awake rats. Endocrinology, 105: 306-310.

24. Takahashi, K., Daughaday, W.H., and Kipnis, D.M., 1971. Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology, 88: 909-917.

25. Thompson, D.A., and Campbell, R.G., 1977. Hunger in humans induced by 2-deoxy-D-glucose: glucoprivic control of taste preference and food intake. Science, 198: 1065-1068.

26. Wegienka, L.C., Grodsky, G.M., Karam, J.H., Grasso, S.G. and Forsham, P.H., 1967. Comparison of insulin- and 2-deoxy-D-glucose-induced glucopenia as stimulators of growth hormone secretion. Metabolism, 16: 245-256.

27. Woolf, P.D., Lee, L.A., Leebaw, W., Thompson, D., Lilavivathana, U., Brodows, R., and Campbell, R., 1977. Intracellular glucopenia causes prolactin release in man. Journal of Clinical Endocrinology and Metabolism, 45: 377-383.

GENERAL REFERENCES

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

2. Alberti, K.G.M.M., Christensen, N.J., Christensen, S.E., Prange Hansen, Aa., Iversen, J., Lundbaek, K., Seyer-Hansen, K., and Orskov, H., 1973. Inhibition of insulin secretion by somatostatin. The Lancet, 2:1299-1301.

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

4. Altszuler, N., Steele, R., Wall, J.S., Dunn, A., and De Bodo, R.C., 1959. Effects of growth hormone on carbohydrate metabolism in normal and hypophysectomized dogs; studies with C^{14} glucose. American Journal of Physiology, 196:121-124.

5. Altszuler, N., Steele, R., Rathgeb, I., and De Bodo, R.C., 1968. Influcence of growth hormone on glucose metabolism and plasma insulin levels in the dog. In: Growth Hormone, Pecile, A., Müller, E.E., (eds), First International Symposium on Growth Hormone, Milan, Italy, pp.: 309-318.

6. Altszuler, N., 1974. Action of growth hormone on carbohydrate metabolism. In: Handbook of Physiology, Sect. 7, Vol. 4, part 2, Knobil, E., Sawyer, W.H., (eds), American Physiological Society, Washington, D.C., pp.: 233-252.

7. Antoni, F.A., Makara, G.B., and Rappay, G., 1981. Growth hormone releasing activity persists in the deafferented medial-basal hypothalamus of the rat. Journal of Endocrinology, 91:415-425.

8. Arimura, A., Sato, H., Coy, D.H., and Schally, A.V., 1975. Radioimmunoassay for GH-release inhibiting hormone. Proceedings of the Society for Experimental Biology and Medicine, 148:784-789.

9. **Arimura, A.,** Smith, W.D., and Schally, A.V., 1976. Blockade of stress-induced decrease in blood GH by antisomatostatin serum in rats. Endocrinology, 98:540-543.

10. **Balagura, S.,** and Kanner, M., 1971. Hypothalamic sensitivity to 2-deoxy-D-glucose and glucose: Effects on feeding behavior. Physiology and Behavior, 7:251-255.

11. **Berelowitz, M.,** Dudlak, D., and Frohman, L.A., 1982. Release of somatostatin-like immunoreactivity from incubated rat hypothalamus and cerebral cortex. Effects of glucose and glucoregulatory hormones. Journal of Clinical Investigation, 69:1293-1301.

12. **Bernardis, L.L.,** and Frohman, L.A., 1970. Effect of lesion size in the ventromedial hypothalamus on growth hormone and insulin levels in weanling rats. Neuroendocrinology, 6:319-328.

13. **Bernardis, L.L.,** and Frohman, L.A., 1971. Plasma growth hormone responses to electrical stimulation of the hypothalamus in the rat. Neuroendocrinology, 7:193-201.

14. **Best, J.,** Catt, K.J., and Burger, H.G., 1968. Non-specificity of arginine infusion as a test for growth hormone secretion. The Lancet, 2:124-126.

15. **Betteridge, A.,** and Wallis, M., 1974. Biosynthesis of growth hormone in the rat anterior pituitary gland. Control of biosynthesis in vitro by glucose. Biochimica et Biophysica Acta, 362:66-74.

16. **Blackard, W.G.,** Boylen, C.T., Hinson, T.C., and Nelson, N.C., 1969. Effect of lipid and ketone infusions on insulin-induced growth hormone elevations in rhesus monkeys. Endocrinology, 85:1180-1185.

17. **Blanco, S.,** Schalch, D.S., and Reichlin, S., 1966. Control of GH secretion by glucoreceptors in the hypothalamic-pituitary unit. Federation Proceedings, 25:191 (Abstract).

18. Bloch, B., Brazeau, P., Ling, N., Böhlen, P., Esch, F., Wehrenberg, W.B., Benoit, R., Bloom, F., and Guillemin, R., 1983. Immunohistochemical detection of growth hormone-releasing factor in brain. Nature, 301:607-608.

19. Bloom, S.R., Mortimer, C.H., Thorner, M.O., Besser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russell, R.C.G., Coy, D.H., Kastin, A.J., and Schally, A.V., 1974. Inhibition of gastrin and gastric acid secretion by growth hormone release-inhibiting hormone. The Lancet, 2:1106-1109.

20. Bluet-Pajot, M.T., and Schaub, C., 1977. Interaction of morphine and hypoglycemia in stimulating the release of growth hormone in the rat. Journal of Endocrinology, 74:335-336.

21. Bluet-Pajot, M.T., and Schaub, C., 1978. Effects of inhibitors of catecholamine synthesis and catecholamine agonists on morphine- and hypoglycemia-induced release of growth hormone in the rat. Journal of Endocrinology, 76:365-366.

22. Boden, G., Sivitz, M.C., Owen, O.E., Essa-Koumar, N., and Landor, J.H., 1975. Somatostatin suppresses secretin and pancreatic exocrine secretion. Science, 190:163-164.

23. Bolli, G.B., Gottesman, I.S., Cryer, P.E., and Gerish, J.E., 1984. Glucose counterregulation during prolonged hypoglycemia in normal humans. American Journal of Physiology, 277:E206-E214.

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

25. Brazeau, P., Ling, N., Böhlen, P., Esch, F., Ying, S.-Y., and Guillemin, R., 1982. Growth hormone-releasing factor, somatocrinin, releases pituitary growth hormone in vitro. Proceedings of the National Academy of Science of the U.S.A., 79:7909-7913.

26. Brodows, R.G., Pi-Sunyer, F.X., and Campbell, R.G., 1973. Neural control of counter-regulatory events during glucopenia in man. Journal of Clinical Investigation, 52: 641-644.

27. Brown, I., and Bachelard, H.S., 1969. Effects of 2-deoxyglucose on blood glucose levels in the rat. Proceedings of the Society for Experimental Biology and Medicine, 130:641-644.

28. Brown, I., 1967. Effects of 2-deoxyglucose on nitrogenous metabolism: Review of the literature and studies in the rat. Metabolism, 11:1198-1212.

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

30. Cahill, Jr., G.F., Herrera, M.G., Morgan, A.P., Soeldner, J.S., Steinke, J., Levy, P.L., Reichard, Jr., G.A., and Kipnis, D.M. 1966. Hormone-fuel interrelationships during fasting. Journal of Clinical Investigation, 45:1751-1769.

31. Cheek, D.B., and Hill, D.E., 1974. Effect of growth hormone on cell and somatic growth. In: Handbook of Physiology, Knobil, E., Sawyer, W.H., (eds), sect 7, vol 4, part 2., American Physiological Society, Washington, D.C., pp.:159-185.

32. Chihara, K., Minamitani, N., Kaji, H., Kodama, H., Kita, T., and Fujita, T., 1983. Human pancreatic growth hormone-releasing factor stimulates release of growth hormone in conscious unrestrained male rabbits. Endocrinology, 113:2081-2085.

33. Coimbra, C.C., Gross, J.L., and Migliorini, R.H., 1979. Intraventricular 2-deoxyglucose, glucose, insulin and free fatty acid mobilization. American Journal of Physiology, 236:E317-E327.

34. Colin-Jones, D.G., Himsworth, R.L., 1970. The location of the chemoreceptor controlling gastric acid secretion during hypoglycemia. Journal of Physiology (London), 206:397-410.

35. Collu, R., Jéquier, J.-C., Letarte, J., Leboeuf, G., and Ducharme, J.R., 1975. Effect of stress and hypothalamic deafferentation on the secretion of growth hormone in the rat. Neuroendocrinology, 1:183-190.

36. Chritchlow, V., Rose, F.W., Abe, K., Van Vugt, D., 1978. Somatostatin content of the median eminence in female rats with lesion-induced disruption of the inhibitory control of growth hormone secretion. Endocrinology, 103:817-823.

37. Chritchlow, V., Abe, K., Urman, S., and Vugt, D., 1981. Effects of lesions in the periventricular nucleus of the preoptic-anterior hypothalamus on growth hormone and thyrotropin secretion and brain somatostatin. Brain Research, 222:267-276.

38. Cryer, P.E., 1981. Glucose counterregulation in man. Diabetes, 30:261-264.

39. Desiraju, T., Banerjee, M.G., and Anand, B.K., 1968. Activity of single neurons in the hypothalamic feeding centers: Effect of 2-deoxy-D-glucose. Physiology and Behavior, 3:757-760.

40. Deuben, R.R., and Meites, J., 1964. Stimulation of pituitary growth hormone release by hypothalamic extract in vitro. Endocrinology, 74:408-414.

41. DiRocco, R.J., and Grill, H.J., 1979. The forebrain is not essential for sympathoadrenal hyperglycemic response to glucoprivation. Science, 204:1112-1114.

42. Dobbs, R., Sakurai, H., Sasaki, H., Faloona, G., Valverde, I., Baetens, D., Orci, L., and Unger, R., 1975. Glucagon: role in the hyperglycemia of diabetes mellitus. Science, 187:544-547.

43. Eikelboom, R., and Tannenbaum, G.S. 1983. Effects of obesity-inducing ventromedial hypothalamic lesions on pulsatile growth hormone and insulin secretion: evidence for the existence of a growth hormone-releasing factor. Endocrinology, 112:212-219.

44. Epelbaum, J., Willoughby, J.O., Brazeau, P., and Martin, J.B., 1977. Effects of brain lesions and hypothalamic deafferentation on somatostatin distribution in the rat brain. Endocrinology, 101: 495-502.

45. Fraser, R., 1965. Circulating levels of growth hormone in man and woman. Nature, 208:68.

46. Berland, L., Labrie, F., Jobin, M., Arimura, A., and Schally, A.V., 1976. Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. Biochemical and Biophysical Research Communications, 68:149:156.

47. Filby, A.B., and Gross, D.S., 1982. Distribution of immunoreactive somatostatin in the primate hypothalamus. Cell and Tissue Research, 233:69-80

48. Friedman, R.C., and Reichlin, S., 1965. Growth hormone content of the pituitary gland of starved rats. Endocrinology, 76:787-788.

49. Frohman, L.A., Bernardis, L.L., and Kant, K.J., 1968. Hypothalamic stimulation of growth hormone secretion. Science, 162:580-582.

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

51. Frohman, L.A., Müller, E.E., and Cocchi, D., 1973. Central nervous system mediated inhibition of insulin secretion due to 2-deoxyglucose. Hormone and Metabolic Research, 5:21-26.

52. Garcia, J.F., and Geschwind, I.I., 1966. Increase in plasma growth hormone level in the monkey following the administration of sheep hypothalamic extracts. Nature, 211:372-374.

53. Gelato, M.C., Pescovitz, O., Cassorla, F., Loriaux, D.L., and Merriam, G.R., 1983. Effects of growth hormone-releasing factor in man. Journal of Clinical Endocrinology and Metabolism, 57:674-676.

54. Gerich, J.E., Lorenzi, M., Schneider, V., Karam, J.H., Rivier, J., Guillemin, R., and Forsham, P.H., 1974. Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. The New England Journal of Medicine, 291:544-547.

55. Glick, S.M., Roth, J., Yalow, R.S., and Berson, S.A., 1965. The regulation of growth hormone secretion. Recent Progress in Hormone Research, 21:241-283.

56. Glick, S.M., and Goldsmith, S., 1968. The physiology of growth hormone secretion. In: Growth Hormone, Pecile, A., Muller, E.E., (eds), First International Symposium on Growth Hormone, Milan, Italy, pp.: 84-89.

57. Glick, S.M., 1969. The regulation of growth hormone secretion. In: Frontiers in Neuroendocrinology, Ganong, W.F., and Martini, L., (eds), New York: Oxford University Press, pp.: 141-182.

58. Glick, S.M., 1970. Hypoglycemic threshold for human growth hormone release. Journal of Clinical Endocrinology, 30:619-623.

59. Goldsmith, S.J., and Glick, S.M. 1970. Rhythmicity of human growth hormone secretion. Journal of Mount Sinai Hospital, 37:501-509.

60. Goodman, H.M., and Knobil, E., 1959. Effects of fasting and of growth hormone on plasma fatty acid concentration in normal and hypophysectomized rhesus monkeys. Endocrinology, 65:451-458.

61. Goodman, H.M., 1963. Effects of chronic growth hormone treatment on lipogenesis in rat adipose tissue. Endocrinology, 72:95-99.

62. Goodman, H.M., and Schwartz, J., 1974. Growth hormone and lipid metabolism. In: Handbook of Physiology, Knobil, E., Sawyer, W.H., (eds), sect 7, vol 4, part 2. American Physiological Society, Washington, D.C. pp.:211-231.

63. Greenwood, F.C., Hunter, W.M., and Glover, J.S. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochemical Journal, 89:114-123.

64. Guillemin, R., Brazeau, P., Böhlen, P., Esch, F., Ling, N., and Wehrenberg, W.B., 1982. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science, 218:585-587.

65. Haito, K., Kagawa, S., Takeda, A., Shimizu, S., Mimura, K., and Matsuoka, A., 1984. Elevation of plasma triglyceride levels due to 2-deoxy-D-glucose in conscious rats. Life Sciences, 35:1821-1827.

66. Harvey, S., Scanes, C.G., and Marsh, J.A., 1984. Stimulation of growth hormone secretion in dwarf chickens by thyrotropin-releasing hormone (TRH) or human pancreatic growth hormone-releasing factor (hpGRF). General and Comparative Endocrinology, 55:493-497.

67. Herbert, V., Lau, K.-S., Gottlieb, C.W., and Bleicher, S.J., 1965. Coated charcoal immunoassay of insulin. Journal of Clinical Endocrinology and Metabolism, 25:1375-1384.

68. Hertelendy, F., and Kipnis, D.M., 1973. Studies on growth hormone secretion: V. Influence of plasma free fatty acid levels. Endocrinology, 92:402-410.

69. Heubi, J.E., Burstein, S., Sperling, M.A., Gregg, D., Subbiah, M.T.R., and Matthews, D.E., 1983. The role of human growth hormone in the regulation of cholesterol and bile acid metabolism. Journal of Clinical Endocrinology and Metabolism, 57:885-891.

70. Himsworth, R.L., 1970. Hypothalamic control of adrenaline secretion in response to insufficient glucose. Journal of Physiology (London), 206:411-417.

71. Himsworth, R.L., Carmel, P.W., and Frantz, A.G., 1972. The location of the chemoreceptor controlling growth hormone secretion during hypoglycemia in primates. Endocrinology, 91:217-226.

72. Hökfelt, B., and Bydgeman, S., 1961. Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat. Proceedings of the Society for Experimental Biology and Medicine, 106:537-539.

73. Hunter, W.M., Rigal, W.M., and Sukkar, M.Y., 1968. Plasma growth hormone during fasting. In: Growth Hormone, Pecile, A., Müller, E.E., (eds), First International Symposium on Growth Hormone, Milan, Italy, pp.: 408-417.

74. Kerr, D.S., Hansen, I.L., and Levy, M.M., 1983. Metabolic and hormonal responses in children and adolescents to fasting and 2-deoxyglucose. Metabolism, 32:951-959.

75. Knopf, R.F., Conn, J.W., Fajans, S.S., Floyd, J.C., Guntsche, E.M., and Rull, J.A., 1965. Plasma growth hormone response to intravenous administration of amino acids. Journal of Clinical Endocrinology, 25:1140-1144.

76. Koerker, D.J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C.J., Ensinck, J., and Gale, C.C., 1974. Somatostatin: Hypothalamic inhibitor of the endocrine pancreas. Science, 184:482-484.

77. Koh, C.S., Kohn, J., Catt, K.J., and Burger, H.G., 1968. Lack of relation between plasma growth hormone levels and small decrements in blood sugar. Lancet, 1:13-14.

78. Kokka, N., Garcia, J.F., Morgan, M., and George, R., 1971. Immunoassay of plasma growth hormone in cats following fasting and administration of insulin, arginine, 2-deoxyglucose and hypothalamic extract. Endocrinology, 88:359-366.

79. Kostyo, J.L., and Nutting, D.F., 1974. Growth hormone and protein metabolism. In: Handbook of Physiology, Knobil, E., Sawyer, W.H. (eds), sect 7, vol 4, part 2., American Physiological Society, Washington, D.C., pp.: 187-210.

80. Krulich, L., Dhariwal, A.P.S., and McCann, S.M., 1968. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology, 83:-783-780.

81. Krulich, L., Illner, P., Fawcett, C.P., Quijada, M., and McCann, S.M., 1971. Dual hypothalamic regulation of growth hormone secretion. In: Growth and Growth Hormone, Pecile, A., Müller, E.E., (eds), Second International Symposium on Growth Hormone. Milan, Italy, pp.: 306-316.

82. Krzemien, K., and Lipowska-Zabawska, L., 1976. Urethane-induced hyperglycemia in rats. Acta Physiologica Polonica, 27:369-376.

83. Lengyel, A.M.J., Grossman, A., Nieuwenhuyzen-Kruseman, A.C., Ackland, J., and Rees, L.H., 1984. Glucose modulation of somatostatin and LHRH release from rat hypothalamic fragments in vitro. Neuroendocrinology, 39:31-38.

84. Lengyel, A.M.J., Nieuwenhuyzen-Kruseman, A.C., Grossman, A., Rees, L.H., and Besser, G.M., 1984. Glucose-induced changes in somatostatin-14 and somatostatin-28 released from rat hypothalamic fragments in vitro. Life Sciences, 35:713-719.

85. Lin, H.D., Bollinger, J., Ling, N., and Reichlin, S., 1984. Immunoreactive growth hormone-releasing factor in human stalk median eminence. Journal of Clinical Endocrinology and Metabolism, 58:1197-1199.

86. Ling, N., Esch, F., Davis, F., Mercado, M., Regno, M., Böhlen, P., Brazeau, P., and Guillemin, R., 1980. Solid phase synthesis of somatostatin-28. Biochemical and Biophysical Research Communications, 85:945-951.

87. Ling, N., Esch, F., Böhlen, P., Brazeau, P., Wehrenberg, W.B., and Guillemin, R., 1984. Isolation, primary structure and synthesis of human hypothalamic somatocrinin: Growth hormone-releasing factor. Proceedings of the National Academy of Science of the U.S.A., 81:4302-4306.

88. Luft, R., and Cerasi, E., 1968. Human growth hormone in blood glucose homeostasis. In: Growth Hormone, Pecile, A., Müller, E.E., (eds), First International Symposium on Growth Hormone, Milan, Italy, pp.: 373-381.

89. Luft, R., Efendic, S., Hökfelt, T., Johansson, O., and Arimura, A., 1974. Immunohistochemical evidence for the localization of somatostatin-like immunoreactivity in a cell population of the pancreatic islets. Medical Biology, 52:428-430.

90. Machlin, L.J., Horino, M., Kipnis, D.M., Phillips, S.L., and Gordon, R.S., 1967. Stimulation of growth hormone secretion by median eminence extracts in the sheep. Endocrinology, 80:205-207.

91. Machlin, L.J., Horino, M., Hertelendy, F., and Kipnis, D.M., 1968. Plasma growth hormone and insulin levels in the pig. Endocrinology, 82:369-376.

92. Machlin, L.J., Takahashi, Y., Horino, M., Hertelendy, F., Gordon, R.S., and Kipnis, D.M., 1968. Regulation of growth hormone secretion in non-primate species. In: Growth Hormone, Pecile, A., Müller, E.E. (eds), First International Symposium on Growth Hormone, Milan, Italy, pp.: 292-305.

93. Malacara, J.M., and Reichlin, S., 1971. Elevation of plasma radioimmunoassayable growth hormone in the rat induced by porcine hypothalamic extracts. In: Growth and Growth Hormone, Pecile, A., Müller, E.E., (eds), Second International Symposium on Growth Hormone, Milan, Italy, pp.: 299-305.

94. Marks, V., Howorth, N., and Greenwood, F.C., 1965. Plasma growth hormone levels in chronic starvation in man. Nature, 208:686-687.

95. Martin, J.B., 1972. Plasma growth hormone (GH) response to hypothalamic or extrahypothalamic electrical stimulation. Endocrinology, 91:107-115.

96. Martin, J.B., Renaud, L.P., and Brazeau, P., 1974. Pulsatile growth hormone secretion: suppression by hypothalamic ventromedial lesions and by long-acting somatostatin. Science, 186:538-540.

97. Martin, J.B., Tannenbaum, G.S., Willoughby, J.O., Renaud, L.P., and Brazeau, P., 1975. Functions of the central nervous system in regulation of pituitary GH secretion. In: Hypothalamic Hormones; Chemistry, Physiology, Pharmacology, and Clinical Uses. Motta, M., Grosignani, P.G., Martin, L., (eds), Academic Press, New York, pp.: 217-236.

98. Martin, J.B., Brazeau, P., Tannenbaum, G.S., Willoughby, J.O., Epelbaum, J., Terry, L.C., and Durand, D., 1978. Neuroendocrine organization of growth hormone regulation. In: The Hypothalamus. Reichlin, S., Baldessarini, R.J., Martin, J.B., (Eds), Raven Press, New York, pp.: 329-357.

99. Martino, E., Bambini, G., Aghini-Lombardi, F., Breccia, M., Baschieri, L., 1984. Effects of 2-deoxy-D-glucose on hypothalamic-pituitary-tyroid axis in rats. Life Sciences, 35:1569-1574.

100. Mayer, J., and Arees, E.A., 1968. Ventromedial glucoreceptor system. Federation Proceedings, 27:1345-1348.

101. McIntyre, H.B., and Odell, W.D., 1974. Physiological control of growth hormone in the rabbit. Neuroendocrinology, 16:8-21.

102. Mendelson, W.B., Jacob, L.S., Gillin, J.C., and Wyatt, R.J., 1979. The regulation of insulin-induced and sleep-related human growth hormone secretion: a review. Psychoneuroendocrinology, 4:341-349.

103. Merchenthaler, I., Vigh, S., Schally, A.V., and Petrusz, P., 1984. Immunocytochemical localization of growth hormone-releasing factor in the rat hypothalamus. Endocrinology, 114:1082-1085.

104. Merimee, T.J., Burgess, J.A., and Rabinowitz, D., 1966. Sex-determined variation in serum insulin and growth hormone response to amino acid stimulation. Journal of Clinical Endocrinology and Metabolism, 26:791-793.

105. Merimee, T.J., 1979. Growth hormone: Secretion and action. In: Endocrinology, vol. 1. DeGroot, L.J., Cahill, G.F., and Martini, L., (eds), Academic Press, New York, pp.: 123-132.

106. Meyer, E., Knobil, E., 1967. Growth hormone secretion in the unanesthetized rhesus monkey in response to noxious stimuli. Endocrinology, 80:163-171.

107. Millard, W.J., Martin, Jr., J.B., Audet, J., Sagar, S.M., and Martin, J.B., 1982. Evidence that reduced growth hormone secretion observed in monosodium glutamate-treated rats is the result of a deficiency in growth hormone-releasing factor. Endocrinology, 110:540-550.

108. Miller, J.D., Tannenbaum, G.S., Colle, E., and Guyda, H.J., 1982. Daytime pulsatile growth hormone secretion during childhood and adolescence. Journal of Clinical Endocrinology and Metabolism, 55:989-994.

109. Mitchell, J.A., Hutchins, M., Schindler, W.J., and Critchlow, V., 1973. Increases in plasma growth hormone concentration and naso-anal length in rats following isolation of the medial basal hypothalamus. Neuroendocrinology, 12:161-173.

110. Müller, E.E., Miedico, D., Giustina, G., and Cocchi, D., 1971. Ineffectiveness of hypoglycemia, cold exposure and fasting in stimulating GH secretion in the mouse. Endocrinology, 38:345-350.

111. Müller, E.E., Cocchi, D., and Forni, A., 1971. A central site for the hyperglycemic action of 2-deoxy-D-glucose in mouse and rat. Life Sciences, 10:1057-1067.

112. Müller, E.E., Cocchi, D., and Mantegazza, P., 1972. Brain adrenergic system in the feeding response induced by 2-deoxy-D-glucose. American Journal of Physiology, 223:945-950.

113. Müller, E.E., Frohman, L.A., and Cocchi, D., 1973. Drug control of hyperglycemia and inhibition of insulin secretion due to centrally administered 2-deoxy-D-glucose. American Journal of Physiology, 224:1210-1217.

114. Okajima, T., Motomatsu, T., Kato, K.-I., and Ibayashi, H., 1980. Naloxone inhibits prolactin and growth hormone release induced by intracellular glucopenia in the rats. Life Sciences, 27:755-760.

115. Oomura, Y., Ono, T., Ooyama, H., and Wayner, M.J., 1969. Glucose and osmosensitive neurones of the rat hypothalamus. Nature, 222:282-284.

116. Patel, Y.C., and Reichlin, S., 1978. Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. Endocrinology, 102:523-530.

117. Patel, Y.C., Cameron, D.P., Bankier, A., Malaisse-Lagae, F., Ravazzola, M., Studer, P., and Orci, L., 1978. Changes in somatostatin concentration in pancreas and other tissues of streptozotocin diabetic rats. Endocrinology, 103:917-923.

118. Pellegrino, L.J., and Cushman, A.J., 1967. A Stereotaxic Atlas of the Rat Brain. Elliott, R.M., Lindzey, G., and MacCorquodale, K., (eds). Appleton-Century-Crofts, New York.

119. Pelletier, G., Leclerc, R., Arimura, A., and Schally, A.V. 1975. Immunohistochemical localization of somatostatin in the rat pancreas. Journal of Histochemistry and Cytochemistry, 23:699-701.

120. Quabbe, H.-J., Schilling, E., and Helge, H., 1966. Pattern of growth hormone secretion during a 24-hour fast in normal adults. Journal of Clinical Endocrinology, 26:1173-1177.

121. Reichlin, S., 1960. Growth and the hypothalamus. Endocrinology, 67:760-763.

122. Reichlin, S., 1961. Growth hormone content of the pituitaries from rats with hypothalamic lesions. Endocrinology, 69:225-230.

123. Reichlin, S., 1974. Regulation of somatotrophic hormone secretion. In: Handbook of Physiology, sect. 7, vol. 4, part 2. Knobil, E., and Sawyer, W.H., (eds), American Physiological Society, Washington, D.C. pp.: 405-447.

124. Ritter, R.C., Slusser, P.G., and Stone, S., 1981. Glucoreceptors controlling feeding and blood glucose: location in the hindbrain. Science, 213:451-453.

125. Rivier, J., Spiess, J., Thorner, M., and Vale, W., 1982. Characterization of growth hormone-releasing factor from a human pancreatic islet tumor. Nature, 300:276-278.

126. Roth, J., Glick, S.M., Yalow, R.S., and Berson, S.A., 1963(a). Hypoglycemia: a potent stimulus to secretion of growth hormone. Science, 140:987-988.

127. Roth, J., Glick, S.M., Yalow, R.S., and Berson, S.A., 1963(b). Secretion of human growth hormone: physiologic and experimental modification. Metabolism, 12:577-579.

128. Roth, J., Glick, S.M., Yalow, R.S. and Berson, S.A., 1964. The influence of blood glucose on the plasma concentration of growth hormone. Diabetes, 13:355-361.

129. Russell, P.J.D., and Mogenson, G.J., 1975. Drinking and feeding induced by jugular and portal infusions of 2-deoxy-D-glucose. American Journal of Physiology, 229:1014-1018.

130. Saito, H., Ogawa, T., Ishimaru, K., Oshima, I., and Saito, S., 1979. Effect of pentobarbital and urethane on the release of hypothalamic somatostatin and pituitary growth hormone. Hormone and Metabolic Research, 11:550-554.

131. Scanes, C.G., Carsia, R.V., Lauterio, T.J., Huybrechts, L., Rivier, J., and Vale, W., 1984. Synthetic human pancreatic growth hormone-releasing factor (GRF) stimulates growth hormone secretion in the domestic fowl (Gallus Domesticus). Life Sciences, 34:1127-1134.

132. Smith, G.P., Gibbs, J., Strohmayer, A.J., and Stokes, P.E., 1972. Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys. American Journal of Physiology, 222:77-81.

133. Spiess, J., Rivier, J., and Vale, W., 1983. Characterization of rat hypothalamic growth hormone-releasing factor. Nature, 303:532-535.

134. Steiner, R.A., Stewart, J.K., Barber, J., Koerker, D., Goodner, C.J., Brown, A., Illner, P., and Gale, C.C., 1978. Somatostatin: A physiological role in the regulation of growth hormone secretion in the adolescent male baboon. Endocrinology, 102:1587-1594.

135. Sun, C.L., Thoa, N.B., and Kopin, I.J., 1979. Comparison of the effects of 2-deoxyglucose and immobilization on plasma levels of catecholamines and corticosterone in awake rats. Endocrinology, 105: 306-310.

136. Szabo, M., Dudlak, D., Thominet, J.L., and Frohman, L.A., 1983. Ectopic growth hormone-releasing factor stimulates growth hormone secretion in the urethane anesthetized rat in vivo. Neuroendocrinology, 37:328-331.

137. Takahashi, Y., Kipnis, D.M., and Daughaday, W.H., 1968. Growth hormone secretion during sleep. Journal of Clinical Investigation, 47: 2079-2090.

138. Takahashi, K., Daughaday, W.H., and Kipnis, D.M., 1971. Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology, 88:909-917.

139. Takano, K., Hizuka, N., Shizume, K., Asakawa, K., Miyakawa, M., Hirose, N., Shibasaki, T., and Ling, N.C., 1984. Plasma growth hormone (GH) response to GH-releasing factor in normal children with short stature and patients with pituitary dwarfism. Journal of Clinical Endocrinology and Metabolism, 58:236-241.

140. Tannenbaum, G.S., and Martin, J.B., 1976. Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology, 98:562-570.

141. Tannenbaum, G.S., Martin, J.B., and Colle, E., 1976. Ultradian growth hormone rhythm in the rat: effects of feeding, hyperglycemia and insulin-induced hypoglycemia. Endocrinology, 99:720-727.

142. Tannenbaum, G.S., Epelbaum, J., Colle, E., Brazeau, P., and Martin, J.B., 1978. Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. Endocrinology, 102:1909-1914.

143. Tannenbaum, G.S., Rorstad, and Brazeau, P., 1979. Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. Endocrinology, 104:1733-1738.

144. Tannenbaum, G.S., 1980. Evidence for autoregulation of growth hormone secretion via the central nervous system. Endocrinology, 107:2117-2120.

145. Tannenbaum, G.S., 1981. Growth hormone secretory dynamics in streptozotocin diabetes: Evidence of a role for endogenous circulating somatostatin. Endocrinology, 108:76-82.

146. Tannenbaum, G.S., Colle, E., Wanamaker, L., Gurd, W., Goldman, H., and Seemayer, T.A., 1981. Dynamic time-course studies of the spontaneously diabetic BB Wistar rat. II. Insulin-, glucagon-, and somatostatin-reactive cells in the pancreas. Endocrinology, 109:1880-1887.

147. Tannenbaum, G.S., Eikelboom, R., and Ling, N., 1983. Human pancreas GH-releasing factor analog restores high-amplitude GH pulses in CNS lesion-induced GH deficiency. Endocrinology, 113:1173-1175.

148. Tannenbaum, G.S., and Ling, N., 1984. The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. Endocrinology, 115:1952-1957.

149. Tannenbaum, G.S., 1985. Physiological role of somatostatin in regulation of pulsatile GH secretion. In: Somatostatin, Patel, Y.C., and Tannenbaum, G.S., (eds). Plenum Press, New York, pp.: 229-260.

150. Terry, L.C., Willoughby, J.O., Brazeau, P., Martin, J.B., and Patel, Y.C., 1976. Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. Science, 192:565-567.

151. Terry, L.C., Epelbaum, J., and Martin, J.B., 1981. Monosodium glutamate: Acute and chronic effects on rhythmic growth hormone and prolactin secretion, and somatostatin in the undisturbed male rat. Brain Research, 217:129-142.

152. Thompson, D.A., and Campbell, R.G., 1977. Hunger in humans induced by 2-deoxy-D-glucose: Glucoprivic control of taste preference and food intake. Science, 198:1065-1068.

153. Thorner, M.O., Rivier, J., Spiess, J., Borges, J.L., Vance, M.L., Bloom, S.R., Rogol, A.D., Cronin, M.J., Kaiser, D.L., Evans, W.S., Webster, J.D., MacLeod, R.M., and Vale, W., 1983. Human pancreatic growth hormone-releasing factor selectively stimulates growth hormone secretion in man. Lancet, 1:24-29.

154. Tse, T.F., Clutter, W.E., Shah, S.D., Miller, J.P., and Cryer, P.E., 1983. Neuroendocrine responses to glucose ingestion in man. Specificity, temporal relationships, and quantitative aspects. Journal of Clinical Investigation, 72:270-277.

155. Tsushima, T., Irie, M., and Sakuma, M., 1971. Radioimmunoassay for canine growth hormone. Endocrinology, 89:685-693.

156. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J., and Rivier, J., 1983. Effects of synthetic human pancreatic (tumor) GH-releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion in vitro. Endocrinology, 112:1553-1555.

157. Wakabayashi, I., Demura, R., Miki, N., Ohmura, E., Miyoshi, H., and Shizume, K., 1980. Failure of naloxone to influence plasma growth hormone, prolactin and cortisol secretions induced by insulin hypoglycemia. Journal of Clinical Endocrinology and Metabolism, 50:597-599.

158. Wegienka, C.L., Grodsky, G.M., Karam, J.H., Grasso, S.G., and Forsham, P.H., 1967. Comparison of insulin and 2-deoxy-D-glucose-induced glucopenia as stimulators of growth hormone secretion. Metabolism, 16:245-256.

159. Wehrenberg, W.B., Ling, N., Brazeau, P., Esch, F., Böhlen, P., Baird, A., Ying, S., and Guillemin, R., 1982. Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. Biochemical and Biophysical Research Communications, 109:382-387.

160. Willoughby, J.O., Terry, L.C., Brazeau, P., and Martin, J.B., 1977. Pulsatile growth hormone, prolactin, and thyrotropin secretion in rats with hypothalamic deafferentation. Brain Research, 127:137-152.

161. Willoughby, J.O., and Martin, J.B., 1978. Pulsatile growth hormone secretion: inhibitory role of the medial preoptic area. Brain Research, 148:240-244.

162. Willoughby, J.O., Koblar, S., Jervois, P.M., Menadue, M.F., and Oliver, J.R., 1983. Evidence that the regulation of growth hormone secretion is mediated predominantly by a growth hormone-releasing factor. Neuroendocrinology, 36:358-363.

163. Winer, B.J., 1971. Statistical Principles in Experimental Design. Second ed., McGraw-Hill Book Company, New York.

164. Woolf, P.D., Lee, L.A., Leebaw, W., Thompson, D., Lilavivathana, U., Brodows, R., and Campbell, R., 1977. Intracellular glucopenia causes prolactin release in man. Journal of Clinical Endocrinology and Metabolism, 45:377-383.