National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

In Vivo Evidence that Preexposure to Somatostatin Enhances Growth Hormone Responsiveness to Growth Hormone-Releasing Factor

Joel P. Turner

Department of Neurology and Neurosurgery
McGill University, Montreal
August 30, 1994

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

ISBN 0-612-05644-9

"It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair..."

Charles Dickens

TABLE OF CONTENTS

Acknowledgments i

Abstract ii

Résumé iv

I. INTRODUCTION

Introduction to Growth Hormone 1

Growth Hormone Secretory Pattern 2

The Hypothalamic/Pituitary Axis and the Role of the Hypothalamus 3

Somatostatin (SRIF) 4

Growth Hormone-Releasing Factor (GRF) 5

Somatostatin Analog - Octreotide 7

SRIF/GRF Interactions at the Level of the Pituitary 8

SRIF/GRF Interactions at the Level of the C.N.S. 10

SRIF Receptors 12

II. AIM OF PRESENT INVESTIGATION 16

III. CHAPTER 1 - MANUSCRIPT 18

In vivo Evidence that Preexposure to Somatostatin Analog Octreotide Enhances Growth Hormone Responsiveness to Growth Hormone-Releasing Factor

Abstract 19

Introduction 20

Materials and Methods 21

Results 23

Discussion 26

Manuscript References 31

Figures and Figure Legends 36

IV. CHAPTER 2 **Effects of Octreotide Preexposure on Somatostatin Receptor mRNA using *in situ* Hybridization** 37

Introduction 38

Materials and Methods 40

Results 44

Discussion 46

V. GENERAL CONCLUSION 49

VI. GENERAL REFERENCES 52

ACKNOWLEDGEMENTS

I am particularly grateful to Dr. Gloria Tannenbaum for her expert supervision, counsel, and guidance during the past two years.

I am very indebted to Wendy Gurd and Martine Lapointe for their skilful and expert teaching of the many experimental methods and techniques used in the lab, to Julie Temko for her expert secretarial help, and to Claudine Payment for her french translation. I am also very grateful to Dr. Alain Beaudet and Dr. Francis McCarthy for their encouragements, advice, and constant good humour.

I wish to express my gratitude to Sandoz Canada Inc for the provision of octreotide, to Dr. Paul Brazeau for the gift of the GRF peptide, to the NIDDK, Bethesda, MD, for the generous supply of rat GH RIA materials, and finally to Dr. Graeme Bell, Howard Hughes Medical Institute, Chicago, IL, for the generous quantity of the mSSTR1 and mSSTR2 plasmids.

Lastly I wish to address a special thanks to my parents and my brothers for providing continuous motivation and encouragement.

ABSTRACT

The ultradian rhythm of growth hormone (GH) secretion from the anterior pituitary gland is ultimately controlled by the complex interaction of two hypothalamic hormones, a stimulatory GH-releasing factor (GRF), and an inhibitory hormone, somatostatin (SRIF). Both are released episodically into the hypophyseal portal circulation. Recent *in vitro* studies suggest that SRIF may not only be inhibitory to GH secretion, but that under different temporal conditions, SRIF may act in a positive manner to influence GH release, although the mechanism(s) is not known. Moreover, there is a paucity of data *in vivo* regarding the temporal interactions of SRIF and GRF in GH regulation.

In the present study, we used the long-acting SRIF analog, octreotide, as a probe in both the normal and mutant dwarf rat to 1) further clarify the temporal nature of the SRIF/GRF interplay in GH regulation *in vivo*, and 2) define possible mechanisms of action of SRIF in generating the ultradian rhythm of GH secretion characteristic of the normal male rat. Administration of octreotide [25 µg (n=6) and 50 µg (n=6) i.v.] to free-moving, chronically cannulated adult male rats resulted in an almost complete obliteration of spontaneous GH pulses for 3 hours, with gradual recovery observed 3-6 hours after injection. Rats pretreated with octreotide i.v. [25 µg (n=6); 50 µg (n=9)] and subsequently challenged with GRF (1 µg i.v.) exhibited reduced GH responsiveness to exogenous GRF at 1 hour post treatment. In contrast, preexposure to octreotide for 3 hours resulted in a 2-3 fold enhancement in GH responsiveness to GRF (expressed as the mean ± SEM GH area under the curve - 25 µg: 4537 ± 428; 50 µg: 3851 ± 367 ng/ml/30 min), compared to controls (n=9) pretreated with normal saline (2032 ± 490 ng/ml/30 min; $P<0.02$). The concomitant administration of native SRIF-14 (50 µg i.v.) and GRF (1 µg i.v.) to rats preexposed for 3 hours to either octreotide [25 µg (n=6); 50 µg (n=6)] or normal saline (n=6) resulted in a similar degree of SRIF-14-mediated inhibition of GRF-induced GH release 5 min post injection. However, this short-acting inhibition was followed by a striking postsuppression rebound release of GH which was 14- to 16-fold higher in the octreotide-pretreated rats (25 µg: 3673 ± 841; 50 µg: 4099 ± 796 ng/ml/40 min) compared

to normal saline-pretreated controls (254 ± 114 ng/ml/40 min; $P<0.001$). In contrast, we report that preexposure to octreotide (n=6) in a strain of dwarf rats, which shows a selective reduction in pituitary GH synthesis and storage, failed to significantly enhance GRF-induced GH release (50 µg: 22.0 ± 5 vs 13.1 ± 4 ng/ml in controls (n=9); NS).

These results demonstrate that, in the normal adult male rat, a 3-hour period of exposure to the long-acting SRIF analog, octreotide, is sufficient to markedly enhance GH responsiveness to GRF *in vivo*. Our findings suggest that SRIF pretreatment promotes the accumulation of pituitary GH stores in a readily releasable pool so that subsequent GRF challenges can exert an accentuated effect on pituitary somatotrophs. Such a cooperative interaction of the two neuropeptides may be important for optimizing pulsatile GH secretion and, consequently, somatic growth. While it is also possible that chronic octreotide exposure produces SRIF receptor downregulation, our preliminary *in situ* hybridization experiments of hypothalamic SRIF receptor subtype mRNAs do not provide support for this view.

RÉSUMÉ

La sécretion de l'hormone de croissance (GH) par l'hypophyse est controlée par l'interaction complexe de deux hormones hypothalamiques: la somatocrinine (GRF), à effet stimulant, et la somatostatine (SRIF), à effet inhibitrice. Ces deux neuropeptides sont déchargés à un rythme régulier dans la circulation hypothalamique hypophysaire.

De récentes analyses *in vitro* ont suggéré que SRIF ne jout pas simplement un rôle inhibitoire dans la sécretion de GH, mais que sous des conditions temporelles différentes, SRIF pourrait influencer d'une manière positive la sécretion de GH, toutefois le (les) méchanisme(s) ne sont pas connus. D'ailleurs il y a peu de resultats *in vivo* concernant ces interactions temporelles entre SRIF et GRF dans la régulation de la sécretion de GH.

Dans ce projet-ci, l'octreotide, un analogue de SRIF, est utilisé comme sonde, chez le rat normal et le rat nain, pour: 1) mieux comprendre l'interaction temporelle entre SRIF et GRF dans la régulation de la secretion de GH *in vivo*; 2) determiner le (les) mechansisme(s) d'action de SRIF pour creer le rythme "ultradien" de GH chez le rat mâle adulte normal. L'administration d'octreotide [25 µg i.v. (n=6) et 50 µg i.v. (n=6)] chez des rats mâles adultes et mobiles, munis de cannules intracardiaques, abaisse complètement les hausses spontannées de la sécrétion de GH pendant 3 heures, suivi d'un retour graduel au cycle normal durant les prochaines 3 à 6 heures après l'injection. Lorsque des rats sont prétraités à l'octreotide [25 µg (n=6) et 50 µg (n=9)], une injection de GRF (1 µg i.v.) une heure après le prétraitement, abaisse la réponse du GH. Par contre, une injection de GRF 3 heures après le prétraitement entrâine un très forte hausse de la sécretion de GH (25 µg: 4537 ± 428; 50 µg: 3851 ± 367 ng/ml/30 min), soit 2 à 3 fois le taux mesuré chez les rats prétraités avec une solution saline (n=10)(2032 ± 490 ng/ml/30 min, $P<0.02$). Cependant, nos études chez le rat nain démontrent qu'il n'y a pas d'augmentation significative de la réponse de GH au GRF chez ceux prétraités à l'octreotide (n=6)(50 µg: 22.0 ± 5) par rapport aux rats prétraités avec la solution saline (n=9)(13.1 ± 4 ng/ml; NS).

Lorsque de la SRIF-14 originale (50 µg i.v.) est administrée de façon concomitante avec GRF chez des rats prétraités pendant 3 heures à l'octreotide [25 µg (n=6) ou 50 µg

(n=6)] ou à une solution saline (n=6), l'action inhibitoire de SRIF-14 sur l'action stimulante de GRF sur GH est comparable pour les trois groupes de rats durant les premières cinq minutes après l'injection. Toutefois, cet effet inhibitoire à court terme est suivi d'une sécrétion post-inhibitoire marquante de GH soit de 14 à 16 fois plus élevée chez les rats prétraités à l'octreotide (25 µg: 3673 ± 841; 50 µg: 4099 ± 796 ng/ml/40 min) que ceux prétraités avec la solution saline (254 ± 114 ng/ml/40 min; $P<0.001$).

Ces résultats nous démontrent qu'un prétraitement d'une durée de 3 heures avec une substance analogue à SRIF est suffisant pour augmenter la réponse du GH au GRF *in vivo* chez des rats mâles adultes normaux. Ceci suggère que le prétraitement avec SRIF favoriserait une accumulation des réserves hypophysaires de GH et qu'ensuite, une stimulation avec GRF aurait un effet plus marqué sur les somatotrophes hypophysaires. Cette interaction coopérative des deux peptides pourait être importante pour optimiser la sécretion de GH, et, donc, la croissance. Tout en reconnaissant la possibilité que le traitement chronique avec octreotide pourrait causer une désensibilisation des récepteurs de SRIF, nos experiences préliminaires par l'hybridisation *in situ* sur deux isotypes de récepteurs hypothalamiques de SRIF ne supportent pas cette conclusion.

INTRODUCTION

The control of normal postnatal body growth and development depends on the correct integration of neuroendocrine, endocrine, paracrine, and autocrine mechanisms, and central to this process is an adequate supply of growth hormone (GH), synthesized and released from the anterior pituitary gland. GH is largely a single chain, 191 amino acid protein (22 kD molecular weight) with 2 intramolecular disulfide bonds. Other minor forms, such as a 20 kD protein also exist and are produced by alternate splicing of the mRNA sequence (Cooke *et al*, 1988; DeNoto *et al*, 1981). The gene encoding human GH (hGH) is found among a 5 gene cluster on chromosome 17, also responsible for encoding human placental lactogen (Owerbach *et al*, 1980; Cooke *et al*, 1986).

Unlike the other anterior pituitary hormones, GH does not function through a distinct target organ. Instead, GH exerts a more generalized action, promoting somatic growth in both skeletal and soft tissue. Early studies on the action of GH suggested that the growth-promoting effects of GH are indirect, mediated by two small GH-dependent peptides exhibiting a high degree of homology with human proinsulin: insulin-like growth factor (IGF)-I and IGF-II, collectively known as somatomedins (Salmon & Daughaday, 1957; Rinderknecht & Humbel, 1976). These two serum factors, secreted mainly by the liver in response to increased plasma GH levels, showed a more potent effect than GH in promoting cell proliferation and protein synthesis in both skeletal and non-skeletal tissues. However, recently it has been suggested that GH may, in fact, act directly on a number of tissue cells. For example, GH has been shown to increase the rate of mitosis and promote differentiation of several lines of precursor cells, such as prechondrocytes and preadipose cells (Green *et al*, 1985).

Aside from general influences on cell growth, GH has many specific metabolic effects such as 1) increasing the rate of protein synthesis in all cell types, 2) increasing the mobilization and use of fatty acids from adipose tissue, and 3) decreasing the rate of glucose utilization (see Strobl & Thomas, 1994 for review).

Growth Hormone Secretory Pattern

Initial studies in healthy adult men revealed that GH is released from the pituitary in bursts lasting between a few minutes to a few hours (Glick & Goldsmith, 1968; Spitz *et al*, 1972), with the maximum GH secretory rate occurring during the sleep cycle, and the most striking elevations in plasma GH occurring at the onset of the first slow wave sleep (within the initial 90 minutes of sleep) (Takahashi *et al*, 1968; Mendelson *et al*, 1979). Plasma GH levels may vary from less than 5 ng/ml during a trough period to as high as 60 ng/ml during a peak (Martin *et al*, 1975; Finkelstein *et al*, 1972).

The advent of chronic sampling in animals led to the discovery that the secretion of GH is in fact pulsatile in nature in all species of animal investigated thus far (Meyer & Knobil, 1967, Machlin *et al*,1968; Takahashi *et al*, 1971; Tsushima *et al*, 1971). In the rat, GH secretion is distinguished by an ultradian rhythm which, in the male, is synchronized to the light-dark cycle of the day (Tannenbaum & Martin, 1976). Specifically, rat pituitary GH secretion is characterized by an endogenous ultradian rhythm composed of high-amplitude secretory bursts occurring at precise 3.3 h intervals throughout the 24-h period; in the intervening trough periods, basal plasma GH levels are undetectable (Tannenbaum & Martin, 1976).

Although the pattern of GH secretion depends on a number of neurogenic and metabolic factors such as stage of development, nutritional status, sleep, stress, and exercise, pituitary GH bursts are, for the most part, spontaneous (Spitz *et al*, 1972). This is further demonstrated in the rat where the GH rhythm is capable of functioning independently of the light-dark cycle and feeding (Tannenbaum *et al*, 1976), indicating that while it can be regulated by environmental influences, it is primarily controlled by endogenous mechanisms, specifically via hypophysiotrophic factors.

The Hypothalamic/Pituitary Axis and the Role of the Hypothalamus

The importance of an intact hypothalamic-pituitary axis in the regulation of GH secretion in the human originated from early observations in patients with pituitary stalk section, who demonstrated reduced plasma GH concentrations with partial or complete loss of the GH response to insulin-induced hypoglycemia (Glick & Goldsmith, 1968; Antony *et al*, 1969). More direct evidence for a hypothalamic influence came from ablation and stimulation studies in several animal species. While lesions of the basal ventral hypothalamus or ventromedial nuclei were found to result in growth retardation and a fall in plasma and pituitary GH levels (Frohman & Bernardis, 1968; Goldman *et al*, 1972; Martin *et al*, 1974; Terry & Martin, 1981; Eikelboom & Tannenbaum, 1983), electrical stimulation of these same regions resulted in an immediate rise in plasma GH (Bernardis & Frohman, 1971; Martin, 1972; Martin, 1973). Other hypothalamic and extrahypothalamic areas also produced increases in plasma GH when stimulated, including the hippocampus and basolateral amygdala (Martin *et al*,1973). In addition, isolated hypothalamic extracts were found to stimulate GH release in cultured anterior pituitaries (Deuben & Meites, 1964; Malacara *et al*, 1972; Stachura *et al*, 1972; Nair *et al*,1978).

In sharp contrast, animals with selective lesions of the medial preoptic or anterior medio-basal hypothalamus exhibited augmented levels of plasma and pituitary GH (Collu *et al*, 1973; Willoughby *et al*,1977; Epelbaum *et al*, 1977; Willoughby & Martin, 1978; Critchlow *et al*, 1981). Moreover, these same areas, when electrically stimulated caused a decrease in plasma GH levels, providing evidence for this region as inhibitory for GH regulation (Martin *et al*, 1975). Through these findings, it became evident that GH secretion from the pituitary gland may be ultimately regulated by two peptides, one excitatory and one inhibitory, produced and released in very specific and localized areas of the hypothalamus. These neuropeptides could be released from nerve terminals in the median eminence, and descend the pituitary stalk in the hypophyseal portal circulation to perfuse and influence the somatotropic cells of the anterior pituitary gland (Harris, 1955). However, the nature of these factors had yet to be elucidated.

Somatostatin (SRIF)

In 1968, while searching for the distribution of GH-releasing factors (GRF's) in brain, Krulich & McCann (1969) reported the discovery of an endogenous factor that surprisingly inhibited GH release. However, it was not until the early 1970's that somatostatin (somatotrophin release-inhibiting factor; SRIF), a 14 amino acid peptide, was isolated and characterized from ovine hypothalami on the basis of its ability to inhibit GH release from pituitary cells in culture (Brazeau *et al*, 1973). It soon became evident that SRIF was a potent inhibitor of GH release to virtually all known stimuli, both *in vivo* and *in vitro*, in a wide variety of species including human (Martin *et al*, 1975). SRIF was also shown to inhibit the release of thyrotropin (Vale *et al*, 1975; Weeke *et al*, 1975; Chihara *et al*, 1978), insulin and glucagon (Alberti *et al*, 1973; Koerker *et al*. 1974; Gerich, 1981; Miller, 1981), and gastric acid (Mogard *et al*, 1985) release. Although SRIF was originally described as a single cyclic tetradecapeptide, current evidence in mammals, and particularly in the rat, points to a single pre-prosomatostatin gene which is enzymatically cleaved to yield four distinct peptides (Goodman *et al*, 1983; Montminy *et al*, 1984). These include the originally identified SRIF-14, an amino-terminal-extended somatostatin-28 (SRIF-28), SRIF-$28_{(1-12)}$, and prosomatostatin (pro-S), a 92-amino acid peptide containing the SRIF-28 sequence at the C-terminus (Pradayrol *et al*, 1980; Benoit *et al*, 1985). SRIF-14 and SRIF-28 are the two principal bioactive forms. Although SRIF-28 is reported to be more potent and longer acting in suppressing pituitary hormone release (Brazeau *et al*, 1981; Tannenbaum *et al*, 1982), extracts of normal rat hypothalamus contain SRIF-14 and SRIF-28 in the approximate molar ratio 15:4 (Patel *et al*. 1981; Charpenet & Patel, 1985).

Immunocytochemical studies have shown that SRIF cells are widely distributed throughout the body of vertebrates. Within the nervous system, somatostatinergic neurons are found in the anterior pituitary regulating system (hypothalamus), the brain stem, the spinal cord and the limbic system, as well as throughout the cerebral cortex (Krisch, 1978; Bennett-Clarke *et al*, 1980; Beal *et al*, 1986; Reubi *et al*, 1986). Within the hypothalamus, the most prominent collection of somatostatin-positive nerve cell bodies

is in the anterior periventricular region (Alpert *et al*, 1976; Krisch, 1978; Finley *et al*, 1981; Johansson *et al*, 1984). These cells project primarily to the median eminence (termed the tuberoinfundibular system) and account for approximately 80% of SRIF immunoreactivity in the hypothalamus (Patel *et al*, 1979; Critchlow *et al*, 1981). A small contingent of axons from the periventricular nucleus also projects to other areas, both within the hypothalamus and to extrahypothalamic regions. SRIF-immunoreactive cells have also been found, in lower quantity, in the paraventricular, arcuate, ventromedial and suprachiasmatic areas of the hypothalamus (Dierickx & Vandesande, 1979; Krisch, 1979; Finley *et al*, 1981).

SRIF induces its biological actions by interacting with high affinity membrane bound receptors. These receptors are coupled by pertussis toxin-sensitive G proteins to adenylate cyclase (Jakobs *et al*, 1983; Mahy *et al*, 1988) and ion gaited channels (Ikeda & Schonfield, 1989; Wang *et al*, 1989; 1990). Consequently, SRIF has been shown to be a potent inhibiter of adenylate cyclase and Ca^{2+} conductance, and can potentiate K^{+} currents.

As described previously, SRIF's role in suppressing GH release had been suggested from early anatomical studies of somatostatinergic input to the anterior pituitary. Subsequent physiological studies concentrated directly on SRIF's role in controlling the pulsatile pattern of GH release. Passive immunization of rats with SRIF antiserum was shown to result in a significant augmentation of basal GH levels, while not affecting the GH pulses (Ferland *et al*, 1976; Steiner *et al*, 1978; Terry & Martin, 1981; Tannenbaum, 1988). These findings provided evidence that SRIF is released episodically into the hypophyseal portal circulation to inhibit GH release and therefore acts as a physiologic regulator of the GH trough periods. Furthermore, the preservation of the spontaneous GH pulses indicated that a stimulatory GH-releasing factor may also be released episodically from the hypothalamus.

Growth Hormone-Releasing Factor (GRF)

Although the existence of a stimulatory hypothalamic hormone for GH secretion

had been well recognized, the identity of this factor had eluded investigators for over a decade. The breakthrough ultimately came from clinical studies with the discovery of a link between carcinoid tumors and acromegaly and the theory that the tumors had stimulatory effects on GH secretion (Buse *et al*,1961; Dabek, 1974). Further studies of carcinoid tissue extracts demonstrated a dose-dependent GH-releasing activity in rat pituitary cell cultures (Saeed *et al*, 1979; Shalet *et al*, 1979). It wasn't until 1982 that GH-releasing factor (GRF) was finally isolated and sequenced by two separate groups (Rivier *et al*, 1982; Guillemin *et al*, 1982) using excised tissue extracts from two patients with pancreatic islet adenomas (Thorner *et al*, 1982; Sassolas *et al*, 1983). From these investigations, three forms of human GRF were identified: $GRF_{1-44}NH_2$, $GRF_{1-40}OH$, $GRF_{1-37}NH_2$. The 44-amino acid peptide is believed to be the native structure of GRF (Ling *et al*,1984). Spiess *et al*,(1983) later isolated and described the 43-amino acid rat GRF (rGRF). All these peptides exhibit potent GH-releasing activity, both *in vitro* (Brazeau *et al*, 1982; Vale *et al*,1983) and *in vivo* (Wehrenberg *et al*, 1982a; Chihara *et al*, 1983; Szabo *et al*, 1983; Gelato *et al*, 1983; Thorner *et al*, 1983; Tannenbaum *et al*, 1983). GRF mediates these effects by activating the adenylate cyclase-cAMP system in the somatotrophs, with intracellular cAMP accumulation occurring within five minutes (Frohman & Jansson, 1986).

As mentioned earlier, even prior to the identification of the GRF peptide, its localization and distribution within the hypothalamus had been predicted through the use of lesion and stimulation studies. Immunocytochemical studies subsequently confirmed the localization of GRF-positive cells (Bloch *et al*,1983; Ling *et al*, 1984). The majority of GRF-containing perikarya in the human and in other primates are in the mediobasal hypothalamus, particularly in the arcuate and ventromedial nuclei. These nerve fibres project primarily to the median eminence to terminate onto capillaries forming the hypothalamic-pituitary portal circulation (Jacobowitz *et al*, 1983; Brazeau *et al*, 1983; Bloch *et al*, 1984). GRF-positive cells have also been identified along the wall of the third ventricle, and anteriorly at the optic chiasm. In the rat, the distribution of GRF immunoreactivity is similar to that in the primate (Jacobowitz *et al*,1983).

The contribution of GRF to the generation of GH secretory pulses has been examined using the technique of passive immunization (Wehrenberg *et al*,1982b; Miki *et al*, 1984; Painson and Tannenbaum, 1991). Immunoneutralization with anti-GRF serum virtually obliterated the spontaneous GH pulses, providing further support for GRF's role in controlling the episodic bursts of GH.

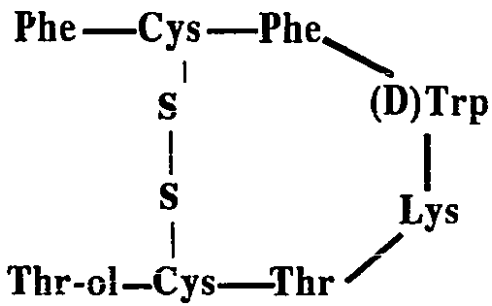
SRIF Analog SMS 201-995 (Octreotide)

Because of its diverse physiologic effects, the use of native SRIF has been extensively investigated for clinical treatment of a variety of pathophysiological states associated with hypersecreting endocrine tumors, such as gastrinomas, insulinomas, glucagonomas, and vipomas (Mogard *et al*, 1985; Kitson *et al*, 1980; Reichlin, 1983; Rene & Bonfils, 1984). In addition, when given to patients with type I diabetes, SRIF lowers blood glucose levels as well as the degree of ketosis after insulin withdrawal (Gerich, 1981; Gottesman *et al*, 1982; Johnson & Bressler, 1984). However, despite its beneficial usefulness, the clinical value of native SRIF is severely limited by two main disadvantages. First, due to a rapid clearance rate by tissue and plasma peptidases (Griffiths *et al*, 1977; Sheppard *et al*, 1979) SRIF has a very short half-life of 2-3 minutes, necessitating continuous intravenous infusion. Secondly, cessation of SRIF infusion is characterized by a rebound hypersecretion of GH, both *in vitro* (Cowan *et al*, 1983; Kraicer *et al*, 1986; Kraicer *et al*, 1988) and *in vivo* (Clark *et al*, 1988; Sugihara *et al*, 1989).

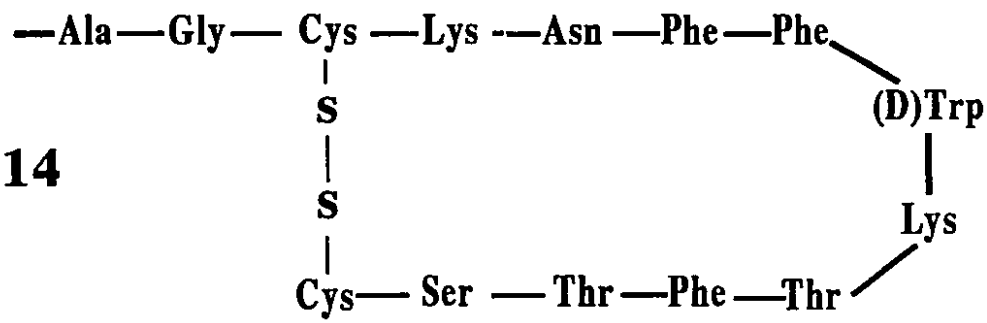
In 1980, scientists at Sandoz Ltd, Basel, Switzerland, developed an 8 amino acid peptide sharing homology with a 4 amino acid sequence in SRIF-14 (see Fig. i)(Pless *et al*, 1986). It was found to be a mimetic of SRIF, exhibiting potent biological effects similar to native SRIF but without the drawbacks. Octreotide (also referred to in the literature as Sandostatin, SMS 201-995) is more resistant to metabolic degradation resulting in a prolonged elimination half-life and therefore providing a much longer duration of activity. Octreotide behaves similarly to native SRIF in its ability to inhibit the release of thyrotropin, insulin, glucagon, and gastrin in normal man (Davies *et al*,

Fig. i. Amino acid composition of Octreotide and SRIF-14

Octreotide



SRIF-14

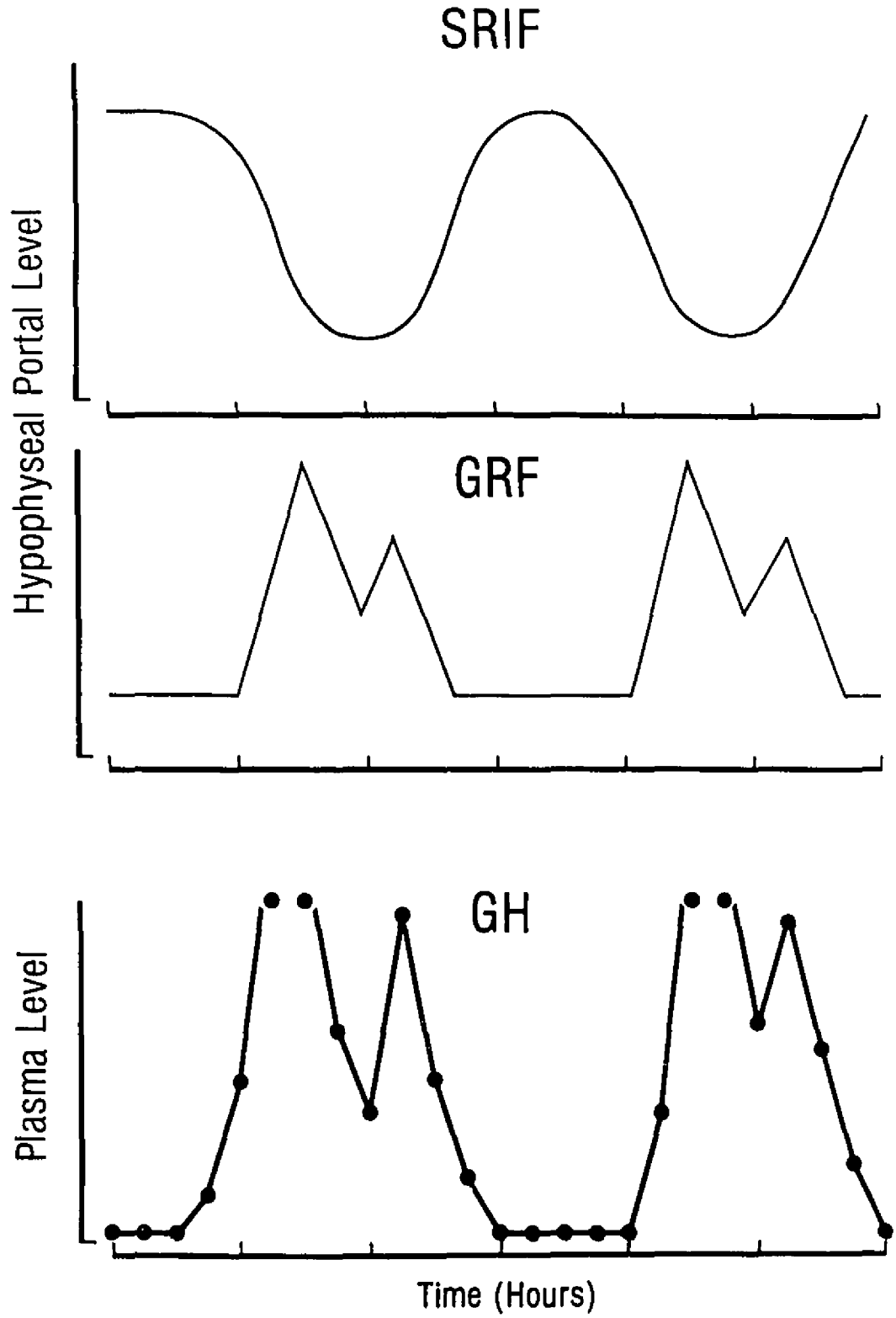


1986; Del Pozo *et al*, 1986; Masuda *et al*, 1989). Consequently, octreotide is currently used in the treatment of a variety of peptide secreting tumors of the gut and pancreas (Clements & Elias, 1985; Kraenzlin *et al*, 1985; O'Dorisio, 1986; Anderson & Bloom, 1986; Vinik *et al*, 1986; Santangelo *et al*. 1986; Longnecker, 1988). Octreotide is more selective and is 70 times more potent than native SRIF-14 for inhibiting GH secretion in the human (Pless *et al*, 1986), thus octreotide has proven to be particularly beneficial in the treatment of acromegaly (Plewe *et al*, 1984; Ch'ng *et al*, 1985; Lamberts *et al*, 1985; Barnard *et al*, 1986). Finally, a rebound hypersecretion does not occur following the administration of the drug, once its inhibitory effect lingers off (Lamberts *et al*, 1985; del Pozo *et al*, 1986).

SRIF/GRF Interactions at the Level of the Pituitary

Individually, the roles of GRF and SRIF in regulating pituitary GH release are well established. In addition, a large body of evidence indicates that SRIF can interact directly with GRF at the level of the pituitary gland to influence GH secretion. Numerous studies have repeatedly documented that SRIF, in fact, antagonizes GRF's stimulatory action on pituitary GH release, both *in vitro* (Brazeau *et al*, 1982; Vale *et al*, 1983; Lamberts *et al*, 1984) and *in vivo* in the rat (Tannenbaum *et al*,1983) and human (Losa *et al*, 1985). When Tannenbaum & Ling (1984) administered exogenous GRF injections to male rats, they demonstrated that GRF-induced GH release was greater during a time of a spontaneous GH peak than during a GH trough period. The finding that this cyclic response was subsequently eliminated following the immunoneutralization of endogenous circulating SRIF provided testimony that i) hypothalamic SRIF release was indeed episodic in nature and ii) the presence of high circulating SRIF during the GH trough period attenuates GH responsiveness to GRF. These various results have led to an overall theory that, in the male rat, SRIF and GRF are released in reciprocal 3-4 h cycles (about 180° out of phase) from the median eminence into the hypophyseal portal blood and act upon the pituitary somatotropes to generate the ultradian rhythm of GH secretion that is observed in the circulation (Tannenbaum & Ling, 1984)(see Fig. ii). This hypothesis was

Fig. ii. Schematic representation of the rhythmic secretion of SRIF and GRF into the hypophyseal portal blood with the net result on GH secretion.


SRIF
Hypophyseal Portal Level
GRF
GH
Plasma Level
Time (Hours)

subsequently confirmed by Plotsky and Vale (1985) who measured immunoreactive levels of SRIF and GRF directly in rat hypophyseal portal blood.

An episodic pattern of hypothalamic SRIF secretion was further supported by studies examining the effects of continuous GRF delivery to the pituitary. In both the male rat (Tannenbaum *et al*, 1990b) and human (Vance *et al*, 1985), constant GRF infusions resulted in a striking augmentation in both GH pulse amplitude and GH trough value, while preserving the ultradian rhythm of GH secretory bursts. In the rat, this effect was abolished following the administration of anti-SRIF serum resulting in tonically high levels of plasma GH with no distinct GH peaks or troughs.

Despite convincing evidence of SRIF's and GRF's episodic pattern of secretion and its importance in regulating pituitary GH release, the nature of the interplay between these two hypothalamic peptides in controlling GH secretion is far from clear.

Earlier studies investigating SRIF's effect on GH responsiveness to GRF demonstrated that continuous infusion of GRF causes a gradual desensitization of GRF-induced GH release, both *in vitro* (Badger *et al*, 1984; Bilezekjian & Vale, 1984; Bilezekjian *et al*, 1986; Ceda & Hoffman, 1985; Fukata *et al*. 1985) and *in vivo* (Wehrenberg *et al*, 1984; Wehrenberg *et al*, 1986), an effect prevented by prior exposure to SRIF (Losa *et al*, 1985; Clayton & Bailey, 1987; Soya & Suzuki, 1988; Soya & Suzuki, 1990). Abrupt withdrawal of SRIF in the rat leads to a significant rebound secretion of GH in a variety of experimental paradigms, such as cultured perifused pituitary cells (Cowan *et al*, 1983; Kraicer *et al*, 1986). Furthermore, *in vitro* studies of rat anterior pituitary cells have suggested that pretreatment with SRIF enhances GRF-induced GH secretion (Weiss *et al*, 1987; Richardson & Twente, 1991; Richardson & Twente, 1993). Thus, SRIF may not only be inhibitory to GH release, but that under certain temporal conditions, SRIF may possess a positive influence on pituitary somatotrophs.

Despite these *in vitro* findings, there is still a paucity of data *in vivo* regarding the temporal interactions of SRIF and GRF in GH regulation. In one study, Clark and Robinson (1988) demonstrated that when SRIF was injected in an intermittent fashion in

female rats, there was a significant increase in the rate of weight gain and pituitary GH content. In another study, Tannenbaum *et al*, (1989) used the starved rat as a model of high circulating SRIF levels, and found a paradoxical enhancement in GH responsiveness to GRF, although the mechanism is not known. Moreover, because numerous endocrine and metabolic changes are associated with nutritional deprivation, it is conceivable that the effect they observed *in vivo* was not directly related to changes in plasma SRIF levels.

GRF/SRIF Interactions at the Level of the CNS

An abundance of physiological evidence suggests that, in addition to the GRF/SRIF interplay at the level of the pituitary, these two neuropeptides may interact within the CNS to regulate hypophyseal GH secretion. Initial studies demonstrated that the intracerebroventricular (icv) administration of SRIF resulted in a paradoxical increase in plasma GH, a response postulated to be mediated, in part, through SRIF autoreceptors (Abe *et al*,1978; Lumpkin *et al*, 1981; Tannenbaum & Patel, 1986). This raised the possibility that SRIF may regulate its own release via an ultrashort feedback loop.

In addition, numerous studies have suggested that SRIF may play a direct role in regulating the release of hypothalamic GRF. The rebound secretion of GH generated by the abrupt withdrawal of SRIF is abolished following the administration of GRF antiserum, suggesting an active role for hypothalamic GRF (Clark *et al*, 1988; Miki *et al*, 1988; Sugihara *et al*, 1989; Okada *et al*, 1991). Rats depleted of endogenous hypothalamic SRIF through lesions to the medial preoptic area (Katakami *et al*, 1988), or via the administration of the thiol agent cysteamine (Tannenbaum *et al*, 1990; Bertherat *et al*, 1991) demonstrated a striking increase in both GRF immunoreactivity, and GRF mRNA in the arcuate nucleus. This hypothesis is further supported by the finding that immunoneutralization of central SRIF results in an increase in the GRF concentration in hypophyseal blood during trough periods (Plotsky & Vale, 1985). Moreover, SRIF administration was found to directly inhibit GRF release, both in a cell dispersion system (Yamauchi *et al*, 1991), as well as into the hypophyseal portal blood of conscious sheep (Guillaume *et al*, 1992). Furthermore, stimulation of the periventricular nucleus was found

to inhibit arcuate neurons. These findings provided strong physiological and morphological evidence to support the concept of a SRIF-mediated central influence on the release of hypothalamic GRF. Such an interaction between SRIF and GRF may play an important role in regulating the secretion of GH. However, the nature of this communication has not yet been fully defined.

In order to confirm such an association between SRIF and GRF, anatomical evidence must be established. Recent anatomical data suggest that the influence of SRIF on GRF release may be exerted through a direct action of SRIF onto GRF-containing neurons within the arcuate nucleus. First, the arcuate nucleus has been shown to contain dense networks of SRIF-immunoreactive neurons (Dierickx & Vandesande, 1979; Makara *et al*, 1983; Johansson *et al*, 1984) as well as a dense network of SRIF-positive axon terminals (Kawano *et al*,1982; Willoughby *et al*,1989), some of which are in direct contact with GRF-immunoreactive cell bodies in the arcuate nucleus (Daikoku *et al*,1988; Liposits *et al*,1988; Horvath *et al*,1989).

In order to fully substantiate the theory of a central influence of SRIF on GRF-containing neurons within the hypothalamus, receptor sites for the peptide need to be established. Early autoradiographic studies have reported the distribution of SRIF binding sites in widespread areas of the rat (Leroux & Pelletier, 1984; Leroux *et al*, 1985; Reubi & Maurer, 1985), and human (Reubi *et al*, 1986) brain, including the hypothalamus (Leroux *et al*, 1988; Najimi *et al*, 1991). More recently, combined autoradiographic/immunocytochemical (McCarthy *et al*, 1992) or autoradiographic/*in situ* hybridization (Bertherat *et al*, 1992) experiments have, respectively, obtained evidence for the presence of ^{125}I-SRIF binding sites on a subpopulation of arcuate neurons that contain and express GRF. While these anatomical findings provide strong support for the concept of a central influence of SRIF on pathways associated with the synthesis and/or release of GRF, their physiological implications in controlling the secretion of pituitary GH are far from clear.

SRIF Receptors

The diverse actions of SRIF are mediated through specific high affinity membrane-bound receptors on target cells. Using radioligand binding studies with ^{125}I-tyr^{11}SRIF, SRIF binding sites were first identified in the GH_4C_1 pituitary cell line (Schonbrunn & Tashjian, 1978). Soon after, SRIF binding sites were found in several mammalian target tissues, such as pancreatic cells (Sakamoto *et al*, 1984; Srikant & Patel, 1986), the gut (Del Valle *et al*, 1990), and particularly the CNS (Reubi *et al*, 1981,1986; Srikant & Patel, 1981; Beal *et al*, 1986) where autoradiographic studies have demonstrated that their distribution in the cortex, limbic system, hypothalamus and brainstem correlated closely with immunohistologically localized SRIF neuropeptides (Leroux & Pelletier, 1984; Tran *et al*, 1984; Uhl *et al*, 1985).

Following the discovery of multiple circulating forms of native SRIF (SRIF-14, SRIF-28, etc), it became apparent, through autoradiography, that SRIF receptors comprised a more heterogenous group. The distribution of radiolabeled SRIF-14 or SRIF-28 binding sites showed small, yet distinct, variations within the CNS, particularly in the hypothalamus (Leroux & Pelletier, 1984; Leroux *et al*. 1985;Patel *et al*, 1986). Subsequently, the specific SRIF analog, octreotide, was used in competition studies and further demonstrated the existence of multiple SRIF receptors in the CNS (Reubi, 1984; Tran *et al*, 1985). While octreotide biphasically inhibited radiolabeled SRIF-14 binding in the CNS, illustrating the presence of at least two distinct binding sites, the pituitary appeared to express only a single SRIF receptor.

However, the use of native SRIF and octreotide as specific ligands to identify receptor subtypes was limited. With the advent of more selective and more metabolically stable SRIF analogs, studies were undertaken to define and characterize these receptors more fully. The potent cyclohexapeptide, MK 678, made it possible to distinguish two specific SRIF receptor subtypes in the CNS, referred to as $SRIF_1$ and $SRIF_2$ (Raynor *et al*. 1992). While $SRIF_1$ possessed high affinity for MK 678, $SRIF_2$ showed no significant affinity for the analog. Further pharmacological studies revealed that $SRIF_1$ interacts selectively with G protein α subunits in brain and pituitary cells (Law *et al*, 1991) which

couple selectively the $SRIF_1$ receptor to adenylate cyclase (Tallent & Reisine, 1992).

In 1992, Yamada *et al.* succeeded in cloning the human and mouse genes for two SRIF receptor subtypes, referred to as SSTR1 and SSTR2, thus confirming the presence of more than one SRIF binding site. SSTR1, a 391 amino acid protein, was isolated from among a group of G-protein-coupled receptor sequences from human pancreatic islet cDNAs. Its identification was confirmed through its ability to specifically bind ^{125}I-Tyr^{11}-SRIF with high affinity. Human and mouse SSTR2 were subsequently revealed by screening corresponding genomic libraries with SSTR1 (Yamada *et al*, 1992a). Recently, it has been demonstrated that two variants of SSTR2 exist: SSTR2A (2.8 kb) and SSTR2B (2.4 kb)(Vanetti *et al.*1992; Patel *et al*, 1993). Shortly thereafter, SSTR1 and SSTR2 in the rat were cloned (Meyerhof *et al*, 1991, Li *et al.* 1992; Kluxen *et al*, 1992).

To determine the functional and pharmacological properties of both cloned receptors, genomic fragments of the two isoforms were transfected into cultured cells and studied using radioligand binding techniques. Both receptor subtypes are coupled to inhibition of adenylate cyclase via pertussis toxin-sensitive G-proteins (Hershberger *et al.* 1994), and both bind SRIF-14 and SRIF-28 with high affinity. However, specific differences have been noted in competition studies using SRIF analogs (Rens-Domiano *et al*, 1992). While SSTR2 shows high affinity for both SMS 201-995 and MK 678, SSTR1 has little or no affinity for either analog. In view of these pharmacological findings, it has been proposed that SSTR2 and SSTR1 may, in fact, correspond to the $SRIF_1$ and $SRIF_2$ family of receptors discussed earlier (Rens-Domiano *et al*, 1992).

As a result of the cloning of the SSTR1 and SSTR2 receptors, the pattern of expression has been extensively investigated in the human, rat and mouse to determine the specific location of somatostatin receptor-synthesizing neurons (Yamada *et al*, 1992a; Meyerhof *et al*, 1991; Breder *et al*, 1992; Bruno *et al.* 1993). SSTR1 has its highest levels of expression in the human stomach and jejunum, as well as in the brain (cortex, hypothalamus, brainstem, hippocampus) of the mouse and rat. Moderate levels are also found in the spleen and pituitary. SSTR2, on the other hand, is primarily localized in the human brain and kidney, while in the rat and mouse, it is found in high concentrations

in the brain (cortex, amygdala, hippocampus, hypothalamus) and pituitary. In the rat, the patterns of SSTR1 and SSTR2 show considerable overlap in the hypothalamus, with the most heavily expressed regions being located in those neurons known to contain and express SRIF and/or GRF; that is, the arcuate, periventricular, medial preoptic, and ventromedial nuclei (Beaudet *et al*, 1994). These findings support the notion that SRIF's role in the regulation of pituitary GH secretion at the level of the CNS may be mediated, in part, through both SSTR1 and SSTR2 receptor subtypes.

Following the cloning of these first two SRIF receptor subtypes, three more isotypes have since been cloned, referred to as SSTR3 (Yasuda *et al*, 1992b), SSTR4 (Bruno *et al*, 1992), and SSTR5 (O'Carroll *et al*, 1992). SSTR3 differs from SSTR1 and SSTR2 in that it is larger than both, at 428 amino acids. However, it shares certain aspects of their pharmacological properties in that it demonstrates low affinity for MK 678 and SMS 201-995, similar to SSTR1, and its action is mediated primarily though its coupling with adenylyl cyclase, a major effector system for SRIF (Yamada *et al*, 1992b; Yasuda *et al*, 1992). The distribution of this third receptor subtype is also widespread, with the highest level of expression located in the cortex, hippocampus, hypothalamus and cerebellum of the mouse and rat (Yasuda *et al*, 1992; Kong *et al*, 1994), as well as peripherally in the liver, stomach, kidney and pituitary of the rat (Bruno *et al* 1993).

SSTR4 (383 amino acids) and SSTR5 (384 amino acids) are the most recent SRIF receptor clones and have been detected primarily in the brain and pituitary, respectively (O'Carroll *et al*, 1992; Bruno *et al*. 1992,1993).

In comparing all five currently characterized SSTR sequences, there is an over 90% identity in amino acid sequence between the same SSTR in different species (Raynor *et al*, 1992), whereas within the same species, there is between 35 and 60% homology between the different SSTR's. All five isoforms bind with high affinity to both SRIF-14 and SRIF-28, and exist in a configuration characteristic of G-protein coupled receptors; they all contain seven putative membrane-spanning domains, the third intracellular loop believed to be the site of G protein interaction (Probst *et al*, 1992).

While the anatomical data concerning these SRIF receptors is rapidly

accumulating, nothing is known about the biological role and the physiological regulation of these receptor subtypes. A major mechanism of regulating the effect of a hormone is through its direct influence on its own receptors. While this phenomenon had been described for other hormonal systems, such as insulin, GH, and TSH (Kahn *et al*, 1973; Lesniak & Roth, 1976), studies on the kinetics of SRIF binding sites have provided conflicting results. With the discovery of the 5 cloned SRIF receptor subtypes, this issue can now be more fully investigated.

II. AIM OF PRESENT INVESTIGATION

The aim of the present study is 1) to further elucidate the nature of the temporal pattern of SRIF/GRF signalling in determining GH responsiveness to GRF *in vivo*, and 2) to define possible mechanisms of action of SRIF in generating the ultradian rhythm of GH secretion. Our working hypothesis is that SRIF preexposure will promote the accumulation of GH in a readily releasable pool so that GRF challenges will exert an accentuated effect on pituitary somatotrophs. It is also possible that the chronic exposure to high SRIF levels will induce a downregulation of SRIF receptors as well as secondary disinhibition of GRF neurons due to desensitization of SRIF receptors. In order to test these hypotheses, a combined physiological/neuroanatomical approach will be taken. In the physiological studies, we will examine the temporal nature of the SRIF/GRF interaction *in vivo* in two animals models, the normal adult male rat, and the mutant dwarf strain. Using chronically cannulated free moving male rats, the first set of experiments will involve the i.v. administration of the long-acting SRIF analog, octreotide (or normal saline as controls), to examine its time course of inhibition on *in vivo* GH release. Next, we will assess the effects of octreotide preexposure on GH responsiveness to exogenous GRF in rats pretreated will either octreotide or normal saline.

In the second set of physiological experiments, we investigated the mechanism(s) of octreotide's effects on GH responsiveness to GRF. Animals will be pretreated with octreotide or normal saline, using the optimal temporal condition (as defined above), then concomitantly administered GRF and native SRIF-14. Plasma GH levels will be monitored immediately following the simultaneous injections in order to 1) assess the ability of the short acting native SRIF-14 to inhibit GRF-induced GH release, and 2) to examine the postinhibitory rebound release of GH following the SRIF-14-mediated inhibition, to investigate whether preexposure to SRIF accumulates GH in readily releasable pools.

Chapter 2 addresses the neuroanatomical investigation, which will consist of *in situ* hybridization histochemistry of two SRIF receptor subtypes in order to determine

whether SRIF regulates its own receptors in the CNS. Rats will be sacrificed at the optimal time following a single injection of octreotide (or normal saline) to test the effects of SRIF preexposure on SRIF receptor mRNA levels in the arcuate nucleus. Image analysis and quantification will be carried out by computer assisted microdensitometry, using the BIOCOM RAG software program.

The results of these experiments should allow us to 1) more clearly define the important temporal interaction between GRF and SRIF in the control of the pulsatile pattern of GH release, and 2) to elucidate the specific role of SRIF in generating the ultradian rhythm of GH secretion. Results from this investigation may not only be of physiological significance but may also be important in the design of therapy in idiopathic-deficient children.

III. MANUSCRIPT

In Vivo Evidence that Preexposure to Somatostatin Analog Octreotide Enhances Growth Hormone Responsiveness to Growth Hormone-Releasing Factor*

Joel P. TURNER† and Gloria S. TANNENBAUM‡

Departments of Pediatrics, Neurology and Neurosurgery, McGill University; and the Neuropeptide Physiology Laboratory, McGill University-Montreal Children's Hospital Research Institute, Montreal, Québec H3H 1P3, Canada.

*Presented in part at the 22nd Annual Meeting of the Society for Neuroscience, Anaheim, CA, 1992. This work was supported by Grant 911437-102 from the Fonds de la recherche en santé de Québec and Grant MT-6837 from the Medical Research Council of Canada.

†Recipient of Studentship Awards from the Fonds pour la formation de chercheurs et l'aide à la recherche and the Fonds de la recherche en santé du Québec.

‡'Chercheur de Carrière' of the Fonds de la recherche en santé du Québec.

Abstract

While *in vitro* studies suggest that the temporal pattern of hypothalamic somatostatin (SRIF) and GH-releasing factor (GRF) signaling to pituitary somatotrophs may be an important determinant for GH secretion, there is a paucity of data *in vivo* regarding the temporal interactions of SRIF and GRF in GH regulation. In the present study, we used the long-acting SRIF analog, octreotide, as a probe in both the normal and mutant dwarf (dw) rat, to 1) further clarify the temporal nature of the SRIF/GRF interaction *in vivo*, and 2) define possible mechanisms of action of SRIF in generating the ultradian rhythm of GH secretion. Administration of octreotide (25 and 50 μg iv) to free-moving adult male rats resulted in an almost complete attenuation of the spontaneous GH pulses during the initial 3 h after injection, with some recovery of GH pulses evident 3-6 h later. Rats pretreated with two different doses of octreotide (25 and 50 μg iv) and subsequently challenged with GRF (1 μg iv) exhibited a markedly blunted GH response to exogenous GRF at 1 h after treatment. In contrast, preexposure to octreotide for 3 h produced a 2- to 3-fold augmentation in GH responsiveness to GRF (Mean ±SEM GH area under the curve: 25 μg: 4537 ± 428; 50 μg: 3851 ± 367 ng/ml/30 min vs. 2032 ± 490 ng/ml/30 min in controls pretreated with normal saline, $P<0.02$). Examination of the postsuppression rebound release of GH following the concomitant administration of native SRIF-14 (50 μg) and GRF (1 μg) showed that preexposure to octreotide for 3 h resulted in a 14- to 16-fold augmentation in the amount of GH released compared to animals pretreated with normal saline (25 μg: 3673 ± 841; 50 μg: 4099 ± 796 ng/ml/40 min vs. 254 ± 114 ng/ml/40 min; $P<0.001$). In contrast, 3 h preexposure to octreotide (n=6) in a strain of mutant dwarf rats, which shows a selective reduction in GH synthesis and storage, failed to significantly alter GRF-induced GH release (50 μg: 22.0 ± 5.2 vs 13.1 ± 4.7 ng/ml in controls; NS). Taken together, these results demonstrate, that in the normal adult male rat, a 3-h period of exposure to the SRIF analog, octreotide, is sufficient to enhance GH responsiveness to GRF. Our findings suggest that this effect is due to a SRIF-mediated build-up of pituitary GH stores in a readily releasable pool thereby preparing the somatotroph to respond optimally to GRF. Such a temporal interaction between the two neuropeptides may be important for optimizing pulsatile GH secretion and, ultimately, somatic growth.

Introduction

It is well established that the ultradian rhythm of GH secretion from the pituitary gland is generated by an interaction of two hypothalamic hormones, the stimulatory GH-releasing factor (GRF) and the inhibitory hormone somatostatin (SRIF) (see 1 for review). In the male rat, both neuropeptides are released rhythmically from the median eminence into the hypophyseal portal circulation, in reciprocal 3- to 4-hour cycles, to act upon pituitary somatotrophs (2, 3). Passive immunization studies have demonstrated that the episodic, high-amplitude bursts of GH are due to the stimulatory influence of GRF (4-6), whereas SRIF is the physiological regulator of the GH trough periods (7-10). In addition, SRIF has been shown to exert potent inhibitory actions on GRF-stimulated GH release, both *in vitro* (11,12) and *in vivo* (2, 13).

Despite convincing evidence that GRF and SRIF, individually, play crucial roles in GH regulation, the nature of the interplay between these two hypothalamic peptides in controlling pituitary GH release is far from clear. In recent years, *in vitro* studies of rat anterior pituitary cells have suggested that SRIF may not only be inhibitory to GH secretion, but that under certain temporal conditions SRIF may act in a positive manner to influence pituitary GH release (14-18). There is a paucity of data *in vivo* regarding the temporal interactions of SRIF and GRF in GH regulation. In one study, Clark & Robinson (19) reported that the infusion of SRIF in a patterned rhythm produced a paradoxical growth-promoting effect in female rats (19). In another study, we used the starved rat as a model of high SRIF tone and found a paradoxical enhancement of GH responsiveness to exogenous GRF (20), however, the mechanism remains unknown. Moreover, because nutritional deprivation is associated with alterations in several endocrine and metabolic parameters, it is conceivable that the *in vivo* effects we observed in that study could be accounted for by variables other than SRIF.

In the present investigation, therefore, we used the more specific, long-acting SRIF analog, octreotide, as a probe in both the normal adult rat and in the mutant dwarf (dw) rat, to 1) further elucidate the temporal nature of the SRIF/GRF interaction in GH

regulation *in vivo* and 2) define possible mechanisms of action of SRIF in generating the striking ultradian rhythm of GH secretion characteristic of the male rat.

Materials and Methods

Animals and experimental procedure

Normal adult male Sprague-Dawley rats (285-330 g) were obtained from Charles River Canada (St. Constant, Quebec, Canada). Adult homozygous male dwarf (*dw*) rats (175-225 g) were a gift from Dr. Ross Clark (Genentech Inc., South San Francisco, CA); this recently discovered mutation, inherited as an autosomal recessive, arose spontaneously in a breeding colony of Lewis rats (21).

All animals were individually housed on a 12-h light, 12-h dark cycle (lights on at 0600 h) in a temperature (22 ± 1 C)- and humidity-controlled room. Chronic intracardiac venous cannulae were implanted under sodium pentobarbitol (50 mg/kg ip) anesthesia using a previously described technique (22). After surgery, the rats were placed directly in isolation test chambers with Purina rat chow (Ralston-Purina, St. Louis, MO) and tap H_2O available *ad libitum* until their body weights returned to preoperative levels (5-7 days for normal rats; 10-14 days for *dw* rats). All animal-based procedures were approved by the McGill University Animal Care Committee.

On the day of the experiments, food was removed 1.5-2 h prior to the start of sampling while H_2O was continuously available. Beginning at 1000 h, blood samples (0.3 ml) were withdrawn every 15 min for periods of up to 6 h. All blood samples were immediately centrifuged and the plasma was separated and stored at -20 C for subsequent assay of GH. To prevent hemodynamic disturbance, the red blood cells were resuspended in normal saline and returned to the animal after removal of the next blood sample.

In the first experiment, we examined the time course of effects of octreotide (kindly provided by Sandoz Canada Inc., Dorval, Quebec) on spontaneous pulsatile GH secretion in the normal rat. Three groups of free-moving chronically cannulated rats were i.v. administered either 0.2 ml normal saline (n=6) or octreotide, in doses of 25 (n=6) and 50 (n=6) µg per 0.2 ml normal saline, after the first blood sample at 1000 h, and

sequential blood samples were obtained until 1600 h.

In the second experiment, we assessed the temporal interaction of octreotide pretreatment and GH responsiveness to exogenous GRF. Three groups of normal rats were i.v. injected with either normal saline (n=10) or the two doses of octreotide (25 µg: n=6; 50 µg: n=9) at 1000 h, and were subsequently challenged with 1 µg rat GRF(1-29)NH_2 i.v. at 1 and 3 h after octreotide administration. The GRF peptide (lot no. CH-23-25-31-10-16, kindly provided by Dr. Paul Brazeau, Notre Dame Hospital, Montreal, Quebec, Canada) was diluted in 0.3 ml normal saline just prior to use. To document the rapidity of the GH response to GRF, an additional blood sample was taken 5 min after each injection of the peptide.

In the third experiment, designed to elucidate the mechanism(s) involved, native SRIF-14 (50 µg) (code no. 8001; Peninsula Laboratories Inc, Belmont, CA) was co-administered with GRF (1 µg) i.v. at 1300 h, to three groups of normal rats pretreated with either normal saline (n=6) or the two doses of octreotide (25 µg: n=6; 50 µg: n=6) 3 h earlier. We assessed both the SRIF-14-mediated inhibition of GRF-induced GH release at 5 min post-injection and the post-suppression rebound release of GH 5 to 45 min after GRF/SRIF-14 co-administration. GRF (1 µg i.v.) was also injected alone at 1 h after octreotide administration.

Finally, in the last two experiments, *dw* rats were subjected to similar experimental paradigms as those described above for the normal rats. First, two groups of *dw* rats were either injected with 50 µg i.v. octreotide (n=9) or not (n=6) at 1000 h, and were subsequently challenged with GRF (1 µg i.v.) 1 and 3 h later. Second, GRF (1 µg i.v.) was administered alone at 1100 h, and then in combination with SRIF-14 (50 µg i.v.) at 1300 h, to two groups of *dw* rats pretreated at 1000 h with either normal saline (n=6) or 50 µg octreotide (n=6).

GH assay

Plasma GH concentrations were determined in duplicate by double antibody RIA using materials supplied by the NIDDK Hormone Distribution Program (Bethesda, MD). The average plasma GH values are reported in terms of the rat GH reference preparation

(rGH RP-2). The minimal detectable level of the GH RIA was 0.62 ng/ml and the standard curve was linear between 0.62 and 320 ng/ml. All samples with values over 320 ng/ml were reassayed at dilutions ranging from 1:2 to 1:10. The intra- and interassay coefficients of variation were 6.6% and 9.5 %, respectively, for duplicate samples of pooled plasma containing a mean GH concentration of 8.3 ng/ml.

Statistical analysis

Statistical analysis across the three groups was performed by one-way ANOVA. Student's unpaired and paired t-tests were used for comparisons between and within experimental groups, respectively. The integrated area under the GH response curves was calculated using the linear trapezoidal method. The results are expressed as the mean ± SE. *P* values below 0.05 were considered significant.

Results

Time course of effects of octreotide on spontaneous GH secretion in normal rats

Free-moving control rats treated with normal saline exhibited the typical pulsatile pattern of GH secretion characteristic of the male rat (Fig. 1A). Individual plasma GH profiles showed two distinct episodes of GH secretion during the 6-h sampling period with most peak GH values greater than 125 ng/ml; in the intervening trough period plasma GH levels were generally undetectable (<1.2 ng/ml). Administration of octreotide, at both doses, resulted in a near elimination of the spontaneous GH pulses during the first 3 h after injection (Fig. 1, B and C). Both GH peak amplitude and the GH integrated area under the curve (AUC) were severely reduced compared to normal saline-treated controls (Fig. 2). Some recovery of GH pulses was evident 3-6 h after octreotide injection in the two groups (Fig. 1, B and C); both GH peak amplitude and GH AUC increased significantly during this time period compared to the 0-3 h interval (Fig. 2). Overall, both octreotide-treated groups exhibited a marked reduction in mean 6-h plasma GH levels (25 μg: 15.1 ± 2.0; 50 μg: 11.4 ± 1.6 *vs.* 35.3 ± 3.4 ng/ml in normal saline controls; $P<0.01$). There were no significant dose-related differences between the two

octreotide-treated groups.

Temporal effects of octreotide on GH responsiveness to GRF in normal rats

Figure 3 illustrates individual representative plasma GH responses to GRF administered 1 and 3 h after pretreatment with either normal saline or the two different doses of octreotide. Normal saline-treated control animals exhibited the typical male-like time-dependent pattern of GH responsiveness to GRF (2), with high GRF-induced GH release observed at the time of a spontaneous peak (1100 h) and only a limited response during the trough period (1300 h) (Fig. 3A). In contrast, animals pretreated with octreotide displayed a reversed pattern of GH responsiveness to GRF (Fig. 3, B and C). While the GH response to GRF was almost abolished in both octreotide-pretreated groups at 1 h after injection (25 μg: 19.6 ± 7.0; 50 μg: 5.4 ± 1.3 *vs.* 467.2 ± 51.6 ng/ml in normal saline controls; $P<0.001$) (Figs 3, 4), there was a 2-3 fold augmentation in both the GH peak response to GRF, within 5 min after injection (25 μg: 300.0 ± 38.7; 50 μg: 246.1 ± 18.8 *vs.* 148.4 ± 29.3 ng/ml in normal saline controls; $P<0.05$), and in the GH AUC over 30 min (Fig. 4), at 3 h after either dose of octreotide. Again, no significant differences were observed between the two octreotide-treated groups.

Effects of octreotide pretreatment on SRIF-14-induced postinhibitory rebound release of GH in normal rats

GH responsiveness to GRF was again significantly suppressed 1 h after the injection of both doses of octreotide (Fig. 5, B and C) compared to normal saline-treated control rats (Fig. 5A). The administration of SRIF-14 in combination with GRF at 1300 h markedly inhibited GRF's stimulatory action on GH release at 5 min post injection, to a similar degree (78-90%), in all three groups of rats. However, in those animals pretreated with either dose of octreotide, this effective SRIF-14-induced short-acting inhibition was followed by a striking post-suppression rebound release of GH (Fig 5, B and C), compared to the modest GH rebound observed in normal saline-pretreated controls (Fig. 5A).

Analysis of the effects of GRF/SRIF-14 co-administration on GH secretory

dynamics in the 3 groups revealed no significant difference in plasma GH levels at 5 min post injection, regardless of whether the animals were preexposed to normal saline (14.0 ± 6.7 ng/ml) or octreotide (25 µg: 44.8 ± 18.0; 50 µg: 53.8 ± 18.1 ng/ml) (Fig 6). In contrast, examination of the post-inhibitory rebound release of GH 5 to 45 min after GRF/SRIF-14 co-administration showed that a 3-h period of preexposure to either dose of octreotide produced a 14- to 16-fold augmentation in GH release (GH AUC: 25 µg: 3673 ± 841; 50 µg: 4099 ± 796 ng/ml/40 min) compared to control animals pretreated with normal saline (254 ± 114 ng/ml/40 min; $P<0.001$) (Fig. 6).

Effects of octreotide pretreatment on GH responsiveness to GRF in dw rats

Figure 7 illustrates the effects of a 1- and 3-h period of exposure to octreotide on GRF-induced GH release in the *dw* rat. Although plasma GH levels were severely diminished in dw control animals, they were not insensitive to GRF (Fig. 7A); however, their responses were approximately 10-fold lower than those observed in the normal Sprague-Dawley rats (see Fig 3A). Of interest, the *dw* rats still showed a time-dependent pattern of GH responsiveness to GRF, with GRF-induced GH secretion significantly greater at 1100 h than at 1300 h (50.0 ± 10 *vs.* 13.1 ± 4 ng/ml; $P<0.05$) (Fig. 7A).

Pretreatment of *dw* rats with octreotide effectively inhibited GRF-induced GH release at 1 h post treatment (1.2 ± 0 *vs* 50.0 ± 10.3 ng/ml; $P<0.001$) (Fig. 7B). However, in marked contrast to that observed in the normal rats described above, a 3-h period of preexposure to octreotide failed to potentiate GH responsiveness to GRF when compared to untreated control *dw* animals (22.0 ± 5.2 vs. 13.1 ± 4.7 ng/ml; NS).

Effects of octreotide pretreatment on SRIF-14-induced postinhibitory rebound release of GH in dw rats

Analysis of the effects of octreotide pretreatment on GH release following GRF/SRIF-14 co-administration in *dw* rats showed that SRIF-14-induced inhibition of GRF-stimulated GH release, at 5 min post injection, was similar in *dw* rats pretreated with

either 50 µg octreotide or normal saline (Fig. 8). While the SRIF-14-induced postinhibitory rebound release of GH 5-45 min after injection was 2-3 fold higher in *dw* rats pretreated with octreotide, compared to their own normal saline-pretreated controls (Fig. 8), this augmentation was far smaller than the 14-16 fold augmentation observed in the normal Sprague-Dawley rats (see Fig. 6).

Figure 9 provides a comparison of the effects of 3-h preexposure to octreotide (50 µg dose) on GH responsiveness to GRF in both strains of rat. In normal rats, pretreatment with octreotide significantly augmented GRF-induced GH release (AUC over 30 min: 3851 ± 367 *vs.* 2032 ± 490 ng/ml/30 min in normal saline-pretreated controls; $P<0.02$). This effect was further enhanced following the co-administration of GRF/SRIF-14 (6164 ± 1180 ng/ml/30 min; $P<0.05$ compared to octreotide+GRF alone).

In sharp contrast, in *dw* rats, 3-h octreotide pretreatment failed to increase pituitary GH responsiveness to GRF in either experimental paradigm when compared to the untreated control group (Fig. 9). While the postinhibitory rebound release of GH was found to be higher in *dw* rats pretreated with octreotide, when compared to their own normal saline-pretreated control group given GRF/SRIF-14 (see Fig. 8), the total amount of GH released in this group of rats (233.2 ± 26.1 ng/ml/30 min) was, in fact, not significantly different from that observed in either the octreotide-pretreated *dw* rats given GRF alone (235.9 ± 51.0 ng/ml/30 min) or in the untreated control group (154.2 ± 45.0 ng/ml/30 min) (Fig. 9).

Discussion

Octreotide is a long acting SRIF analog which, in the rat, is a more potent inhibitor of GH release than native SRIF-14, with greater selectivity for GH suppression (23). Although octreotide's suppressive effects on both basal and stimulated rat GH release have been well documented *in vitro*, the time course of its inhibitory action on *in vivo* GH release in the normal adult rat is not known. The findings of the present study clearly demonstrate that the i.v. administration of octreotide, at doses of 25 and 50 µg, resulted in an immediate and almost complete attenuation of the spontaneous GH pulses

during the first 3 hours after injection, and plasma GH levels remained significantly suppressed for up to 6 hours. A decrease in octreotide's bioactivity was illustrated by the gradual recovery of plasma GH levels 3-6 hours after injection. On the basis of these findings, we selected a 3 h period of preexposure to octreotide for the ensuing experiments.

Animals preexposed to octreotide showed a reversed pattern of responsiveness to GRF compared to that observed in rats pretreated with normal saline. The exogenous administration of GRF during the period of high octreotide bioactivity (i.e. at 1 hour) resulted in a markedly blunted GH response, consistent with octreotide's known biological action on GRF-induced GH secretion in man (24). However, in marked contrast, preexposure to octreotide for 3 hours produced a 2-3 fold augmentation in pituitary GH responsiveness to the GRF challenge, compared to normal saline pretreated controls, indicating that a 3 hour period of exposure to the SRIF analog was sufficient to enhance the sensitivity of the pituitary somatotroph to the stimulatory actions of GRF. Although this paradoxical stimulatory influence of SRIF on GRF-induced GH secretion parallels our earlier observations in the starved rat model (20), the findings reported here demonstrate for the first time a direct positive effect of SRIF on GH responsiveness to GRF *in vivo*. These *in vivo* results are congruent with previous *in vitro* (14-18) studies showing that preexposing dispersed pituitary cells to SRIF can enhance GH responsiveness to GRF. Taken together, these findings suggest that the temporal pattern of SRIF signalling to pituitary somatotrophs is an important determinant for optimizing pulsatile GH secretion.

Various mechanisms might account for this synergistic interaction between octreotide and GRF. *In vitro* studies have demonstrated that one of the functions of SRIF is to temporarily block the release of stored intracellular GH, but not to prevent GH from accumulating in an immediately releasable pool (25). It is therefore conceivable that the role of octreotide in our experiment is to build up pituitary GH stores and that a GRF challenge 3 hours later will amplify pituitary GH secretion.

Support for this concept comes from our experiments examining the post-suppression rebound release of GH following the concomitant administration of GRF/SRIF-14. Earlier studies have reported that the withdrawal of SRIF induces a

spontaneous release of GH, both *in vitro* (14,26) and *in vivo* (27,28). In the normal saline-pretreated rats of the present study, a limited, yet measurable amount of rebound GH secretion was observed 5-45 min following the SRIF-mediated inhibition of GRF-induced GH release. However, in those animals preexposed for 3 h to octreotide, at both doses, the amount of GH released was 14-16 fold higher, an effect that could not be due solely to the withdrawal of SRIF. This episode is most likely due to a continual accumulation of GH in a readily releasable pool over the 3 hour preexposure period.

Additional evidence for such a role for SRIF comes from our studies in the mutant dwarf (*dw*) rat, a strain of rats which shows a selective reduction in pituitary GH synthesis and storage (21). Because pituitary GH concentrations are approximately 10% of normal in males, we postulated that these animals would be unable to accumulate GH to any large extent. As expected, and in contrast to the normal rats, preexposure to octreotide for 3-h failed to enhance GRF-induced GH secretion. Moreover,while the GH rebound release following the coadministration of GRF and SRIF-14 was 2-3 fold higher in the octreotide-pretreated *dw* rats than their own normal saline-pretreated controls, the total amount of GH released was not different than that observed in *dw* animals either preexposed to octreotide and given GRF alone or in untreated *dw* controls (See Fig. 9B). This is in marked contrast to the 14-16 fold increase in GH rebound release observed in the normal rats. Taken together, these findings provide strong support for the thesis that, in the normal rat, SRIF potentiates GRF-induced GH release through a direct SRIF-mediated build-up of pituitary GH stores in a readily releasable pool.

The findings reported here may have important implications for understanding the role of SRIF and its interaction with GRF in the generation of pulsatile GH secretion. It would appear that exposure to high SRIF concentrations, as is known to occur during a normal GH trough period, prepares the somatotroph to respond maximally to a subsequent pulse of hypothalamic GRF. Such a cooperative interaction of the two peptides may be necessary to optimize pulsatile GH release and, subsequently, somatic growth.

It is also possible that chronic octreotide exposure resulted in SRIF receptor desensitization, thus causing a decrease in SRIF's antagonistic action on GRF-induced GH

release. While downregulation of pituitary SRIF receptors has been demonstrated in certain cell types *in vitro* by direct exposure to high concentrations of SRIF (29-31), other studies have failed to find significant changes in either SRIF receptor number or cellular responsiveness following continuous SRIF exposure (32,33). Furthermore, in humans, long-term treatment of acromegaly with octreotide continues to actively suppress GH secretion for over 2 years (34,35).

To address this issue, we measured the ability of SRIF-14 to effectively inhibit GRF's stimulatory action on GH release following a 3-h period of preexposure to octreotide or normal saline. In both the normal and *dw* rats, those animals pretreated with octreotide exhibited almost identical SRIF-14-mediated inhibition of GRF-induced GH release to that of animals pretreated with normal saline (ranging from 78-90%). These preliminary findings suggest that the response to SRIF was not desensitized *in vivo*, although full dose-response studies are needed to thoroughly address this question. Neuroanatomical studies using *in situ* hybridization are currently underway in this laboratory to determine whether SRIF receptor mRNA levels are altered in rats pretreated with octreotide.

Although our findings support the notion of a SRIF-mediated build-up of a readily releasable GH pool at the level of the pituitary, we cannot discount the possibility that octreotide may have acted centrally within the hypothalamus to affect GRF release, since octreotide is able to penetrate the blood brain barrier (36). An abundance of physiological and anatomical evidence suggests that SRIF may interact directly with GRF within the CNS to regulate pituitary GH release (3,37-40). More recently, exogenously administered SRIF was shown to directly inhibit GRF release, both *in vitro* (41) and *in vivo* (42). All these findings suggest that SRIF may directly affect the activity of hypothalamic GRF neurons to inhibit GRF release.

Clinical diagnosis of GH deficiency in short children and adolescents has proven to be difficult, due to inconsistent, poorly reproducible GH responses to a single i.v. challenge of GRF (43,44). This variability is presumably caused by the temporal variations in SRIF levels and its antagonistic effects on GRF in the human. We have

recently reported that pretreatment of short children with octreotide significantly enhanced their GH responsiveness to GRF (45). On the basis of our observations reported here in the rat, it is possible that SRIF behaves in a similar manner in the human to regulate GH release, thus supporting the use of octreotide as a potential diagnostic tool to facilitate the discrimination between normal short children and those with truly diminished GH secretion.

In conclusion, the present study indicates that, in the normal male rat, preexposure to the SRIF analog, octreotide, for 3 hours significantly enhances GH responsiveness to exogenous GRF; and our findings suggest that this effect is due to a SRIF-mediated build-up of pituitary GH stores in a readily releasable pool. These results provide good support for the concept that SRIF may not simply act as an inhibitor of GH release during a GH trough period, but in fact plays a prominent positive role in the generation of the GH pulses by poising the somatotroph to respond optimally to GRF. This temporal interplay between SRIF and GRF may be necessary to optimize pulsatile GH release and, ultimately, somatic growth.

Manuscript References

1. **Tannenbaum GS** 1991 Neuroendocrine control of growth hormone secretion. Acta Paediatr Scand [Suppl] 372:5-16
2. **Tannenbaum GS, 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
3. **Plotsky PM, Vale W** 1985 Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. Science 230:461-463
4. **Wehrenberg WB, Brazeau P, Luben R, Böhlen P, Guillemin R** 1982 Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). Endocrinology 111:2147-2148
5. **Miki N, Ono M, Shizume K** 1984 Evidence that opiatergic and α-adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). Endocrinology 114:1950-1952
6. **Painson J-C, Tannenbaum GS** 1991 Sexual dimorphism of somatostatin and growth hormone-releasing factor signaling in the control of pulsatile growth hormone secretion in the rat. Endocrinology 128:2858-2866
7. **Ferland L, Labrie F, Jobin M, Arimura A, Schally AV** 1976 Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. Biochem Biophys Res Commun 68:149-156
8. **Steiner RA, Stewart JK, Barber J, Koerker D, Goodner CJ, Brown A, Illner P, Gale CC** 1978 Somatostatin: A physiological role in the regulation of growth hormone secretion in the adolescent male baboon. Endocrinology 102:1587-1594
9. **Terry LC, Martin JB** 1981 The effects of lateral hypothalamic-medial forebrain stimulation and somatostatin antiserum on pulsatile growth hormone secretion in freely behaving rats: evidence for a dual regulatory mechanism. Endocrinology 109:622-627

10. **Tannenbaum GS** 1988 Somatostatin as a physiological regulator of pulsatile growth hormone secretion. Hormone Res 29:70-74
11. **Brazeau P, Ling N, Bohlen P, Esch F, Ying SY, Guillemin R** 1982 Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone *in vitro*. Proc Natl Acad Sci USA 79:7909
12. **Vale W, Vaughan J, Yamamoto G, Spiess J, 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
13. **Tannenbaum GS, Eikelboom R, Ling N** 1983 Human pancreas GH-releasing factor analog restores high-amplitude GH pulses in CNS lesion-induced GH deficiency. Endocrinology 113:1173-1175
14. **Kraicer J, Cowan JS, Sheppard MS, Lussier B, Moor BC** 1986 Effect of somatostatin withdrawal and growth hormone (GH)-releasing factor on GH release *in vitro*: Amount available for release after disinhibition. Endocrinology 119:2047-2051
15. **Weiss J, Cronin MJ, Thorner MO** 1987 Periodic interactions of GH-releasing factor and somatostatin can augment GH release *in vitro*. Am J Physiol 253: E508-E514
16. **Soya H, Suzuki M** 1988 Somatostatin rapidly restores rat growth hormone (GH) release response attenuated by prior exposure to human GH-releasing factor *in vitro*. Endocrinology 122:2492-2498
17. **Stachura ME, Tyler JM, Farmer PK** 1988 Combined effects of human growth hormone (GH)-releasing factor-44 (GRF) and somatostatin (SRIF) on post-SRIF rebound release of GH and prolactin: A model for GRF-SRIF modulation of secretion. Endocrinology 123:1476-1482
18. **Richardson SB, Twente S** 1991 Pre-exposure of rat anterior pituitary cells to somatostatin enhances subsequent growth hormone secretion. J Endocrinol 128:91-95
19. **Clark RG, Robinson ICAF** 1988 Paradoxical growth-promoting effects induced by patterned infusions of somatostatin in female rats. Endocrinology 122:2675-2682.

20. **Tannenbaum GS, Painson J-C, Lengyel AMJ, Brazeau P** 1989 Paradoxical enhancement of pituitary growth hormone (GH) responsiveness to GH-releasing factor in the face of high somatostatin tone. Endocrinology 124:1380-1388

21. **Charlton HM, Clark RG, Robinson ICAF, Porter Goff AE, Cox BS, Bugnon C, Bloch BA** 1988 Growth hormone-deficient dwarfism in the rat: A new mutation. J Endocrinol 119:51-58

22. **Tannenbaum GS, Martin JB** 1976 Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98:562-570

23. **Pless J, Bauer W, Briner U, Doepfner W, Marbach P, Maurer R, Petcher TJ, Reubi J-C, Vonderscher J** 1986 Chemistry and pharmacology of SMS 201-995, a long-acting octapeptide analog of somatostatin. Scand J Gastroenterol 21:54-64

24. **Losa M, Müller OA, Sobieszczyk S, Von Werder K** 1985 Interaction between growth hormone releasing factor (GRF) and somatostatin analogue (SMS 201-995) in normal subjects. Clinical Endocrinol 23:715-720

25. **Stachura ME** 1977 Interaction of somatostatin inhibition and dibutyryl cyclic AMP or potassium stimulation of growth hormone release from perifused rat pituitaries. Endocrinology 101:1044-1053

26. **Cowan JS, Moor B, Chow A, Kraicer J** 1983 Characteristics of the post-somatostatin rebound in growth hormone secretion from perifused somatotrophs. Endocrinology 113:1056-1061

27. **Clark RG, Carlsson LMS, Rafferty B, Robinson ICAF** 1988 The rebound release of growth hormone (GH) following somatostatin infusion in rats involves hypothalamic GH-releasing factor release. J Endocrinol 119:397-404.

28. **Miki N, Ono M, Shizume K** 1988 Withdrawal of endogenous somatostatin induces secretion of growth hormone-releasing factor in rats. J Endocrinol 117:245-252.

29. **Smith M, Yamamoto G, Vale W** 1984 Somatostatin desensitization in rat anterior pituitary cells. Mol Cell Endocrinol 37:311-318

30. **Reisine T, Axelrod J** 1983 Prolonged somatostatin pretreatment desensitizes somatostatin's inhibition of receptor-mediated release of adrenocorticotropin hormone and sensitizes adenylate cyclase. Endocrinology 113:811-813

31. **Heisler S, Srikant CB** 1985 Somatostatin-14 and somatostatin-28 pretreatment down-regulate somatostatin-14 receptors and have biphasic effects on forskolin-stimulated cyclic adenosine, 3',5'-monophosphate synthesis and adrenocorticotropin secretion in mouse anterior pituitary tumor cells. Endocrinology 117:217-225

32. **Presky DH, Schonbrunn A** 1988 Somatostatin pretreatment increases the number of somatostatin receptors in GH_4C_1 pituitary cells and does not reduce cellular responsiveness to somatostatin. J Biol Chem 263:714-721

33. **Thermos K, Reisine T** 1988 Somatostatin receptor subtypes in the clonal anterior pituitary cell lines AtT-20 and GH_3 cells. Mol Pharmacol 33:370-376

34. **Lamberts SWJ, Uitterlinden P, del Pozo E** 1987 SMS 201-995 induces a continuous decline in circulating growth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. J Clin Endocrinol Metab 65:703-710

35. **Chiodini PG, Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Petroncini M, Liuzzi A, del Pozo E** 1987 Medical treatment of acromegaly with SMS 201-995, a somatostatin analog: A comparison with bromocriptine. J Clin Endocrinol Metab 64:447-453

36. **Begley DJ** 1992 The interaction of some centrally active drugs with the blood-brain barrier and circumventricular organs. Prog Brain Res 91:163-169

37. **Murakami Y, Kato Y, Kabayama Y, Inoue T, Koshiyama H, Imura H** 1987 Involvement of hypothalamic growth hormone (GH)-releasing factor in GH secretion induced by intracerebroventricular injection of somatostatin in rats. Endocrinology 120:311-316

38. **Willoughby JO, Brogan M, Kapoor R** 1989 Hypothalamic interconnections of somatostatin and growth hormone releasing factor neurons. Neuroendocrinology 50:584-591

39. **Liposits SZ, Merchenthaler I, Paull WK, Flerko B** 1988 Synaptic communication between somatostatinergic axons and growth hormone-releasing factor (GRF) synthesizing neurons in the arcuate nucleus of the rat. Histochemistry 89:247-252

40. **Tannenbaum GS, McCarthy GF, Zeitler P, Beaudet A** 1990 Cysteamine-induced enhancement of growth hormone-releasing factor (GRF) immunoreactivity in arcuate neurons: morphological evidence for putative somatostatin/GRF interactions within hypothalamus. Endocrinology 127:2551-2560

41. **Yamauchi N, Shibasaki T, Ling N, Demura H 1991** In vitro release of growth hormone-releasing factor (GRF) from the hypothalamus: somatostatin inhibits GRF release. Reg Peptides 33:71-78

42. **Guillaume V, Magnan E, Cataldi M, Conte-Devolx B, Thomas F, Chihara K, Oliver C** BIM 23014, a long acting somatostatin analog inhibits GH-RH release into hypophysial portal blood of conscious sheep. 9th International Congress of Endocrinology, Nice, France, 1992 (Abstract P-08.01.076)

43. **Butenandt O** 1989 Diagnostic value of growth hormone-releasing hormone tests in short children. Acta Pediatr Scand (Suppl) 349:93-99

44. **Devesa J, Lima L, Lois N, Fraga C, Lechuga MJ, Arce V, Tresguerres JAF** 1989 Reasons for the variability in growth hormone (GH) responses to GHRH challenge: the endogenous hypothalamic-somatotroph rhythm. Clin Endocrinol 30:367-377

45. **Dickerman Z, Guyda H, Tannenbaum GS** 1993 Pretreatment with somatostatin analog SMS 201-995 potentiates growth hormone responsiveness to growth hormone-releasing factor in short children. J Clin Endocrinol Metab 77:652-657

Figures and Figure Legends

Fig. 1. Individual representative 6-h plasma GH profiles in male Sprague-Dawley rats i.v. administered either normal saline (A) or octreotide, in doses of 25 µg (B) and 50 µg (C), at 1000 h. A. Normal saline-treated control animals displayed the characteristic ultradian rhythm of GH release. B and C. Both doses of octreotide virtually obliterated the spontaneous GH pulses for approximately 3 h, with partial recovery evident 3 to 6 h after injection.

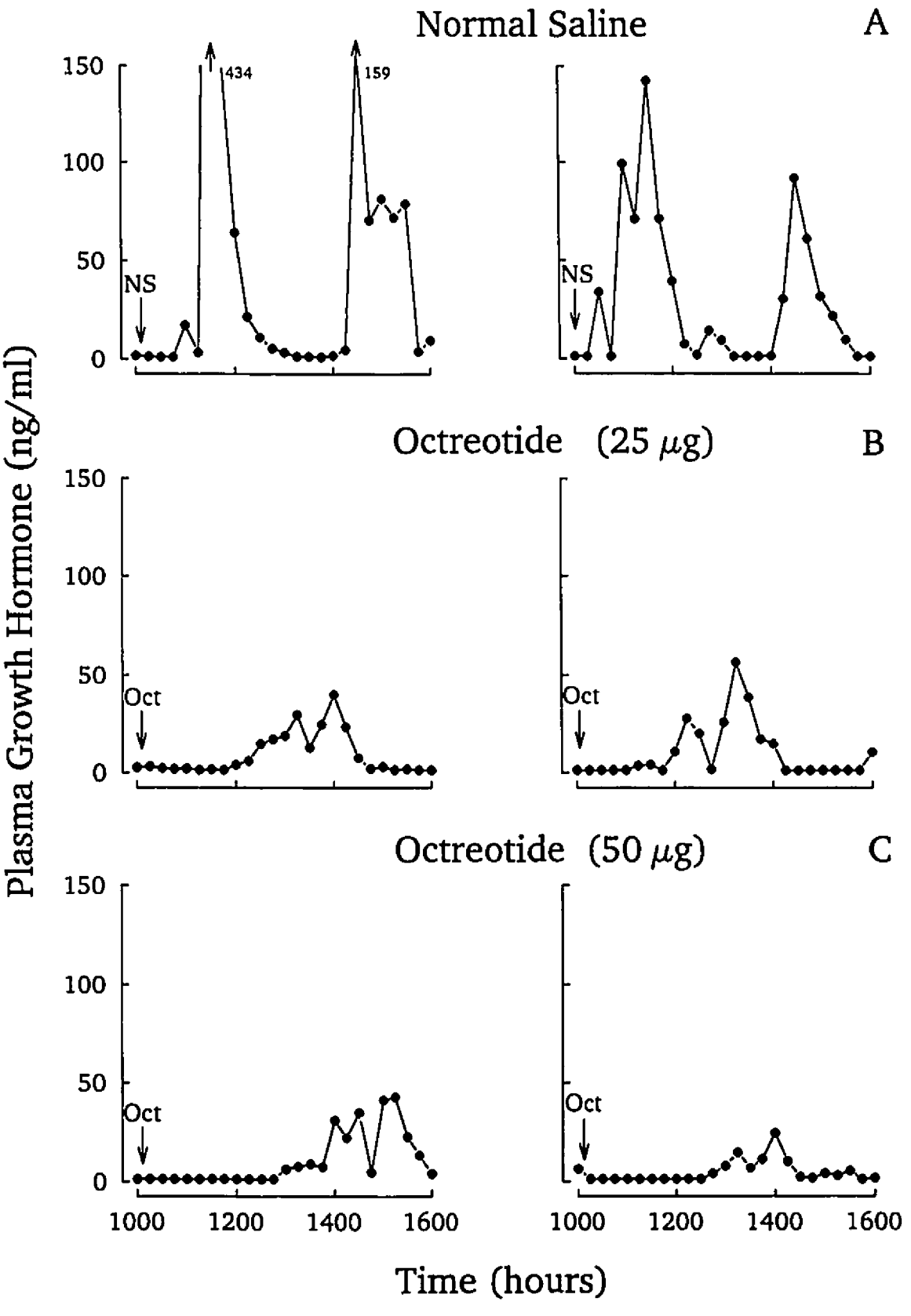
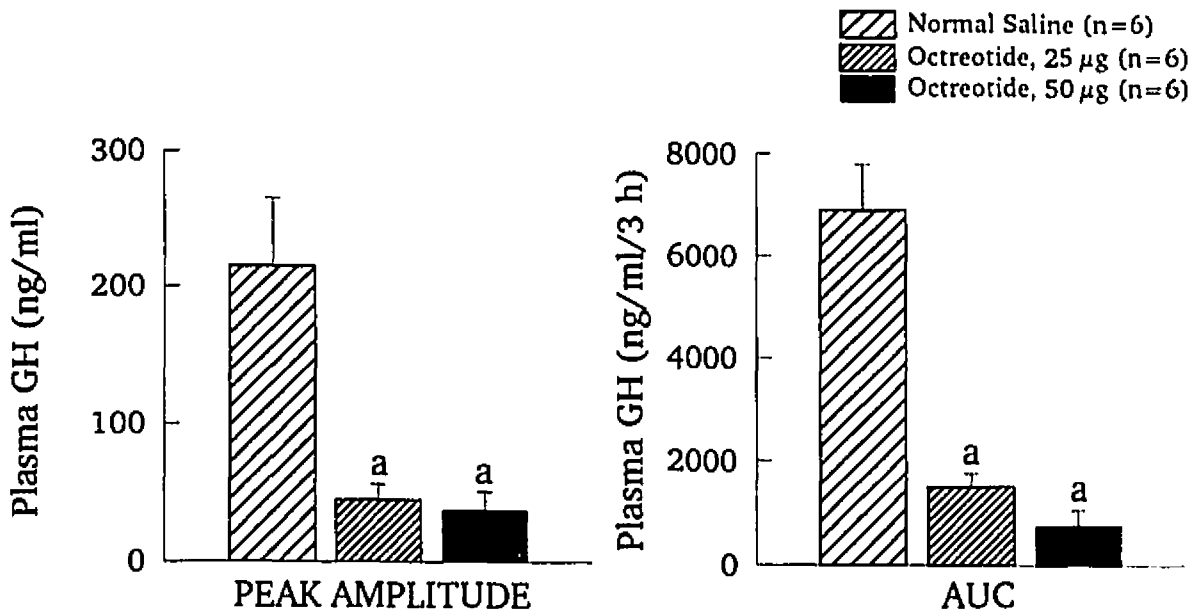

Normal Saline
A
Octreotide (25 μg)
B
Octreotide (50 μg)
C
Plasma Growth Hormone (ng/ml)
150
100
50
0
NS
434
159
Oct
1000
1200
1400
1600
Time (hours)

Fig. 2. Summary of time course of effects of octreotide on pulsatile GH secretion. AUC represents the integrated GH release calculated over 3 h. Each bar represents the mean + SEM. The number of animals in each group is shown in parentheses.

a, $P<0.01$; b, $P<0.05$ compared to normal saline-treated controls during the same time periods;

c, $P<0.05$ compared to the 0-3 h time period within each group.

0-3 Hours Post Octreotide Injection



3-6 Hours Post Octreotide Injection

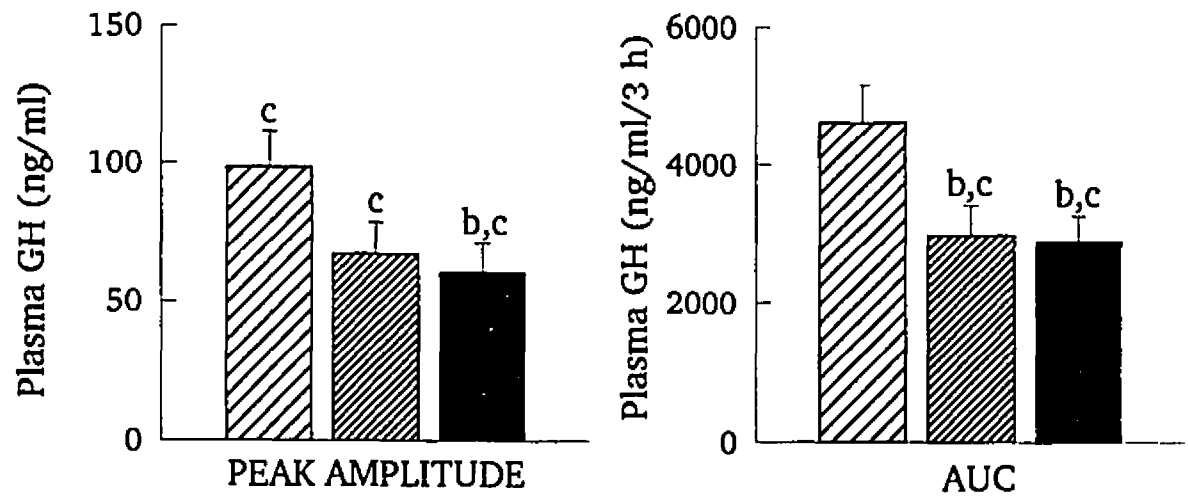


Fig. 3. Plasma GH response to GRF (1 μg i.v.) in individual rats pretreated with either normal saline (A) or octreotide in doses of 25 μg (B) and 50 μg (C). While GRF-induced GH release was severely suppressed 1 h post octreotide injection, a 3-h period of preexposure to either dose of octreotide markedly enhanced GH responsiveness to GRF, compared to normal saline-treated controls. Arrows indicate times of injections.

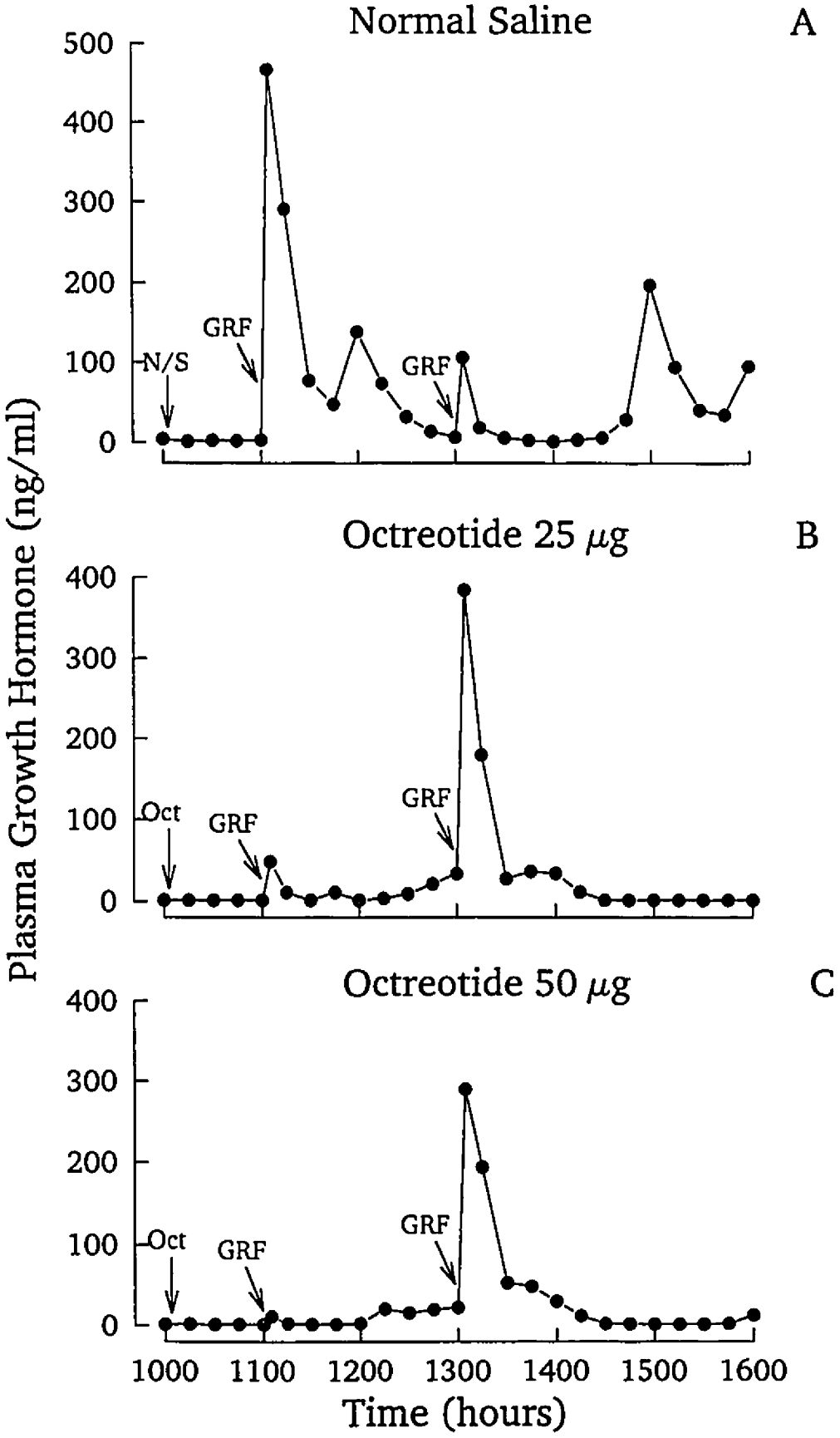

Normal Saline
A
500
400
300
200
100
0
N/S
GRF
GRF
Octreotide 25 μg
B
400
300
200
100
0
Oct
GRF
GRF
Octreotide 50 μg
C
400
300
200
100
0
Oct
GRF
GRF
1000
1100
1200
1300
1400
1500
1600
Time (hours)
Plasma Growth Hormone (ng/ml)

Fig. 4. Summary of temporal effects of octreotide on GRF-induced GH release. There was a 2- to 3-fold augmentation in GH AUC at 3 h after the administration of either dose of octreotide compared to normal saline-pretreated controls. Each bar represents the mean + SEM of the integrated GH area calculated over 30 min after each GRF injection. The number of animals in each group is shown in parentheses.

a, $P<0.001$; b, $P<0.02$ compared to normal saline-treated controls at the same time point.

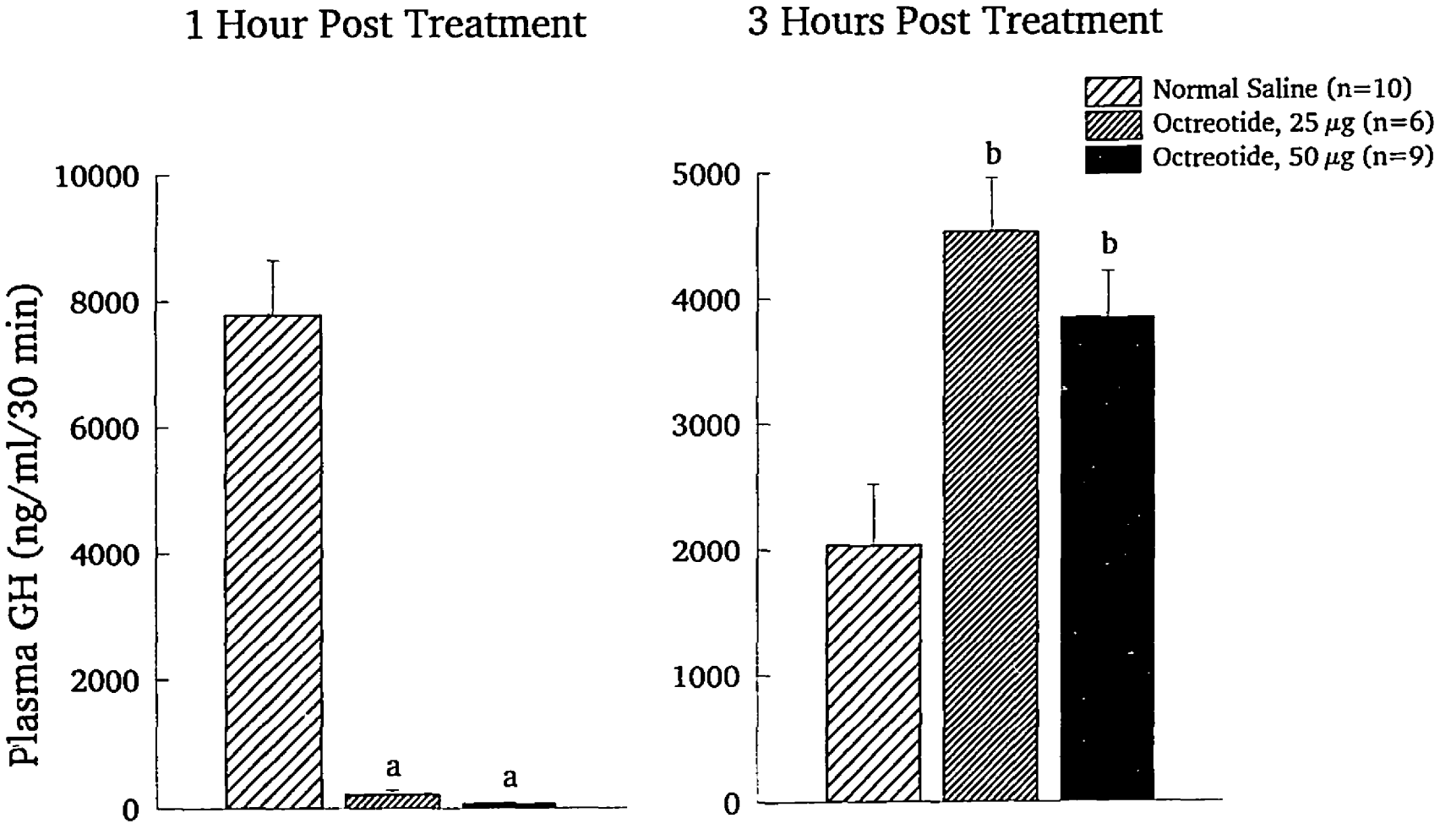

1 Hour Post Treatment
3 Hours Post Treatment
Normal Saline (n=10)
Octreotide, 25 μg (n=6)
Octreotide, 50 μg (n=9)
Plasma GH (ng/ml/30 min)
10000
8000
6000
4000
2000
0
a
a
5000
4000
3000
2000
1000
0
b
b

Fig. 5. Individual representative plasma GH responses following the co-administration of SRIF-14 (50 μg i.v.) and GRF (1 μg i.v.) in animals pretreated with either normal saline or octreotide 3 h earlier. Administration of SRIF-14 in combination with GRF resulted in a marked inhibition of GRF's stimulatory action on GH release 5 min post injection in all three groups. However, this short-acting inhibition was followed by a striking postsuppression rebound release of GH in both octreotide-pretreated groups compared to normal saline pretreated controls.

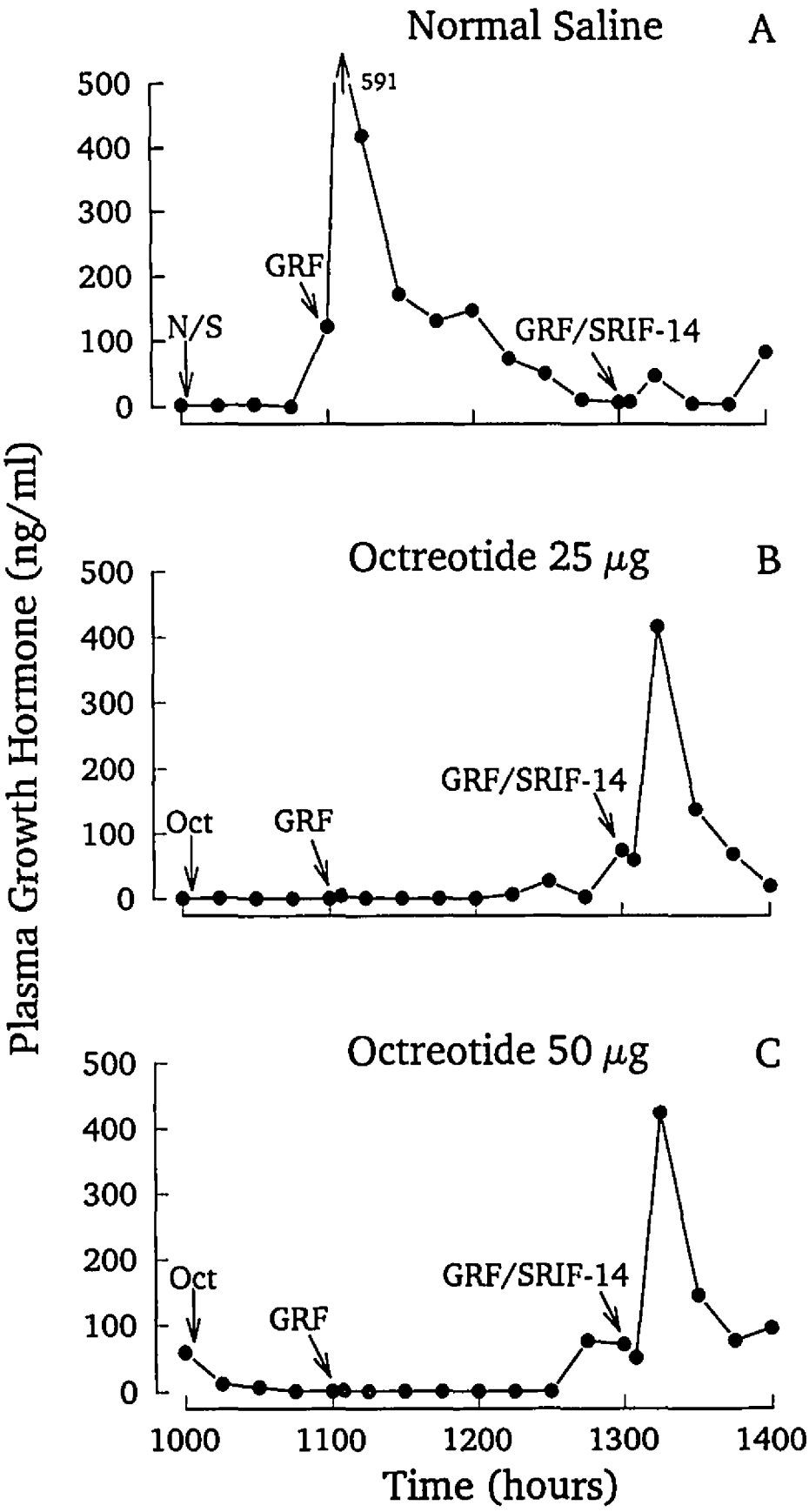

Normal Saline
A
591
500
400
300
200
100
0
GRF
N/S
GRF/SRIF-14
Octreotide 25 μg
B
Oct
GRF
GRF/SRIF-14
Octreotide 50 μg
C
Oct
GRF
GRF/SRIF-14
Plasma Growth Hormone (ng/ml)
1000
1100
1200
1300
1400
Time (hours)

Fig. 6. Summary of effects of 3-h preexposure to octreotide on GH response to co-injection of SRIF-14 and GRF.

Left Panel: SRIF-14-mediated inhibition of GRF-induced GH release was similar in all groups 5 min post injection.

Right Panel: The SRIF-14-mediated postinhibitory rebound release of GH was 14-16 fold higher in octreotide-pretreated animals compared to normal saline-pretreated controls: AUC measured 5-45 min post GRF/SRIF-14 injection. Each bar represents the mean + SEM. The number of animals in each group is shown in parentheses.

a, $P<0.001$ compared to normal saline-pretreated controls.

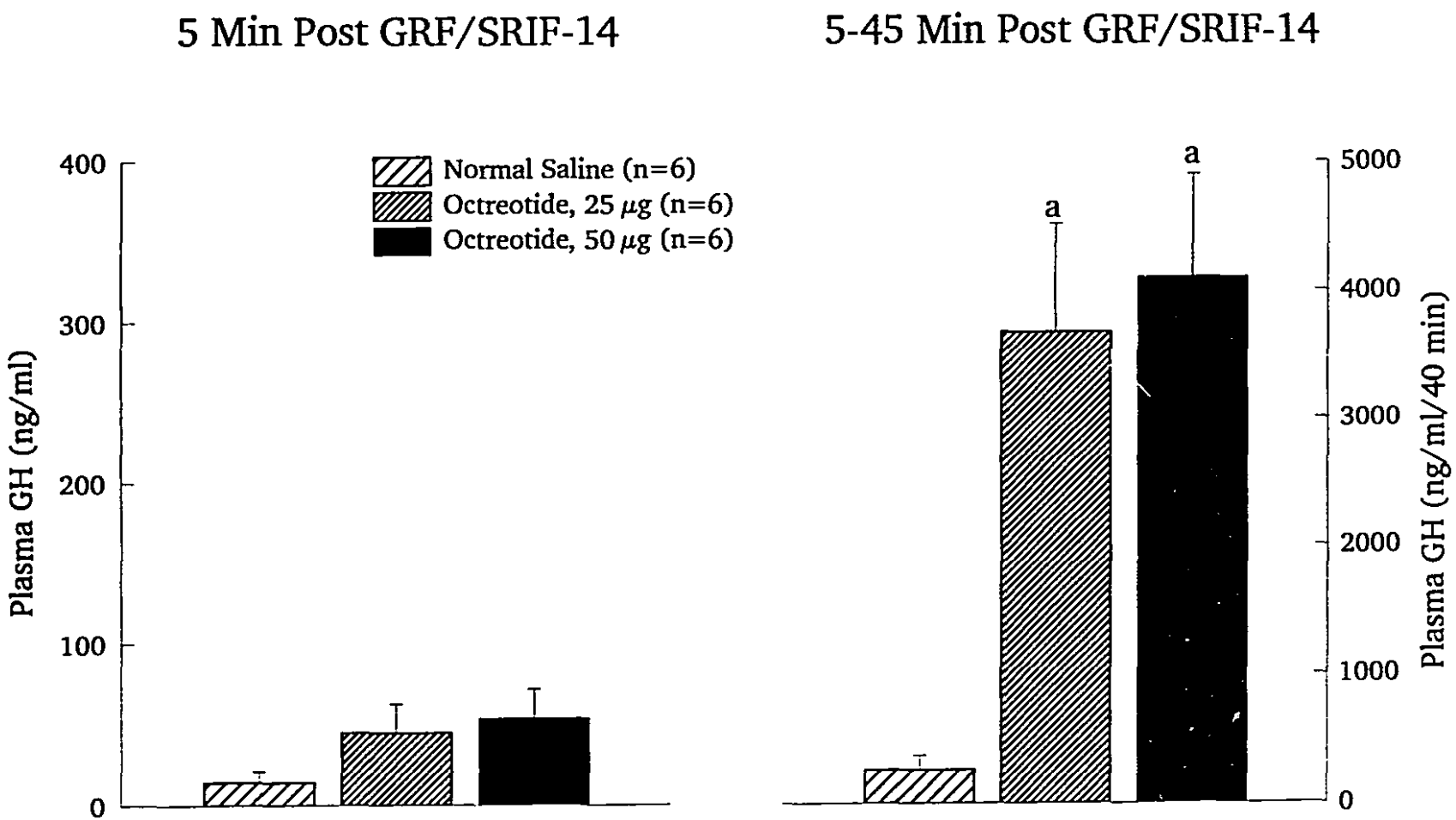

5 Min Post GRF/SRIF-14
5-45 Min Post GRF/SRIF-14
Normal Saline (n=6)
Octreotide, 25 μg (n=6)
Octreotide, 50 μg (n=6)
Plasma GH (ng/ml)
Plasma GH (ng/ml/40 min)
400
300
200
100
0
5000
4000
3000
2000
1000
0
a
a

Fig. 7. Effects of octreotide pretreatment (50 µg i.v.) on mean plasma GH response to GRF (1 µg i.v.) in *dw* rats. While octreotide pretreatment effectively inhibited GRF-induced GH release 1 h after injection, it failed to enhance GH responsiveness to GRF at 3 h compared to control *dw* rats. Vertical lines represent the SEM; the number of animals in each group is shown in parentheses. Note 8-fold reduction in scale of y-axis compared to Fig 3.

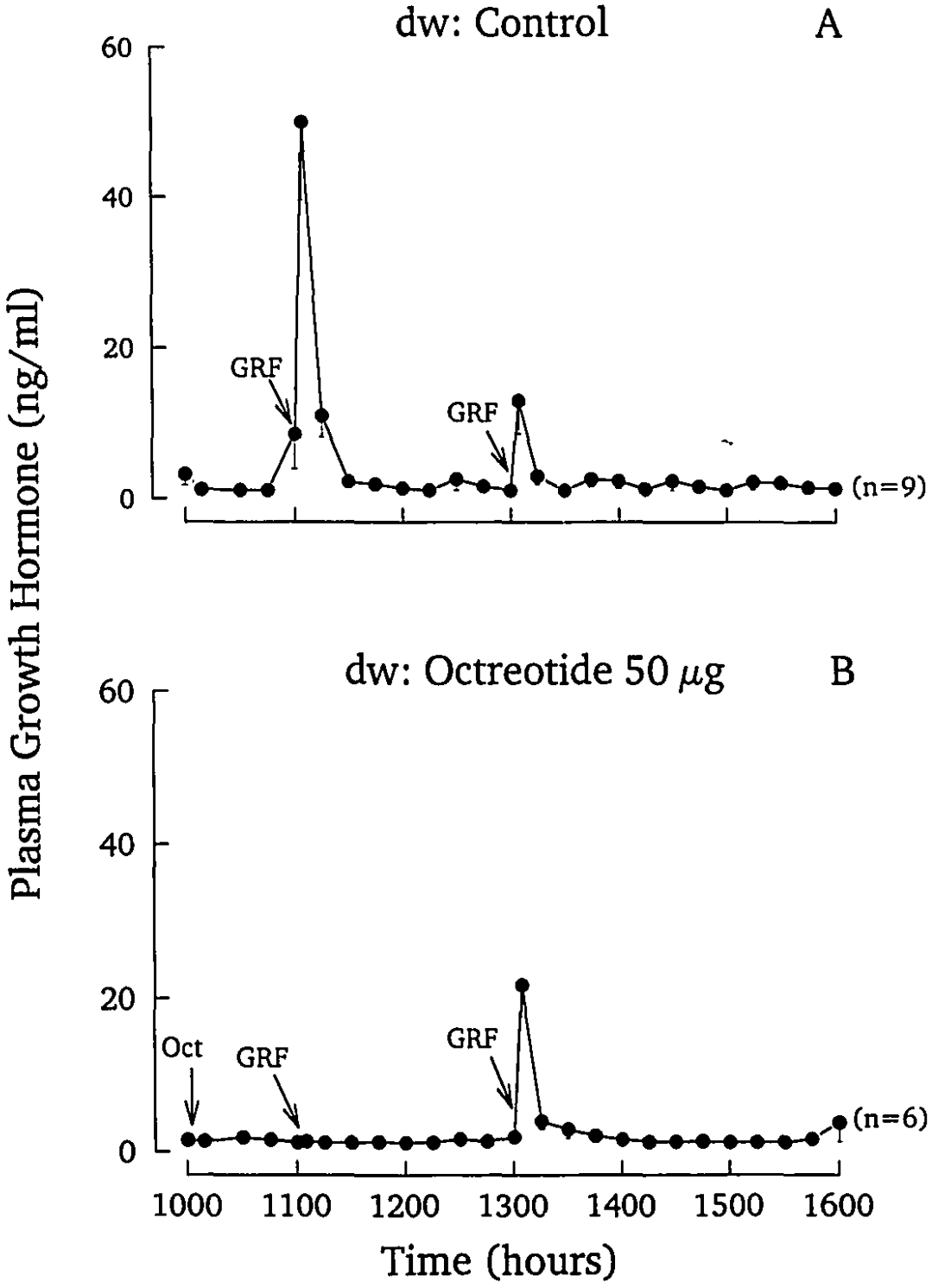

dw: Control
A
60
40
20
0
GRF
GRF
(n=9)
dw: Octreotide 50 μg
B
60
40
20
0
Oct
GRF
GRF
(n=6)
1000
1100
1200
1300
1400
1500
1600
Time (hours)
Plasma Growth Hormone (ng/ml)

Fig. 8. Effects of 3-h preexposure to octreotide on GH release following co-administration of SRIF-14 and GRF in *dw* rats. The postinhibitory rebound release of GH was only 2-3 fold higher in *dw* rats pretreated with octreotide compared to normal saline controls. Each bar represents the mean +SEM. The number of animals in each group is shown in parentheses. Note 10- to 14-fold difference in y-axis compared to Fig 6.

a, $P<0.01$ compared to normal saline-pretreated animals.

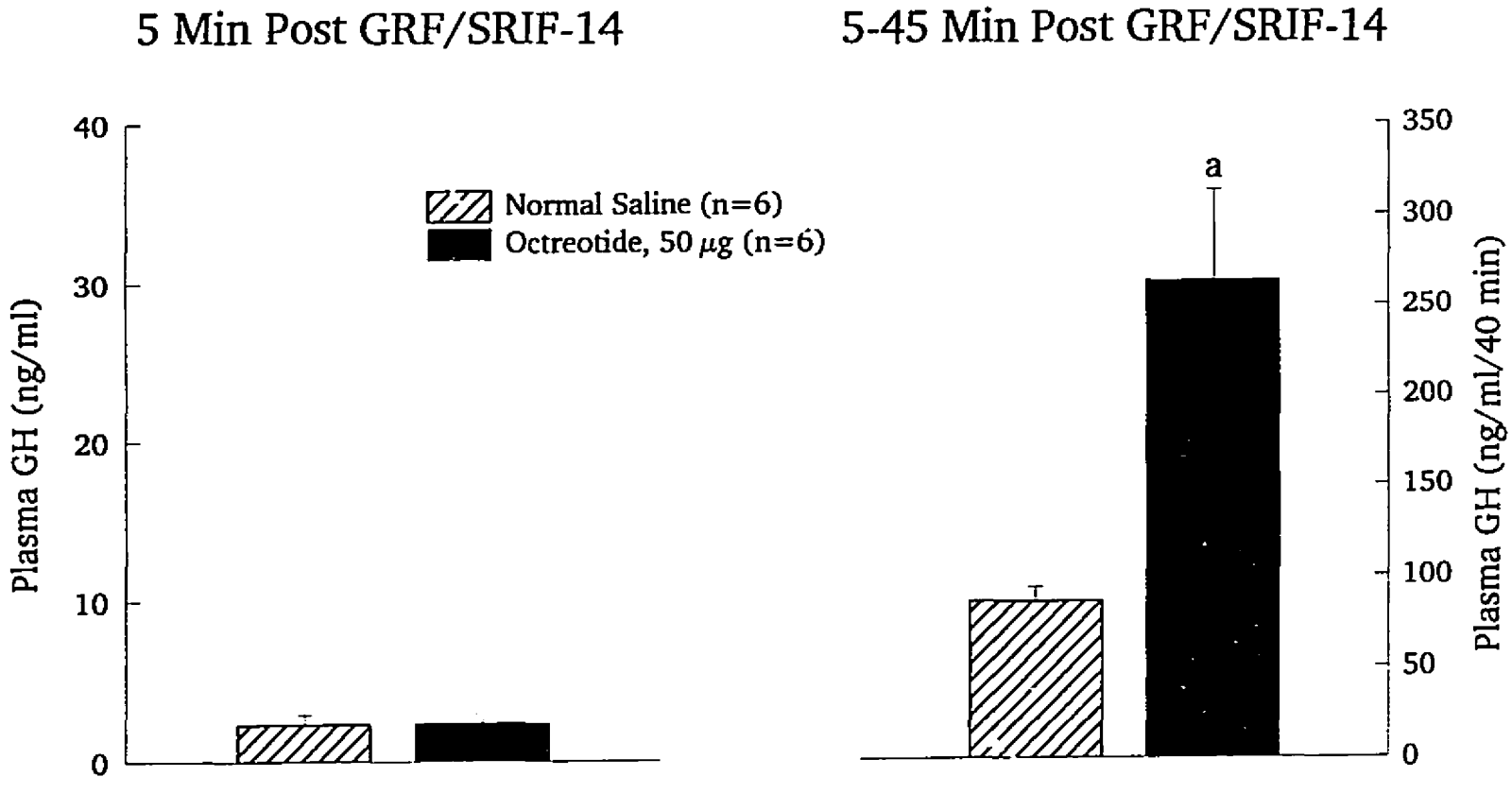
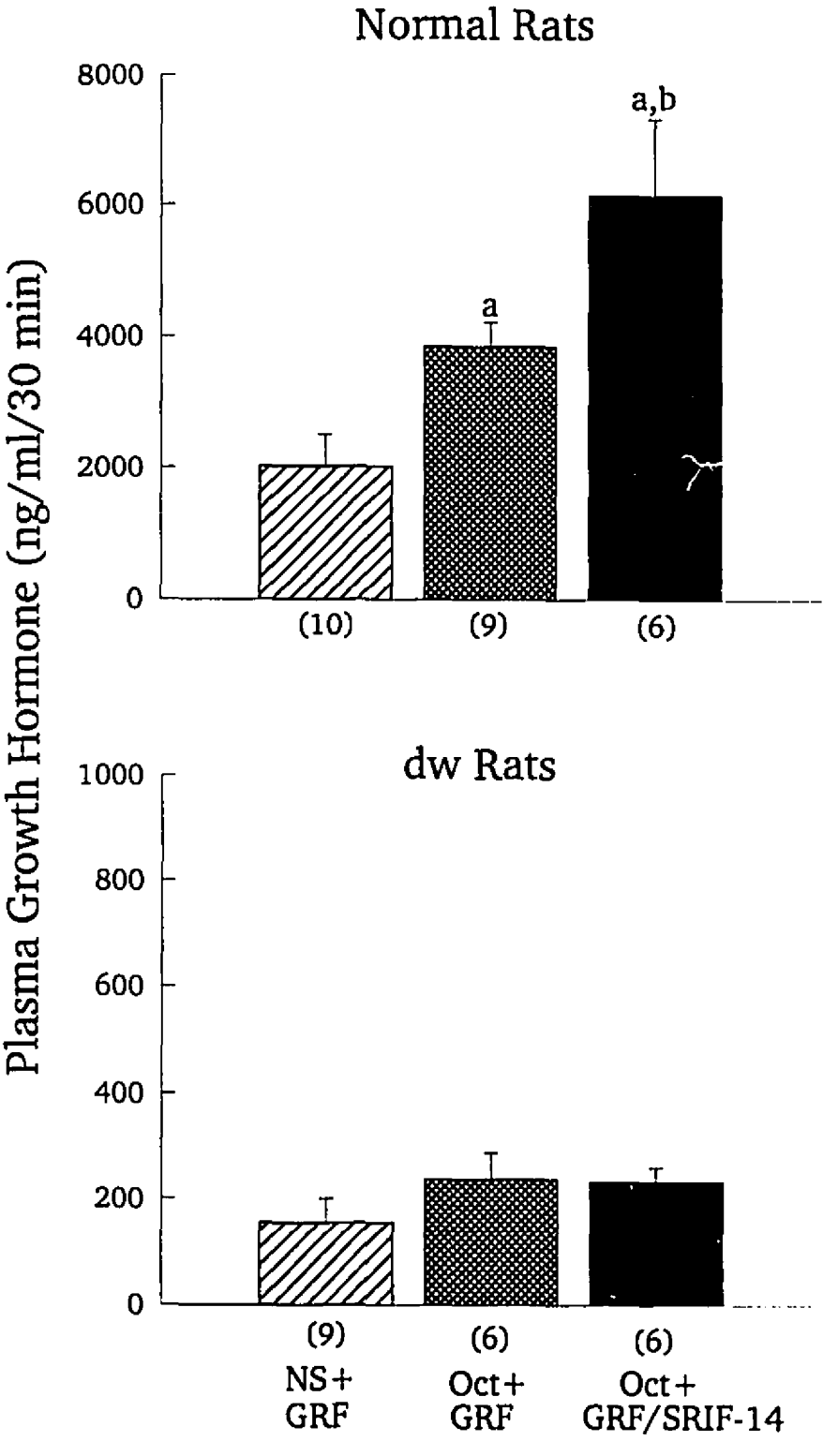

5 Min Post GRF/SRIF-14
5-45 Min Post GRF/SRIF-14
Normal Saline (n=6)
Octreotide, 50 μg (n=6)
Plasma GH (ng/ml)
Plasma GH (ng/ml/40 min)
40
30
20
10
0
350
300
250
200
150
100
50
0
a

Fig. 9. Comparison of effects of 3-h octreotide preexposure on GRF-induced GH release in normal and *dw* rats. While octreotide pretreatment (50 μg) significantly augmented GH responsiveness to GRF in normal rats under both experimental paradigms, it failed to alter GRF's stimulatory action on GH in *dw* rats. Each bar represents the mean + SEM of the integrated GH area calculated over 30 min. The number of animals in each group is shown in parentheses. Note 8-fold difference in scale of y-axis between the 2 groups.

a, $P<0.02$ compared to normal saline-pretreated controls.

b, $P<0.05$ compared to octreotide + GRF alone.


Normal Rats
8000
6000
4000
2000
0
a,b
a
(10)
(9)
(6)
Plasma Growth Hormone (ng/ml/30 min)
dw Rats
1000
800
600
400
200
0
(9)
NS+
GRF
(6)
Oct+
GRF
(6)
Oct+
GRF/SRIF-14

IV. CHAPTER 2

Effects of Octreotide Preexposure on Somatostatin Receptor mRNA Using *In Situ* Hybridization

Introduction

Within the last decade, physiological and morphological data have been rapidly accumulating to support the notion that SRIF may have a vital central influence on the regulation of GRF synthesis and release. However, while the discoveries of SRIF binding sites located on GRF cells in the arcuate nucleus, as well as the localization of multiple SRIF receptor subtypes throughout the hypothalamus, have supported the view of a central SRIF/GRF interaction, very little is known about the regulation of these SRIF receptors.

In vitro studies have demonstrated SRIF receptor desensitization in both normal pituitary cells, as well as in AtT-20 cells pretreated with SRIF (Reisine & Axelrod, 1983; Reisine, 1984; Smith *et al*, 1984). In addition, Srikant & Patel (1984) demonstrated that depletion of endogenous brain SRIF results in an upregulation of SRIF receptors. In contrast, GH_3 and GH_4C_1 cells exposed to SRIF showed no change in cellular responsiveness to SRIF (Thermos & Reisine, 1988; Presky & Schonbrunn, 1988). Moreover, the long term effectiveness of SRIF analogs for the treatment of acromegaly is well established, suggesting that the responses to SRIF do not desensitize *in vivo*.

The principle aims of the *in situ* hybridization study were two-fold. First, we needed to verify that this technique could be developed in our laboratory using fresh frozen tissues. Perfused tissue, which is often used for *in situ* hybridization, requires the animals to be anesthetized prior to sacrificing. However, anesthetics have been shown to have deleterious effects on GH hormonal balance in the rat. Consequently, the use of fresh frozen tissue would enable us to sacrifice the animals swiftly and would therefore offer a more physiological approach to our question. Thus it was essential to map the distribution of SRIF receptor mRNA in the rat brain in order to confirm that fresh frozen tissues gave us comparable results. The results of this first experiment are presented here.

Our second goal was to determine whether octreotide preexposure produces alterations in SSTR1 and SSTR2 mRNA levels in the rat brain. While our physiological findings strongly support the notion of a SRIF-induced build up of a readily releasable GH pool,

we wished to determine whether the enhanced GH responsiveness to GRF that we observed *in vivo* was due, in part, to an octreotide-induced down-regulation in SRIF receptor message either in those hypothalamic nuclei implicated in GH regulation or at the level of the pituitary gland. Since the *in situ* hybridization technique was being developed for the first time in our lab to determine the pattern of expression of SSTR1 and SSTR2 mRNA in the rat hypothalamus, we decided to initiate our investigation at the level of the hypothalamus, with the intention of proceeding to the pituitary gland at a later time. The ability of octreotide to cross the blood brain barrier justified its use in this experiment (Begley, 1992). The results of these initial studies are reported here.

Materials and Methods

Animals and Experimental Procedure

Adult male Sprague-Dawley rats, similar in weight to those in our physiological studies, were used here. To obtain fresh frozen sections, animals were sacrificed by decapitation and the brains were rapidly dissected out of the skull, and frozen in isopentane for 1 min at -40°C. The frozen brains were subsequently wrapped in aluminum foil and stored at -80°C until use. The entire procedure took less than 2.5 minutes.

For perfused tissue, animals were anaesthetized with a combination of ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip), followed by cardiac perfusion with a 4% w/v paraformaldehyde solution, supplemented with 10% 1M PBS buffer and 10 ml 4% NaOH (600-750 ml/animal). Brains were subsequently removed from the skull, post-fixed for 1 hour, then immersed overnight in 20% sucrose. The following day, the brains were frozen in isopentane for 1 min at -40°C, then wrapped in aluminum foil and stored at -80°C until used. Cutting perfused brains involved the same protocol as with the fresh frozen tissue.

To cut sections, the brains were first allowed to equilibrate in the cryostat chamber (-20°C), and were then trimmed and mounted onto the specimen holder with OCT embedding medium (Tissue Tek, Miles Inc, Elkhart, IN). 20 µm thick coronal sections were obtained in a rostro-caudal direction, beginning at the decussation of the anterior commissure and terminating at the disappearance of the third ventricle. Alternate sections were thaw-mounted onto Poly-L-Lysine (50 µg/ml)-coated glass slides (for processing both probes at comparable levels), air dried over a hot plate at 37°C for 2 min, and then stored at -80°C until hybridization.

In experiment 1, to determine whether fresh frozen and perfused preparations gave comparable results, we sacrificed 6 rats using both techniques and performed *in situ* hybridization for SSTR1 and SSTR2 on both tissue preparations. Our results indicated that both tissue preparations gave qualitatively comparable results and therefore we used fresh frozen tissue sections in the second experiment.

In the second experiment, 6 Sprague Dawley rats were pretreated at 1000 h with

either 0.2 ml normal saline (n=3) or a single 50 µg dose of octreotide (n=3), i.v, and sacrificed three hours later in accordance with the time course adopted in our *in vivo* study. The animals were sacrificed and brains removed, as described above, and *in situ* hybridization using both SSTR1 and SSTR2 probes was performed to measure SRIF receptor mRNA levels in both groups of animals.

Riboprobe Preparation

Hybridization of tissue sections was performed with an RNA riboprobe complementary to the two subtypes of the mouse SRIF receptor: SSTR1 and SSTR2 (generously provided by Dr. Graeme Bell, Howard Hughes Medical Institute, University of Chicago). Both probes were obtained by inserting the specific cDNA fragment into the plasmid vector pGEM-3Z. The insertion region is flanked by two different RNA polymerase promoter sequences, T7 and SP6. Therefore, to obtain antisense probes, plasmids were linearized by restriction endonuclease digestion (EcoRI) and transcribed with the Gemini II system (Promega) using SP6 RNA polymerase and [^{35}S]-UTP. Transcription of sense strands were prepared from Hind III linearized plasmids using T7 RNA polymerase.

***In Situ* Hybridization**

For fresh frozen sections, slides were removed from the freezer and brought to room temperature (30-45 min). To maximize RNA retention in the tissue, sections were first post-fixed in freshly prepared 4% w/v paraformaldehyde solution (10 min), rinsed in 1 M phosphate buffer (3 washes, 10 min each), then in H_2O (2 min). In the case of the perfused sections, the considerable cross-linking requires an additional step to increase the penetrability of the riboprobe into the tissue. Consequently, upon removal from the freezer, slides were rinsed in 1 µg/ml proteinase K in TE buffer (0.1 M Tris, 50 mM EDTA, pH=8.0) at 37 C for 30 min, followed by a 2 min wash in H_2O. All subsequent steps of the procedure were identical for both fresh and perfused sections.

All sections were rinsed in solutions of 0.25% acetic anhydride to reduce

electrostatic attraction between the hybridization probe and non-specific basic proteins. Slides were then incubated for 30 min in 0.1 M tris-glycine and washed three times in 2 X SSC (standard saline citrate: 1 X SSC in 0.15 M NaCl and 15 mM Citric acid, pH=7.0). Finally, sections underwent a gradual dehydration process with consecutive rinses in 70%, 95%, and 99% ethanol (3 min each). A final prehybridization step, designed to saturate non-specific binding sites in the tissue, constituted a 1 h incubation in a prehybridization buffer containing 4 X SSC, 1 X Denhardt's solution (ficoll, bovine serum albumin, polyvinyl pyrrolidone), and 0.1 mM mercaptoethanol.

Hybridization of the ^{35}S-labelled riboprobe involved the administration of 80 µl/slide of the hybridization mixture, made up of 10% sarcosyl, 50% deionized formamide, 200 mM dithiothreitol (DTT), 4 X SSC, 1 X Denhardt's, 50% dextran sulphate, 0.1 mM mercaptoethanol, and 80 µL of radiolabeled probe. Optimal probe concentrations are difficult to predict, but should generally yield the highest signal to noise ratio. It is best to use the lowest concentration required to saturate target nucleic acids, since non-specific binding is directly related to probe concentration. Consequently, probe was added to the hybridization buffer for a final activity of 2 X 10^6 cpm/ml. Ten out of every 12 slides were hybridized with the antisense probes (SSTR1 or SSTR2). The remaining two slides were treated with the sense probe and used as a control. Each slide was covered with a glass coverslip, and placed in a moist chamber to allow hybridization to proceed overnight (approximately 19 h) at 60 C.

The second day of the procedure comprised of steps needed to minimize background labelling of the probe to the tissue. Sections were first rinsed in 4 X SSC for 1 h to remove the coverslips, then underwent 3 successive 10 min washes in 4 X SSC. Due to the "stickiness" of the RNA probe, sections were treated with 20 µg/ml RNase A in 4 X SSC for 30 min at 37 C in order to selectively remove non-base paired RNA from the tissue section. Since hybridizations are performed at relatively low stringency (high temperature) to enhance dissociation of the nucleic acid, post-hybridization washings at increasing stringency were necessary to induce dissociation of non-homologous hybrids. Consequently, slides were subjected to a series of increasingly stringent washes from 2 -

0.1 X SSC baths supplemented with 0.25% DTT. After dehydration through a graded ethanol series (70%, 95%, 99%), the slides were placed on ßmax hyperfilm (Amersham Corp, Arlington Heights, IL), loaded into light-tight X-ray film cassettes and stored at 4°C for 4 weeks. Exposed films were developed using Kodak GBX developer and Kodak Rapid fixer.

Quantitative Analysis

Quantification of the hybridization signal was carried out by computer-assisted microdensitometry, using the BIOCOM RAG software program. Specific grey values were calculated by subtracting background values. Data was compared to a standard curve prepared by mixing increasing quantities of radionucleotide to a brain paste mixture.

Results

Distribution of SSTR1 in Fresh Frozen Brain Tissue

Figure 10(A-C) illustrates the distribution of SSTR1 mRNA in coronal sections of fresh frozen rat brain. Gross examination of autoradiographs revealed intense hybridization signal throughout the cortex and hypothalamic area. Cortical distribution showed a selectivity to the outermost and innermost layers. Little or no signal was detected in the medial habenular nucleus, the hippocampus, and the dentate gyrus. Within the hypothalamus, labelling was detected most intensely in the arcuate and the suprachiasmatic nuclei. The paraventricular and ventromedial nuclei showed only weak to moderate signal.

The specificity of the probe was established in a control section prepared with a sense probe (Fig 10D). Specific labelling was eliminated from all regions.

Distribution of SSTR2 in Fresh Frozen Brain Tissue

While the distributional pattern of SSTR2 mRNA overlapped to some degree with that of SSTR1, distinctive variations were found (Fig 11A). Once again, hybridization signal was intense throughout the cerebral cortex, this time in a more unilaminar fashion. In addition, the habenular nucleus and hippocampus were heavily stained. Distribution within the hypothalamus was similar to that of SSTR1, however the hybridization signal was generally less distinct throughout (Fig 11B). Nevertheless SSTR2 mRNA signal was specifically located in the arcuate, ventromedial, suprachiasmatic, and dorsomedial nuclei. However, the signal to noise ratio of our SSTR2 probe was not as high as that of SSTR1. Once again, the probe specificity was confirmed using a control slide prepared with a sense probe for SSTR2 (Fig 11C).

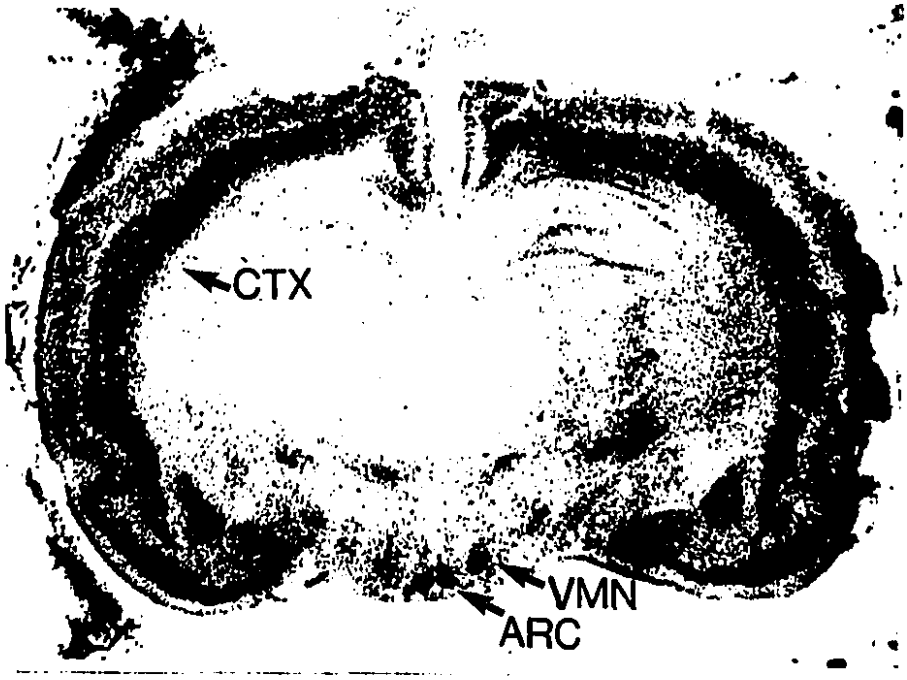
Effects of Octreotide Preexposure on SSTR1 and SSTR2 mRNA Levels in the Rat Brain

Figure 12 illustrates our preliminary data of the effects of a 3-hour period of preexposure to octreotide on SSTR1 and SSTR2 mRNA levels in 4 regions of the rat brain - the arcuate nucleus (ARC), the ventromedial nucleus (VMN), the suprachiasmatic

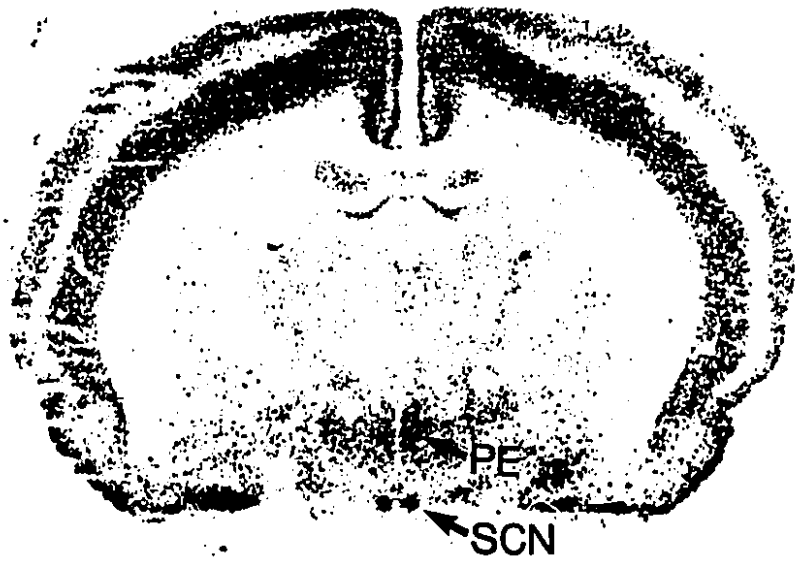
nucleus (SCN), and the cortex (CTX). With respect to SSTR1, octreotide pretreatment did not significantly alter mRNA density levels in any of the brain regions examined thus far. However, SSTR2 mRNA levels tended to be increased, in all four brain areas, in those animals pretreated with octreotide when compared to normal saline-pretreated controls, and this difference reached significance for the arcuate nucleus.

Fig. 10. Regional distribution of SSTR1 mRNA in coronal sections of fresh frozen rat brain tissue. A & B. Whole brain; C. Hypothalamic area; D. Sense probe. CTX=Cortex; Arc=Arcuate nucleus; VMN=Ventromedial nucleus; SCN=Suprachiasmatic nucleus; PE=Periventricular area

A.



B.



C.

D.

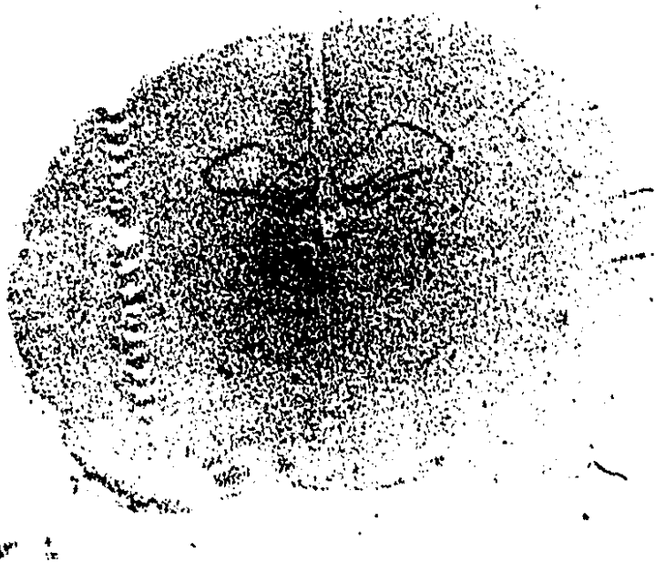
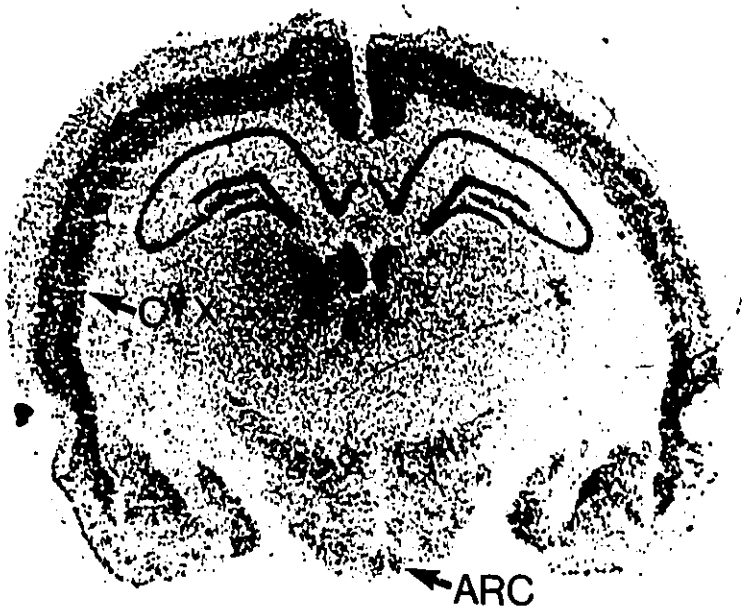


Fig. 11. Regional distribution of SSTR2 mRNA in coronal sections of fresh frozen rat brain tissue. A Whole brain; B. Hypothalamic area; C. Sense probe. CTX=Cortex; Arc=Arcuate nucleus; VMN=Ventromedial nucleus; SCN=Suprachiasmatic nucleus; PE=Periventricular area

A.



B.

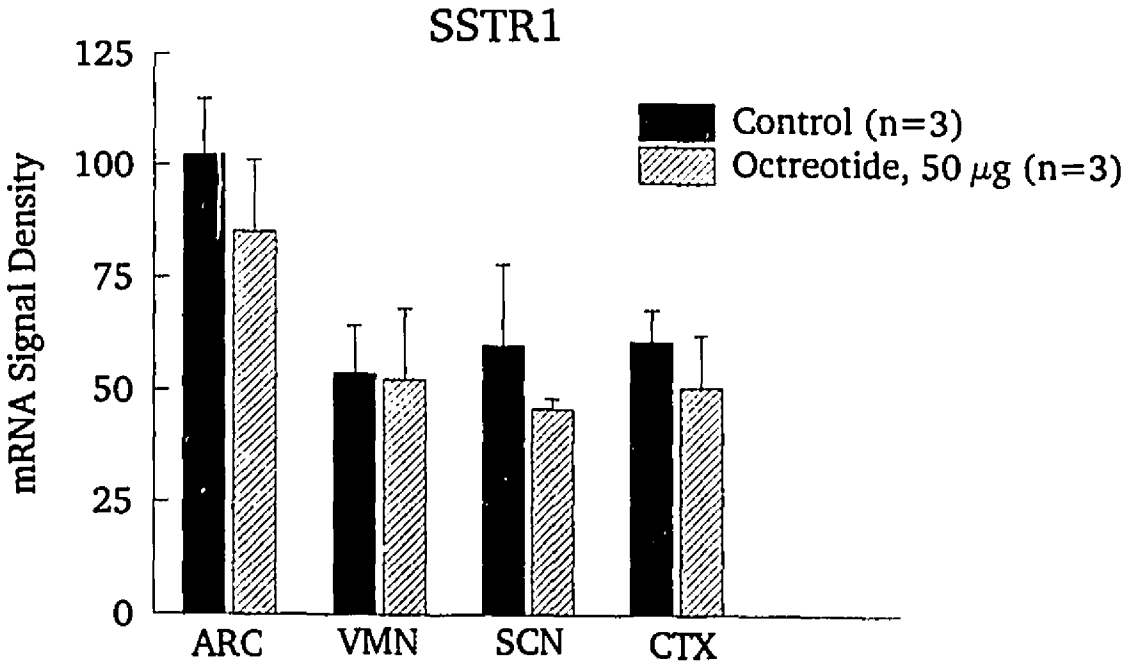
C.

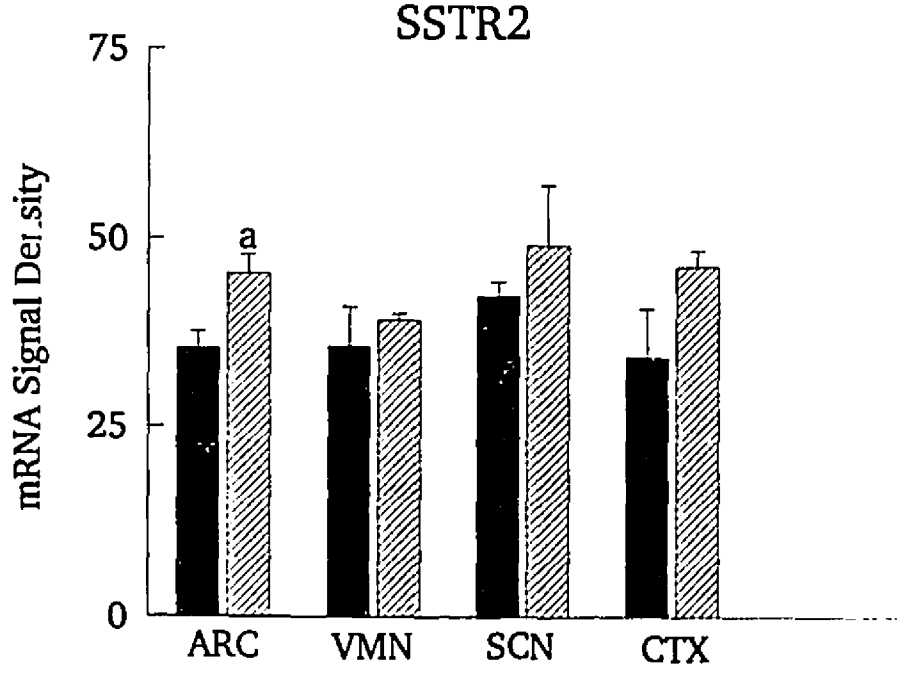
Fig. 12. Preliminary data on the effects of octreotide preexposure on SSTR1 and SSTR2 mRNA density levels in the rat brain.

ARC=Arcuate nucleus; VMN=Ventromedial nucleus;

SCN=suprachismatic nucleus; CTX=Cortex

$P<0.05$, compared to control rats.


SSTR1
125
100
75
50
25
0
mRNA Signal Density
Control (n=3)
Octreotide, 50 μg (n=3)
ARC
VMN
SCN
CTX


SSTR2
75
50
25
0
mRNA Signal Density
a
ARC
VMN
SCN
CTX

Discussion

In spite of the new wealth of information regarding the anatomical distribution of SRIF receptors in the central nervous system, very little is known regarding the control of SRIF receptor expression. Consequently, we seized the opportunity to use *in situ* hybridization to investigate another possible mechanism of action of SRIF in the regulation of pituitary GH release. We tested the hypothesis that chronic exposure to the SRIF analog, octreotide, results in dynamic changes in SRIF's own receptors within the hypothalamus, thus altering octreotide's ability to antagonize GRF stimulation.

The goal of the first part of our study was to determine whether our two probes would be capable of specifically hybridizing to SRIF receptor mRNA on fresh frozen tissue sections. While a previous study of mRNA levels of the SRIF peptide in rat brain demonstrated that fresh frozen tissue gave comparable *in situ* hybridization results to those obtained using perfused tissue (Chowen, *et al*, 1991), this had not been established for the newly cloned SRIF receptors. Gross examination of our data revealed that both SSTR1 and SSTR2 mRNA were found in very distinct areas of the brain. The specificity of our probes was established following the hybridization of certain sections using a sense probe. SSTR1 was intensely labeled in the cortex, as well as the arcuate and suprachiasmatic nuclei of the hypothalamus. Moderate levels were also detected in the paraventricular and ventromedial nuclei. SSTR2 was also distributed in the cortex and similar hypothalamic nuclei as SSTR1, as well as in several limbic structures, namely the hippocampus and habenular nucleus. These distributions of SSTR1 and SSTR2 mRNA in the rat brain, and particularly in the hypothalamus, corresponded very closely with those we found in perfused tissue in the present study and also with that described by others (Beaudet *et al*, 1994). This encouraging result demonstrated that *in situ* hybridization of SRIF receptor isotypes could be carried using fresh frozen tissue, and allowed us to proceed to our next experiment, that is, to determine the effects of octreotide preexposure on SRIF receptor mRNA levels in the brain.

Our preliminary results show that octreotide failed to alter SSTR1 mRNA levels

in any of the four regions that were measured, the arcuate, ventromedial and suprachiasmatic nuclei of the hypothalamus, as well as the cortex. However, this result was more or less expected. Octreotide is an analog that is subtype selective (Raynor *et al*, 1992). While octreotide has very high affinity for SSTR2, it has little or no affinity for SSTR1. In addition, the lack of a positive result may be due to an intrinsic property of the receptor itself. It is possible that SSTR1 is simply not sensitive to prolonged SRIF exposure, as suggested in a recent hybridization/nuclease protection study (Bruno *et al*, 1994), and that autoregulation of SRIF receptors may not occur in the SSTR1 receptor subtype.

In contrast, SSTR2 mRNA levels showed a tendency to increase in all four regions following octreotide treatment, with a significant elevation observed in the arcuate nucleus. Although these are only preliminary results, they do allow for the speculation that the octreotide-induced alteration in SRIF receptor message may be a generalized phenomenon affecting several SRIF-responsive regions of the brain.

Secondly, it was surprising to find a paradoxical increase in mRNA levels following octreotide exposure. Based on previous studies, we would have predicted a downregulation of SRIF receptors following chronic SRIF exposure. Thus the significance of our results is not yet known. However, a recent *in vitro* study examining the SSTR subtype gene expression in GH_3 cells following a 2 hour exposure to SRIF has recently produced similar findings (Bruno *et al*, 1994). While SRIF preexposure increased mRNA levels of SSTR2 (as well as SSTR3, 4 & 5) in that study, there was no change in SSTR1 mRNA levels. The findings of this study also support the time course of effects of octreotide observed in our investigation, suggesting that the 3 hour period of exposure may in fact be adequate to cause a measurable change in SRIF receptor mRNA levels.

There is no evidence, to date, that directly links changes in mRNA levels to changes in peptide expression. Therefore, although octreotide may mediate effects on SSTR2 mRNA, we have no way of knowing that this has measurable consequences on the level of SRIF receptor expression on the cell membrane. However, it has been recently shown that the levels of both hypothalamic SRIF peptide mRNA binding sites

(Zeitler *et al*, 1989) and SRIF bining sites (Tannenbaum *et al*, 1993), as well as SRIF receptor subtype mRNA (Bruno *et al*, 1994), are indeed capable of fluctuating in such a short 2-3 hour time period. From these studies, it may be postulated that the changes in mRNA levels following a 3 hour exposure to octreotide may in fact translate into a direct change in receptor number or density. Further study is certainly merited to fully address this question.

GENERAL CONCLUSIONS

Normal postnatal somatic growth depends on an adequate supply of GH from the anterior pituitary. In addition, many major physiological and metabolic functions are also affected by circulating levels of plasma GH. Consequently, it is of particular interest to understand the mechanisms regulating its secretion. It is well established that the ultradian pattern of GH secretion is governed by the episodic release of two hypothalamic hormones, GRF and SRIF, each playing specific individual roles on pituitary somatotrophs. While GRF is responsible for the episodic GH bursts, SRIF acts as the regulator of GH troughs (see Tannenbaum, 1991, for review). However, considerably less is understood regarding their complex interactions in regulating GH release.

Physiological evidence is accumulating to indicate that, at the level of the pituitary, SRIF functions is a variety of ways to govern the responsiveness of GH to GRF. SRIF acts to antagonize GRF's stimulatory action on GH release, both *in vitro* and *in vivo* (Vale *et al*, 1983; Lamberts *et al*, 1984; Tannenbaum *et al*, 1983), and consequently plays a vital role in generating the ultradian rhythm of GH secretion.

However, it has been suggested that the role of SRIF extends beyond its ability to simply suppress GH release and GH responsiveness to GRF. Studies investigating the importance of the episodic secretion of SRIF have suggested that, under certain temporal conditions, SRIF may possess a paradoxically positive influence on pituitary somatotrophs, however the mechanism(s) is not known.

SRIF prevents the gradual desensitization of GRF-induced GH release following chronic GRF administration (Clayton & Bailey, 1987; Soya & Suzuki, 1988; Losa *et al*, 1985). Secondly, GRF-induced GH release is augmented by a concurrent withdrawal of SRIF (Kraicer *et al*, 1986; Weiss *et al*, 1987). In addition, *in vitro* investigations of the interactions between SRIF and GRF in generating the GH pulses have recently found that preexposure to SRIF surprisingly enhanced pituitary responsiveness to GRF (Richardson & Twente, 1991).

However, due to the lack of *in vivo* evidence to support these statements, we

attempted in the present study to further explore the role of SRIF in controlling GH release in the adult male rat. We used the long-acting SRIF analog, octreotide, to 1) further investigate the temporal patterning of the SRIF/GRF interplay *in vivo*, and 2) to define possible mechanism(s) of action of SRIF in generating the ultradian rhythm of GH secretion.

The findings of the present study reveal several important points: 1) the i.v. infusion of octreotide at a dose of 25 and 50 µg significantly suppressed spontaneous GH secretion for up to six hours with an immediate and complete reduction in plasma GH levels during the initial 3 hours. These data provide, for the first time, a time course of effects of the long-acting SRIF analog on GH release in the male rat. 2) Preexposure to octreotide for three hours significantly augmented GRF-induced GH release, demonstrating for the first time a direct positive effect of SRIF on GH responsiveness to GRF *in vivo*. 3) In examining the various mechanisms that could account for octreotide's potentiating role in GH release, our results suggest that SRIF may regulate GRF-induced GH release through a direct SRIF-mediated build up of pituitary stores in a readily releasable pool so that a GRF challenge 3 hours later will amplify the release of GH. The implications of our findings shed new light on the role of SRIF in the generation of the ultradian rhythm of GH secretion; exposure to high SRIF concentrations, as is known to occur during a normal trough period potentiates the somatotrophs to respond maximally to a subsequent pulse of hypothalamic GRF.

In addition, there is growing physiological data to suggest that SRIF can act centrally, at the level of the hypothalamus, to regulate GRF release (Plotsky & Vale, 1985; Tannenbaum *et al*, 1990; Guillaume *et al*, 1992). Results from recent anatomical studies strongly support these findings. GRF-immunoreactive neurons in the arcuate nucleus exhibit localized ^{125}I-labelled SRIF binding sites (McCarthy *et al*, 1992). Furthermore, in the rat, it has just recently been shown that the hypothalamus displays highly expressed levels of newly cloned SRIF receptor subtype mRNAs in those neurons known to contain and express GRF (Beaudet *et al*, in press). Thus it is possible that chronic octreotide exposure results in dynamic changes in SRIF receptor levels on these

GRF neurons, causing a decrease in SRIF's inhibitory action on hypothalamic GRF release. While our results suggest that octreotide does produce some effect on SSTR2 mRNA levels, these preliminary experiments addressing the question of SRIF receptor kinetics have not yet been carried out to fruition.

These numerous findings give credence to the hypothesis that SRIF, in addition to its role as the generator of GH troughs, may play an important interactive function in GH release by determining the ability of the somatotroph to respond maximally to stimulations by GRF. This temporal interplay between SRIF and GRF at the level of the pituitary and the hypothalamus may be necessary to optimize the pulsatile pattern of GH secretion, and hence somatic growth.

GENERAL REFERENCES

Abe H, Kato Y, Iwasaki Y, Chihara K, Imura H 1978 Central effect of somatostatin on the secretion of growth hormone in the anesthetized rat. Proc Soc Exp Biol Med 159:346-349

Alberti KGMM, Christensen NJ, Christensen SE 1973 Inhibition of insulin secretion by somatostatin. Lancet 2:40-41

Alpert LC, Brawer JM, Patel YC, Reichlin S 1976 Somatostatinergic neurons in anterior hypothalamus: immunohistochemical localization. Endocrinology 98:255-258

Anderson JV, Bloom SR 1986 Neuroendocrine tumours of the gut: long term therapy with the somatostatin analog SMS 201-995. Scand J Gastroenterol 21:115-128

Antony GJ, Van Wyk JJ, French FS, Weaver RP, Dugger GS. Timmons RL, Newsome JF 1969 Influence of pituitary stalk section on growth hormone, insulin and TSH secretion in women with metastatic breast cancer. J Clin Endocrinol Metab 29:1238-1250

Badger TM, Millard WJ, McCormick GF, Bowers CY, Martin JB 1984 The effects of growth hormone (GH)-releasing peptides on GH secretion in perifused pituitary cells of adult male rats. Endocrinology 115:1432-1438

Barnard LB, Granthan WG, Lamberton P, O'Dorisio TM, Jackson IMD 1986 Treatment of resistant acromegaly with a long-acting somatostatin analog (SMS 201-995). Ann Intern Med 105:856-862

Beaudet A, Greenspun D, Raelson J, Tannenbaum GS Patterns of expression of SSTR1 and SSTR2 somatostatin receptor subtypes in the hypothalamus of the adult rat: relationship to neuroendocrine function. J Neuroscience, In press

Beal M, Tran V, Majurek M, Chattha G, Martin J 1986 Somatostatin binding sites in human and monkey brain: localization and characterization. J Neurochem 46:359-365

Begley DJ 1992 The interaction of some centrally active drugs with the blood-brain barrier and circumventricular organs. Prog Brain Res 91:163-169

Benoit R, Bohlen P, Ling N, Esch F, Baird A, Ying SY, Wehrenberg WB, Guillemin R, Morrison JH, Bakhit C 1985 Somatostatin-$28_{(1-12)}$-like peptides. Adv Exp Med Biol 188:89-107

Bennett-Clarke C, Romagnano MA, Joseph SA 1980 Distribution of somatostatin in the rat brain: telencephalon and diencephalon. Brain Res 188:473-486

Bernardis LL, Frohman LA 1971 Plasma growth hormone responses to electrical stimulation of the hypothalamus in the rat. Neuroendocrinology 7:193-201

Bertherat J, Berod A, Normand E, Bloch B, Rostene W, Kordon C, Epelbaum J 1991 Somatostatin depletion by cycteamine increases somatostatin binding and growth hormone-releasing factor messenger ribonucleic acid in the arcuate nucleus. J Neuroendocrinology 3:115-118

Bertherat J, Doumaud P, Berod A, Normand E, Bloch B, Rostene W, Kordon C, Epelbaum J 1992 Growth hormone-releasing hormone-synthesizing neurons are a subpopulation of somatostatin receptor-labelled cells in the rat arcuate nucleus: a combined *in situ* hybridization and receptor light-microscopic radioautographic study. Neuroendocrinology 56:25-31

Bilezikjian LM, Vale WW 1984 Chronic exposure of cultured rat anterior pituitary cells to GRF causes partial loss of responsiveness to GRF. Endocrinology 115:2032-2034

Bilezikjian LM, Seifert H, Vale W 1986 Desensitization to growth hormone-releasing factor (GRF) is associated with down-regulation of GRF-binding sites. Endocrinology 118:2045-2052

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

Bloch B, Gaillard RC, Brazeau P, Lin HD, Ling N 1984 Topographical and ontogenetic study of the neurons producing growth hormone-releasing factor in human hypothalamus. Regul Pept 8:21-31

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

Brazeau P, Ling N, Esch F, Bohlen P, Mougin C, Guillemin R 1982 Somatocrinin (growth hormone releasing factor) *in vitro* bioactivity; Ca^{++} involvement, cAMP mediated action and additivity of effect with PGE_2. Biochem Biophys Res Commun 109:588-594

Brazeau P, Ling N, Esch F, Bohlen P, Benoit R, Guillemin R 1981 High biological activity of the synthetic replicates of somatostatin-28 and somatostatin-25. Regul Pept 1:255-265

Brazeau P, Ling N, Bohlen P, Esch F, Ying SY, Guillemin R 1982 Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone *in vitro*. Proc Natl Acad Sci USA 79:7909-7913

Brazeau P, Bloom F, Ling N 1983 Topographical study of the neurons containing hpGRF immunoreactivity in monkey hypothalamus. Neurosci Lett 37:23-28

Breder CD, Yamada Y, Yasuda K, Seino S, Saper CB, Bell GI 1992 Differential expression of somatostatin receptor subtypes in brain. J Neurosci 12:3920-3924

Bruno JF, Xu Y, Song J, Berelowitz M 1992 Molecular cloning and functional expression of a brain-specific somatostatin receptor. Proc Natl Acad Sci USA 89:11151-11155

Bruno JF, Xu Y, Song J, Berelowitz M 1993 Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. Endocrinology 133:2561-2567

Bruno JF, Xu Y, Berelowitz M 1994 Somatostatin regulates somatostatin receptor subtypes mRNA expression in GH_3 cells. 76th Annual Meeting of The Endocrine Society, June, 1994, Anaheim, CA, Abstract

Buse J, Buse MG, Roberts WJ 1961 Eosinophilic adenoma of the pituitary and carcinoid tumors of the rectosigmoid area. J Clin Endocrinol Metab 21:735-737

Ceda GP, Hoffman AR 1985 Growth hormone-releasing factor desensitization in rat anterior pituitary cells *in vitro*. Endocrinology 116:1334-1340

Ch'ng LJC, Snadler LM, Kraenzlin ME, Burrin JM, Joplin JF, Bloom SR 1985 Long-term treatment of acromegaly with the somatostatin analog SMS 201-995. N Engl J Med 290:284-285

Charpenet G, Patel YC 1985 Characterization of tissue and releasable molecular forms of somatostatin-28_{1-12}-like immunoreactivity in rat median eminence. Endocrinology 116:1863-1868

Chihara K, Arimura A, Chihara M, Schally AV 1978 Studies on the mechanisms of growth hormone and thyrotropin responses to somatostatin antiserum in anesthetized rats. Enodocrinology 103:1916-1923

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

Chowen JA, Steiner RA, Clifton DK 1991 Semiquantitative analysis of cellular somotostatin mRNA levels by *in situ* hybridization histochemistry. Methods Neurosci 5:137-161

Chneiweiss H, Bertrand P, Epelbaum J, Kordon C, Glowinski J, Premont J, Enjalbert A 1987 Somatostatin receptors on cortical neurons and adenohypophysis: comparisons between specific binding and adenylate cyclase inhibition. Eur J Pharmacol 138:249-255

Clark RG, Robinson ICAF 1988 Paradoxical growth-promoting effects induced by patterned infusions of somatostatin in female rats. Endocrinology 122:2675-2682

Clark RG, Carlsson LMS, Rafferty B, Robinson ICAF 1988 The rebound release of growth hormone (GH) following somatostatin infusion in rats involves hypothalamic GH-releasing factor release. J Endocrinol 119:397-404

Clayton RN, Bailey LC 1987 Somatostatin partially reverses desensitization of somatotrophs induced by growth hormone-releasing factor. J Endocrinol 112:69-76

Clements D, Elias E 1985 Regression of metastatic VIPoma with somatostatin analog SMS 201-995. Lancet 1:874-875

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

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

Cooke NE, Szpirer C, Levan G 1986 The related genes encoding growth hormone and prolactin have been dispersed to chromosomes 10 and 17 in the rat. Endocrinology 119:2451-2454

Cooke NE, Ray J, Watson MA, Estes PA, KUO BA, Liebhaber SA 1988 Human growth hormone gene and the highly homologous growth hormone variant gene display different splicing patterns. J Clin Invest 82:270-275

Cowan JS, Moor B, Chow A, Kraicer J 1983 Characteristics of the post-somatostatin rebound in growth hormone secretion from perifused somatotrophs. Endocrinology 113:1056-1061

Dabek FT 1974 Bronchial carcinoid tumor with acromegaly in two patients. J Clin Endocrinol Metab 38:329-333

Daikoku S, Hisano S, Kawano H, Chikamori-Aoyama M, Kagotani Y, Zhang RJ, Chihara K 1988 Ultrastructural evidence for neuronal regulation of growth hormone secretion. Neuroendocrinology 47:405-415

Davies RR, Miller M, Turner SJ, Goodship TH, Cook DB, Watson M, McGill A, Orskov H, Alberti KG, Johnston DG 1986 Effects of somatostatin analogue SMS 201-995 in normal man. Clin Endocrinol 24:665-674

Del Pozo E, Neufeld M, Schluter K, Tortosa F, Clarenbach P, Bieder E, Wendel L, Nuesch E, Marbach P, Cramer H, Kerp L 1986 Endocrine profile of a long acting somatostatin derivative SMS 201-995. Study in normal volunteers following subcutaneous administration. Acta Endocrinologica 111:433-439

Del Valle J, Park J, Chiba T, Yamada T 1990 Cellular mechanisms of somatostatin actions in the gut. Clin Exp Metabolism 39:134-137

DeNoto FM, Moore DD, Goodman HM 1981 Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. Nucleic Acids Res 9:3719-3730

Deuben RR, Meites J 1964 Stimulation of pituitary growth hormone release by a hypothalamic extract *in vitro*. Endocrinology 74:408-414

Dierickx K, Vandesande F 1979 Immunocytochemical localization of somatostatin containing neurons in the rat hypothalamus. Cell Tissue Res 201:349-359

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

Epelbaum J, Willoughby JO, Brazeau P, Martin JB 1977 Effects of brain lesions and hypothalamic deafferentation on somatostatin distribution in the rat brain. Endocrinology 101:1495-1502

Ferland L, Labrie F, Jobin M, Arimura A, Schally AV 1976 Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. Biochem Biophys Res Commun 68:149-156

Finkelstein JW, Roffwarg HP, Booyar RM, Kream J, Hellman L 1972 Age-related changes in twenty-four-hour spontaneous secretion of growth hormone. J Clin Endocrinol Metab 35:665-670

Finley JCW, Maderdrut JL, Roger LJ, Petrusz P 1981 The immunocytochemical localization of somatostatin-containing neurons in the rat central nervous system. Neuroscience 6:2173-2192

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

Frohman LA, Jansson JO 1986 Growth hormone-releasing hormone. Endocr Rev 7:223-257

Fukata J, Diamond DJ, Martin JB 1985 Effects of rat growth hormone (rGH)-releasing factor and somatostatin on the release and synthesis of rGH in dispersed pituitary cells. Endocrinology 117:457-467

Gerich JE 1981 Somatostatin and diabetes. Am J Med 70:619-626

Glick SM, Goldsmith S 1968 The physiology of growth hormone secretion. In: Growth Hormone, Pecile A, Muller EE (eds) Excerpta Medica, Amsterdam, pp 84-88

Goldman JK, Schnatz JD, Bernardis LL, Frohman LA 1972 Effects of ventromedial hypothalamic destruction in rats with preexisting streptozotocin-induced diabetes. Metabolism 21:132-136

Goodman R, Aron DC, Ross BA 1983 Rat preprosomatostatin: structure and processing by microsomal membranes. J Biol Chem 258:5570-5573

Gottesman IS, Mandarino LJ, Gerich JE 1982 Somatostatin: its role in health and disease. Spec Top Endocrinol Metab 4:177-243

Green H, Morikawa M, Nixon T 1985 A dual effector theory of growth hormone action. Differentiation 29:195-198

Griffiths EC, Jeffcoate SL, Holland DT 1977 Inactivation of somatostatin by peptidases in different areas of the rat brain. Acta Endocrinol 85:1-4

Guillaume V, Magnan E, Cataldi M, Conte-Devolx B, Thomas F, Chihara K, Oliver C, BIM 23014, a long acting somatostatin analog inhibits GH-RH release into hypophyseal portal blood of conscious sheep. 9th International Congress of Endocrinology, Nice, France, 1992, Abstract P-08.01.076

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

Harris GW. The Pituitary Gland. Edward Arnold, London, 1955

Hershberger RE, Newman BL, Florio T, Bunzow J, Civelli O, Li X, Forte M, Stork PJS 1994 The somatostatin receptors SSTR1 and SSTR2 are coupled to inhibition of adenylate cyclase in chinese hamster ovary cells via pertussis toxin-sensitive pathways. Endocrinology 134:1277-1285

Horvath S, Palkovits M, Gorcs T, Arimura A 1989 Electron microscopic immunocytochemical evidence for the existence of bidirectional synaptic connections between growth hormone-releasing hormone- and somatostatin-containing neurons in the hypothalamus of the rat. Brain Res 481:8-15

Ikeda SR, Schofield GG 1989 Somatostatin blocks a calcium current in rat sympathetic ganglion neurons. J Physiol 409:221-240

Jacobowitz DM, Schulte H, Chrousos GP, Loriaux DL 1983 Localization of GRF-like immonoreactivity in the rat brain. Peptides 4:521-524

Jakobs K, Aktories K, Schultz G 1983 A nucleotide regulatory site for somatostatin inhibition of adenylate cyclase in S49 lymphoma cells. Nature 303:177-178

Johansson O, Hokfelt T, Elde RP 1984 Immunohistochemical distribution of somatostatin-like immunoreactivity in the central nervous system of the adult rat. Neuroscience 13:265-339

Johnson DG, Bressler R 1984 New pharmacological approaches to the treatment of diabetes. Spec Top Endocrinol Metab 6:163-192

Kahn CR, Neville DM, Roth J 1973 Insulin-receptor interaction in the obese-hyperglycemic mouse. A model of insulin resistance. J Biol Chem 248:244-250

Katakami H, Arimuura A, Frohman LA 1986 Growth hormone releasing hormone stimulates hypothalamic somatostatin release: an inhibitory feedback effect on growth hormone secretion. Endocrinology 118:1872-1877

Katakami H, Downs TR, Frohman LA 1988 Inhibitory effect of hypothalamic medial preoptic area somatostatin on growth hormone-releasing factor in the rat. Endocrinology 123:1103-1109

Katakami H, Hidaka H, Romanelli RJ, Mayo KE, Matsukura S Inhibition by somatostatin of growth hormone-releasing factor gene expression in the hypothalamic arcuate nucleus. 9th International Congress of Endocrinology, Nice, France, 1992, Abstract

Kawano H, Daikoku S, Saito S 1982 Immunohistochemical studies of intrahypothalamic somatostatin-containing neurons in the rat. Brain Res 242:227-232

Kitson HF, McCrossin RB, Jimenez M, Middleton A, Silink M 1980 Somatostatin treatment of insulin excess due to beta-cell adenoma in neonate. J Pediatr 86:145-149

Kluxen FW, Bruns C, Lubbert H 1992 Expression cloning of a rat brain somatostatin receptor cDNA. Proc Natl Acad Sci USA 89:4618-4622

Koerker DJ, Ruch W, Chideckel E, Palmer J, Goodner CJ, Ensinck J, Gale CC 1974 Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science 184:482-484

Kong H, DePaoli AM, Breder CD, Yasuda K, Bell GI, Reisine T 1994 Differential expression of messenger RNAs for somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3 in adult rat brain: analysis by RNA blotting and *in situ* hybridization histochemistry. Neuroscience 59:175-184

Kraenzlin ME, Ch'ng JCG, Wood SM, Carr DH 1985 Long-term treatment of a VIPoma with somatostatin analog resulting in remission of symptoms and possible shrinkage of metastases. Gastroenterology 88:185-187

Kraicer J, Cowan JS, Sheppard MS, Lussier B, Moor BC 1986 Effect of somatostatin withdrawal and growth hormone (GH)-releasing factor on GH release *in vitro*: Amount available for release after disinhibition. Endocrinology 119:2047-2051

Kraicer J, Sheppard MS, Luke J, Lussier B, Moor BC, Cowan JS 1988 Effect of withdrawal of somatostatin and growth hormone (GH)-releasing factor on GH release in vitro. Endocrinology 122:1810-1815

Krisch B 1978 Hypothalamic and extrahypothalamic distribution of somatostatin-immunoreactive elements in the rat brain. Cell Tissue Res 195:499-513

Krisch B 1979 Immunohistochemical results on the distribution of somatostatin in the hypothalamus and in the limbic system structures of the rat. J Histochem Cytochem 27:1389-1390

Krulich L, McCann S 1969 Effect of GH-releasing factor and GH-inhibiting factor on the release and concentration of GH in pituitaries incubated *in vitro*. Endocrinology 85:319-324

Lamberts SWJ, Verleun T, Oosterom R 1984 The interrelationship between the effects of somatostatin and pancreatic growth horome-releasing factor on growth hormone release by cultured pituitary tumor cells from two patients with acromegaly. J Clin Endocrinol Metab 58:250-254

Lamberts SWJ, Uitterlinden P, Verschoor L, Van Dongen KJ, del Pozo E 1985 Long-term treatment of acromegaly with the somatostatin analog SMS 201-995. N Engl J Med 313:1576-1580

Law S, Manning D, Reisine T 1991 Identification of the subunits of GTP binding proteins coupled to somatostatin receptors. J Biol Chem 268:17885-17897

Leroux P, Pelletier G 1984 Radioautographic localization of somatostatin-14 and somatostatin-28 binding sites in the rat brain. Peptides 5:503-506

Leroux P, Quirion R, Pelletier G 1985 Localization and characterization of somatostatin receptors as studied with somatostatin-14 and somatostatin-28 receptor radioautography. Brain Res 347:74-84

Leroux P, Gonzalez BJ, Laquerriere A, Bodenant C, Vaudry H 1988 Autoradiographic study of somatostatin receptors in the rat hypothalamus: Validation of a GTP-induced desaturation procedure. Neuroendocrinology 47:533-544

Lesniak MA, Roth J 1976 Regulation of receptor concentration by homologous hormone. Effect of human growth hormone on its receptors in IM-9 lymphocytes. J Biol Chem 251:3720-3729

Li XJ, Forte M, North RA, Ross CA, Synder SH 1992 Cloning and expression of a rat somatostatin receptor enriched in brain. J Biol Chem 267:21307-21312

Ling N, Esch F, Bohlen P, Brazeau P, Wehrenberg WB, Guillemin R 1984 Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: growth hormone-releasing factor. Proc Natl Acad Sci USA 81:4302-4306

Liposits Z, Merchenthaler I, Paull WK, Glerko B 1988 Synaptic communication between somatostatinergic axons and growth hormone-releasing factor (GRF) synthesizing neurons in the arcuate nucleus of the rat. Histochemistry 89:247-252

Longnecker SM 1988 Somatostatin and Octreotide: Literature review and description of therapeutic activity in pancreatic neoplasia. Drug Intell Clin Pharm 22:99-106

Losa M, Müller OA, Sobieszczyk S, Von Werder K 1985 Interaction between growth hormone releasing factor (GRF) and somatostatin analogue (SMS 201-995) in normal subjects. Clin Endocrinol 23:715-720

Lumpkin MD, Negro-Vilar A, McCann SM 1981 Paradoxical elevation of growth hormone by intraventricular somatostatin: possible ultrashort-loop feedback. Science 211:1072-1074

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

Mahy N, Woolkalis M, Thermos K, Carlson K, Manning D, Reisine T 1988 Pertussis toxin modifies the characteristics of both the inhibitory GTP binding proteins and the somatostatin receptor in anterior pituitary tumour cells. J Pharmacol Exp Ther 246:779-785

Makara GB, Palkovitz M, Antoni FA, Kiss JZ 1983 Topography of the somatostatin-immunoreactive fibers to the stalk-median eminence of the rat. Neuroendocrinology 37:1-8

Malacara JM, Valverde C, Reichlin S, Bollinger J 1972 Elevation of plasma radioimmunoassayable growth hormone in the rat induced by porcine hypothalamic extract. Endocrinology 91:1189-1198

Martin JB 1972 Plasma growth hormone (GH) responses to hypothalamic or extrahypothalamic electrical stimulation. Endocrinology 91:107-115

Martin JB 1973 Neural regulation of growth hormone secretion. N Engl J Med 288:1384-1393

Martin JB, Kontor J, Mead P 1973 Plasma GH responses to hypothalamic, hippocampal and amygdaloid electrical stimulation: effects of variation in stimulus parameters and treatment with methyl-p-tyrosine (-MT). Endocrinology 92:1354-1361

Martin JB, Renaud LP, Tannenbaum GS 1974 Pulsatile growth hormone secretion: suppression by hypothalamic ventromedial lesions and by long-acting somatostatin. Science 186:538-540

Martin JB, Tannenbaum G, Willoughby JO, Renaud, LP, 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 PG, Martin L (eds), Academic Press, New York, pp: 217-236

Masuda A, Shibasaki T, Kim YS, Imaki T, Hotta M, Demura H, Ling, N Shizume K 1989 The somatostatin analog octreotide inhibits the secretion of growth hormone (GH)-releasing hormone, thyrotropin, and GH in man. J Clin Endocrinol Metab 69:906-909

McCarthy GF, Beaudet A, Tannenbaum GS 1992 Colocalization of somatostatin receptors and growth hormone-releasing factor immunoreactivity in neurons of the rat arcuate nucleus. Neuroendocrinology 56:18-24

Mendelson WB, Jacobs LS, Gillin JC, Wyatt RJ 1979 The regulation of insulin-induced and sleep-related human growth hormone secretion: a review. Psychoneuroendocrinology 4:341-349

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

Meyerhof W, Paust HJ, Schonrock C, Richter D 1991 Cloning of a cDNA encoding a novel putative G-protein-coupled receptor expressed in specific rat brain region. DNA Cell Biol 10:689-694

Miki N, Ono M, Shizume K 1984 Evidence that opiatergic and α-adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). Endocrinology 114:1950-1952

Miki N, Ono M, Shizume K 1988 Withdrawal of endogenous somatostatin induces secretion of growth hormone-releasing factor in rats. J Endocrinol 117:245-252.

Miller RE 1981 Pancreatic neuroendocrinology: peripheral neural mechansims in the regulation of the islets of Langerhans. Endocr Rev 2:471-494

Mogard MH, Maxwell V, Kovacs T, Van Deventer G, Elashoff JD, Yamada T, Kauffman GL jr, Walsh JH 1985 Somatostatin inhibits gastric acid secretion after gastric mucosal prostaglandin synthesis inhibition by indomethacin in man. Gut 26:1189-1191

Montminy MR, Goodmann RH, Horovitch SJ, Habener JF 1984 Primary structure of the gene encoding rat preprosomatostatin. Proc Natl Acad Sci 81:3337-3340

Najimi M, Jordan D, Chigr F, Champier J, Kopp N, Slama A, Bertherat J, Videau C, Epelbaum J 1991 Regional distribution of somatostatin binding sites in the human hypothalamus: a quantitative autoradiographic study. Neuroscience 40:321-335

Nair RMG, DeVillier C, Barnes M, Antalis J, Wilbur DL 1978 A bovine hypothalamic peptide possessing immunoreactive growth hormone-releasing activity. Endocrinology 103:112-120

O'Carroll AM, Lolait SJ, Konig M, Mahan LC 1992 Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. Mol Pharmacol 42:939-946

O'Dorisio TM 1986 Neuuroendocrine disorders of the gastroenteropancreatic system. Clinical applications of the somatostatin analog SMS 201-995. Am J Med 81(6B):100-101

Okada K, Wakabayashi I, Sugihara H, Minami S, Kitamura T, Yamada J 1991 Electrical stimulation of hypothalamic periventricular nucleus is followed by a large rebound secretion of of growth hormone in unanesthetized rat. Neuroendocrinology 53:306-312

Owerbach D, Rutter WJ, Martial JA, Baxter JD, Shows TB 1980 Genes for growth hormone, chorionic somatommammotropin, and growth hormone-like gene on chromosome 17 in humans. Med J S Dakota 209:289-292

Painson J-C, Tannenbaum GS 1991 Sexual dimorphism of somatostatin and growth hormone-releasing factor signaling in the control of pulsatile growth hormone secretion in the rat. Endocrinology 128:2858-2866

Patel YC, Hoyte K, Martin JB 1979 Effect of anterior hypothalamic lesions on neurohypophysial and peripheral tissue concentrations of somatostatin in the rat. Endocrinology 105:712-715

Patel YC, Wheatley T, Ning C 1981 Multiple forms of immunoreactive somatostatin: comparison of distribution in neural and nonneural tissues and portal plasma of the rat. Endocrinology 109:1943-1949

Patel Y, Baquiran G, Srikant CB 1986 Quantitative *in vivo* autoradiographic localization of ^{125}I-tyr^{11} somatostatin-14 and ^{125}I-Leu^{8}trp^{22} somatostatin-28 binding sites in rat brain. Endocrinology 119:2262-2269

Patel Y, Greenwood M, Kent G, Panetta R, Srikant C 1993 Multiple gene transcripts of the somatostatin receptor SSTR2: Tissue selective distribution and cAMP regulation. Biochem Biophys Res Commun 192: 288-294

Pless J, Bauer W, Briner U, Doepfner W, Marbach P, Maurer R, Petcher TJ, Reubi JC, Vonderscher J 1986 Chemistry and pharmacology of SMS 201-995, a long-acting octapeptide analogue of somatostatin. Scand J Gastroenterol Suppl 119:54-94

Plewe G, Beyer J, Krause U, Neufeld M, del Pozo E 1984 Long acting and selective suppression of growth hormone secretion by somatostatin analog SMS 201-995 in acromegaly. Lancet 2:782-784

Plotsky PM, Vale W 1985 Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. Science 230:461-463

Pradayrol L, Jornvall H, Mutt V 1980 N-terminally-extended somatostatin: The primary structure of somatostatin-28. FEBS Lett 109:55-58

Presky DH, Schonbrunn A 1988 Somatostatin pretreatment increases the number of somatostatin receptors in GH_4C_1 pituitary cells and does not reduce cellular responsiveness to somatostatin. J Biol Chem 263:714-721

Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon ST 1992 Sequence alignment of the G-protein coupled receptor family. DNA Cell Biol 11:1-20

Raynor K, Coy DC, Reisine T 1992 Analogs of somatostatin bind selectively to brain somatostatin receptor subtypes. J Neurochem 59:1241-1250

Reichlin S 1983 Somatostatin. N Eng J Med 309:1495-1501

Reichlin S 1983 Somatostatin (2nd of two parts). N Eng J Med 309:1556-1563

Reisine T, Axelrod J 1983 Prolonged somatostatin pretreatment desensitizes somatostatin's inhibition of receptor mediated release of adrenocorticotropin hormone and sensitizes adenylate cyclase. Endocrinology 113:811-813

Reisine T 1984 Somatostatin desensitization: loss of the ability of somatostatin to inhibit cyclic AMP accumulation and adrenocorticotropin hormone release. J Pharmacol Exp Ther 229:14-20

Rene E, Bonfils S 1984 Control of severe diarrhea with somatostatom (letter). N Engl J Med 311:598-599

Rens-Domiano S, Lay SF, Yamada Y, Seino S, Bell GI, Reisine T 1992 Pharmacological properties of two cloned somatostatin receptors. Mol Pharmacol 42:28-34

Reubi J, Perrin M, Rivier J, Vale W 1981 High affinity binding sites for a somatostatin-28 analog in rat brain. Life Sci 28:2191-2198

Reubi J 1984 Evidence for two somatostatin-14 receptor types in rat brain cortex. Neuroscience Lett 49:259-263

Reubi JC, Maurer R 1985 Autoradiographic mapping of somatostatin receptors in the rat central nervous system and pituitary. Neuroscience 15:1183-1193

Reubi JC, Cortes R, Maurer R, Probst A, Palacios JM 1986 Distribution of somatostatin receptors in the human brain: an autoradiographic study. Neuroscience 18:329-346

Richardson SB, Twente S 1991 Pre-exposure of rat anterior pituitary cells to somatostatin enhances subsequent growth hormone secretion. J Endocrinol 128:91-95

Richardson SB, Twente S 1993 Timing of exposure to somatostatin relative to growth hormone-releasing factor dictates the rat anterior pituitary cell growth hormone response. J Endocrinol 138:369-377

Rinderknecht E, Humbel RE 1976 Amino terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: evidence for structural homology with insulin B chain. Proc Natl Acad Sci USA 73:4379-4381

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

Saeed Uz Zafar M, Mellinger RC, Fine G, Szabo M, Frohman LA 1979 Acromegaly associated with a bronchial carcinoid tumor: evidence for ectopic production of growth hormone-releasing activity. J Clin Endocrinol Metab 46:66-71

Sakamoto C, Goldfine I, Williams J 1984 The somatostatin receptor on isolated pancreatic acinar cell plasma membranes. J Biol Chem 259:9623-9627

Salmon WD Jr, Daughaday WH 1957 A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vivo*. J Lab Clin Med 49:825-836

Santangelo WC, O'Dorisio TM, Kim JG, Severino G, Krejs G 1986 VIPoma syndrome: effect of synthetic somatostatin analogue. Scand J Gastroenterol 21:187-190

Sassolas G, Chayvialle JA, Partensky C, Berger G, Trouillas J, Berger F, Claustrat B, Cohen R, Girod C, Guillemin R 1983 Acromegalie, expression clinique de la production de facteurs de liberation de l'hormone de croissance (GRF) par une tumeur pancreatique. Ann Endocrinol (Paris) 44:347-354

Schonbrunn A, Tashjian jr AH 1978 Characterization of functional receptors in rat pituitary cells in culture. J Biol Chem 253:6473-6483

Shalet SM, Beardwell CG, McFarlane IA, Ellison ML, Norman CM, Rees LH, Highes M 1979 Acromegaly due to the production of growth hormone-releasing factor by a bronchial carcinoid tumour. Clin Endocrinol (Oxf) 10:61-67

Sheppard M, Shapiro B, Pimstone B, Kronheim MB, Gregory M 1979 The metabolic clearance and plasma half-life disappearance time of exogenous somatostatin in man. J Clin Endocrinol Metab 48: 50-53

Smith M, Yamamoto G, Vale W 1984 Somatostatin desensitization in rat anterior pituitary cells. Mol Cell Endocrinol 37:311-318

Soya H, Suzuki M 1988 Somatostatin rapidly restores rat growth hormone (GH) release response attenuated by prior exposure to human GH-releasing factor *in vitro*. Endocrinology 122:2492-2498

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

Spitz I, Gonen B, Rabinowitz D 1972 Growth hormone release in man revised: Spontaneous vs stimulus-initiated tides. In: Growth and Growth Hormone. Pecile A, Muller EE (Eds) Excerpta Medica, Amsterdam, pp. 371-381

Srikant CB, Patel Y 1981 Somatostatin receptors: identification and characterization in rat brain membrane. Proc Natl Acad Sci USA 78:3930-3934

Srikant CB, Patel Y 1984 Cyteamine-induced depletion of brain somatostatin is associated with up-regulation of cerebrocortical somatostatin receptors. Endocrinology 115:990-995

Srikant CB, Patel Y 1986 Somatostatin receptors on rat pancreatic acinar cells. J Biol Chem 261:7690-7696

Stachura ME, Dhariwal APS, Frohman LA 1972 Growth hormone synthesis and release *in vitro*: effects of partially purified ovine hypothalamic extracts. Endocrinology 91:1071-1078

Steiner RA, Stewart JK, Barber J, Koerker D, Goodner CJ, Brown A, Illner P, Gale CC 1978 Somatostatin: A physiological role in the regulation of growth hormone secretion in the adolescent male baboon. Endocrinology 102:1587-1594

Strobl JS, Thomas MJ 1994 Human growth hormone. Pharmacol Rev 46:1-34

Sugihara H, Minami S, Wakabayashi I 1989 Post-somatostatin rebound secretion of growth hormone is dependent on growth hormone-releasing factor in unrestrained female rats. J Endocrinol 122:583-591

Szabo M, Dudlak D, Thominet J, Frohman LA 1983 Ectopic growth hormone-releasing factor (GHRF) stimulates growth hormone secretion in the urethane-anesthetized rat *in vivo*. Neuroendocrinology 37:328-331

Takahashi Y, Kipnis DM, Daughaday WH 1968 Growth hormone secretion during sleep. J Clin Invest 47:2079-2090

Takahashi Y, Daughaday WH, Kipnis DM 1971 Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology 88:909-917

Tallent M, Reisine T 1992 $G_{i\alpha1}$ selectively couples somatostatin receptors to adenylate cyclase in AtT-20 cell membranes. Mol Pharmacol 41:452-255

Tannenbaum GS, Martin JB 1976 Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98:562-570

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

Tannenbaum GS, Ling N, Brazeau P 1982 Somatostatin-28 is longer acting and more selective than somatostatin-14 on pituitary and pancreatic hormone release. Endocrinology 111:101-107

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

Tannenbaum GS, 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

Tannenbaum GS, Patel YC 1986 On the fate of centrally administered somatostatin in the rat: massive hypersomatostatinemia resulting from leakage into the peripheral circulation has effects on growth hormone secretion and glucoregulation. Endocrinology 118:2137-2143

Tannenbaum GS 1988 Somatostatin as a physiological regulator of pulsatile growth hormone secretion. Hormone Res 29:70-74

Tannenbaum GS, Painson J-C, Lengyel AMJ, Brazeau P 1989 Paradoxical enhancement of pituitary growth hormone (GH) responsiveness to GH-releasing factor in the face of high somatostatin tone. Endocrinology 124:1380-1388

Tannenbaum GS, McCarthy GF, Zeitler P, Beaudet A 1990a Cysteamine-induced enhancement of growth hormone-releasing factor (GRF) immunoreactivity in arcuate neurons: morphological evidence for putative somatostatin/GRF interactions within hypothalamus. Endocrinology 127:2551-2560

Tannenbaum GS, Painson JC, Gurd W, Reeves I, Brazeau P Growth hormone secretory dynamics in response to a sustained-release preparation of a growth hormone-releasing factor analog: evidence for an ultradian rhythm of somatostatin secretion. 72nd Annual Meeting of The Endocrine Society, 1990b, Abstract

Tannenbaum GS 1991 Neuroendocrine control of growth hormone secretion. Acta Paediatr Scand [Suppl] 372:5-16

Tannenbaum GS, Farhadi-Jou F, Beaudet A 1993 Ultradian oscillation in somatostatin binding in the arcuate nucleus of adult male rats. Endocrinology 133:1029-1033

Terry LC, Martin JB 1981 The effects of lateral hypothalamic-medial forebrain stimulation and somatostatin antiserum on pulsatile growth hormone secretion in freely behaving rats: Evidence for a dual regulatory mechanism. Enocrinology **109**:622-627

Thermos K, Reisine T 1988 Somatostatin receptor subtypes in the clonal anterior pituitary cell lines AtT-20 and GH_3 cells. Mol Pharmacol 33:370-377

Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K 1982 Somatotroph hyperplasia: successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. J Clin Invest **70**:965-977

Thorner MO, Rivier J, Spiess J, Borges JL, Vance ML, Bloom SR, Rogol AD, Cronin MJ, Kaiser DL, Evans WS, Webster JD, Macleod RM, Vale W 1983 Human pancreatic growth hormone-releasing factor selectively stimulates growth hormone secretion in man. Lancet **1**:24-29

Tran VT, Uhl GR, Perry DC, Manning DC, Vale WW, Perrin MH, Rivier JE, Martin JB, Snyder SH 1984 Autoradiographic localization of somatostatin receptors in rat brain. Eur J Pharmacol **101**:307-309

Tran VT, Beal F, Martin J 1985 Two types of somatostatin receptors differentiated by cyclic somatostatin analogs. Science **22**:492-495

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

Uhl G, Tran V, Snyder S, Martin J 1985 Somatostatin receptors: distribution in rat brain. Eur J Neurol **240**:288-304

Vale W, Rivier C, Brazeau P, Guillemin R 1975 Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology **95**:968-977

Vale W, Vaughan J, Yamamoto G, Spiess J, 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

Vanetti M, Kouba M, Wank X, Vogt G, Hollt V 1992 Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). Fedn Eur Biochem Socs Lett 311:290-294

Vance ML, Kaiser DL, Evans WS, Furlanetto R, Vale W, Rivier J, Thorner MO 1985 Pulsatile growth hormone secretion in normal man during a continuous infusion of human growth hormone releasing factor (1-40). Evidence for intermittent somatostatin secretion. J Clin Invest 75:1584-1590

Vinik AI, Tsai ST, Moattari AR, Cheung P, Eckhauser FE, Cho K 1986 Somatostatin analog (SMS 201-995) in the management of gastroenteropancreatic tumors and diarrhea syndromes. Am J Med 81(6B):23-41

Wang H, Bogen C, Reisine T, Dichter M 1989 Somatostatin-14 and somatostatin-28 induce opposite effects on pottassium currents in rat neocortical neurons. Proc Natl Acad Sci USA 86:9616-9620

Wang H, Reisine T, Dichter M 1990 Somatostatin-14 and somatostatin-28 inhibit calcium currents in rat neocortical neurons. Neuroscience 342:335-342

Weeke J, Hansen AP, Lundaek K 1975 Inhibition by somatostatin of basal levels of serum thyrotropin in normal men. J Clin Endocrinol Metab 41:168-171

Wehrenberg WB, Ling N, Brazeau P, Esch F, Bohlen P, Baird A, Ying S, Guillemin R 1982a Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. Biochem Biophys Re Comm 109:382-387

Wehrenberg WB, Brazeau P, Luben R, Böhlen P, Guillemin R 1982b Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). Endocrinology **111**:2147-2148

Wehrenberg WB, Brazeau P, Ling N, Testor G, Guillemin R 1984 Pituitary growth hormone response in rats during a 24-hour infusion of growth hormone-releasing factor. Endocrinology **114**:1613-1616

Wehrenberg WB, Seifert H, Bilezikjian LM, Vale W 1986 Down-regulation of growth hormone releasing factor receptors following continuous infusion of growth hormone releasing factor *in vivo*. Neuroendocrinology **43**:266-268

Weiss J, Cronin MJ, Thorner MO 1987 Periodic interactions of GH-releasing factor and somatostatin can augment GH release *in vitro*. Am J Physiol 253: E508-E514

Willoughby JO, Terry LC, Brazeau P, Martin JB 1977 Pulsatile growth hormone, prolactin, and thyrotropin secretion in rats with hypothalamic deafferentation. Brain Res **127**:137-152

Willoughby JO, Martin JB 1978 Pulsatile growth hormone secretion: inhibitory role of medial preoptic area. Brain Res **148**:240-244

Willoughby JO, Brogan M, Kapoor R 1989 Hypothalamic interconnections of somatostatin and growth hormone releasing factor neurons. Neuroendocrinology **50**:584-591

Yamada Y, Ito S, Matsubara Y, Kobayashi S, 1977 Immunohistochemical demonstration of somatostatin-containing cells in the human, dog, and rat thyroids. Toohuku J Exp Med **122**:87-92

Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S 1992a Cloning and functional characteristics of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. P.N.A.S. USA 89:251-255

Yamada Y, Resine T, Law SF, Ihara Y, Kubota A, Kagimoto S, Seino M, Seino Y, Bell GI, Seino S 1992b Somatostatin receptors, an expanding gene family: Cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. Mol Endocrinol 92:2136-2142

Yamauchi N, Shibasaki T, Ling, N, Demura H 1991 In vitro release of growth hormone-releasing factor (GRF) from the hypothalamus: somatostatin inhibits GRF release. Reg Peptides 33:71-78

Yasuda K, Rens-Domiano S, Breder CD, Law S, Saper CB, Reisine T, Bell GI 1992 Cloning of a novel somatostatin receptor, SSTR3, coupled to adenylcyclase. J Biol Chem 267:20422-20428

Zeitler P, Tannenbaum GS, Clifton DK, Steiner RA 1991 Ultradian oscillations in somatostatin and growth hormone-releasing hormone mRNAs in the brains of adult male rats. P.N.A.S. USA 88:8920-8924