Hypothalamic Inhibition of Growth Hormone Secretion

by

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DEDICATION

This dissertation is dedicated to my wife Marty, whose constant support, encouragement and understanding made the successful completion of this work possible.

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ABBREVIATIONS

BHPI	basal hypothalamus-pituitary island
C	degrees Celsius
cc	cubic centimeters
cm	centimeter
g	gram(s)
g	gravity (when preceded by x)
GH	growth hormone (somatotropin)
h	hour(s)
id	inside diameter
im	intramuscular
ip	intraperitoneal
mA	milliamperes
MBH	medial basal hypothalamus
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
N	normal (concentration)
ng	nanogram(s)
NS	not significant
od	outside diameter
p	probability
pg	picogram(s)
PO-AHA	preoptic-anterior hypothalamic area

PV	periventricular
SE	standard error of mean
sec	second(s)
SRIF	somatotropin-release inhibiting hormone (somatostatin)
TRF	thyrotropin-releasing factor
TSH	thyroid-stimulating hormone (thyrotropin)
U	unit(s)
µg	microgram(s)
µl	microliter(s)

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THESIS ABSTRACT

Evidence suggests that the medial preoptic-anterior hypothalamic area (PO-AHA) plays a key role in the inhibitory regulation of growth hormone (GH) secretion and that this inhibition is mediated by somatostatin (SRIF)-containing neurons in this part of the brain. Thus, large lesions of the medial PO-AHA or interruption of connections between this area and the median eminence produced marked depletion of SRIF in the median eminence, chronic elevation of nonstress plasma GH levels and blockade of the GH response to stress. SRIF has also been shown to inhibit thyrotropin (TSH) secretion, although it is not known whether SRIF neurons in the PO-AHA are involved in this inhibition.

This picture was complicated by the results of recent experiments in this laboratory which showed that small lesions confined to the periventricular (PV) nucleus of the PO-AHA, the location of the majority of SRIF-containing neuron perikarya in the hypothalamus, caused marked depletion of median eminence SRIF content but only transient elevations of nonstress plasma GH and TSH levels and had no effect on the GH response to stress. In order to reproduce and extend these seemingly paradoxical findings, electrolytic lesions which were larger than those studied

previously, were placed in the PV nucleus of female rats (Manuscript 1). Blood samples collected at various times after placement of PV lesions showed that nonstress plasma GH levels were only transiently elevated and the GH response to stress was normal. In contrast, PV lesions produced long-term elevation of nonstress plasma TSH levels and blockade of stress-induced suppression of TSH secretion. Content and concentration of SRIF in the median eminence were reduced 90% in the PV-lesioned group. Thus, it appears that PV lesions which markedly deplete SRIF content in the median eminence can cause long-term elevations of plasma TSH levels and block the TSH response to stress without producing alterations in GH secretion.

Studies suggest that the PO-AHA and SRIF are involved in the suppression of the pulsatile pattern of GH secretion. The purpose of the experiment described in the second manuscript was to determine the effects of destroying the SRIF neurons of the PV nucleus and depletion of median eminence SRIF on the pulsatile pattern of GH secretion. At 6-10 days after placement of lesions in female rats, blood samples collected every 15 min for 3-4h through indwelling atrial cannulae showed an elevation in baseline GH levels and an increase in the amplitude of GH secretory peaks. These changes caused an increase in mean circulating plasma GH levels. However, these alterations were transient

because blood samples collected 6-17 weeks after surgery demonstrated that GH levels in PV-lesioned rats were similar to those in control. At autopsy, nonstress GH levels were similar in all treatment groups, although median eminence content of SRIF was 85% depleted in the PV-lesioned group. These results suggest that the hypothalamic PV nucleus is essential for the maintenance of most of the SRIF in the median eminence, but it does not seem to be necessary for long-term suppression of the pulsatile pattern of GH secretion.

Evidence suggests that the medial basal hypothalamus (MBH) is responsible for the pulsatile pattern of GH secretion. In the experiment present in the third manuscript a basal hypothalamus-pituitary island (BHPI) was isolated by partial forebrain removal and brainstem transection to determine whether these tissues can support pulsatile GH secretion. The effects of physical removal of brain structures which inhibit GH secretion on the pulsatile pattern of such secretion and content of SRIF in basal hypothalamic tissue were also studied. Thirty hours after forebrain removal, sequential blood samples collected through indwelling atrial cannulae every 15 min for approximately 3h showed that BHPI rats had marked increases in both baseline plasma GH levels and amplitude of peaks. These changes were associated with markedly elevated

circulating GH levels. Immobilization stress caused a paradoxical rise in plasma GH levels in the BHPI group. At autopsy, nonstress plasma GH levels in BHPI rats were elevated, although SRIF content in MBH-median eminence tissue was similar to that in sham-operated controls. Thus, it appears that the BHPI is capable of supporting pulsatile GH secretion and that brain structures outside the MBH inhibit GH secretion by affecting both the baseline and amplitude of secretory pulses. In addition, removal of SRIF neuron cell bodies outside of the MBH does not lead to depletion of SRIF in the MBH-median eminence at 30h after surgery.

Collectively, these studies show that destruction of many of the hypothalamic SRIF neurons and the subsequent 80-90% depletion of median eminence SRIF are not necessarily associated with elevated nonstress plasma GH levels, blockade of stress-induced inhibition of GH secretion or long-term alterations in the pulsatile pattern of GH secretion. These studies also show that the MBH is an adequate neural substrate for pulsatile GH secretion, that GH secretion under nonstress and stress conditions is inhibited by brain structures lying outside of the MBH and that the hypothalamic PV nucleus inhibits nonstress TSH levels and is involved in the TSH respnse to stress. It is possible that SRIF is involved in the inhibition of GH and TSH secretion although the site and mechanism of action remain to be determined.

CHAPTER I

HISTORICAL REVIEW OF LITERATURE

A. Introduction: Growth Hormone

1. Discovery of Pituitary Growth Hormone

In 1921, Evans and Long became the first to show that the anterior lobe of the pituitary gland contained growth promoting substances. They demonstrated that repeated intraperitoneal injections of anterior pituitary extract produced gigantism in rats (1). Smith and Smith (2,3) later showed that the acidophilic and not the basophilic-rich portions of the pituitary gland stimulated growth in hypophysectomized tadpoles. Thus, it appeared that the pituitary gland was involved in regulating growth and that the acidophilic portions of the anterior lobe contained a 'growth hormone' (GH). Great advances in growth hormone research followed the development of a sensitive bioassay for GH (4), including the purification of bovine GH in 1944 (5). It was shown that mammalian GH is a simple globular protein with a molecular weight of approximately 22,000.

2. Hypothalamo-pituitary Interactions

Information concerning the regulation of GH secretion was limited until relatively recently.

It was known since the early 1950's that the hypothalamus exerted a trophic influence on the secretion of most hormones from the pituitary including GH. Pituitaries grafted to sites distant from the hypothalamus, lost their GH-secreting acidophilic cells (6,7) and the rate of growth in these animals was much lower than in intact controls (8,9). It was subsequently shown that hypophysectomized rats receiving pituitary implants to the ventral portion of the hypothalamus had a significantly greater rate of growth than those receiving implants to other sites (10). These studies suggested that the brain exerts some control on the secretion of GH. The first direct evidence that the diencephalon was involved in the regulation of GH secretion came from the studies of Cahane and Cahane (11). These investigators demonstrated growth retardation in combination with regressive changes in pituitary acidophilic cells in rats subjected to massive lesions of the ventral hypothalamus. Similar findings were made in a number of subsequent studies (see 12) but an accurate description of the areas in the brain that were presumably essential for maintaining normal GH secretion and growth was lacking. It seemed that hypothalamic regulation of pituitary function was probably not mediated

by direct nervous connections since the only nerve fibers in the anterior pituitary gland are peripheral autonomic fibers innervating pituitary vasculature (13). However, it was known that functional hypothalamo-hypophysial portal vessels had to be present in order to maintain normal pituitary function (14,15,16). Furthermore, it had been shown that nerve fibers to the pituitary did not regenerate after section (17,18) but portal vessels had an amazing capacity to regenerate (15,19) following their transaction. In fact, the return of normal pituitary hormone secretion in hypophysectomized animals that had received pituitary implants, depended upon the regeneration of these vessels. These findings were best explained by the view that nerve fibers of the hypothalamus liberate some humoral substance into the primary plexus of the hypophysial portal vessels and that these vessels transmit the substance to the adenohypophysis where it exerts an activating effect on the gland cells (20). It was Reichlin (21) in the late 1950's who first systematically localized areas in the hypothalamus that are involved in the regulation of growth. Reichlin found that severe growth retardation was produced in rats with lesions placed in the anterior half of the median eminence, the arcuate nuclei and the supraoptico-hypophyseal tract as it courses through the median

eminence. The concept of the brain communicating with the pituitary by the release of substances into the blood rather than by direct nervous connections was a radical one. Nonetheless, an increasing body of literature was accumulating in support of this concept. The following passage from a review of the neural control of the pituitary gland by G.W. Harris in 1948 (13), reflects the view of endocrinologists at that time.

"The slowly acting endocrines are apparently regulated by humoral means. Some activities of the adenohypophysis are clearly influenced by the nervous system, and it may be that nervous control of the endocrine system as a whole is enacted through the mediation of this gland. This surmise would place the hypothalamicoadenohypophysial unit as a key link in the chain of neural endocrine regulation..."

B. Hypophysiotrophic Hormones

1. Growth Hormone-Releasing Hormone

The dependence of the secretion of pituitary tropic hormones on the hypothalamus stimulated the search for hypothalamic 'releasing factors' that affect anterior pituitary secretion (22). In the early 1960's, it was shown that pituitary fragments were able to maintain their cellular structure and secretory function

only when they were implanted directly into the basal hypothalamic region. This region was subsequently named the hypophysiotrophic area (23,24). These observations led a number of investigators to prepare extracts from basal hypothalamic tissue. Before the introduction of radioimmunoassay (RIA) techniques for the direct measurement of GH, investigators relied on less sensitive and technically difficult bioassays in order to detect GH-releasing activity. Brain extracts were incubated with rat pituitaries in vitro and the incubation medium was then injected into hypophysectomized rats. Early GHRH studies produced conflicting results (25). Finally, after the introduction of practical RIA methods in the early 1960's, a more accurate understanding of the neural regulation of GH secretion began to emerge. Krulich and collaborators found that various fractions of hypothalamic extracts produced both GH-releasing (29,30,31) and release-inhibiting activity (30,31,32) from pituitary cells in vitro. GH-releasing activity in hypothalamic extracts and portal blood (33) has been demonstrated, although the structure of the GH-releasing factor has not yet been determined. The anatomical distribution of GHRF activity in the rat hypothalamus appears to be restricted to the area of the ventromedial nucleus (34).

It appears that this region is similar in location to the area where lesion placement produced growth retardation in young rats (21).

2. Growth Hormone-Release-Inhibiting Hormone.

Following the initial identification of GH-release inhibiting activity in hypothalamic extracts, Guillemin and collaborators in 1973, isolated, identified and synthesized the GH-release-inhibiting factor and renamed it somatostatin (SRIF) (35,36,37). SRIF is a 14 amino acid peptide with widespread inhibitory effects on the secretion of a number of hormones in many species. SRIF has been found throughout the central nervous system with highest concentrations in the hypothalamic median eminence (38,39,40,40). SRIF-containing cell bodies have been found in the periventricular (PV) nucleus of the anterior hypothalamus and preoptic area (POA) (40,41,42,43,44) and they are also located in a number of extrahypothalamic areas such as amygdala, hippocampus and cerebral cortex (40,41).

The SRIF-containing neuron cell bodies in the hypothalamic periventricular area seem to be responsible for most of the SRIF in the median eminence. Lesions in the PV area or knife cuts placed between this area and the median eminence reduce the content of SRIF in

the median eminence by 80-90% (41,45,46,47,48). These procedures were not associated with changes in SRIF-fiber content of arcuate or ventromedial nuclei (40,41 47). Such findings indicate that other somatostatinergic neurons, probably of extrahypothalamic origin, project to these nuclei in the basal hypothalamus. These projections may represent neural connections through which extrahypothalamic areas affect hypothalamic function and pituitary hormone secretion. In this regard, it has been shown that medial basal amygdaloid lesions result in a reduction of median eminence SRIF by about 30% (48). Thus, limbic forebrain structures may be directly involved in the inhibitory regulation of GH secretion through connections of this type.

C. Neural Regulation of Growth Hormone Secretion

1. Hypothalamic Regulation

It is generally accepted that the hypothalamus regulates the secretion of pituitary GH by the secretion of a GH-releasing and GH-release-inhibiting hormone. Many of the hypothalamic peptidergic neurons that synthesize these factors have their cell bodies in the hypophysiotropic area and their axons project to other brain regions and to the primary plexus of the hypophysial portal vessles located in the external or

palisade layer of the median eminence. After gaining access to hypophysial-portal circulation, these factors reach pituitary sinusoids by way of the portal veins and the secretion of GH from pituitary somatotrophs is either stimulated or inhibited. Although this view was formulated during the pre-RIA era, it is still accepted today but in a much expanded form. In contrast to the early view that the GH control system is slow to react to exogenous stimuli, and primarily concerned with growth, more recent studies have revealed that GH secretion is a highly labile function with diverse physiologic roles. GH is secreted in an episodic manner and is rapidly and markedly influenced by many stimuli including psychic factors, exercise, food intake and a number of stressful, metabolic and endocrine factors (25).

In the rat, the ventromedial (VM) nuclei seem to be of primary importance in the regulation of GH secretion. Lesions in this area resulted in growth retardation and a fall in plasma and pituitary GH levels (49), while electrical stimulation of the VM-arcuate complex produced a prompt rise in plasma GH levels (50,51). These studies suggest that the VM region can stimulate GH secretion, a finding which is consistent with studies showing that extracts of tissue from this

region contain GH-releasing activity (34). Growth hormone secretion in the rat occurs in episodic pulses. In the female rat, these pulses occur at intervals of approximately 70 min with peak GH levels reaching about 70 ng/ml plasma and trough values declining to less than 10 ng/ml (52). The pulsatile pattern of GH secretion is entrained to the light-dark cycle (53) and occurs independent of fluctuations in plasma concentrations of corticosterone, prolactin or thyrotropin (54). Lesions of the VM nuclei have been shown to block pulsatile GH secretion (55), indicating that this area is essential for the production of episodic GH secretory pulses. Because pretreatment of rats with anti-SRIF serum elevated GH secretory peaks and increased basal levels without abolishing the pulsatile secretory pattern, Ferland et al. (56) suggested that pulsatile GH release probably results from the intermittent release of GHRF. Other investigators have arrived at similar conclusions using these techniques (57,58).

2. Extrahypothalamic Regulation

Many neuroanatomic pathways have been described (59,60,61,62) connecting extrahypothalamic structures with the medial basal hypothalamus (MBH). However, it is unknown whether these structures played a major role in the regulation of GH secretion since isolation of the

MBH did not suppress plasma GH levels or abolish pulsatile GH release (63,64,65). It was not until 1969 when Eleftheriou et al. (66) reported that amygdaloid lesions in the deermouse enhanced GHRF activity in the hypothalamus and increased pituitary GH content that a possible role of extrahypothalamic structures in GH control was first suggested. Further investigations showed that various extrahypothalamic structures can have differential excitatory or inhibitory effects on GH secretion. For example, in a series of experiments by Martin and coworkers, the effects of electrical stimulation of various limbic forebrain structures on plasma GH levels were investigated. It was shown that stimulation of the hippocampal formation or basolateral amygdala caused a prompt GH release which could be blocked by placement of bilateral VM lesions (51,67,68). These findings suggest that the GHRF-producing area of the MBH is involved in this rise in plasma GH levels induced by stimulation of extrahypothalamic structures. In contrast, stimulation of the corticomedial (CM) amygdala produced a fall in plasma GH levels (68) comparable to that observed with POA stimulation (69). Since many of the efferent connections of the CM amygdala travel in the stria terminalis to the POA, it is possible that inhibitory limbic system input to the

MBH may involve relays in the POA. Studies have implicated a number of other extrahypothalamic neural areas in the regulation of GH secretion. The ventral tegmental area of Tsai (51) and locus ceruleus (68) have been reported to be stimulatory to GH secretion, while the raphe nucleus (54) and septal nuclei (70) appear to be inhibitory.

Extrahypothalamic projections to the MBH from the preoptic-anterior hypothalamic area (PO-AHA) are of particular interest with respect to the regulation of GH secretion since input from this area seems to be important in suppressing nonstress plasma GH levels and in mediating the GH response to stress. It first became apparent that areas located anterior to the MBH were important in suppressing nonstress plasma GH concentrations and growth when it was observed that complete surgical isolation of the MBH and anterior hypothalamic deafferentation caused augmentation of linear growth (71,72,73) and elevation of nonstress plasma GH levels (71,72) in female rats. Later it was shown (74) that the medial and not the lateral PO-AHA is the source of input to the MBH essential for suppression of nonstress plasma GH levels. In addition to the effects of lesions in the PO-AHA or disruption of neural connections from this region to the MBH on nonstress

plasma GH levels, others have shown that similar procedures elevate the baseline and increase the frequency of peaks of pulsatile GH secretion (63,75). Passive immunization of rats with anti-SRIF serum produced alterations in the pulsatile pattern of GH secretion (56,57,58) and elevations of nonstress plasma GH concentrations (56,76) similar to those produced by the placement of lesions and knife cuts. These findings suggest that SRIF is involved in the inhibitory physiological control of nonstress plasma GH levels and the pulsatile pattern of GH secretion. Immunocytochemical localization of SRIF-containing neuron perikarya revealed that the PV nucleus of the PO-AHA houses a fairly prominent group of SRIF neurons (40,41, 42,43,44). As discussed previously, the effects of lesions indicate that this area suppresses the secretion of GH under nonstress conditions. Furthermore, the placement of lesions in this area or knife cuts which sever connections between this area and the MBH (41,45, 46,47,48) caused marked depletion of the content of SRIF in the median eminence. These observations suggest that the medial PO-AHA and the SRIF-containing neurons in its PV zone are most important for maintaining the content of SRIF in the median eminence and in suppressing nonstress GH secretion.

In the rat, stressful stimuli cause a prompt fall in plasma GH levels (77). Early studies demonstrated that extrahypothalamic input to the MBH is essential for this response (72) because rats with MBH-isolation fail to show the normal stress-induced inhibition of GH secretion. Rice and Critchlow (78) found that ablation of the POA blocked the stress-induced inhibition of GH secretion and later it was shown that the medial and not the lateral PO-AHA was essential for inhibiting GH secretion in response to stress (74). Because of the location of SRIF-containing neuron cell bodies in this area of the brain, it seemed possible that SRIF might be involved in the suppression of plasma GH levels in response to stress as well as in the suppression of nonstress plasma GH levels. In support of this possibility, it was subsequently shown that passive immunization of rats with anti-SRIF serum blocked stress-induced inhibition of GH secretion (58,76). Further support for the involvement of the POA and SRIF in the GH stress reponse came from studies showing that electrical stimulation of the POA produced a rapid fall in plasma GH levels (69) similar to that produced by stress and raised hypophysial portal blood levels of SRIF (79). These data suggest that the medial PO-AHA is essential for stress-induced inhibition of GH release in addition to its involvement in the suppression of

nonstress plasma GH levels. Furthermore, it appears that such inhibition is mediated by the effects of SRIF.

Recent studies in this laboratory (80,81) have shown that whereas large medial PO-AHA lesions caused a prolonged elevation in plasma GH levels, blockade of stress-induced inhibition of GH secretion and reduction in median eminence SRIF content (45), lesions confined to the PV nucleus of the PO-AHA produced a similar marked reduction of median eminence SRIF content but only transient elevation of GH levels and did not alter the GH reponse to stress. Thus, a marked reduction in median eminence content of SRIF in lesioned animals was accompanied by normal nonstress plasma GH concentrations and GH stress reponses. Prior to these studies, it was assumed by us and others (48) that the effects of medial PO-AHA lesions or anterior hypothalamic cuts on GH secretion reflected disruption of SRIF projections to the median eminence because such lesions also depleted median eminence content of SRIF (46,47) and produced alterations of GH secretion similar to those produced by the treatment of rats with anti-SRIF serum (56,58,76). However, in view of the most recent findings, this assumption now appears to have been made prematurely since it seems that much of the SRIF in the median

eminence and most SRIF neurons in the PO-AHA are not essential for maintenance of normal nonstress secretion of GH or for stress-induced suppression of GH secretion.

The aims of the experiments presented in this thesis are to determine whether these preliminary findings are reproducible and to extend the observations of previous experiments in order to clarify the relationship between SRIF neurons in the PO-AHA, the content of SRIF in the median eminence and the inhibitory regulation of GH secretion under nonstress and stress conditions.

D. Introduction: Thyrotropin

1. Discovery of Pituitary Thyrotropin

Early studies of the regulation of thyroid function emphasized control by the autonomic nervous system, since the thyroid gland was shown to receive nerve fibers from the cervical sympathetic system and the vagi (82,83). It was not until investigators showed that thyroid function was unaffected following vagotomy, sympathectomy or transplantation that humoral control of the thyroid was considered as a possible means of regulation (20). In 1916, Smith found that ablation of the adenohypophysis in the tadpole prevented metamorphosis and growth of the thyroid gland (84). He

suggested that the defect was primarily due to the lack of some pituitary trophic substance since treatment of these animals with bovine anterior pituitary extracts were able to restore their thyroids to the normal state (85). Similar findings were obtained independently by Allen (86,86). The existence of a pituitary 'thyreotropic' hormone was first suggested (88) on the basis of studies which reported stimulatory effects of anterior pituitary extracts on thyroid gland histology and function (89,90). The search for a thyroid-stimulating hormone (TSH) began in the 1930's and extended for a period of 40 years. Mammalian TSH was isolated and purified and has been shown to be a glycoprotein with a molecular weight of approximately 28,500 consisting of two nonidentical, carbohydrate containing subunits (91). This hormone is synthesized and stored by the basophilic pituitary thyrotrops.

2. Hypothalamo-pituitary Interactions

Early investigators used a number of approaches to investigate whether the brain is involved in the regulation of pituitary TSH secretion. Studies utilizing techniques such as pituitary stalk section (92,93) and pituitary transplantation (8,94,95) showed in the rat that the hypothalamus exerts some control over TSH secretion. These procedures reduced TSH activity in the

blood but none of them totally abolished TSH activity to the extent that hypophysectomy did. Thus, it was concluded by a number of investigators that pituitary TSH release is, to some extent, independent of neural influences exerted via the hypothalamus and that only certain reflex changes in TSH secretion depend upon hypothalamic influences. Aron and coworkers were the first to observe that TSH secretion was in balance with the secretion of thyroid hormones (96) and later Kuschinsky (97) first recognized the self-regulating character of this classic negative feedback system. The role of the hypothalamus in the regulation of this system was still obscure. Nonetheless, Halasz et al. (23) demonstrated a hypophysiotropic area in the hypothalamus for TSH. Studies of this type showed that only pituitary transplants placed in the basal tuberal region maintained their normal histology and were able to stimulate thyroid function. This was taken to mean that the baseline level of TSH function was determined by hypothalamic influences (98).

Cahane and Cahane (99) were the first to show that diencephalic lesions produced changes in thyroid histology. However, it was not until 1951 when Greer demonstrated that midline anterior hypothalamic lesions interfered with the normal feedback regulation of TSH

in response to lowering of blood thyroid hormone levels (100,101) that a definite role for the hypothalamus in the regulation of TSH secretion was shown to exist. Subsequent studies showed that the hypothalamus is involved in the TSH response to thyroid hormone deficiency and in establishing the baseline level of thyroid function (98). The topographical localization of a 'thyrotropic area' has come from numerous studies in which lesions have been shown to reduce thyroid function. The results of these studies (102,103) indicate that the thyrotropic area of the hypothalamus is near the midline, between the paraventricular nuclei and the rostral infundibulum. Although some early conflicting reports appeared in the literature (98), more recent studies have shown that electrical stimulation of an area in the hypothalamus corresponding fairly well to the thyrotropic area, elevates blood TSH levels and produces histologic changes in the thyroid suggestive of activation (104,105,106,107).

E. Hypophysiotropic Hormones

1. Thyrotropin-Releasing Factor

As the accumulating evidence suggested that the hypothalamus is involved in the regulation of TSH secretion, the existence of a thyrotropin-releasing factor (TRF) became increasingly probably. The first

attempts to identify TRH were made in the 1950's by testing the TSH-stimulating activity of a number of substances that were known to exist in the hypothalamus and neurohypophysis (108,109). Subsequent studies attempted to identify TRH activity in various hypothalamic extracts (110). Most of the problems that were encountered arose from the lack of a sensitive bioassay to detect TRH activity. With the development of a number of new assay and chromatographic separatory techniques, the isolation and purification of TRH simultaneously by the groups of Guillemin (111) and of Schally (112) was completed in 1966. TRF was structurally characterized as a tripeptide (113,114) and has been found throughout the central nervous system (40,41). The highest concentrations of TRF-positive nerve terminals are found in the median eminence, with moderate numbers in the periventricular region, dorsomedial nuclei and perifornical area. The location of these cell bodies seems to correspond well with the thyrotropic area described previously (102,103).

2. Thyrotropin-Release-Inhibiting Factor

A number of stressful stimuli and physiological states cause a decrease in plasma TSH levels in rats (98). Although this may be primarily due to inhibition of TRF secretion, recent experiments suggest that a thyrotropin-release-inhibiting factor may be involved.

It has been postulated that this factor may be SRIF, because this peptide inhibits basal and TRH-induced TSH secretion (115). Furthermore, passive immunization of rats with SRIF antiserum increased basal as well as TRF-stimulated TSH secretion and potentiated the TSH response to cold exposure (56,116,117). The location of SRIF-positive cell bodies and fibers has been reviewed in previous sections. It therefore seems possible in the rat that hypothalamic control of TSH secretion involves both a release and release-inhibiting hormone.

F. Neural Regulation of Thyrotropin Secretion

1. Hypothalamic and Extrahypothalamic Regulation

TSH is secreted in an episodic manner (63) and mean circulating plasma TSH levels oscillate with a circadian periodicity; mean plasma TSH levels are highest in the morning and fall to the lowest levels during the dark (118,119). Complete hypothalamic deafferentation abolished the circadian periodicity and produced alterations in the pulsatile pattern of TSH secretion (63,119,120). Thus, it appears that input to the MBH from extra-MBH areas is necessary to maintain the circadian periodicity of plasma TSH levels, but such input is not essential for the pulsatile secretion of TSH. Early experiments (98,100,101) showed that the

hypothalamus is necessary for establishing the 'setpoint' of plasma TSH levels and is involved in normal feedback regulation of TSH in response to changes in peripheral plasma concentrations of thyroid hormones. More recent studies have shown that extra-MBH input is not involved in this regulation since the removal of all forebrain structures in rats except the MBH or disconnection of all projections to the MBH were compatible with normal thyroid function and TSH release in response to thyroid hormone deficiency (121,122,123).

Although many studies support the existence of a thyrotropic area in the hypothalamus, evidence for a region in the hypothalamus which inhibits TSH secretion is lacking. Recently, a report that small midline retrochiasmatic knife cuts produced elevated levels of TSH in plasma at 90 days after surgery (124) furnished evidence for hypothalamic inhibition of TSH secretion and similar inhibition was postulated (125) on the basis of transient elevation in plasma TSH levels in hypothyroid rats following the placement of medial preoptic lesions. In both studies, it was suggested that SRIF is involved in this inhibition and the results of passive immunization studies support this possibility (56,115,116,117). Recent studies in this laboratory suggest that the periventricular (PV) nucleus in the preoptic-anterior hypothalamic area (PO-AHA), the

location of many SRIF-containing neuron cell bodies (40,41,42,43,44), may be involved in suppressing non-stress plasma TSH levels (80). Following the placement of discrete lesions in the PV nucleus in female rats, nonstress plasma TSH levels were elevated transiently and median eminence content of SRIF was markedly depleted. These results suggest that the PV nucleus of the PO-AHA and SRIF from neurons in this region may be involved in the suppression of nonstress plasma TSH levels by the hypothalamus.

It is possible that some extrahypothalamic input to the MBH may be involved in the control of TSH secretion. Extrahypothalamic structures that have been implicated in the regulation of TSH secretion include the habenula, pineal gland, septal area, globus pallidus, hippocampus and amygdala (110,126,127). However, any conclusions concerning the role or importance of these structures in the regulation of TSH secretion must remain tentative.

Input from extra-MBH areas appears to be essential for the release of TSH in response to acute exposure to cold because complete hypothalamic deafferentation blocks this reponse (128). Other studies have shown that cold-induced release of TSH is primarily due to the release of TRF since anti-TRF serum completely blocks this reponse (129).

In the rat, TSH secretion is inhibited during stress (127,130,131,132,133). Conflicting reports have appeared in the literature, but the discrepant findings are thought to arise from differences in the type of stressor and the methods used to quantify the response of TSH to stressful stimuli (110,127). It is not known whether projections from extra-MBH areas are important for the TSH response to stress, but evidence suggests that they are (127). Furthermore, it has been postulated that SRIF is involved in stress-induced inhibition of TSH secretion (127). It is possible that stress-induced suppression of plasma TSH levels results from inhibition of tonic TRF secretion by SRIF (127). A similar mechanism is reported to be involved in the suppression nonstress plasma TSH levels (134).

As discussed previously, large lesions of the PO-AHA produced elevated nonstress plasma GH levels, blockade of the GH response to stress and caused marked depletion of SRIF content of the median eminence. Since evidence suggests that SRIF is physiologically involved in inhibiting nonstress TSH secretion (56,115, 116,117), one aim of the first experiment presented in this thesis was to determine whether discrete lesions placed in the PV nucleus of the PO-AHA which cause depletion of SRIF in the median eminence produce changes in plasma TSH levels that are consistent with the postulated inhibitory role of SRIF in the regulation of TSH secretion.

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CHAPTER II

STATEMENT OF PROBLEM

Evidence suggests that the medial preoptic-anterior hypothalamic area (PO-AHA) plays a key role in the inhibitory regulation of growth hormone (GH) secretion under nonstress and stressful conditions, and the somatostatin (SRIF)-containing neurons in this part of the brain (1,2,3,4) are involved in this inhibition. Thus, destruction of the medial PO-AHA or surgical disruption of connections between this area and the median eminence have been shown to markedly reduce the content of SRIF in the median eminence (5,6,7) elevate nonstress plasma GH concentrations (5,8,9), alter the pulsatile pattern of GH secretion (10,11,12) and block stress-induced suppression of GH secretion (5,8,20). Likewise, passive immunization experiments suggest that SRIF is involved in the regulation of physiological GH secretion since treatment of rats with SRIF antiserum has been shown to produce similar effects on GH secretion (13,14,15) to those which accompany the surgical procedures described above. SRIF may play a role in the inhibitory regulation of thyrotropin (TSH) as well as GH secretion since SRIF was shown to inhibit TSH secretion (16) and passive immunization of rats with anti-SRIF serum elevated plasma TSH concentrations (13,17,18).

The periventricular (PV) nucleus in the PO-AHA houses a prominent group of SRIF-containing neuron perikarya (1,2,3,4). Recently, experiments were undertaken to investigate the effect of placing discrete lesions confined largely to the PV nucleus of the PO-AHA on median eminence content of SRIF and GH and TSH secretion under nonstress and stress conditions (19). Such lesions produced marked depletion of median eminence SRIF content but cause only transient elevations in nonstress plasma GH and TSH levels and had no effect on the response of these hormones to stress. These findings suggest that many of the SRIF neurons in the PV nucleus and the majority of SRIF in the median eminence are not essential for normal GH and TSH secretion under nonstress conditions.

Prior to our recent findings (19), it was assumed that the PV nucleus or the PO-AHA and the SRIF neurons located there were not only the major source of SRIF in the median eminence but were also essential for inhibiting GH secretion under nonstress and stress conditions. Because of the paradoxical nature of these preliminary findings, further experiments were designed to investigate the functional relationships between the PV nucleus of the PO-AHA, SRIF content of the median eminence and inhibitory regulation of GH and TSH secretion. Therefore, some aims of the experiments presented in this thesis were to determine whether or not the hypothalamic PV nucleus is essential

for the maintenance of normal nonstress plasma GH and TSH levels, the production of the normal nonstress pattern of pulsatile GH secretion, stress-induced inhibition of GH secretion and maintaining the normal content of SRIF in the median eminence. This was done by determining whether:

I) the placement of discrete lesions in the PV nucleus of the PO-AHA, larger than those used in previous studies, which markedly deplete the content of SRIF in the median eminence would cause long- rather than short-term elevations of nonstress plasma levels of GH and TSH and block the response of these hormones to stress.

II) lesions placed in the PV nucleus of the PO-AHA which cause marked depletion of the median eminence content of SRIF also produce alterations of the pulsatile pattern of GH secretion.

III) an isolated basal hypothalamus-pituitary island is capable of supporting pulsatile GH secretion and what effect removal of neural input to the medial basal hypothalamus (MBH) has on the pulsatile pattern of GH secretion, the GH response to stress and MBH-median eminence content of SRIF.

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CHAPTER III

Long-term Elevations in Plasma Thyrotropin but Not Growth Hormone Concentrations Associated With Lesion-induced Depletion of Median Eminence Somatostatin

ABSTRACT

Evidence suggests that somatostatin (SRIF) inhibits nonstress GH and TSH secretion and suppresses GH secretion in response to stress. The aims of this study were to determine whether placement of lesions in the hypothalamic periventricular (PV) nucleus, the location of SRIF neurons which seem to be responsible for most median eminence SRIF, causes elevation of nonstress plasma GH and TSH levels and blocks stress-induced suppression of these hormones. Electrolytic lesions were placed in female rats and blood was obtained for assessing nonstress and stress plasma levels of GH and TSH at several intervals after surgery until autopsy, 56 weeks after surgery, when median eminences were collected. PV lesions produced only transient elevation of nonstress plasma GH levels and failed to block the suppression of GH secretion by stress. In contrast, PV lesions caused long-term elevation of nonstress plasma TSH levels and blockade of stress-induced suppression of TSH secretion. Content and concentration of SRIF in the median eminence were reduced 90% in the PV-lesioned group. These data demonstrate that PV lesions which result in marked depletion of median eminence SRIF can cause long-term elevations of plasma TSH levels and disruption of the TSH response to stress without producing alterations in GH secretion. Thus, the hypothalamic PV nucleus, its SRIF neurons and most median eminence SRIF are not essential for maintaining normal GH secretion but seem to be involved in the regulation of TSH release. It appears that different brain structures are involved in inhibiting GH and TSH secretion.

INTRODUCTION

In the rat, it appears that somatostatin (SRIF) suppresses nonstress plasma GH and TSH concentrations and is essential for stress-induced inhibition of GH secretion. Thus, passive immunization of rats with anti-SRIF serum elevates basal nonstress plasma levels of GH (1,2) and TSH (1,3,4) and blocks stress-induced inhibition of GH secretion (5,2).

SRIF has been found throughout the central nervous system (6) with the highest concentrations in the hypothalamic median eminence (7,8). A major group of SRIF-containing neuron perikarya is located in the periventricular (PV) nucleus of the preoptic-anterior hypothalamic area (PO-AHA) (9,10,11,12). Whereas little is known about their role in controlling TSH secretion, these neurons, which appear to be the primary source of SRIF in the median eminence, seem to be involved in the suppression of nonstress plasma GH levels and in the GH response to stress because placement of large lesions which destroyed the medial PO-AHA as well as the PV nucleus or interruption of connections between the PO-AHA and the median eminence produced marked depletion of SRIF in the median eminence (13,14,15), chronic elevation of nonstress plasma GH levels (14,16,17,18) and blockade of the GH response to stress (14,16,18). However, this seemingly coherent relationship between SRIF neurons of the PV nucleus and the inhibitory control of GH secretion was complicated by results from recent experiments in this laboratory (19). These studies showed that small lesions which destroyed a portion of the PV nucleus in the PO-AHA caused depletion of median eminence SRIF similar in magnitude to that which accompanied large medial PO-AHA

lesions, but plasma levels of GH were elevated only transiently and the GH response to stress was unaffected; similarly, plasma TSH concentrations were affected transiently and in parallel with GH.

The aim of the present experiment was to determine whether PV lesions which are larger than those studied previously and which produce a major reduction in median eminence SRIF would cause long- rather than short-term deficits in the inhibitory control of GH and TSH and block the responses of these hormones to stress.

MATERIALS AND METHODS

Adult female rats (Charles-River, CD) weighing 180-200 g were housed 4 per cage, kept under conditions of controlled light (fluorescent, 0400-1800 h) and temperature (22 $\pm$ 2 C) and provided with rat chow and water ad libitum. Each animal was assigned to one of three treatment groups, lesioned, sham-lesioned or intact. Rats in the lesioned or sham-lesioned groups were anesthetized with ether and a triple-barreled platinum electrode with a 2 mm uninsulated tip was oriented 30^{o} posterior to vertical and lowered into the third ventricle through midline to the base of the brain. Lesions were produced by passing 2 mA of anodal DC current for 15 sec at 0.3 and 1.3 mm from the base of the brain. The sham-lesioning procedure consisted of lowering the electrode to the level of the anterior commissure and withdrawing it without passage of current. Following surgery, the rats were given 0.1 cc Bicillin (60,000 U, im, Wyeth) and body temperatures were monitered for 4 to 5 h. Animals with body temperature that exceeded 39 C were immersed in cold

water. All animals were then placed in individual cages, and the animal room was locked 24 h after surgery in preparation for blood collection at 48 h.

In order to study the effects of PV lesions on nonstress and stress plasma concentrations of GH and TSH, blood samples were obtained at 48 h and at 2, 4, 30 and 46 weeks after surgery using a 3 min immobilization-blood withdrawal stress bleeding procedure. This procedure consisted of removing rats from their individual cages and transferring them to an adjacent laboratory where they were immobilized in the supine position for 3 min. The skin over an external jugular vein was infiltrated with procaine, the vein was exposed and blood (1.2 ml) was collected in an EDTA-rinsed syringe within 3 min of cage opening. Following 3 min of immobilization, the animals were placed in holding cages. Thirteen minutes after initial handling, the rats were anesthetized with ether and a second blood sample was collected at 15 min. Plasma from the first blood sample was used to estimate nonstress GH and TSH concentrations and that from the second was used to assess the effects of stress. The percent change (stress levels/nonstress levels X 100) was used as an index of the response to stress. As shown previously, these sampling times are appropriate for estimating nonstress plasma GH concentrations and the effects of stress (16). Preliminary studies showed that these procedures are also valid for assessing nonstress plasma concentrations of TSH and the suppressive effects of stress. To reduce environmental disturbances and inadvertent stress, rats were placed into individual cages 3 days and the animal room locked 24 h prior to each of the sampling

sessions subsequent to the first blood collection at 48 h.

At autopsy, 56 weeks after surgery, rats were killed by decapitation within 1 min of cage opening and trunk blood was collected to determine nonstress levels of GH and TSH in plasma. Brains were rapidly removed and inverted on a chilled glass plate. The median eminence was visualized with a Zeiss operating microscope, removed, immersed in 200 µl of 2 N acetic acid and flash frozen within 4 min of cage opening. This tissue was later assayed for SRIF. Brains of lesioned rats were fixed in 10% neutral buffered formalin and processed for histological examination. Pituitaries were removed and the anterior lobes weighed, immersed in phosphate buffered saline, snap frozen and subsequently assayed for GH and TSH content.

Blood samples were immediately chilled and centrifuged. Plasma was rapidly frozen and stored at -20 C until assayed. GH and TSH were measured by radioimmunoassay (RIA). Materials were supplied by the NIAMDD through Dr. A. Parlow. Results are expressed in terms of NIAMDD-Rat GH, RP-1 and NIAMDD-Rat TSH, RP-1 standards. All samples were assayed at four dilutions, and the mean concentration of the four aliquots was used for each estimate. The sensitivity of the GH assay was 0.2 ng/tube and that of the TSH assay was approximately 5 ng/tube. Inter- and intra-assay coefficients of variation were 9% and 8%, respectively, for GH and 7% and 11% for TSH.

Each median eminence was extracted by sonication in 200 µl of 2 N acetic acid, immersed in a boiling water bath for 5 min and quickly chilled to 0 C. A small aliquot of homogenate was removed and assayed for total protein using the method of Schaffner and

Weissman (20). A 1% aqueous solution of fraction V bovine serum albumin (Sigma) was used as the reference standard. The homogenates were then centrifuged at 4 C for 60 min at 700 x g. The supernatant of each sample was neutralized with 2 N NaOH followed by a 1:10 dilution in assay buffer. All samples were assayed for SRIF in duplicate at 5 dilutions with the RIA described by Arimura et al. (21) using synthetic somatostatin (Beckman) as the reference standard. The mean concentration of 10 aliquots was used for each SRIF value. This assay employed rabbit anti-somatostatin serum (#101) provided by Dr. A. Arimura and (^{125}I-Tyr^{1})-SRIF as a tracer. The sensitivity of the assay was approximately 3 pg/tube. Inter- and intra-assay coefficients of variation were 13.5% and 7%, respectively.

The rats were assigned to treatment groups according to a completely randomized design. One-way analysis of variance (ANOVA) and the Newman-Keuls' test were used to compare data from single point measurements. Two-way ANOVA for repeated measures (22) was used to compare data obtained by serial sampling. Statistical analysis was performed after log transformation when heterogeneity of variance was encountered, except for the body weight data obtained at autopsy which was analyzed with the Kruskal-Wallis H test.

RESULTS

The location and extent of the PV lesions are illustrated in Figs. 1 and 2. The parasagittal diagram of the rat forebrain in Fig. 1 shows the common area of destruction resulting from PV lesions in 5 rats. The lesions extended rostrally to the anterior commissure and posteriorly to the caudal diencephalon; they did not involve the

preoptic area. Most of the PV nucleus of the anterior, tuberal and posterior hypothalamic areas was destroyed. Only the parts of the PV nucleus in the preoptic area and in the basal hypothalamus near the arcuate and posterior mammillary nuclei were spared. Figures 2A and B, respectively, show representative thionin-stained coronal sections of PV-lesioned and sham-lesioned rats to demonstrate the lateral extent of the lesions. Histological examination showed that the PV lesions destroyed an area approximately 0.5 mm on each side of midline and that the magnocellular cells of the paraventricular nuclei, the suprachiasmatic nuclei and approximately 70% of the ventromedial-arcuate nuclear complex remained intact. Data from rats with asymmetrical lesions or those which did not fit the above description were not used.

The effects of PV lesions on nonstress plasma levels of GH and TSH are shown in Figs. 3A and 3B, respectively. Nonstress plasma GH levels were elevated above those of intact controls in rats with PV ($p < 0.01$) and sham ($p < 0.05$) lesions 48 h after surgery, but GH levels were similar in all three groups from 2 weeks after surgery until autopsy at 56 weeks. In contrast, nonstress plasma TSH levels of lesioned rats were similar to those of the control groups at 48 h after surgery. At 2 weeks, however, plasma TSH levels in rats with PV lesions were higher ($p < 0.01$) than those in both control groups. These elevated TSH concentrations persisted through the 46th week after lesioning ($p < 0.05$) until autopsy, at which time TSH levels in the lesioned rats were similar to those of the control groups.

PV lesions had only a transient effect on stress-induced

inhibition of GH secretion (Fig. 4A). Although the GH response to stress in rats with PV lesions was reversed and differed ($p < 0.01$) from those of controls at 48 h, no difference between groups was apparent thereafter. In contrast (Fig. 4B), the stress-induced fall in plasma TSH concentrations observed in the controls was repeatedly blocked in rats with PV lesions; the stress response in the PV lesion group differed ($p < 0.01$) from those of the control groups throughout the experiment.

The effect of PV lesions on median eminence content of SRIF at autopsy is shown in Fig. 5. Median eminence content of SRIF was 90% lower ($p < 0.01$) in rats with PV lesions than in both groups of controls. The results were similar when expressed as ng SRIF/mg protein (intacts, 1223.2; sham-lesioned, 1057.2; PV lesioned, 178.5).

At autopsy, nonstress plasma concentrations of GH (ng/ml plasma; mean $\pm$ SE) in the lesioned group (14.2 ± 1.6) were lower ($p < 0.05$) than in sham-lesioned (40 ± 5.7) and intact controls (51.9 ± 18.8). The concentrations of TSH in plasma were similar in all groups at autopsy. The pituitary content of GH (µg/pituitary) in PV-lesioned rats (207 ± 67) was lower ($p < 0.05$) than in sham-lesioned (435 ± 57) or intact (447 ± 60) controls. Likewise, the content of TSH in pituitaries (µg/pituitary) of lesioned rats (638 ± 106) was lower than that of the sham-lesioned (1048 ± 62, $p < 0.01$) and intact controls (951 ± 75, $p < 0.05$). The thyroids of rats with PV lesions (20.0 mg) weighed more than those of sham-lesioned (14.5 mg, $p < 0.05$) or intact (13.6 mg, $p < 0.01$) controls. However, no differences between groups were apparent when thyroid weights were expressed relative to body weights. Although PV-lesioned rats were somewhat heavier (490 ± 64 g) than sham-lesioned

(361±8 g) or intact (365±13 g) control groups at autopsy, body weights were similar when subjected to nonparametric statistical analysis.

DISCUSSION

These results demonstrate that lesions which destroyed much of the PV nucleus of the hypothalamus but which spared the PV zone of the preoptic region produced a marked depletion of SRIF in the median eminence that was not accompanied by either long-term elevations of nonstress levels of GH in plasma or blockade of the GH response to stress. Such depletion was, however, associated with prolonged elevation of circulating TSH levels and disruption of the inhibition of TSH release induced by stress. As reported previously (19), small lesions confined largely to the PV zone of the hypothalamus are as effective in depleting median eminence SRIF as are large medial PO-AHA lesions (13,14) and anterolateral hypothalamic cuts (13,14,15,23). Because immunocytochemical studies have shown that most of the SRIF-containing neuron cell bodies that project to the median eminence are located in the PV nucleus of the preoptic and anterior hypothalamic areas (6,9,10,11,12,24), a considerable reduction in median eminence content of SRIF was expected. That depletion of SRIF of such magnitude was produced by lesions that spared the PV nucleus of the preoptic area suggests that either the lesions destroyed fibers of passage from this area or that SRIF neurons of the preoptic area do not contribute significantly to median eminence stores of SRIF.

The hypothalamic PV lesions had no apparent long-term effect

on GH secretion despite the severe reduction in median eminence content of SRIF. Because the lesions in the present study destroyed most of the hypothalamic PV nucleus, it appears that the SRIF neurons located in this nucleus are not required for the inhibition of GH secretion under nonstress or stress conditions. In contrast, the medial preoptic area seems essential in this regard because lesions which involved this area produced long-term elevations in nonstress plasma GH levels and blockade of stress-induced suppression of GH secretion (14,16). Furthermore, electrical stimulation of this area produced a dramatic fall in plasma GH levels (25). Heretofore, we and others (26) assumed that the effects of medial preoptic lesions on GH secretion reflected disruption of SRIF projections to the median eminence because such lesions or disruption of caudal projections from this area depleted median eminence content of SRIF (13,15) and produced alterations of GH secretion similar to those produced by the treatment of rats with anti-SRIF serum (1,2,5). Moreover, electrical stimulation of the medial preoptic area elevated portal SRIF concentrations in hypophysial portal blood (27). However, this assumption now appears somewhat tenuous in view of the present and previous (19) findings which show that SRIF neurons in the preoptic and hypothalamic PV nucleus and most of the SRIF in the median eminence are not essential for maintenance of normal GH secretion under nonstress and stress conditions. One possible explanation is that some as yet unidentified neural factor suppresses GH secretion. Such a factor may exist, but passive immunization studies provide strong evidence that SRIF plays a prominent

role in the physiological suppression of GH release (1,2,5). As discussed previously (19), the persistence of apparently normal inhibitory control of GH secretion under nonstress and stress conditions in conjunction with lesion-induced depletion of SRIF in the median eminence does not preclude a role for SRIF in these processes. It is possible that the amount of SRIF that reached the adenohypophysis in rats with PV lesions was normal despite a 90% reduction of median eminence content of SRIF. This would imply that residual SRIF neurons can maintain normal levels of SRIF in the hypophysial portal circulation and that this function is not necessarily related to the content of SRIF in the median eminence; residual SRIF neurons could be located in parts of the PV nucleus that were not destroyed by the lesions or in other areas where SRIF neurons have been observed (6,12,24). The functional role of the 90% of SRIF that was depleted from the median eminence following placement of the PV lesions is unknown. However, it was suggested (28) that most of the SRIF in the median eminence exists in a non-releasable form. Another possibility is that the concentration of SRIF in portal blood in the rats with PV lesions was 90% lower than normal. This situation could imply redundancy in the system and that approximately 10% of the SRIF normally made available to the anterior pituitary is sufficient to maintain normal nonstress plasma GH concentrations and the GH response to stress. Alternatively, it is possible that pituitary somatotropes develop increased sensitivity to SRIF in response to a deficiency in SRIF, the converse to what was observed after prolonged in vitro exposure of dispersed pituitary cells to SRIF (29).

These results also demonstrate for the first time that hypothalamic PV lesions produce long-term elevations of nonstress plasma TSH concentrations and blockade of stress-induced suppression of plasma TSH levels, suggesting that the PV zone of the hypothalamus contains neural elements that suppress plasma TSH levels under nonstress and stress conditions. A recent report that small midline retrochiasmatic knife cuts produced elevated levels of TSH in plasma at 90 days after surgery (30) furnishes additional evidence for hypothalamic inhibition of TSH secretion, and similar inhibition was postulated (31) because of the transient elevation in plasma TSH levels that occurred in hypothyroid rats following the placement of medial preoptic lesions. Although the basis for these effects of hypothalamic lesions is unknown, it appears from the present results that brain structures which inhibit TSH secretion are anatomically dissociated from those which inhibit GH secretion. Thus, rats with hypothalamic PV lesions had normal GH secretion under nonstress and stress conditions associated with evidence of increased TSH secretion. Consistent with such dissociation, we found in unpublished observations that large medial PO-AHA lesions which caused prolonged elevations in nonstress plasma GH levels were accompanied by normal circulating TSH levels. Since the lesions in the present study were located more caudally than those which caused only transient elevations in nonstress plasma TSH levels (19), we postulate that the neurons responsible for inhibiting TSH secretion reside in or their axons traverse the portions of the PV zone located caudal to the preoptic area.

In view of the evidence indicating that SRIF is physiologically

involved in inhibiting nonstress TSH secretion (1,3,4,32), it is possible that the lesion-induced elevation of plasma TSH concentrations observed in this study resulted from disruption of SRIF neurons or their projections. Although such projections may inhibit TSH secretion directly via an action on the pituitary or indirectly by affecting hypothalamic release of TRF, available evidence, in our opinion, favors the latter because the increase in TSH secretion produced by administration of SRIF antiserum was blocked by pretreatment with TRF antiserum (34). Whether SRIF is involved in stress-induced suppression of TSH secretion, as suggested previously (35), is unknown. Because of the apparent anatomical dissociation of neural elements inhibiting GH and TSH secretion discussed above, it seems unlikely that hypothalamic lesions cause increases in both GH and TSH secretion through a common effect such as compromising the release of SRIF into the hypophysial portal circulation.

The significance of the finding that plasma TSH levels returned to normal at autopsy 56 weeks after surgery is unknown. Because pituitary content of TSH was reduced at this point of time after signs of increased secretion for approximately 46 weeks, it is possible that an 'exhaustion' phenomenon was involved. In view of the finding that the PV-lesioned rats had an increase in absolute but not relative thyroid weight, these data offer little insight as to whether the prolonged elevation in TSH concentrations resulted in hyperstimulation of the thyroid.

In summary, these results indicate that destruction of the hypothalamic PV nucleus and an associated 90% depletion of median

eminence SRIF content are compatible with normal nonstress plasma GH levels and stress-induced inhibition of GH secretion. Thus, it appears that the majority of somatostatinergic neurons of the hypothalamic PV nucleus and 90% of the SRIF in the median eminence are not essential for the maintenance of normal secretion of GH under nonstress and stress conditions. However, the prolonged elevation in nonstress plasma TSH and inhibition of the TSH responses to stress observed in the PV-lesioned rats suggest that neural elements residing in or near the PV zone of the hypothalamus inhibit TSH secretion under nonstress and stress conditions. Such inhibition may be mediated by the inhibitory effects of SRIF on TRF secretion.

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Figure 1. Parasagittal diagram of the rat forebrain showing the common area of destruction produced by PV lesions. The shaded area indicates the extent of the lesions. Abbreviations: AHA, area anterior hypothalami; AR, nucleus arcuatus hypothalami; CA, commissura anterior; CO, chiasma opticum; MM, nucleus mamillaris medialis; PD, pars distalis; POA, area preoptica; P, nucleus paraventricularis hypothalami; S, nucleus suprachiasmaticus; VM, nucleus ventromedialis hypothalami.

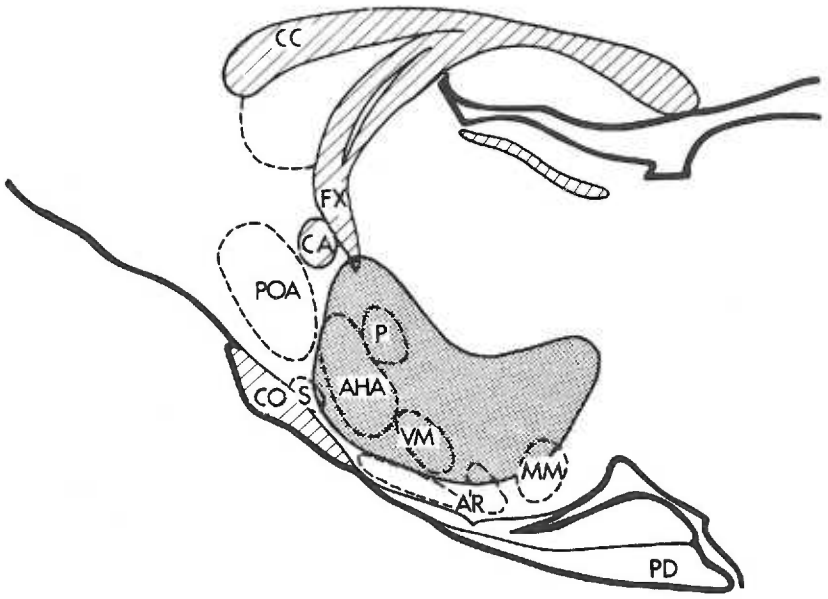
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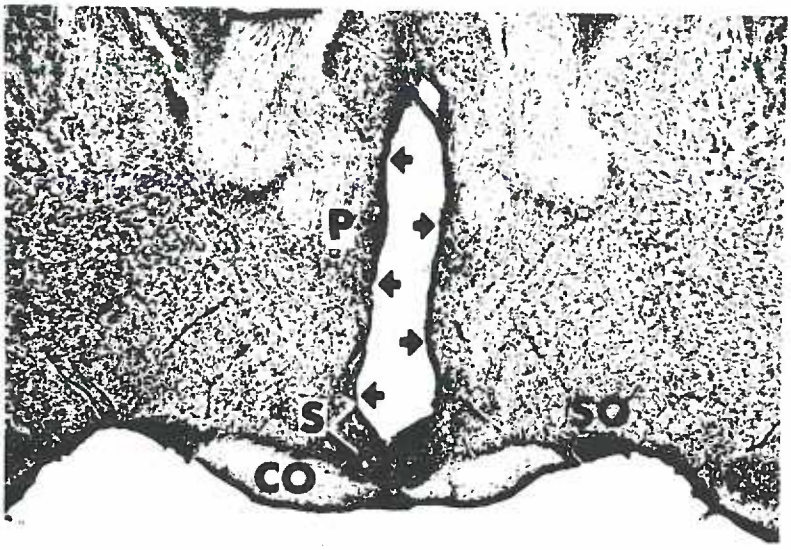
Figure 2. Photomicrographs of representative coronal sections of the rat forebrain in a PV-lesioned rat (A) and a sham-operated control (B). Abbreviations: CO, chiasma opticum; P, nucleus paraventricularis hypothalami; S, nucleus suprachiasmaticus; SO, nucleus supraopticus hypothalami. Arrows indicate the borders of the lesion.

A.



B.

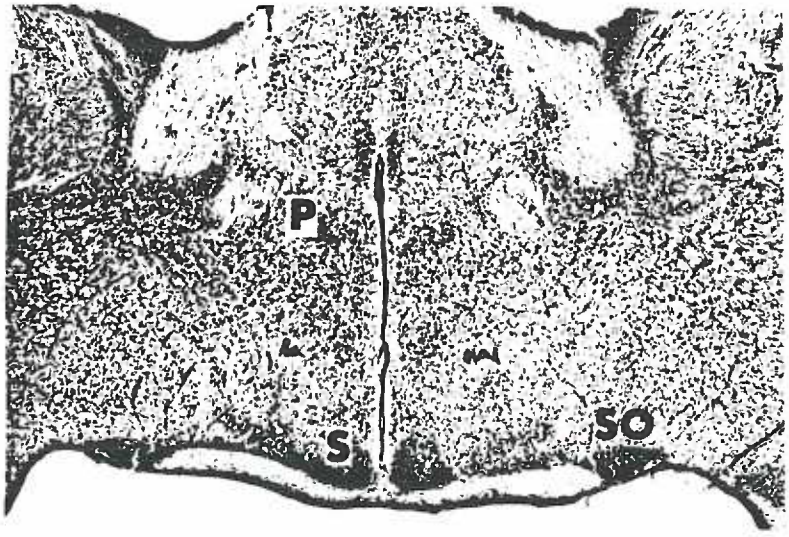


Figure 3. Effects of PV lesions on nonstress plasma GH concentrations (A) and nonstress plasma TSH concentrations (B) at 48 h and 2,4,30,46, and 56 weeks after surgery. Means $\pm$ SE are indicated. *, $P < 0.05$ vs. intact controls at the time; **, $P < 0.01$ vs. intact controls at that time.

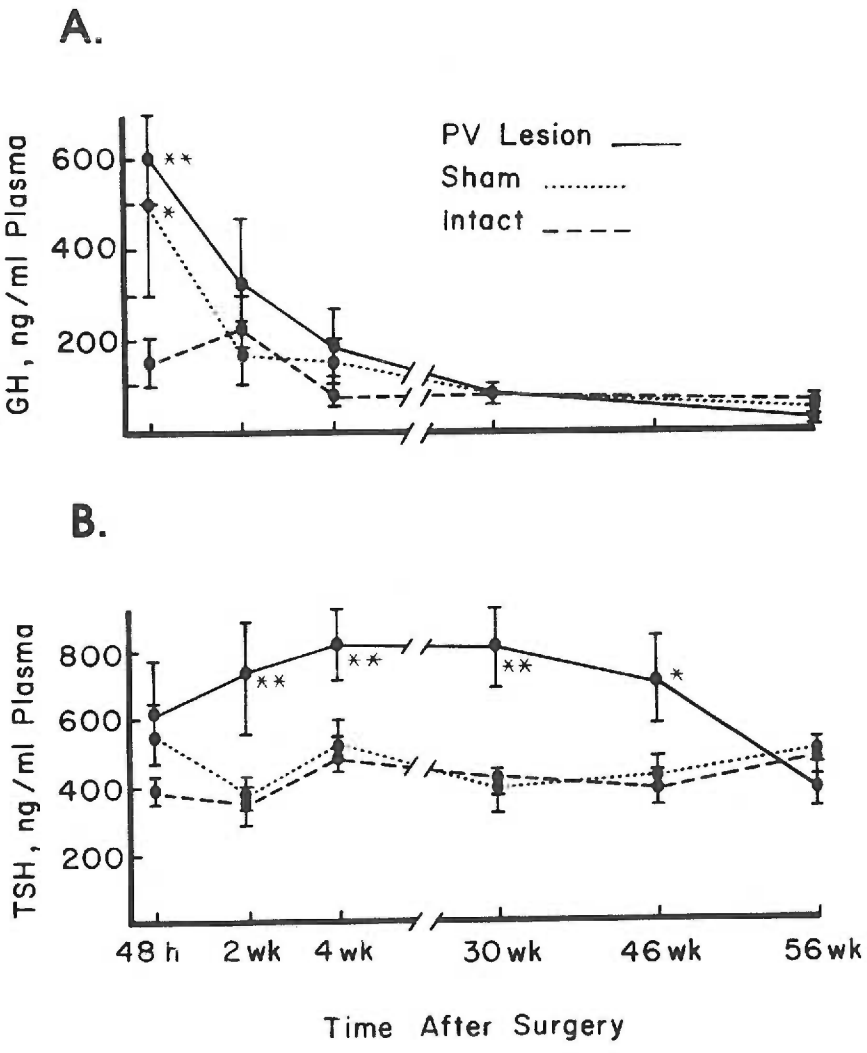

A.
PV Lesion
Sham
Intact
GH, ng/ml Plasma
600
400
200
**
*
B.
TSH, ng/ml Plasma
800
600
400
200
**
**
**
*
48 h
2 wk
4 wk
30 wk
46 wk
56 wk
Time After Surgery

Figure 4. Effects of PV lesions on the GH response to stress (A) and the TSH response to stress (B) at various times after surgery. Means of group responses to stress expressed as % change in hormone levels from nonstress levels ± SE are indicated. Numbers in brackets indicate rats/group. **, $P < 0.01$ vs. sham-lesioned and intact controls. The TSH response to stress in PV-lesion group differed ($P < 0.01$) from that of both control groups at all times tested.

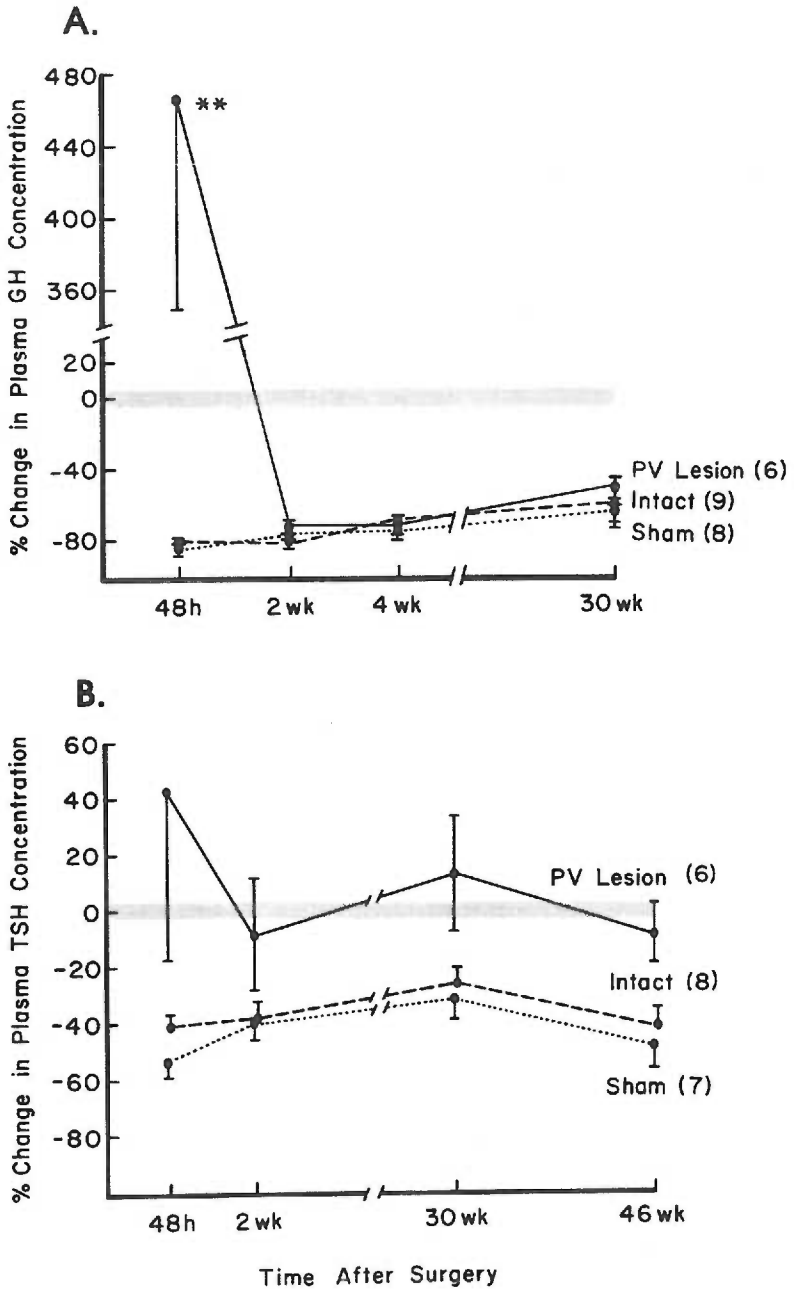
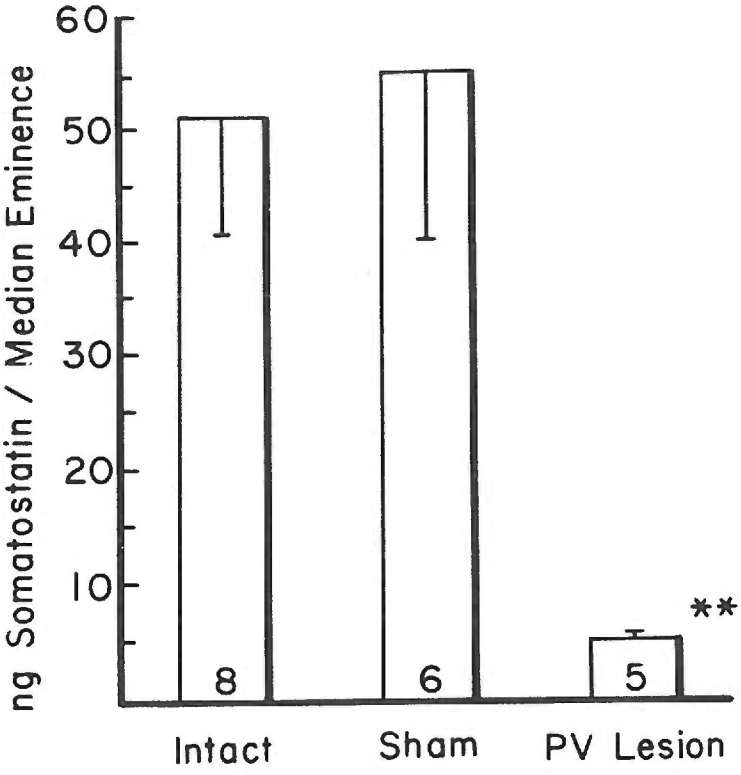

A.
480
440
400
360
20
0
-40
-80
**
% Change in Plasma GH Concentration
PV Lesion (6)
Intact (9)
Sham (8)
48h
2wk
4wk
30wk
B.
60
40
20
0
-20
-40
-60
-80
% Change in Plasma TSH Concentration
PV Lesion (6)
Intact (8)
Sham (7)
48h
2wk
30wk
46wk
Time After Surgery

Figure 5. Effects of PV lesions on somatostatin content of the median eminence. Means $\pm$ SE are indicated. Numbers at bases of columns indicate rats/group. **, $P < 0.01$ vs. sham-lesioned and intact controls.


60
50
40
30
20
10
ng Somatostatin / Median Eminence
8
6
5
**
Intact
Sham
PV Lesion

ACKNOWLEDGEMENTS

We thank Jessie Kroning and Robin Ruppel for their technical assistance. A preliminary report of this work appeared as an abstract in the Program of the Endocrine Society, 63rd Annual Meeting (Abstract 90). This work was supported by NIH grant AM 16794.

CHAPTER IV

The Effects of Hypothalamic Periventricular Lesions on Pulsatile Growth Hormone Secretion and Median Eminence Somatostatin in the Female Rat

ABSTRACT

The purpose of the present experiment was to determine the effects of destroying the somatostatin (SRIF) neurons of the periventricular (PV) nucleus of the hypothalamus and depletion of median eminence SRIF on the pulsatile pattern of growth hormone (GH) secretion in female rats.

At 6-10 days after placement of PV lesions, blood samples collected every 15 min for 3-4 h showed an elevation in baseline GH levels and an increase in the amplitude of GH secretory peaks; frequency of pulses was not affected. These changes were associated with an increase in mean integrated plasma GH levels. These alterations appeared transient because GH levels were normal in blood samples collected 6-17 weeks after surgery. Stress-induced suppression of GH secretion was also unaffected by the PV lesions. At autopsy, nonstress GH levels in PV-lesioned rats were similar to those in intact and sham-lesioned controls. However, the median eminence content and concentration of SRIF was approximately 85% depleted in the group with the PV lesions.

The results suggest that the hypothalamic PV nucleus is essential for maintaining most of the SRIF in the median eminence but this nucleus and most of the SRIF in the median eminence are not necessary for long-term suppression of plasma GH levels under nonstress and stress conditions. Whether changes in the amount of SRIF in portal blood are responsible for the alterations in the pulsatile pattern of GH secretion and reinstatement of normal inhibitory control of GH secretion remains to be determined.

INTRODUCTION

In the rat, growth hormone (GH) is secreted in an episodic manner (1,2) and in the female, the pulsatile pattern of GH secretion is similar during the different stages of the estrous cycle (3). Lesions placed in the ventromedial (VM) region of the hypothalamus abolish pulsatile GH secretion (1), whereas procedures that destroy most of the medial preoptic area (4) or interrupt connections between this area and the median eminence (5) were reported to elevate the baseline and increase the frequency of peaks in the pulsatile pattern of GH secretion; amplitude of secretory pulses was apparently not affected. Thus, it appears that the VM area is essential for pulsatile GH secretion and that the preoptic area inhibits some aspects of the secretory pattern. It was suggested (6,7,8) that somatostatin (SRIF) is involved in this inhibitory regulation since passive immunization of rats with anti-SRIF serum altered the pulsatile pattern of GH secretion by increasing baseline levels and amplitude of GH pulses. Moreover, large lesions and knife cuts (4,5) that involved the periventricular (PV) nucleus of the preoptic-anterior hypothalamic area (PO-AHA), an area that houses many SRIF-containing neuron cell bodies (9,10,11), or destruction of PV-projections to the medial basal hypothalamus caused long-term elevation in plasma GH concentrations (12) and markedly reduced the content of SRIF in the median eminence (12,13,14). Whereas these findings collectively suggest that the PO-AHA and median eminence SRIF inhibit GH secretion under physiologic conditions, the results of subsequent experiments have complicated this view. Recent studies in this laboratory (15,16) have shown

that whereas large medial PO-AHA lesions caused a prolonged elevation in plasma GH levels and reduction in median eminence SRIF content (12), lesions confined to the PV nucleus of the PO-AHA produced a similar marked reduction of median eminence SRIF content but only transiently-elevated GH levels. Thus, the functional relationship between SRIF neurons in the PV nucleus, median eminence content of SRIF and inhibition of GH secretion is unclear. The present study was undertaken to determine whether or not destruction of SRIF neurons of the PV nucleus and depletion of SRIF in the median eminence alters the pulsatile pattern of GH secretion.

MATERIALS AND METHODS

Adult female rats (Charles-River, CD) weighing 180-200 g were housed 4/cage, kept under conditions of controlled light (fluorescent, 0400-1800 h) and temperature (22 $\pm$ 2 C) and provided with rat chow and water ad libitum. Each rat was implanted with an indwelling atrial cannula and assigned to one of three treatment groups, lesioned, sham-lesioned or intact, using a randomized block design. The cannulae were constructed and implanted with a modification of the technique described by Harms and Ojeda (17), using single lumen clear vinyl tubing (0.5 mm id, 0.8 mm od, Dural Plastics). A 2 cm^2 piece of organza (polyester) was attached to the distal end of each cannula to facilitate securing to subcutaneous structures. The cannulae were sterilized and filled with a mixture of heparin (500 U/ml saline) and polyvinylpyrrolidone (1 g/2 ml solution, Calbiochem) to prevent clot formation within the cannulae. The cannulae were implanted under ether anesthesia. Body weight

gain was used as an index of recovery from operative procedures. Most intact rats regained or surpassed their preoperative body weights by the 3rd postoperative day.

Immediately following the implantation of cannulae, rats assigned to the lesioned or sham-lesioned groups were positioned in a stereotaxic apparatus and a triple-barreled platinum electrode oriented 30° posterior to vertical was lowered into the third ventricle to the base of the brain using deGroot coordinates (18). The electrode, constructed by soldering 3 platinum tubes (each with 0.5 mm diameter) together, had an uninsulated tip that extended 2 mm in the sagittal plane, 2 mm high and 0.5 mm wide. Two lesions, produced by passing 2 mA of anodal DC current for 15 sec, were placed in midline at a height of 0.3 mm and 1.3 mm from the base of the brain. The sham procedure consisted of lowering the electrode to the level of the corpus callosum and withdrawing it without passage of current. The lesioned and sham-lesioned rats were given 0.1 cc Bicillin (60,000 U, im, Wyeth), and their body temperatures were monitored for 4 to 5 h after surgery. Animals with body temperatures that exceeded 39 C were immersed in cold water. Following surgery, the rats were placed in individual cages measuring 5"x11" and 14" deep. These cages were designed so that the animals could obtain food and water at will, had freedom of movement and could not see the investigator as blood samples were being withdrawn. Body weights were measured daily and the cannulae flushed daily with saline to maintain patency. The animal room was locked 24 h before blood sampling to prevent inadvertent disturbances. At 0800-0900 h on the day of blood collection, 6-10 days following surgery, a 30 cm

piece of PE-50 polyethylene tubing (0.023" id, 0.038" od) was attached to the externalized end of the cannula with a 2-3 cm piece of 24 gauge stainless steel tubing. The polyethylene tubing had been previously coiled to impart a spring-like quality that kept it out of the animal's reach. The distal end of the cannulae was attached to a swivel device to allow freedom of movement. The room was locked again for 4-5 h to minimize environmental disturbances. Sequential blood sampling was initiated at 1300 h and 0.4 ml blood samples were collected in heparinized syringes every 15 min for 3-4 h. The blood cells of each sample were suspended in warm saline and infused following the removal of the next blood sample. Each sampling session involved 2-3 rats that were taken in the order of their randomized block treatment assignments. Animals were returned to group cages at the completion of the experiment and cannulae were removed within 4 days of sampling.

To assess further the effects of the lesions on the control of GH secretion, single nonstress and stress blood samples were collected from each rat at 6-15 weeks after surgery. The blood samples were collected between 1400 and 1600 h using an immobilization-blood withdrawal stress procedure. This procedure consisted of rapidly transferring individual rats from their cages to an adjacent laboratory where they were immobilized in the supine position for 3 min. During immobilization and within 3 min of cage opening, blood (1.2 ml) was collected in EDTA-rinsed syringes from an exposed external jugular vein after infiltrating the overlying skin with procaine. At 13 min after initial handling, the rats were anesthetized with ether and a second blood sample collected

at 15 min. The first and second blood samples were used to measure nonstress and stress GH levels, respectively. The percent change (stress levels/nonstress levels x 100) was used as an index of the response to stress. These sampling times were chosen on the basis of the time-course of stress-induced changes in circulating plasma GH levels that was reported previously by this laboratory (19). To minimize environmental disturbances before and during the bleeding procedures, the rats were placed in individual cages for 3 days and the animal quarters were locked 20 h preceding each study.

Autopsy was performed at approximately 17 weeks after the placement of lesions. Rats were killed by decapitation within 1 min of cage opening and trunk blood was collected to determine nonstress levels of GH in plasma. The brain of each animal was rapidly removed from the skull and inverted on a chilled glass plate. The median eminence was visualized with a Zeiss operating microscope, dissected, immersed in 200 µl of 2 N acetic acid and flash frozen within 4 min of cage opening. This tissue was stored at -20 C until assayed for content and concentration of SRIF. Each brain was fixed in 10% neutral buffered formalin and processed for histological examination. The uteri, ovaries, adrenals, thyroids and pituitaries were removed, weighed and fixed in Bouin's solution.

Blood samples were chilled immediately after collection and centrifuged. Plasma was snap-frozen and stored at -20 C until assayed. Plasma GH was measured by radioimmunoassay as described by Schindler et al. (20) using materials supplied by NIAMDD through Dr. A. Parlow. Results are expressed in terms of NIAMDD-Rat GH, RP-1 standard. Samples were assayed at four dilutions, and the

mean concentration of the four aliquots was used for each estimate. The sensitivity of the assay was 0.2 ng/tube and the inter-and intra-assay coefficients of variation were 9% and 8%, respectively.

At the time of SRIF assay, each median eminence was extracted by sonication in 200 µl of 2 N acetic acid, immersed in a boiling water bath for 5 min and quickly chilled to 0 C. A small aliquot of homogenate was removed and assayed for total protein using the method of Schaffner and Weissmann (21). A 1% aqueous solution of fraction V-bovine serum albumin (SIGMA) was used as a reference standard. The homogenates were centrifuged for 60 min at 700 xg and 4 C. Supernatants were neutralized with 2 N NaOH and diluted 1:10 in assay buffer. Samples were assayed at 6 dilutions using the method of Arimura et al. (72) and the mean concentration of the 6 aliquots was used for each SRIF value. The assay used synthetic somatostatin (Bechman), (^{125}I-Tyr^{1})-SRIF as a tracer and anti-SRIF serum (#101) provided by Dr. Arimura. The sensitivity of the assay was approximately 3 pg/tube. Inter- and intra-assay coefficients of variation were 13.5% and 7%, respectively.

To facilitate analysis of data obtained by repeated sampling, the following definitions are used. Baseline GH levels are defined as the mean of the lowest 6 values recorded for each animal during a 3 h sampling period. Peaks in GH levels are those which exceed baseline GH levels by at least 2 standard deviations. The amplitude of each peak is the difference between the peak and the baseline plasma GH concentration. Mean integrated plasma GH levels were calculated using a polar planimeter. One-way analysis of variance (ANOVA), the Newman-Keuls test and Duncans multiple range

test were used to compare data from single point measurements and means. Statistical analysis was performed on log-transformed data when heterogeneity of variance was encountered.

RESULTS

The location and extent of the PV lesions are illustrated in Fig. 1. In the sagittal plane (Fig. 1A) the lesions involved most of the PV nucleus to the plane of the anterior commissure rostrally and to the plane of the posterior mammillary nucleus caudally. None of the lesions involved the preoptic area. Tissue extending laterally about 0.5 mm on each side of the midline was destroyed (Figs. 1B, 1C); most of the magnocellular paraventricular nuclei suprachiasmatic nuclei and approximately 70% of the ventromedial-arcuate nuclear complex remained intact in all lesioned animals. Data from rats with lesions which did not fit the above description were not used.

Figure 2 illustrates representative patterns of plasma GH concentrations of control and PV-lesioned rats at 6-10 days after surgery. The mean amplitude of the GH peaks in intact rats was 62.6 $\pm$ 8.2 ng/ml plasma and mean baseline levels were 20.7 $\pm$ 2.3 ng/ml plasma. Surges of GH occurred with a mean interpeak interval of 53.5 $\pm$ 7.2 minutes. These data are in general agreement with pulsatile patterns of GH secretion in intact female rats reported previously (3). Analysis of these patterns (Fig. 3) showed that the baseline of plasma GH concentrations in PV-lesioned animals was elevated above those in sham-operated ($p < 0.01$) and intact controls ($p < 0.01$). In addition, the amplitude of peaks in plasma

GH levels in the PV-lesioned group was greater than that in sham-lesioned ($p < 0.05$) and more than 2-fold greater ($p < 0.01$) than that in intact rats. Two rats had exceptional patterns and these are also shown in Fig. 2; one had markedly elevated baseline levels with no definite peaks and one showed an extremely high GH peak. Interpeak intervals were similar (approximately 51 min) in all groups, so PV lesions had no apparent effect on the frequency of GH pulses. Planimetric integration of the GH data (Fig. 4) revealed that rats with PV lesions had higher mean integrated plasma GH levels than sham-operated ($p < 0.05$) and intact controls ($p < 0.01$). Likewise, overall group means of plasma GH levels during the period of repeated sampling demonstrated similar differences; the mean plasma GH level in rats with PV lesions (97.9 $\pm$ 10.8) was higher than those in sham-lesioned (64.3 $\pm$ 8.7, $p < 0.01$) and intact (41.0 $\pm$ 3.9, $p < 0.01$) rats.

Although baseline and peak GH levels were elevated at 6-10 days after surgery in PV-lesioned rats, nonstress and stress plasma GH concentrations were similar in all treatment groups in the blood samples collected at 6-15 weeks after surgery (Fig. 5).

At autopsy, nonstress plasma GH concentrations were somewhat but not significantly lower in the PV-lesioned group than in the controls (Fig. 6A). The SRIF content in the median eminence (Fig. 6B) of PV-lesioned rats was more than 85% lower ($p < 0.01$) than in both groups of controls. Similarly, the concentration of median eminence SRIF (ng SRIF/mg protein) in PV-lesioned rats (207.2 $\pm$ 79.1) was lower than that of sham-operated (1177.9 $\pm$ 198.9, $p < 0.01$) and intact (841 $\pm$ 170.7, $p < 0.05$) controls.

DISCUSSION

These results show that relatively descrete lesions placed in the hypothalamic portion of the PV nucleus which caused an eventual 85% reduction in median eminence SRIF content produced an elevation in baseline plasma GH concentrations and an increase in the amplitude of GH secretory pulses at 6-10 days after surgery; the frequency of the GH pulses did not appear to be affected. These changes in the pulsatile pattern of GH secretion in the PV-lesioned rats were associated with an increase in circulating GH levels during the 3-4 h sampling period as demonstrated by comparison of integrated areas under the GH curves and of overall means of plasma GH concentrations. However, these alterations in GH secretion were transient as we observed previously (15,16) in the PV-lesioned rats because plasma GH concentrations were not elevated in the two nonstress blood samples collected at 6-17 weeks after surgery. In contrast to the effects of PV lesions on episodic GH secretion observed in the present study, others reported that large lesions placed in the medial preoptic area (4) and complete surgical isolation of the medial basal hypothalamus (5) increased the frequency and failed to affect the amplitude of the GH pulses. The basis for these differences in results is unknown but it may be due to differences in size or placement of lesions or to the sex of the rats.

Whereas our present and previous (15,16) results show that placement of discrete lesions in the PV nucleus caused only transient changes in GH secretion, long-term elevations of nonstress plasma GH levels and blockade of the GH response to stress were

observed in rats with more extensive destruction of the medial PO-AHA (12,19). These differences in the effects of lesions suggest that the PV nucleus is not essential for inhibiting nonstress or stress plasma GH concentrations and that the portion of the medial PO-AHA lateral to the PV zone is important in this regard. It is possible that the transient alterations of the pulsatile pattern of GH secretion in PV-lesioned rats observed in this study reflect a temporary loss of function of inhibitory neurons resulting from edema or other trauma to adjacent tissues by the PV lesions.

Because of our previous findings (12,15), it was expected that lesions involving the PV nucleus of the anterior hypothalamus, the location of many of the SRIF neurons that project to the median eminence (9,13), would result in a marked reduction in median eminence content of SRIF. The degree to which median eminence content of SRIF was reduced, approximately 85%, is similar to that observed in our prior studies. The extent to which this reduction was responsible for the effects noted on the pulsatile pattern of GH secretion remains problematic. Because the increases in baseline levels and amplitude of GH pulses in PV-lesioned rats are consistent with the changes produced by passive immunization with SRIF antisera (6,7), it is possible that the effects of the lesions were due to the disruption of the inhibitory influence of SRIF neurons on the processes responsible for pulsatile GH secretion. However, this interpretation is complicated, as in previous studies (15,16), by the apparent transiency of the effects of these discrete PV lesions on the regulation of GH secretion under nonstress and stress conditions, despite the reduction in median eminence SRIF content at

autopsy. As discussed earlier (15,16), the seemingly normal control of GH secretion that coexists with markedly depleted SRIF levels in the median eminence raises the unanswered questions as to whether depletion of median eminence SRIF of such large magnitude is accompanied by a commensurate reduction in the release of SRIF into the hypophysial portal system or whether the lesions reduced normal median eminence stores of SRIF without affecting such release. Regardless of how these questions are resolved, it appears that the PV nucleus, with its constituent SRIF neurons, does not play an important role in suppressing plasma levels of GH under nonstress and stress conditions. However, the PV nucleus or connections traversing this area appear to be of primary importance in maintaining most of the SRIF contained in the median eminence.

In summary, these results demonstrate that lesions placed in the hypothalamic portion of the PV nucleus cause elevated baseline concentrations of GH in plasma and increased amplitude of GH secretory pulses without an apparent effect on the frequency of such pulses. The effects of PV lesions appeared transient since nonstress plasma GH concentrations were normal when examined at more than 6 weeks after surgery. The lesions successfully destroyed many of the SRIF neuron cell bodies or fibers responsible for maintaining SRIF in the median eminence as evidenced by the 85% reduction in median eminence SRIF content at autopsy. Thus, it appears that the hypothalamic PV nucleus is essential for the maintenance of most of the SRIF in the median eminence but it does not seem necessary for long-term suppression of nonstress GH secretion or stress-induced inhibition of GH secretion. Whether SRIF is involved

in the reinstatement of apparently normal inhibitory control of GH secretion remains to be determined.

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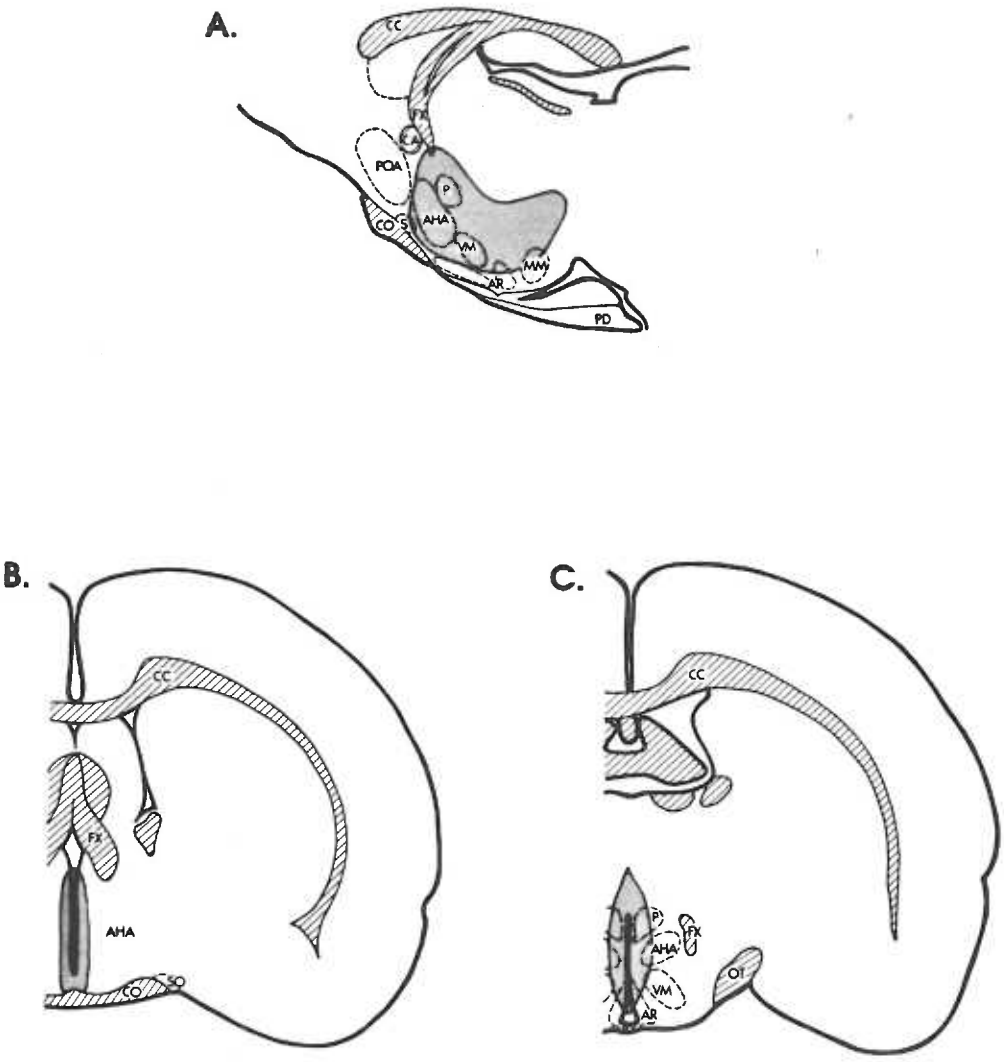
Figure 1. Diagrams of the location of PV lesions. A) The common area of destruction produced by PV lesions in the sagittal plane. B & C) Coronal sections of the rat forebrain showing the lateral extent of PV lesions. The shaded area in each diagram indicates the extent of the lesions. Abbreviations: AHA, area anterior hypothalami; AR, nucleus arcuatus hypothalami; CA, commissura anterior; CC, corpus callosum; CO, chiasma opticum; FX, fornix; MM, nucleus mamillaris medialis; OT, tractus opticus; P, nucleus paraventricularis hypothalami; PD, pars distalis; POA, area preoptica; S, nucleus suprachiasmaticus; SO, nucleus supraopticus hypothalami; VM, nucleus ventromedialis hypothalami.


A.
CC
POA
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AHA
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CO
PD
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CC
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C.
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Figure 2. The pulsatile pattern of plasma GH concentrations in individual intact, sham-lesioned and PV-lesioned rats at 6-10 days after surgery. The interval between each point represents 15 min.

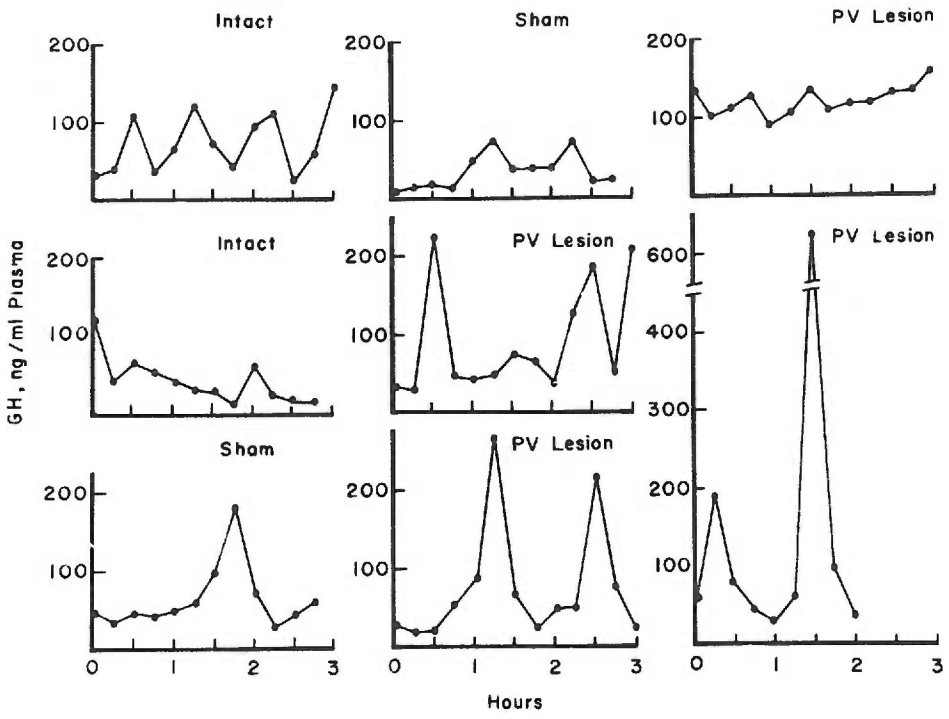

Intact
Sham
PV Lesion
Intact
PV Lesion
PV Lesion
Sham
PV Lesion
200
100
600
400
300
0
1
2
3
GH, ng/ml Plasma
Hours

Figure 3. Effects of PV lesions on the baseline and amplitude of peaks of the pulsatile pattern of GH secretion. Means ± SE are indicated. X, $p < 0.01$ vs. baselines of sham-lesioned and intact groups; *, $p < 0.05$ vs. amplitude of peaks in PV-lesioned group; **, $p < 0.01$ vs. amplitude of peaks in PV-lesioned group.

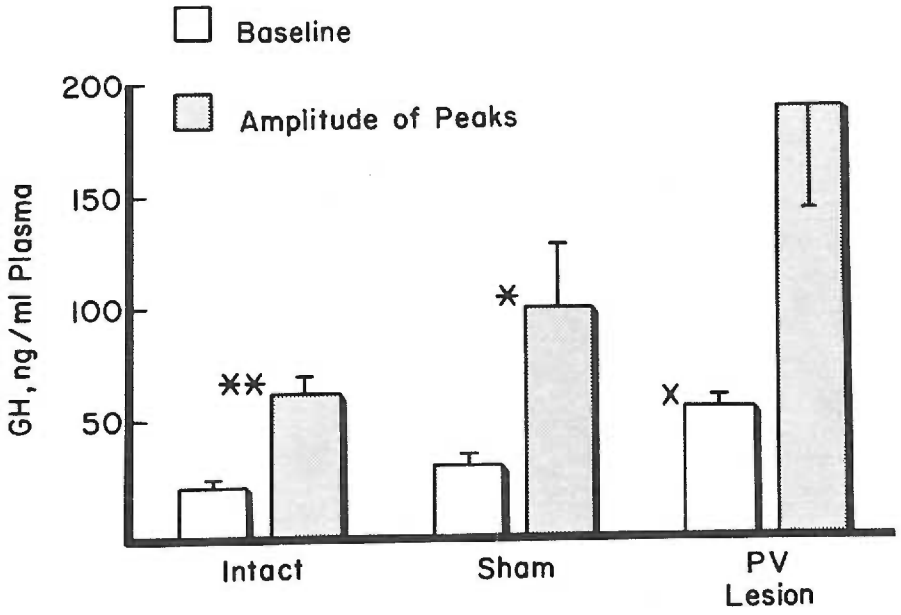

Baseline
Amplitude of Peaks
200
150
100
50
GH, ng/ml Plasma
**
*
X
Intact
Sham
PV
Lesion

Figure 4. Effects of PV lesions on mean integrated plasma GH levels per hour. Mean $\pm$ SE are indicated. Numbers at bases of columns indicate rats/group. *, $p < 0.05$ vs. PV lesioned group; **, $p < 0.01$ vs. PV lesioned group. Differences between sham-lesioned and intact controls were not significant.

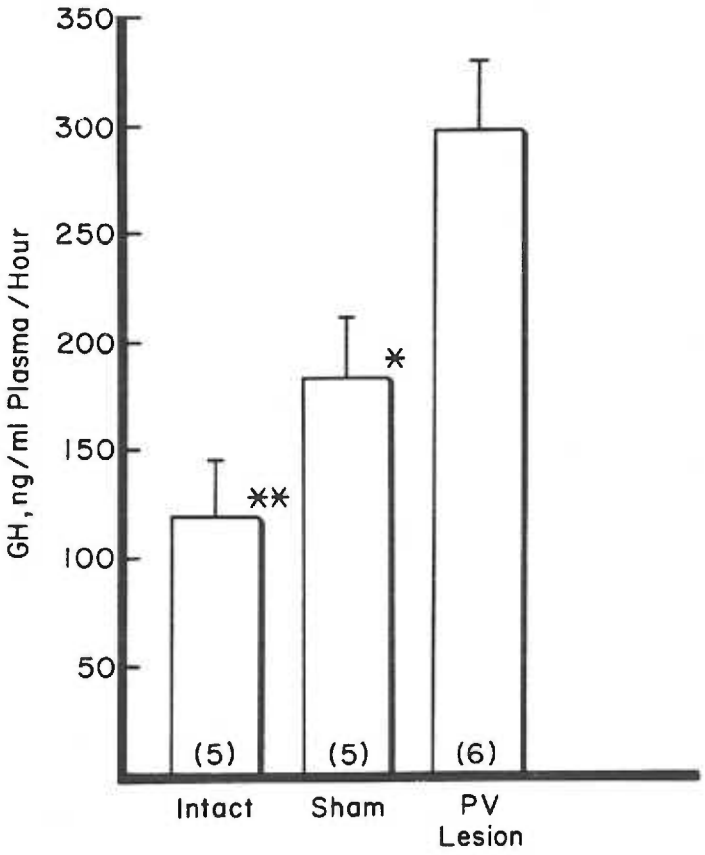

350
300
250
200
150
100
50
GH, ng/ml Plasma / Hour
**
*
(5)
(5)
(6)
Intact
Sham
PV
Lesion

Figure 5. Effects of PV lesions on nonstress plasma GH levels and plasma GH levels following 15 min immobilization-blood withdrawal stress at 6-15 weeks after surgery. No significant differences in nonstress or stress levels of GH were apparent between treatment groups. Stress produced a significant fall ($p < 0.05$) in plasma GH levels in all groups. Means $\pm$ SE are indicated. Numbers at bases of columns indicate rats/group.

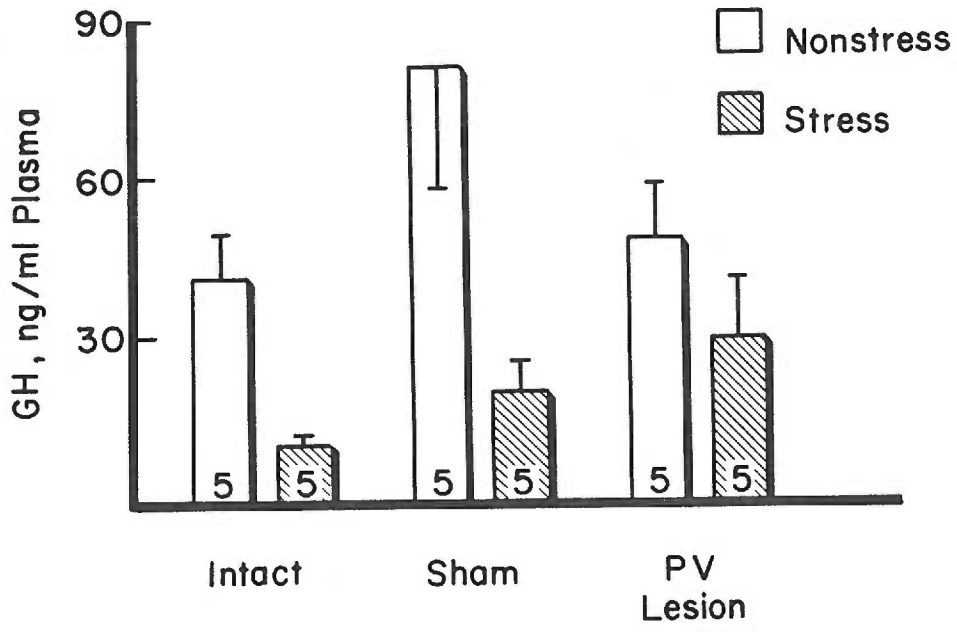
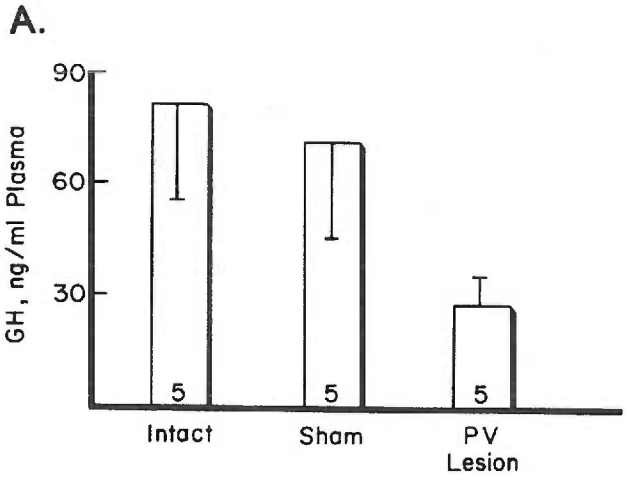
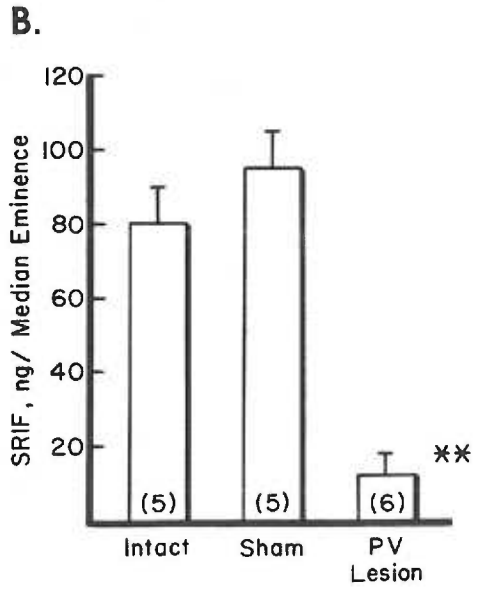

90
60
30
GH, ng/ml Plasma
Nonstress
Stress
5
5
5
5
5
5
Intact
Sham
PV
Lesion

Figure 6. A) Effects of PV lesions on nonstress plasma GH levels at autopsy. No significant differences in plasma GH levels were apparent between treatment groups. B) Effects of PV lesions on somatostatin content of the median eminence at autopsy. **, $p < 0.01$ vs. sham-lesioned and intact controls. Means $\pm$ SE are indicated. Numbers at bases of columns indicate rats/group.


A.
90
60
30
GH, ng/ml Plasma
5
5
5
Intact
Sham
PV
Lesion


B.
120
100
80
60
40
20
SRIF, ng/ Median Eminence
(5)
(5)
(6)
**
Intact
Sham
PV
Lesion

CHAPTER V

The Effects of Medial Basal Hypothalamic Isolation on Pulsatile Growth Hormone Secretion

ABSTRACT

Evidence suggests that the medial basal hypothalamus is responsible for the pulsatile pattern of growth hormone (GH) secretion, that the preoptic-anterior hypothalamic area (PO-AHA) suppresses this episodic pattern and that this inhibition is mediated by somatostatin (SRIF)-containing neurons in this part of the brain. However, various experimental approaches designed to remove the inhibitory influence of the PO-AHA or SRIF on episodic GH secretion have produced incongruous results. In the present experiment, partial forebrain removal and brainstem transection were performed to isolate a basal hypothalamus-pituitary island (BHPI) in order to determine whether these tissues are sufficient to support pulsatile changes in plasma GH concentrations and to explore the effects of removing brain structures which inhibit GH secretion. At 30 h after atrial cannulae placement and partial forebrain removal, BHPI rats showed marked increases in both baseline plasma GH levels and amplitude of GH peaks; these changes were associated with markedly elevated circulating GH levels. Immobilization stress caused a paradoxical rise in plasma GH levels in the BHPI group. Despite marked elevation of nonstress GH levels in rats with BHPI, the content of SRIF in these tissues was similar to that in sham-operated controls.

These results indicate that the BHPI is capable of supporting pulsatile GH secretion and that brain structures outside of the basal hypothalamus inhibit GH secretion by affecting both the baseline and amplitude of pulses. Removal of many of the known SRIF-containing perikarya of the forebrain does not lead to depletion of SRIF in the basal hypothalamus and median eminence at 30 h after surgery.

INTRODUCTION

Growth hormone (GH) is secreted in an episodic manner in female (1) and male (2) rats under nonstress conditions. Exposure of rats to stressful stimuli produces a cessation of pulsatile secretion (3) and a dramatic fall in plasma GH levels (4). Because surgical isolation of the medial basal hypothalamus (MBH) (5,6) is compatible with pulsatile changes in circulating GH levels and lesions placed in the MBH (7) abolish pulsatile GH secretion, it appears that the structures responsible for episodic GH secretion in the rat reside in the MBH. The preoptic-anterior hypothalamic area (PO-AHA) seems to be the part of the brain that tonically inhibits pulsatile release of GH under nonstress conditions; lesions placed in the preoptic area (8) or surgical cuts that interrupt connections between this area and the MBH (5) increased the frequency of GH peaks and produced a tendency toward elevated trough levels. It was suggested (5,8) that the alterations in the pattern of pulsatile GH secretion resulted from interruption of the projections from somatostatin (SRIF) neurons in the PO-AHA to the MBH. However, passive immunization with SRIF antiserum (9,10) produced elevations in both trough and amplitude of GH peaks without affecting the frequency of secretory episodes. Despite these discrepant findings it appeared that the inhibitory effects of the PO-AHA on pulsatile GH secretion were mediated by the SRIF-containing neurons contained in this part of the brain (11,12,13,14).

The major aims of the present experiment were to use another approach, partial forebrain removal, to test the hypothesis that the isolated MBH is sufficient to support pulsatile changes in cir-

culating GH levels and to explore further the effects of removing structures located outside of the MBH which inhibit episodic GH secretion. This alternate method of isolating the MBH was used in order to avoid some of the problems of interpretation inherent in the hypothalamic deafferentation technique, such as assessing the completeness of the surgical isolation, the possible effects of regeneration of neural connections to the MBH or the effects of communication with the MBH-pituitary unit via ventricular cerebrospinal fluid.

MATERIALS AND METHODS

Adult female rats (Charles-River CD) weighing 200-250 g were maintained under conditions of controlled lighting (fluorescent, 0400-1800 h) and temperature (22 $\pm$ 2 C). All animals were provided with rat chow and water ad libitum except where specified. Using a completely randomized design, animals were assigned to basal hypothalamic-pituitary island (BHPI) or sham-operated treatment groups. Each animal was implanted with an indwelling jugular cannula. The cannulae were designed and implanted with a modification of the techniques described by Harms and Ojeda (15) using single lumen clear vinyl tubing (0.5 mm id, 0.8 mm od, Dural Plastics). A 2 cm^2 piece of organza (polyester) was attached 5 cm from the distal end of each cannula to facilitate securing to subcutaneous structures. The cannulae were sterilized and filled with a mixture of heparin (500 U/ml saline) and polyvinylpyrrolidone (1 g/2ml solution, Calbiochem) to prevent clot formation within the cannulae. The rats were anesthetized with ether, the right external jugular vein was exposed and the cannula was implanted.

Immediately following implantation of cannulae, the rats were maintained under ether anesthesia and placed in a stereotaxic apparatus. Portions of frontal and parietal bones were removed bilaterally, leaving the superior sagittal sinus and its overlying bone intact. The dura was removed and brain structures were visualized with an operating microscope and removed by suction. All of the telencephalon and most of diencephalon were removed, and the brainstem was transected at the rostral border of the superior colliculi. The forebrain tissue retained in these preparations consisted of a hypothalamic island extending from the optic chiasm anteriorly to the caudal border of the mammillary bodies posteriorly. The island was approximately 2 mm in height, and it extended approximately 1.5 mm on each side of midline; its connections with the pituitary were left intact. The sham procedure consisted of removing a 2x5 mm patch of dorsal cortex and underlying white matter bilaterally to the level of the corpus callosum. Hemostasis was achieved in both preparations by irrigation of tissue with warm physiological saline and placement of Gelfoam sponge over exposed brain surfaces. Following surgery, the rats were placed in a 95% O_2-5% CO_2 environment and rectal temperatures were monitored periodically. Animals with body temperatures that exceeded 39 C were immersed in cold water. Because BHPI rats do not eat spontaneously, food and water were withheld from all animals after surgery. All rats were given 5 ml of 5% dextrose-saline, ip at 1700 h and at 0800 h on the following morning. At 1700 h on the day of surgery, the rats were placed into individual sampling cages, and the animal quarters were locked to prevent disturbances and inadvertent stress. At 0800 h of the first postoperative day,

each cannula was flushed and a 30 cm piece of PE-50 polyethylene tubing (0.023" id, 0.038" od) was attached to the externalized end of the cannula with a 2-3 cm piece of 24 gauge stainless steel tubing. The distal end of the tubing was attached to a swivel device to allow the animal freedom of movement. The room was then locked for an additional 4-5 h to restrict stressful stimuli. Sequential blood sampling was initiated at 1300 h; 0.4 ml blood samples were collected in heparinized syringes every 15 min for approximately 3 h. The blood samples were centrifuged and the cells suspended in warm saline and infused following the removal of the next blood sample. Twenty seconds before collection of the last nonstress blood sample, the rats were immobilized in the supine position for 3 min. The final nonstress blood sample was collected within 90 sec of initiating this immobilization procedure. Following immobilization, the animal was returned to the sampling cage and a stress blood sample was collected after decapitation at 15 min following initiation of stress. As shown previously (16), these sampling times are appropriate for estimating nonstress plasma GH concentrations and the changes induced by stress. All blood samples were chilled and centrifuged, and the plasma was snap-frozen in dry ice and ethanol and stored at -20 C until assayed for GH.

Except for the 9 rats described below, heads of BHPI rats were fixed by immersion in 10% neutral buffered formalin and processed for histological examination. Four randomly-selected BHPI rats and 5 sham-operated rats were subjected to the operative procedures described above, but sequential blood samples were not withdrawn. At about 1700 h on the day following surgery, these rats were decap-

itated within 30 sec of cage opening and trunk blood was collected to determine nonstress levels of GH in plasma. In BHPI rats, a block containing the hypothalamic island and median eminence was dissected, immersed in 500 µl of 2 N acetic acid and flash frozen within 3 min of initiation of handling. These tissues were stored at -20 C until assayed for SRIF. The brain of each sham-operated control was inverted on a chilled glass plate and, using an operating microscope, a block of hypothalamic and median eminence tissue, approximating that in the BHPI rats, was dissected and treated as described above.

Plasma GH was measured with the radioimmunoassay described by Schindler et al. (17) using materials supplied by the NIAMDD through Dr. A. Parlow. Results were expressed in terms of NIAMDD-Rat GH, RP-1 standard. All samples were assayed at four dilutions and the mean concentration of the four aliquots was used for each estimate. The sensitivity of the GH assay was 0.2 ng/tube and the inter- and intra-assay coefficients of variation were 9% and 8%, respectively.

Each hypothalamic fragment and attached median eminence was extracted by sonication in 500 µl of 2 N acetic acid, immersed in a boiling water bath for 5 min and quickly chilled to 0 C. The homogenates were then centrifuged at 4 C for 60 min at 700 xg. The supernatant of each sample was neutralized with 2 N NaOH followed by a 1:8 dilution in assay buffer. All samples were assayed at 6 dilutions with the method of Arimura et al. (18) and using synthetic somatostatin (Beckman) as a reference standard. The mean concentration of 6 aliquots was used for each SRIF value. This assay employed rabbit anti-somatostatin serum (#101) provided by Dr. A.

Arimura and (^{125}I-Tyr^{1})-SRIF as a tracer. The sensitivity of the assay was approximately 3 pg/tube. Inter- and intra- assay coefficients of variation were 13.5% and 7%, respectively.

In order to standardize the analysis of data which were collected by repeated sampling, the following definitions were used. Baseline GH levels were defined as the lowest 6 values recorded for each animal during a 3 h sampling period. Peak GH levels were those which exceeded baseline GH levels by at least 2 standard deviations. The amplitude of each peak was defined as the difference between the peak and baseline plasma GH concentrations. Mean integrated plasma GH levels were determined using a polar planimeter. One-way analysis of variance (ANOVA) and the Newman-Keuls test were used to compare data from single point measurements. Two-way ANOVA for repeated measures was used to analyze data involving both nonstress and stress plasma levels of hormone from individual rats. Statistical analysis was performed on data which had undergone log transformation when heterogeneity of variance was encountered.

Following fixation, the heads of BHPI rats were placed in 10% formalin-30% sucrose for 24 h. Bone and surrounding tissues were removed until the BHPI was completely exposed dorsally but remained in situ on the petrous temporal bone. Ninety µl of 25% glutaraldehyde was added to 5 ml of a solution of 30 g albumin-gelatin per 100 ml of 0.9% saline-25% sucrose. This solution was poured over the tissue and allowed to harden. The tissues were then immersed in 10% neutral buffered formalin for 24 h, and the remaining bone and associated tissues were removed, leaving the BHPI, and median eminence in their normal positions embedded in the albumin-gelatin

matrix. Frozen sagittal sections were cut, mounted and stained with thionin. Data were discarded from rats which had evidence of marked hypothalamic or pituitary infarction or damage of hypothalamo-pituitary connections.

RESULTS

The structures retained in common in the BHPI preparations are illustrated in Fig. 1. The hypothalamic island included most of the suprachiasmatic nuclei, major portions of the anterior hypothalamic area and paraventricular nuclei, all of the ventromedial-arcuate nuclear complex and much of the dorsomedial and mammillary nuclei. The representative parasagittal section of a BHPI in Fig. 2 illustrates the histological appearance of hypothalamic nuclei, median eminence, pituitary and the transection of the brainstem.

Nonstress pulsatile patterns of GH secretion in 2 BHPI rats and 2 sham-operated controls are shown in Fig. 3. Although the baseline levels were slightly higher and amplitudes of GH peaks above baseline levels were somewhat smaller in the sham-operated rats than in intacts, these data are in general agreement with the pulsatile patterns of plasma GH levels observed by others in intact female rats (1). As suggested by the individual patterns shown in Fig. 3, analysis of the group data revealed (Fig. 4) that BHPI rats had baseline GH levels that were higher ($p < 0.0005$) and GH peaks that were greater ($p < 0.0005$) in amplitude than those in sham-operated controls. These changes in the pulsatile pattern in BHPI rats were associated with an elevation ($p < 0.025$) in mean integrated plasma GH concentration per hour (Fig. 5). Although GH secretory pulses were evi-

dent in BHPI rats, they appeared to be less frequent than in sham-operated rats; usually only 1 peak appeared during the 3 h sampling period, precluding calculation of mean interpeak intervals. The interpeak interval in the sham-operated group was 67.5 $\pm$ 6.4 min, similar to that found in intact female rats (1).

As shown in Fig. 6, GH concentrations in the last nonstress blood sample were elevated ($p < 0.05$) above those in sham-operated controls. Whereas the controls showed a decrease ($p < 0.05$) in GH levels in response to stress, the BHPI rats showed a paradoxical increase ($p < 0.05$).

Figure 7 shows nonstress plasma GH concentrations in blood collected at decapitation approximately 32 h after surgery and hypothalamic-median eminence content of SRIF. Although the nonstress plasma GH level in BHPI rats was elevated ($p < 0.025$) above that of controls (Fig. 7A), content of SRIF in basal hypothalamus-median eminence units was similar in both treatment groups (Fig. 7B).

DISCUSSION

This study demonstrates that the BHPI is capable of supporting pulsatile GH secretion. However, the normal GH secretory pattern was altered in that marked increases in both baseline GH levels and amplitude of peaks were observed. The frequency of GH pulses appeared to be somewhat decreased. In addition, BHPI rats responded to immobilization stress, with a paradoxical rise in plasma GH levels. Finally, despite marked elevation of circulating plasma GH levels in BHPI rats, MBH-median eminence content of SRIF was similar to that in the controls.

The finding that BHPI rats demonstrated pulsatile secretion of GH corroborates the finding of Willoughby et al. (5,6) that complete hypothalamic deafferentation is compatible with pulsatile GH secretion. Although these findings suggest that the MBH-median eminence unit is adequate to support pulsatile GH secretion, it appears that complete isolation of the MBH in BHPI rats removed some inhibitory input to the MBH which normally suppresses pulsatile GH secretion. Thus, BHPI rats had elevated baseline GH levels and increased amplitude of peaks; these changes resulted in markedly elevated circulating plasma GH levels. A number of studies suggest that neurons in the preoptic area are important for inhibiting nonstress plasma GH levels. Lesions placed in the preoptic area (8,16,19) elevated nonstress plasma GH concentrations and electrical stimulation of this region (20) produced a dramatic fall in nonstress plasma GH levels. With respect to the pulsatile pattern of GH secretion, destruction of most of the medial preoptic area (8) or interruption of connections between this area and the median eminence (5) increased the frequency of GH peaks and produced a tendency towards elevated trough levels, but the amplitude of the secretory pulses was apparently not affected. Because amplitude and not frequency of peaks was altered in BHPI rats, the present results differ from those observed with more discrete ablations. The bases for these differences are unknown, but they may relate to sex differences or to variations in experimental approach.

In the present study, BHPI rats demonstrated a paradoxical stress-induced rise in plasma GH concentrations. Similar reversals of the GH stress response have been described previously in rats

with varying degrees of telencephalon removal (21,22). Such findings indicate that structures outside of the basal hypothalamus are essential for the usual stress-induced decrement in the GH levels, and projections from the PO-AHA (16,21) to the MBH appear important in this regard. The paradoxical increase in plasma GH levels in response to stress may reflect the release of the presumptive GH-releasing hormone, the effects of which are normally obscured by the simultaneous release of SRIF. Because stress caused an increase in plasma GH levels in rats with median eminence-pituitary islands (22), in the absence of the MBH, it is possible that vascular connections to the median eminence are involved in the abnormal stimulation of GH secretion in response to stress in the BHPI rats.

The present results also show that whereas nonstress plasma levels of GH were markedly higher in BHPI rats than in sham-operated controls at 30 h after surgery, MBH-median eminence content of SRIF was similar in both groups. It is somewhat surprising that SRIF content of the MBH-median eminence unit was not reduced in the BHPI rats because the brain tissue removed in these preparations contains many of the forebrain SRIF-containing perikarya (11,12,13,14), and these neurons appear responsible for most of the SRIF content of the median eminence (23,24). It may take more than 30 h for the axonal terminals of the ablated neurons to undergo depletion of stored SRIF. Assuming SRIF is involved in the suppression of plasma GH levels under nonstress and stress conditions, as the results of passive immunization studies suggest (3,9,25), SRIF neurons in the preoptic area may be important in such suppression. However, as indicated by recent results (23,24), these SRIF neurons may not reside in the PV zone,

and the role of median eminence stores of SRIF is unclear; rats with lesion-induced destruction of the PV zone and marked reduction of SRIF in the median eminence showed normal nonstress plasma GH levels and normal suppression of circulating GH levels in response to stress. Thus, elevated nonstress plasma levels of GH in the BHPI rats coupled with normal median eminence content of SRIF may provide further evidence demonstrating the lack of a functional relationship between median eminence SRIF content and hypothalamic regulation of GH secretion or may reflect the absence of release of SRIF from the terminals of isolated axons in the process of degeneration.

In summary, these results suggest that the pulsatile pattern of GH secretion persists, although in altered form, in BHPI rats following removal of adjacent forebrain structures. The alterations consisted primarily of increases in both the baseline and amplitude of the GH pulses. The net effect of these changes in pulsatile GH secretion was an elevation in circulating GH levels. The BHPI rats also showed paradoxical increases in plasma GH levels in response to stress. Finally, it was found that the elevated plasma GH levels in BHPI rats were associated with normal median eminence stores of SRIF.

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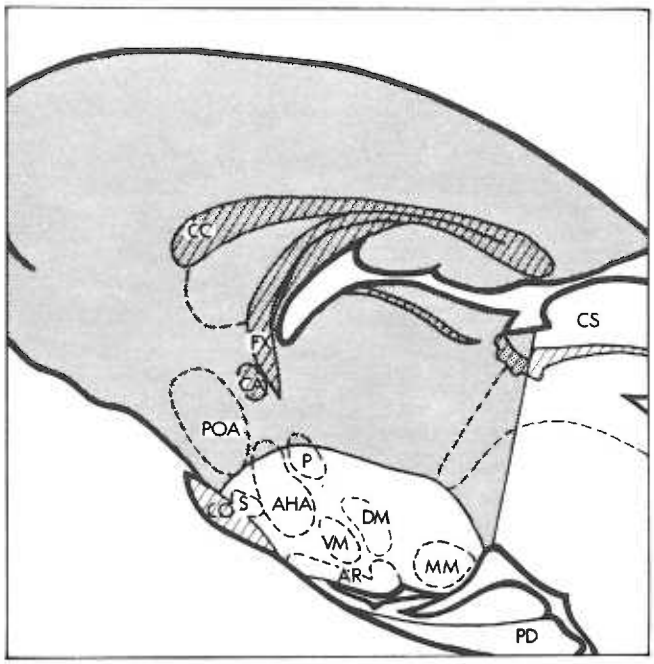
Figure 1 Diagrams of the rat forebrain in the sagittal (A) and coronal (B) planes showing the size and location of a basal hypothalamus-pituitary island (BHPI). The shaded area in each diagram represents the ablated tissue. Abbreviations: AHA, area anterior hypothalami; AR, nucleus arcuatus hypothalami; CA, commissura anterior; CC, corpus callosum; CO, chiasma opticum; CS, colliculus superior DM, nucleus dorsomedialis hypothalami; FX, fornix; MM, nucleus mamillaris medialis; P, nucleus paraventricularis hypothalami; PD, pars distalis; POA, area preoptica; S, nucleus suprachiasmaticus; VM, nucleus ventromedialis hypothalami.

A.



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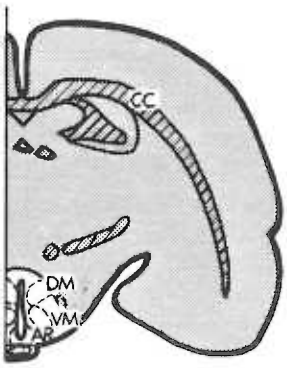


Figure 2 A representative photomicrograph of a BHPI in the sagittal plane demonstrating the histological appearance of this preparation. AR, nucleus arcuatus hypothalami; S, nucleus suprachiasmaticus; VM, nucleus ventromedialis hypothalami.

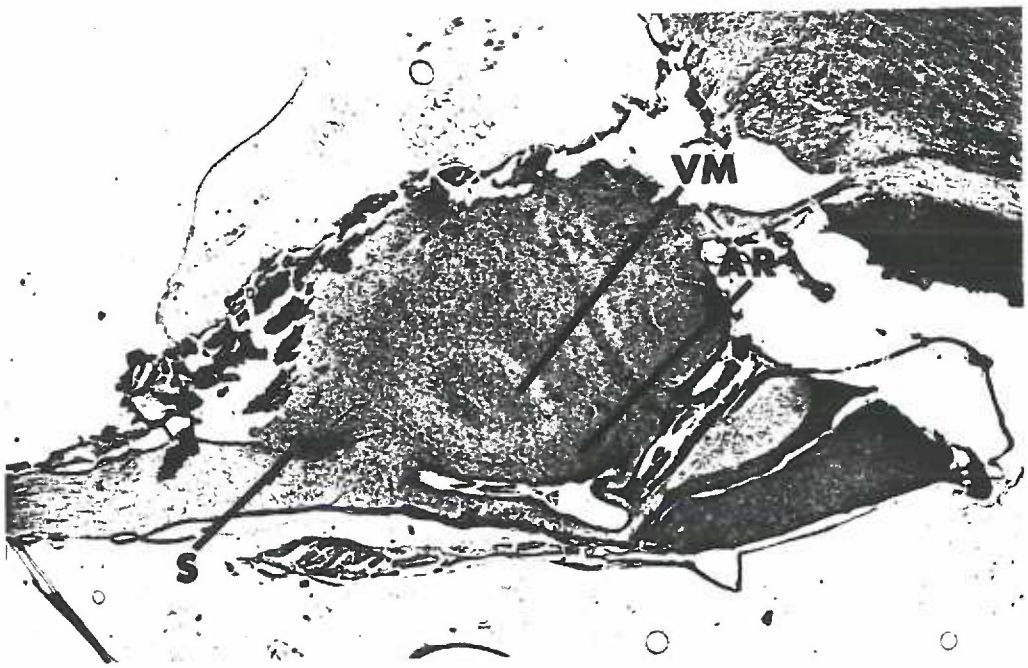

VM
AR
S

Figure 3 Pulsatile patterns of plasma GH concentrations in sham-operated and BHPI rats.

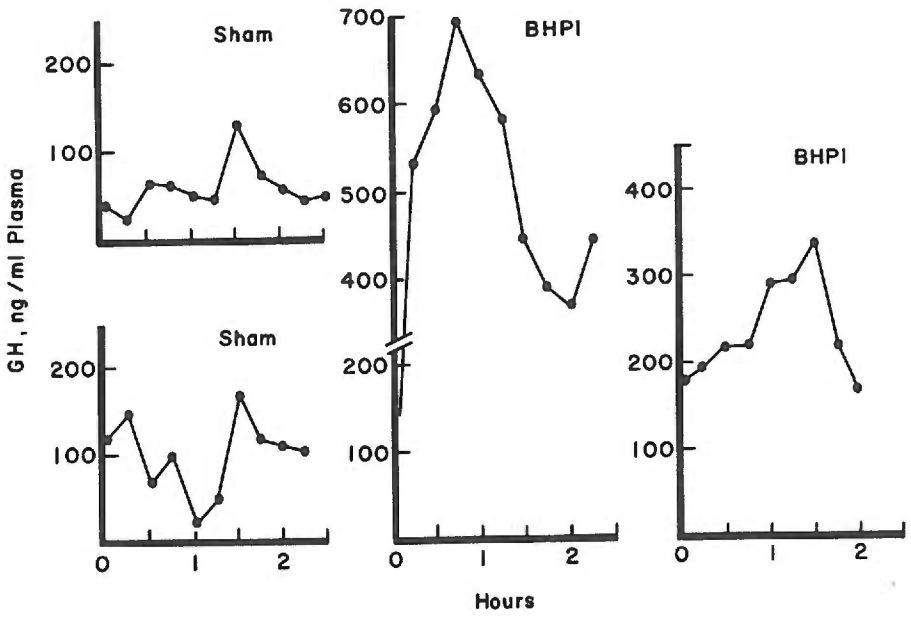

Sham
Sham
BHPI
BHPI
GH, ng/ml Plasma
Hours
100
200
400
500
600
700
0
1
2

Figure 4 Effects of isolation of the medial basal hypothalamus on the baseline and amplitude of peaks of the pulsatile pattern of GH secretion. Means ± SE are indicated. **, $p < 0.0005$ vs baseline of sham-operated group; ***, $p < 0.0005$ vs amplitude of peaks in sham-operated group. Numbers at bases of columns indicate number of samples.

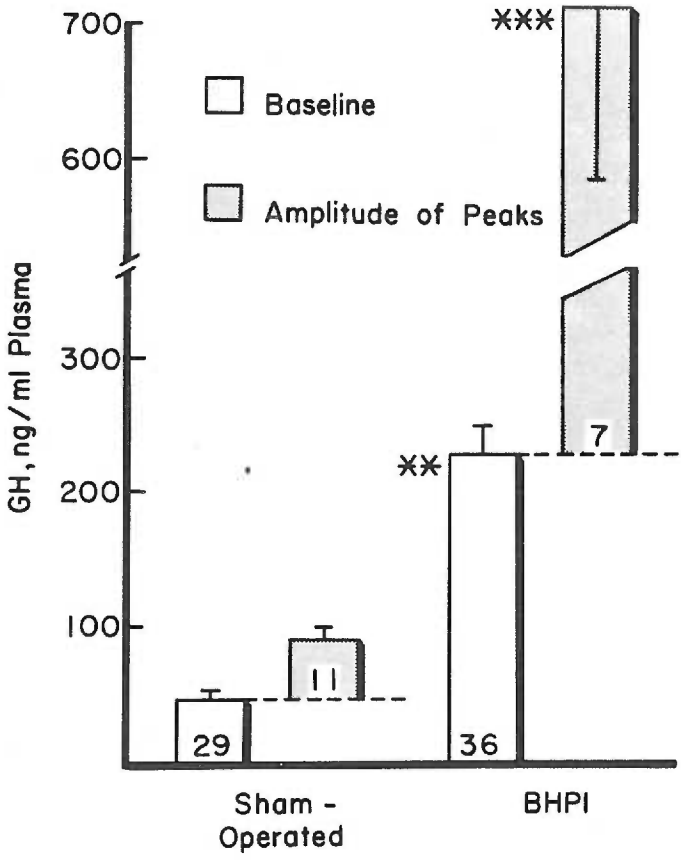

700
600
300
200
100
GH, ng/ml Plasma
Baseline
Amplitude of Peaks
***
**
29
11
36
7
Sham - Operated
BHPI

Figure 5 Effects of isolation of the medial basal hypothalamus on mean integrated plasma GH levels per hour. Means ± SE are indicated. Numbers at the bases of columns indicate number of rats.

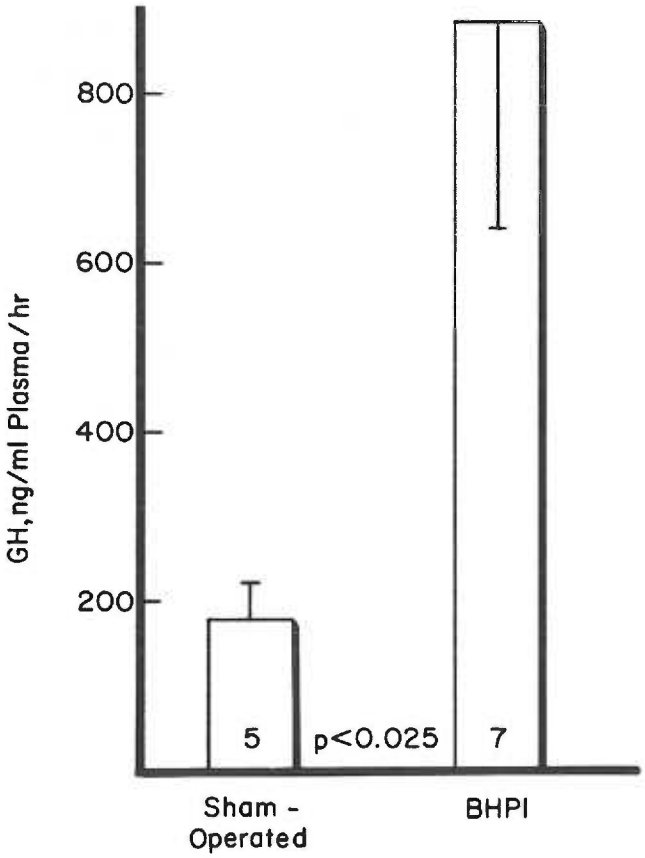

800
600
400
200
GH, ng/ml Plasma / hr
5
p<0.025
7
Sham - Operated
BHPI

Figure 6 Effects of isolation of the medial basal hypothalamus on nonstress plasma GH levels and plasma GH levels following 15 min immobilization stress. Means $\pm$ SE are indicated. *, $p < 0.05$ vs nonstress plasma GH levels in the sham-operated group; **, $p < 0.0025$ vs stress plasma GH levels in the sham-operated group. There was a significant difference ($p < 0.05$) between nonstress and stress plasma GH levels in both groups. Numbers at the bases of columns indicate number of rats.

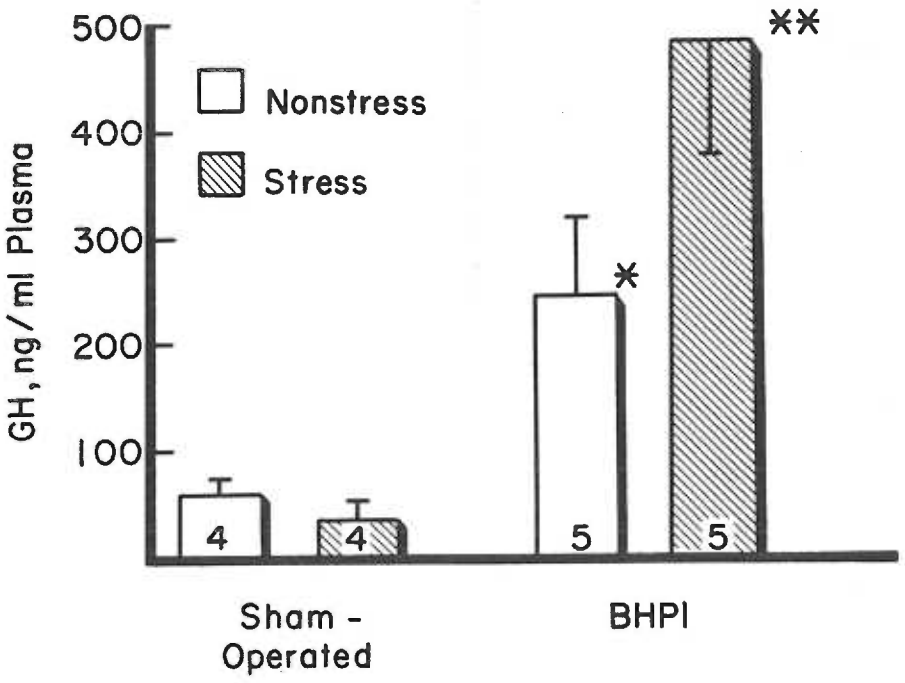
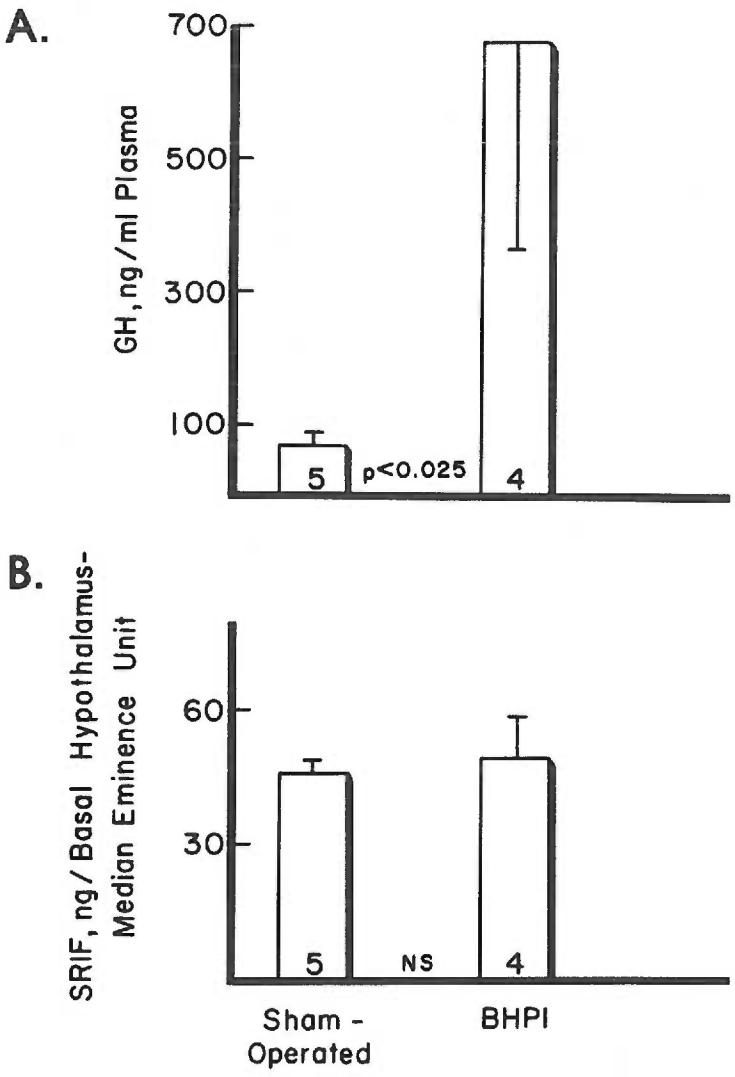

500
400
300
200
100
GH, ng/ml Plasma
Nonstress
Stress
4
4
5
5
*
**
Sham - Operated
BHPI

Figure 7 Effects of isolation of the medial basal hypothalamus on nonstress plasma GH levels (A) and somatostatin content of the basal hypothalamus-median eminence (B). Means $\pm$ SE are indicated. Numbers at bases of columns indicate number of rats.


A.
700
500
300
100
GH, ng/ml Plasma
5
p<0.025
4
B.
60
30
SRIF, ng/Basal Hypothalamus-Median Eminence Unit
5
NS
4
Sham - Operated
BHPI

SUMMARY

The first manuscript describes a study to determine the effects of lesions placed in the hypothalamic periventricular (PV) nucleus, the location of somatostatin (SRIF)-containing neuron cell bodies which seem to be responsible for most of the SRIF in the median eminence, on nonstress plasma GH and TSH levels and the response of these hormones to stress in female rats. PV lesions produced only transient elevation of nonstress plasma GH levels and failed to block stress-induced suppression of GH secretion. In contrast, PV lesions caused long-term elevation of nonstress plasma TSH levels and blockade of stress-induced suppression of TSH secretion. Rats with PV lesions also demonstrated a 90% reduction in the content and concentration of SRIF in the median eminence. These data demonstrate that destruction of the PV nucleus can produce a marked depletion of median eminence SRIF and cause long-term elevation of plasma TSH levels and disruption of the TSH reponse to stress without producing alterations in GH secretion. Thus, the hypothalamic PV nucleus, the SRIF neurons located there and the majority of SRIF in the median eminence are not essential for maintaining normal GH secretion but may be involved in the regulation of TSH secretion. It appears

that different parts of the preoptic-anterior hypothalamic area (PO-AHA) may be involved in inhibiting GH and TSH secretion. It is possible that such inhibition is mediated by the inhibitory effects of SRIF, although further studies are necessary to provide direct evidence for this possibility.

Evidence suggests that SRIF is involved in the suppression of pulsatile GH secretion. The second manuscript describes experiments that were performed to determine the effects of destruction of the hypothalamic PV nucleus and subsequent depletion of median eminence SRIF content, on the pulsatile pattern of GH secretion. At 6-10 days after the placement of PV lesions, sequential blood samples showed an elevation in baseline GH levels and an increase in the amplitude of GH secretory peaks; frequency of pulses was not affected. These alterations in the pulsatile pattern of GH secretion resulted in an increase in mean circulating plasma GH levels. However, the alterations appeared transient because nonstress and stress plasma GH levels were normal in blood samples collected 6-17 weeks after surgery. Despite similar nonstress plasma GH levels in all treatment groups at autopsy, the content and concentration of SRIF in median eminences of PV-lesioned rats were 85% lower than in sham or intact controls. These findings show that destruction of much of the hypothalamic PV nucleus causes an elevation in baseline plasma GH

concentrations and an increase in amplitude of GH secretory pulses without having an apparent effect on the frequency of such pulses. It appears that the PV nucleus is not essential for hypothalamic suppression of GH secretion since PV lesions produced only short-term alterations of plasma GH concentrations. However, the present data support previous findings by showing that the PV nucleus is essential for maintaining most of the SRIF in the median eminence. It remains to be determined whether the effects of PV lesions on episodic GH secretion were due to an initial but temporary cessation of hypothalamic SRIF release and whether SRIF was involved in reinstatement of apparently normal inhibitory control of GH secretion.

Previous studies suggest that the medial basal hypothalamus (MBH) is responsible for episodic GH secretion and that the PO-AHA and SRIF-containing neuron cell bodies in this part of the brain suppress the pulsatile pattern of GH secretion. In the experiment described in the third manuscript, partial forebrain removal and brainstem transection were used to isolate a basal hypothalamus-pituitary island (BHPI) and test the hypothesis that the isolated MBH is sufficient to support pulsatile GH secretion. This approach was used in order to avoid some of the problems of interpretation which are inherent in assessing the extent of lesions and knife cuts and to further study the effects of physically removing brain

structures which inhibit GH secretion. At 30h after surgery, BHPI rats showed marked increases in both baseline plasma GH levels and amplitude of GH peaks. These changes were associated with markedly elevated circulating GH levels. Immobilization stress caused a paradoxical rise in plasma GH levels in BHPI rats. At autopsy, the content of SRIF in the MBH-median eminence unit was similar to that in sham-operated controls despite marked elevation of nonstress GH levels in the BHPI group. These results show that the BHPI is capable of supporting pulsatile GH secretion and that brain structures outside of the MBH suppress nonstress plasma GH concentrations by affecting both baseline GH levels and amplitude of GH peaks. It is not known whether this inhibition is mediated by the inhibitory effects of SRIF, although these results show that removal of many of the known SRIF-containing perikarya located outside of the MBH does not lead to depletion of SRIF in the MBH or median eminence at 30h after surgery.

The experiments in this thesis were designed to investigate various aspects of hypothalamic inhibition of GH secretion. At the beinning of this work, it was assumed that the inhibition exerted by the PO-AHA on GH secretion was mediated by SRIF-containing neurons in this part of the brain because medial PO-AHA lesions or disruption of caudal projections from this area depleted median eminence content of SRIF and produced alterations of GH secretion that were

similar to those produced by treatment of rats with SRIF antiserum. However, this assumption now appears to be inaccurate since there seems to be no firm reciprocal relationship between median eminence content of SRIF and plasma GH concentrations. Recent experiments in this laboratory, including studies presented in this thesis, have shown that destruction of many of the hypothalamic SRIF neurons and the subsequent 80-90% depletion of median eminence SRIF are not necessarily associated with elevated nonstress plasma GH levels, blockade of stress-induced inhibition of GH secretion or long-term alterations in the pulsatile pattern of GH secretion. In addition, these studies have shown that the MBH is an adequate neural substrate for pulsatile GH secretion, that GH secretion under nonstress and stress conditions is inhibited by brain structures lying outside of the MBH and that the hypothalamic PV nucleus appears to inhibit nonstress TSH levels and to be involved in the TSH response to stress. It is possible that hypothalamic inhibition of GH and TSH is mediated by SRIF, although the present findings do not preclude the possibility that another substance is involved in the inhibitory regulation of these hormones. In view of the evidence which suggests that SRIF is involved in the physiological suppression of plasma GH and TSH levels, it is now necessary to determine the effect of surgical procedures which markedly reduce median eminence content of SRIF on the

concentration of SRIF in pituitary portal blood. In order to gain a better understanding of the role of SRIF in the inhibitory regulation of GH and TSH secretion, several questions remain to be answered. These include; 1) Do most SRIF-containing neuron cell bodies in various regions of the brain that send projections to the median eminence release SRIF into portal circulation? 2) Does marked depletion of median eminence SRIF content reflect a parallel fall in the concentration of SRIF reaching the anterior pituitary via portal circulation? 3) Is SRIF the only growth hormone-release-inhibiting factor? 4) Does SRIF inhibit GH and TSH secretion via hypothalamic as well as pituitary sites of action? Answers to these questions will help to further define the role of SRIF in the regulation of GH and TSH secretion.

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