RELATION OF BIOGENIC AMINES, TEMPERATURE AND STRESS TO THE RELEASE OF ANTERIOR PITUITARY HORMONES

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ABSTRACT

RELATION OF BIOGENIC AMINES, TEMPERATURE AND STRESS TO THE RELEASE OF ANTERIOR PITUITARY HORMONES

By

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1. A single injection of synthetic thyrotropin-releasing hormone (TRH) significantly elevated serum levels of prolactin and thyroid-stimulating hormone (TSH) in proestrous female and in normal and estrogen-primed male rats by 10 minutes after injection. Graduated doses of TRH in proestrous female rats stimulated TSH release in a dose-related fashion whereas no definite dose-response relationship in the prolactin response was observed. Estrogen-priming in male rats significantly elevated serum prolactin, reduced TSH and had little influence on the prolactin and TSH responses by 10 or 60 minutes after injection of 1 μg TRH. It is concluded that synthetic TRH can significantly increase serum prolactin as well as TSH in proestrous female, and in normal and estrogen-primed male rats.

2. When mature male rats were placed in a chamber at 40°C for 30 minutes, there was a significant decrease in serum TSH (0.46 ± 0.08 μg/ml vs. 0.12 ± 0.02 μg/ml) and a fivefold elevation of serum prolactin. A temperature of 4°C for 120 minutes increased TSH and resulted in a significant fall in serum prolactin (25 ± 3 μg/ml vs. 6 ± 1 ng/ml).

Plasma levels of growth hormone were not altered under these conditions. Removal of the pituitary from hypothalamic influence by transplantation under the kidney capsule eliminated the ability of warm or cold temperature to influence the release of TSH and prolactin. Thyroidectomy (10 days) maximally elevated TSH but had no influence on the cold-induced suppression of prolactin. Pimozide, a dopamine receptor blocker, significantly elevated resting levels of TSH and prolactin and prevented cold temperature from further increasing TSH or depressing prolactin. Plasma levels of growth hormone tended to be reduced by pimozide; however, this effect was not significant due to animal variation. These findings indicate that TSH and prolactin respond oppositely to the same temperature changes. The effects of temperature on TSH and prolactin release are mediated by the hypothalamus and are not due to associated changes in thyroid function. Dopaminergic neurons tonically inhibit TSH and prolactin secretion and appear to be involved in the cold-induced suppression of prolactin release.

3. The dose response effects of apomorphine and ET-495 (piribedil), two specific dopamine receptor stimulators, and haloperidol, a dopamine receptor blocker, were tested on the secretion of prolactin, TSH, growth hormone and luteinizing hormone (LH) in male rats. Both apomorphine and piribedil reduced serum levels of prolactin and TSH, stimulated growth hormone release at low but not at high doses and either had no effect or tended to reduce serum LH levels.

The minimal effective dose of apomorphine for reducing prolactin by 30 min was 0.01 mg/kg, whereas TSH inhibition was observed at a dose of 0.1 mg/kg - 0.3 mg/kg. The inhibitory effects of apomorphine (1.0 mg/kg) on prolactin and TSH levels were maximal by 15 min and diminished by 120 min, whereas plasma growth hormone was highest by 120 min after injection. Thyroidectomy (10 days) markedly elevated serum TSH, but had no effect on serum prolactin and inhibited the ability of apomorphine (0.1 mg/kg or 0.3 mg/kg) to reduce TSH but not prolactin levels. These observations may indicate the existence of separate dopaminergic control mechanisms for TSH and prolactin secretion. Administration of haloperidol elevated serum prolactin, tended to lower TSH, dramatically reduced growth hormone and had no effect on LH levels. Haloperidol pretreatment blocked the effects of apomorphine on prolactin, TSH and growth hormone secretion. The overall results of this study indicate that dopamine agonists, and thus dopaminergic mechanisms, inhibit prolactin and TSH, stimulate growth hormone and do not alter release of LH in male rats.

4. The dose response and time course effects of L- and D-tryptophan, 5-hydroxytryptophan (5-HTP) and restraint stress on the metabolism of serotonin and release of TSH, prolactin and growth hormone were tested in male rats. All treatments increased serotonin levels in the hypothalamus and remaining brain tissue minus the cerebellum (brain) and significantly elevated brain levels of 5-hydroxyindoleacetic acid

(5-HIAA), indicating stimulation of serotonin turnover. L-tryptophan and restraint stress increased serotonin turnover in the hypothalamus and brain as determined by the accumulation of serotonin after monoamine oxidase inhibition. Both L-tryptophan and restraint stress inhibited TSH and growth hormone and stimulated prolactin release. The effects of D-tryptophan and 5-HTP on TSH, prolactin and growth hormone release generally followed a similar pattern although changes in hormone levels were not as marked, presumably due to the reduced potency and multiple actions of the two drugs, respectively. These findings indicate that enhanced rates of serotonin turnover produced by administration of serotonin precursors and physical restraint are associated with inhibition of TSH and growth hormone and stimulation of prolactin release from the anterior pituitary.

RELATION OF BIOGENIC AMINES, TEMPERATURE AND STRESS TO THE RELEASE OF ANTERIOR PITUITARY HORMONES

By

Gregory Paul Mueller

A DISSERTATION

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DEDICATION

I dedicate this thesis to my parents
Alice and Gerald C. Mueller
and to this institution,
Michigan State University.

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I wish to thank Dr. Joseph Meites for his constant guidance, advice and support throughout my stay at Michigan State University. Dr. Meites has worked to create and maintain an academic environment for which he asks only that his students use this setting wisely--to benefit both themselves and the laboratory. His efforts on a personal level are always directed towards the well-being of his students. For all these reasons I think Dr. Meites is truly a teacher of graduate students. I am honored to have had the opportunity to be a student under his guidance, and have developed a deep and lasting respect for him.

I would like to thank the fellow members of our laboratory for their help and friendship, and Dr. K. E. Moore who was essential in designing and carrying out some of the work presented in this thesis.

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INTRODUCTION

Neural regulation of hormone secretion by the anterior pituitary gland (AP) appears to involve specific relationships between neuronal activity in the hypothalamus and the release of hypophysiotrophic hormones into the hypophyseal portal circulation. These hypothalamic hormones originate within specialized neurosecretory cells and act directly on the AP to modify the synthesis and release of pituitary hormones. The work presented in this thesis was carried out to further investigate hypothalamic mechanisms controlling release of prolactin, thyroid stimulating hormone (TSH), growth hormone and luteinizing hormone (LH) in the rat.

Extensive investigation into the biochemical nature of hypothalamic hormones has revealed the structure of three of these agents; thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LRH) and somatostatin. All of these substances have proven to be small peptides. Synthetic and natural TRH and LRH stimulate the release of TSH and the gonadotropins (LH and follicle stimulating hormone) respectively whereas, somatostatin inhibits the release of growth hormone. Under experimental conditions, TRH also stimulates the release of prolactin and somatostatin blocks TRH-induced release of TSH but not prolactin. One aspect of this thesis is an investigation of the possible role TRH may have in mediating the prolactin and TSH responses to changes in ambient temperature and physical restraint. Findings

presented here and reports of others indicate that TRH is not involved in the physiological control of prolactin release in the rat whereas, the possible role of somatostatin in regulating TSH secretion has not been determined.

Several neurotransmitters including the catecholamines, dopamine and norepinephrine, and the indoleamine, serotonin, are found in high concentration in the hypothalamus. Recent in vitro evidence indicates that dopamine may be the prolactin release-inhibiting factor (PIF), or possibly one of several PIFs present in crude hypothalamic extracts. However, the ability of hypothalamic extracts to inhibit prolactin release in vivo cannot be accounted for solely on the basis of the small amount of dopamine present in the extracts. This difference suggests that another substance, presumably a small peptide (like TRH, LRH and somatostatin), is responsible for the in vivo inhibition of prolactin release by hypothalamic extracts. There is general agreement that dopamine neurons located in the median eminence region of the hypothalamus act to inhibit prolactin secretion by stimulating the release of PIF. Recent evidence clearly demonstrates that various drugs and physiological conditions which enhance hypothalamic dopamine activity, also elevate PIF activity in the hypothalamus and inhibit the release of pituitary prolactin. Treatments that reduce dopamine activity have the opposite effects on PIF and prolactin. Accumulating evidence indicates that serotonin neurons mediate stimulation of prolactin release; however, a PIF and/or prolactin-releasing factor (PRF) mechanism for this stimulation has not been elucidated. Thus, prolactin release in

the rat is under stimulatory control by serotonin and inhibitory control by dopamine. By contrast, the possible role of other neurotransmitters in control of prolactin, and neurotransmitter regulation of TSH, growth hormone and LH release are less clearly understood.

The findings of some, but not all investigators suggest that in the rat, TRH-TSH and LRH-gonadotropin release may be stimulated by norepinephrine and inhibited by serotonin and possibly dopamine. Stimulation of growth hormone release appears to be mediated by a dopaminergic mechanism. Development of specific and highly potent dopamine receptor agonists and antagonists has made it possible to more carefully define the influence of dopamine neurons on the release of hypothalamic and AP hormones. In work presented here, several of these agents were used alone and in combination to determine the effects of dopamine receptor activity on the release of prolactin, TSH, growth hormone, and LH in male rats. The influence of serotonin on the release of AP hormones was evaluated by correlating changes in brain serotonin metabolism as produced by the administration of serotonin precursors and physical restraint with associated changes in the release of prolactin, TSH and growth hormone. The results of these studies support the established view on neurotransmitter control of prolactin release and provide new evidence on the roles of dopamine and serotonin in the control of TSH, growth hormone and LH release.

Findings presented here and reports of many others indicate that exteroceptive and pharmacological stimuli act through the hypothalamus to selectively alter the release of AP hormones. The objective of this

thesis was to clarify and extend current understanding of mechanisms in the hypothalamus controlling the release of prolactin, TSH, growth hormone and LH in the rat.

LITERATURE REVIEW

I. Hypothalamic Control of Anterior Pituitary Hormone Secretion

A. Early Observations on the Functional Relationship Between the Hypothalamus and Anterior Pituitary Gland

Control of anterior pituitary hormone secretion is mediated by the central nervous system (CNS) and influenced by blood concentrations of target gland hormones. In 1797 Haigton observed that mating induces ovulation in rabbits, subsequently this phenomena was determined to be a neuroendocrine reflex involving relay of sensory information to the brain which led to the release of pituitary luteinizing hormone (LH) and subsequent ovulation. Today many exteroceptive stimuli (e.g., temperature, light, odor and touch) are known to affect pituitary hormone secretion by acting through the CNS (Marshall, 1942; Harris, 1955). Study of the functional relationship between the brain and pituitary has developed as the field of neuroendocrinology.

The pituitary gland lies immediately below yet connected to the hypothalamus by the hypophyseal or pituitary stalk. The hypothalamus constitutes a region of densely clustered nuclei located in the ventralmost portion of the diencephalon (Netter, 1968; Jenkins, 1972). The pituitary stalk consists of: 1) nerve tracts which transport oxytocin and vasopressin from their site of origin in the anterior hypothalamus to the posterior pituitary; 2) structural elements; and 3) blood vessels

which communicate between the hypothalamus and anterior pituitary gland. Virtually no nerve fibers pass directly from the hypothalamus to the anterior pituitary. Although the anterior pituitary does receive some fibers which arise from outside the hypothalamus, this innervation is thought to function solely in vasomotion (Harris, 1955; Szentagothai et al., 1972).

Early investigations demonstrated that the hypothalamus was of major importance in the regulation of pituitary hormone secretion. In 1912 Aschner reported that localized lesions in the anterior hypothalamus caused gonadal atrophy in dogs. Similar observations were made in rats (Camus and Roussy, 1920) and guinea pigs (Dey, 1943). Hypothalamic lesions were also reported to produce atrophy of the thyroid (Cahane and Cahane, 1938; Greer, 1952; Bogdenove and Hamli, 1953) and adrenal cortex (de Groot and Harris, 1950). By contrast electrical stimulation of the hypothalamus (Harris, 1937; Haterius and Derbyshire, 1937) but not the pituitary (Markee et al., 1946; Harris, 1948a) was found to induce ovulation in rabbits. Prolonged hypothalamic stimulation enhanced thyroid (Harris, 1948b) and adrenal cortical (de Groot and Harris, 1950) activity in rabbits. These observations indicate that experimental alterations of hypothalamic function can dramatically affect pituitary hormone-target organ physiology.

Removing the pituitary gland from the direct influence of the brain by stalk section or transplantation to a distant site in the body decreased whole body metabolism and caused atrophy of pituitary hormone target blands with the exception of corpora lutea (Harris, 1948b and

1955; Everett, 1954, 1956). Pituitary histology shifts from a normal makeup of acidophils, basophils and chromophobes to a predominantly chromophobe cell type with maintenance of a substantial number of acidophils (Harris, 1955; Everett, 1956). By contrast transplantation of pituitary hormone target organs (gonads, thyroid and adrenal cortex) does not dramatically affect the histology or function of the transplants (Harris, 1948b, 1955), indicating that the pituitary is under a trophic influence by the CNS which usually is not carried to the systemic circulation.

Some of the first pituitary stalk section experiments were carried out by Dott (1923) who observed that placement of small platinum plates between the pituitary and hypothalamus resulted in lowered body temperature and degeneration of the thyroid and gonads in dogs. Later Mahoney and Sheehan (1936) made similar observations after placing silver clips on the pituitary stalk. Harris (1937) found that stalk section caused gonadal atrophy in rabbits. Contrary to these findings many others reported that stalk transection did not appreciably affect the endocrine status of their experimental animals (see Harris, 1955). This conflict was resolved when Harris (1948b) demonstrated that the portal blood vessels transcending the pituitary stalk must be intact for hypothalamic regulation of anterior pituitary hormone secretion. Following stalk section these vessels often regenerated to restore the functional connection between the pituitary and hypothalamus (Harris, 1948b; Harris and Jacobsohn, 1950).

B. Hypophyseal Portal Vessels

Popa and Fielding (1930) first observed that the vessels transcending the human pituitary stalk are true portal vessels connecting a capillary bed in the median eminence region of the basal hypothalamus to sinusoids in the anterior pituitary. The anatomy of the portal vessels was confirmed by Wislocki and King (1936) who (contrary to Popa and Fielding) concluded on the basis of morphological evidence that blood flow was directed from the hypothalamus into the pituitary. The presence of this vascular connection, termed the hypophyseal portal vessels, was observed to be a common feature among vertebrates (Green and Harris, 1947, 1949; Harris, 1972). Direct microscopic observation demonstrated that blood flow in amphibians (Houssay et al., 1935) and in rats (Green and Harris, 1949) was from the hypothalamus to the pituitary.

Two types of portal vessels, long and short, have been described (Adams et al., 1965; Daniel, 1966) on the basis of the relative distances they transcend from the hypothalamic plexus to the pituitary, the region of the pituitary they supply and the origin of their arterial blood flow. Arterial blood to the hypothalamic plexus is carried by the superior and inferior hypophyseal arteries which arise from the internal carotid and posterior communicating arteries and supply the long and short portal vessels, respectively. Venous flow from the pituitary is by way of small venules which drain into sinuses lying adjacent to the anterior pituitary gland (Adams et al., 1965; Daniel, 1966).

C. Chemotransmission and Hypothalamic Releasing Factors

The possibility that specialized hypothalamic neurons might secrete hormones into blood was first considered by Scharrer and Scharrer (1940). Subsequent investigations in several vertebrate species demonstrated the phenomenon of neurosecretion (Bargmann and Scharrer, 1951; Scharrer, 1952; Scharrer and Scharrer, 1954). These workers observed that oxytocin and vasopressin are synthesized in cell bodies of neurons located in the anterior hypothalamus and transported down long axons to the posterior pituitary from which these hormones are released into the general circulation. Based on the anatomy and the importance of the hypophyseal portal system on the control of anterior pituitary hormone secretion, Harris (1948b) proposed the "chemotransmitter hypothesis". He suggested that nervous stimuli induced release of hypothalamic humors into the capillary plexus of the median eminence. These humors were then transported via the hypophyseal portal vessels to the anterior pituitary, where they either stimulate or inhibit hormone secretion. Subsequent demonstrations that extracts of hypothalamic tissue contained substances which acted directly on the pituitary to alter hormone release supported this hypothesis.

Saffran and Schally (1955) and Guillemin *et al.* (1957) reported that rat, bovine and ovine hypothalamic extracts contained a corticotropin-releasing factor (CRF) which stimulated the *in vitro* release of adrenocorticotropic hormone (ACTH). Other laboratories demonstrated that mammalian hypothalamic extracts stimulated release of thyroid stimulating hormone (TSH) (Shibusawa *et al.*, 1956, 1959; Guillemin *et al.*,

1963), LH (McCann et al., 1960), prolactin (Meites et al., 1960), follicle stimulating hormone (FSH) (Igarashi and McCann, 1964; Mittler and Meites, 1964) and growth hormone (Deuben and Meites, 1964). Hypothalamic extracts were also reported to inhibit in vitro release of prolactin (Talwalker et al., 1961, 1963; Pasteels, 1961) and growth hormone (Krulich et al., 1968). Presently there is general agreement that specific substances, probably small polypeptides and perhaps some catecholamines, are responsible for each of the above activities attributed to hypothalamic extracts (McCann and Dhariwal, 1966; Burgus and Guillemin, 1970; Schally et al., 1973; Vale et al., 1973b; Rippel, 1975; Shaar and Clemens, 1974).

Three hypothalamic hormones have been isolated, structurally analyzed and synthesized. Thyrotropin-releasing hormone (TRH) which stimulates TSH release was first isolated from porcine hypothalami and reported to contain the amino acids histidine, proline and glutamic acid in equimolar ratios (Schally et al., 1966). In 1969 the laboratories of Schally (Schally et al., 1969; Folkers et al., 1969) and Guillemin (Burgus et al., 1969, 1970) working on porcine and ovine TRH, respectively, reported the structure of TRH to be the tripeptide amide, (pyro)-Glu-His-Pro-NH_2. Shortly thereafter the structure of porcine luteinizing hormone-releasing hormone (LRH) which stimulates the release of both LH and FSH was proposed (Matsuo et al., 1971a) and the decapeptide was synthesized (Matsuo et al., 1971b). Full activity for both TRH and LRH requires the C-terminal of these peptides to be present as the amide and the N-terminal present as the cyclic pyroglutamyl residue (Schally et al., 1973).

Recently ovine somatotropin release-inhibiting factor (SRIF) was isolated, determined to be a tetradecapeptide and named somatostatin (Brazeau et al., 1973). Synthetic somatostatin was first prepared by Rivier et al. (1973). The capacity of synthetic somatostatin, TRH and LRH to influence release of their respective hormones was found to be dose-related and equipotent to purified native materials (Schally et al., 1973; Vale et al., 1975).

D. General Physiology of Hypothalamic Releasing Hormones

Synthetic releasing hormones as well as hypothalamic extracts do not appear to be species specific in their actions on pituitary hormone secretion. However, a single hypothalamic hormone can influence the release of more than one pituitary hormone (McCann and Dhariwal, 1966; Schally et al., 1973; Vale et al., 1975; Rippel et al., 1975; Convey et al., 1975). Initially it was thought that LH and FSH were under control of separate releasing factors (Schally et al., 1968). However, the isolation and synthesis of LRH was followed by many demonstrations that this agent stimulates the release of both LH and FSH (see Schally et al., 1973; Convey et al., 1975; Yen et al., 1975). Separate hypothalamic hormones which selectively stimulate LH and FSH release have not been isolated and LRH is presently thought to be the physiological releaser for both gonadotropins (Schally et al., 1976). The occasional divergence of LH and FSH release observed in humans and other mammals may be due in part to modification of their secretion by sex steroids and/or different biological half lifes of LH and FSH (Schally et al., 1973; Yen et al., 1975).

Synthetic TRH was shown to rapidly stimulate the in vitro and in vivo release of prolactin as well as TSH in many experimental animals and humans (Jacobs et al., 1971; Tashjian et al., 1973; Meites, 1973; Convey et al., 1973). In addition TRH has been reported to stimulate growth hormone release in the rat (Takahara et al., 1974b; Kato et al., 1975), bovine (Convey et al., 1973) and in humans (Schalch et al., 1972; Maeda et al., 1975).

Somatostatin is the most diverse hypothalamic hormone in terms of its varied biological actions. Somatostatin was reported to inhibit TRH induced release of TSH in vitro and in vivo in the rat (Vale et al., 1973a, 1975) and in humans (Hall et al., 1974). By contrast somatostatin did not inhibit TRH stimulation of prolactin release (Vale et al., 1974a, 1975). In addition to having multiple effects on pituitary hormone secretion, somatostatin was reported to act directly on the pancreas to inhibit secretion of both insulin and glucagon (Fujimoto et al., 1974; see Vale et al., 1975) and in the gut to inhibit gastrin secretion (Blood et al., 1974). The physiological significance of the ability of TRH to stimulate prolactin and growth hormone release and for somatostatin to block TRH induced TSH release and to inhibit the endocrine pancreas and gut gastrin secretion remain to be determined.

E. Anatomy of the Hypothalamus and Location of Hypothalamic Hormones

Further consideration of hypothalamic function requires a more detailed description of the anatomy of the hypothalamus. This review is based on the works of Harris (1955), Netter (1968), Szentagothai et al. (1972), and Jenkins (1972).

From the ventral aspect of the brain the hypothalamus extends from the anterior border of the optic chiasm to the caudal border of the mammillary bodies and medial from the optic tracts. Located in the middle of this region is the tuber cinereum which gives rise to the hypophyseal stalk. The tuber cinereum contains the primary plexus of the hypophyseal portal system and the median eminence. The dorsal border of the hypothalamus is marked by the anterior commissure rostrally and hypothalamic sulcus caudally.

In a rostral-caudal sequence there are three major gray regions termed anterior, intermediate and posterior hypothalamic areas. Hypothalamic nuclei are bilaterally located on each side of the third ventricle with the exception of the arcuate nucleus which lies in the midline below the ventricle and above the median eminence. The hypothalamus receives afferent fibers from the fornix, medial forebrain bundle, thalamus, stria terminalis, and mammillary peduncle. Major efferents leave by way of the hypophyseal, periventricular and mammillary tracts.

The median eminence is a region of densely packed nerve terminals which surrounds the capillaries of the primary plexus. This area contains very high concentrations of hypothalamic hormones (Harris, 1955, 1972; Szentagothai et al., 1972; McCann and Moss, 1975; Brownstein et al., 1976) and neurotransmitters, especially dopamine (Brownstein et al., 1976). The median eminence has a profound influence on the secretion of all anterior pituitary hormones and represents the site at which chemotransmission occurs (Harris, 1948b, 1955, 1972).

The preoptic-suprachiasmatic area of the anterior hypothalamus regulates the ovulatory release of gonadotropins. Electrolytic lesion (Hillarp, 1949) or isolation of this region by a Halász knife cut (Halász, 1969) resulted in failure of ovulation and loss of estrous cycles in rats. By contrast, electrical stimulation of this area induced release of LH and FSH and thereby caused ovulation (Harris, 1937; Haterius and Derbyshire, 1937; Markee et al., 1946; McCann, 1974; Sawyer, 1975). Other hypothalamic sites which function to control individual pituitary hormones have not been clearly defined (Szentagothai et al., 1972) and the influence of higher brain centers, e.g., brain stem, limbic system, cortex) on hypothalamic-pituitary function is largely unknown (Szentagothai et al., 1972; McCann, 1974; Reichlin, 1974; Neill, 1974).

Hypothalamic Hormones

Hypothalamic hormones are concentrated in the median eminence although measurable amounts of immunoreactive TRH, LRH and somatostatin were found to be present throughout the hypothalamus (Brownstein et al., 1976) and in other regions of the rat brain (Oliver et al., 1974; Jackson and Reichlin, 1974). Recently Burt and Snyder (1975) demonstrated the presence of specific high affinity TRH receptors in membrane fractions prepared from a variety of brain regions indicating a possible physiological role for TRH in neuronal function.

High concentrations of LRH were measured in the medial basal hypothalamus of the rat (Palkovits et al., 1974a; McCann and Moss, 1975; Setalo et al., 1975). LRH was found by some (White et al., 1974;

Barry and Dubois, 1975) but not by others (Winters et al., 1974; Setalo et al., 1975) to be located in brain regions outside of the hypothalamus. By contrast 96% of bioassayable somatostatin present in rat brain was reported to be located outside of the hypothalamus (Vale et al., 1974b, 1975). It should be noted that bioassay of somatostatin measures a composite of both GH inhibitory and stimulatory-release activity and thus does not provide an accurate measure of somatostatin.

Radioimmunoassayable somatostatin was found to be concentrated in the median eminence and present to a lesser extent throughout the hypothalamus, diencephalon and mesencephalon (Brownstein et al., 1975a, 1976).

Synthetic TRH (Prange et al., 1972), LRH (Moss and McCann, 1973; Pfaff, 1973) and somatostatin (Vale et al., 1975) were reported to produce behavioral effects in rats and humans (also see McCann and Moss, 1975). Iontophoretic application of LRH was reported to alter firing rate of central neurons (Kawakami and Sakuma, 1974; McCann and Moss, 1975) and intraperitoneal injections of TRH enhanced brain (central) norepinephrine turnover (Keller et al., 1974) in rats. The possibility that hypothalamic hormones and other small peptides may act as neurotransmitters or neuromodulators is under investigation (Plotnikoff et al., 1975).

Another hypothesis proposed for the wide spread central distribution of hypothalamic hormones is that cerebral spinal fluid may function in the transport of these agents to the pituitary (Löfgren, 1959). Specialized ependymal cells (tanycytes) lining the floor of the third

ventricle were observed to send foot processes to the vessel walls of the hypophyseal plexus and to contain granules which appear to serve a secretory function (Bleier, 1971; Mitchell, 1975). Hypothalamic hormones have been reported to be present in cerebral spinal fluid (Shambanch et al., 1975) and localized within tanycytes (Zimmerman et al., 1974). Radiolabeled TRH (Oliver et al., 1975), dopamine (Ben-Jonathan et al., 1975a) and steroid and protein hormones (Ondo et al., 1972) were found to emerge in hypophyseal portal blood shortly after their injection into the third ventricle of rats. Together these reports suggest that tanycyte transport of substances between the cerebral spinal fluid and hypophyseal portal circulation may be involved in regulation of the anterior pituitary. However, the physiological importance of this mechanism has not been determined.

II. Hypothalamic Neurotransmitters

A. General

Neurotransmitters are chemically active substances which mediate the transmission of nerve impulses across synapses. In this respect, these agents are directly involved in neuroendocrine regulation. Drugs and other treatments which affect the function of neurotransmitters can have a profound effect on pituitary hormone secretion. Several established and putative neurotransmitters are reported to be concentrated in different regions of the hypothalamus.

B. Catecholamines

The catecholamines dopamine and norepinephrine are present in the hypothalamus at concentrations which are among the highest found in the brain (Palkovits et al., 1974b). Vogt (1954) first demonstrated the presence of norepinephrine in the hypothalamus of dogs and cats. Following development of the Falck-Hillarp histofluorescence method (Flack et al., 1962) for identifying brain catecholamines and serotonin, high concentrations of norepinephrine were demonstrated in the anterior hypothalamus and internal layer of the median eminence of rats (Carlsson et al., 1962; Dahlstrom and Fuxe, 1964; Fuxe, 1965). Dopamine was found to be highly concentrated in the external layer of the median eminence, arcuate nucleus, dorsomedial nucleus and zona incerta of the hypothalamus (Carlsson et al., 1962; Fuxe, 1963, 1964, 1965; Fuxe and Hökfelt, 1966, 1969; Jonsson et al., 1972; Björkland et al., 1973).

Recent development of sensitive enzymatic isotopic assays for catecholamines (Cuello et al., 1973; Coyle and Henry, 1973) and a method for removal of individual nuclei (Palkovits, 1973) has made possible the precise mapping of hypothalamic catecholamines. However, one drawback in these assays is that norepinephrine cannot be distinguished from epinephrine. This may be of potential importance since both epinephrine (Vogt, 1954) and the enzyme, phenylethanolamine-N-methyltransferase, which converts norepinephrine to epinephrine have been found in the hypothalamus (Hökfelt, 1974; Hökfelt et al., 1974). Using these techniques the highest concentration of brain dopamine was found in the median eminence (65 ng/mg protein). Dopamine was also concentrated in

the arcuate nucleus (28 ng/mg) and to a lesser extent (4-15 ng/mg) in several nuclei located in the anterior and intermediate hypothalamic areas. Norepinephrine-epinephrine content was highest in the retrochiasmatic area of the anterior hypothalamus (48 ng/mg) and high in the dorsomedial nucleus (39 ng/mg), periventricular nucleus (34 ng/mg) and in the median eminence (30 ng/mg). Measurable amounts of dopamine and norepinephrine-epinephrine were present throughout the hypothalamus; however, nuclei in the posterior area contained the lowest concentrations (Palkovits et al., 1974b; Brownstein et al., 1976).

C. Serotonin

Regional distribution of brain serotonin was first reported by Amin et al. (1954) who found concentrations of this indoleamine to be highest in the hypothalamus and lowest in the cerebellum of the dog. Histofluorescence techniques revealed a dense population of serotonin nerve terminals located in the suprachiasmatic nuclei of the anterior hypothalamus whereas little serotonin fluorescence was observed in other portions of the rat hypothalamus (Fuxe, 1965; Loizou, 1972). Saavedra et al. (1974a) have reported the regional distribution of serotonin in individual hypothalamic nuclei as measured by an enzymatic-isotopic technique. Their report confirms the earlier observations of high serotonin concentrations in the suprachiasmatic nuclei (Fuxe, 1965; Loizou, 1972). In addition, many nuclei of the basal and posterior hypothalamus, including the arcuate nucleus and median eminence contained relatively large amounts of serotonin which had not been detected by histofluorescence (Fuxe, 1965; Loizou, 1972).

These findings indicate that dopamine, norepinephrine, serotonin and possibly epinephrine are concentrated in the hypothalamus; however, they are not evenly distributed throughout the hypothalamic nuclei. The median eminence is rich in dopamine and also contains significant amounts of norepinephrine-epinephrine and serotonin. Recently Brownstein et al. (1974) reported that the hypothalamic concentration of histamine (a putative neurotransmitter) was highest in the median eminence-arcuate nucleus region. Enzymes which synthesize epinephrine (Saavedra et al., 1974b) acetylcholine (Brownstein et al., 1975b) and gamma aminobutyric acid (Kuryama and Kimura, 1976) were also present in the hypothalamus. Little is known about the roles these putative brain neurotransmitters (epinephrine, histamine, acetylcholine and gamma aminobutyric acid) play in neuroendocrine regulation.

D. Monoamine Pathways Innervating the Hypothalamus

Norepinephrine containing cell bodies in the medulla oblongata and serotonin containing cell bodies in the midbrain raphae give rise to axons which ascend in the medial forebrain bundle and terminate in the hypothalamus (Fuxe et al., 1968; Fuxe and Hökfelt, 1969; Ungerstedt, 1971; Fuxe and Jonsson, 1974). Brainstem cell bodies containing phenylethanolamine-N-methyltransferase also appeared to project onto the hypothalamus (Hökfelt, 1974), indicating possible adrenergic innervation of this region by extrahypothalamic loci. By contrast extrahypothalamic dopamine systems (nigro-striatal and mesolimbic) do not innervate the hypothalamus (Ungerstedt, 1971). The tubero-infundibular dopamine

system has cell bodies located in the arcuate nucleus and terminals in the external layer of the median eminence (Carlsson et al., 1962; Fuxe, 1965; Fuxe and Hökfelt, 1966, 1969; Björklund, 1973). Recently a new hypothalamic dopamine system, the incerto-hypothalamic, was observed to have cell bodies located in the posterior hypothalamus and medial zona incerta with diffuse terminal projections to anterior and dorsal hypothalamic areas (Björklund et al., 1975).

Hypothalamic deafferentation with a Halász knife resulted in a dramatic reduction of norepinephrine and serotonin but not dopamine concentrations in the rat hypothalamus. Choline acetyl-transferase and phenylethanolamine-N-methyltransferase activities were also reduced following deafferentation (Brownstein et al., 1976). These findings suggest that only dopamine neurons have their cell bodies located within the hypothalamus; whereas, norepinephrine, epinephrine, serotonin, and acetylcholine are for the most part present in terminals of neurons having cell bodies located outside of the hypothalamus.

E. Catecholamine and Serotonin Synthesis and Turnover

Neurotransmitters are continuously being synthesized, released and metabolized and the rate at which these processes occur is termed turnover. The biosynthesis of catecholamines occurs by the following steps: tyrosine $\xrightarrow{1}$ dopa $\xrightarrow{2}$ dopamine $\xrightarrow{3}$ norepinephrine $\xrightarrow{4}$ epinephrine. The enzymes involved are 1. tyrosine hydroxylase, 2. L-aromatic amino acid decarboxylase, 3. dopamine beta hydroxylase and 4. phenylethanolamine-N-methyltransferase (PNMT). Tyrosine hydroxylase is the rate-limiting enzyme for catecholamine synthesis and its

activity is regulated by feedback inhibition of dopamine and norepinephrine (Levitt et al., 1965, 1967). This enzyme which requires a reduced pteridine cofactor and molecular O_2 and Fe^{++} for activity (Nagatsu, 1964; Levitt, 1967), is stereospecific and under normal conditions is saturated by its substrate L-tyrosine (Nagatsu et al., 1964; Cooper et al., 1971). There is considerable evidence that treatments which increase the firing rate of catecholamine neurons also stimulate tyrosine hydroxylase activity acutely (Sedvall et al., 1968; Dairman et al., 1968) and elevate neuronal concentrations of this enzyme when given chronically (Jon et al., 1973).

L-aromatic amino acid decarboxylase displays little substrate specificity and catalyzes decarboxylation steps in the synthesis of catecholamines, and serotonin (Carlsson et al., 1972; Goldstein et al., 1974). This enzyme is widely distributed throughout the brain and other tissues and requires pyridoxal phosphate as a cofactor (see Nagatsu, 1973, Cooper et al., 1971). Dopamine is converted to norepinephrine by dopamine beta hydroxylase. This enzyme, a copper containing protein, is not highly substrate specific and can oxidize most phenylethylamines in the presence of ascorbic acid (Kaufman, 1966; Levitt et al., 1969; Cooper et al., 1971). Epinephrine is mainly present in the adrenal medulla and is synthesized from norepinephrine by PNMT. Although the brain contains measurable amounts of epinephrine (Vogt, 1954; Gunne, 1962) and PNMT (Hökfelt et al., 1974) the function of this monoamine as a central neurotransmitter remains to be demonstrated.

Serotonin synthesis occurs in a two step process beginning with the conversion of L-tryptophan to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. Serotonin is formed from 5-HTP by L-aromatic amino acid decarboxylase. Tryptophan hydroxylase is the rate limiting enzyme in the synthesis of serotonin and its activity is mainly dependent on the intraneuronal concentration of its substrate L-tryptophan (Lovenberg et al., 1968; Fernstrom and Wurtman, 1971, 1972; Héry et al., 1972) and on the firing rate of serotonin neurons (Aghajanian and Weiss, 1968; Weiss and Aghajanian, 1971). Brain concentrations of tryptophan were reported to be below the saturation point for tryptophan hydroxylase (Lovenberg et al., 1968; Lovenberg and Victor, 1974) and increasing brain tryptophan enhanced formation of central serotonin (Fernstrom and Wurtman, 1971, 1972; Graham-Smith, 1971). It is generally accepted that tryptophan hydroxylase activity is not subject to feedback inhibition by serotonin under normal conditions (Lin et al., 1969; Héry et al., 1972; Millard et al., 1972). This enzyme appears to be located only in serotonin neurons and requires molecular O_2 and a reducing agent, probably tetrahydopteridine, for activity (Friedman et al., 1972; Lovenberg and Victor, 1974).

The physiological actions of central neurotransmitters appear to be terminated mainly by the active process of reuptake into the presynaptic terminal (Glowinski et al., 1965; Glowinski and Axelrod, 1966; Wurtman, 1972; Wilson, 1974; Kuhar et al., 1974). Catecholamines are to a minor extent metabolized by catechol-O-methyl transferase (COMT) in the synaptic cleft (Axelrod, 1959; Acton-Tay and Wurtman, 1971).

Following reuptake, monoamines (catecholamines and serotonin) may be incorporated into synaptic vesicles or metabolized by monoamine oxidase (MAO). MAO is widely distributed throughout the brain and other tissues and is located on the outer surface of mitochondria (Schnaitman et al., 1967; Wurtman, 1972). Administration of MAO inhibitors markedly elevated brain concentrations of serotonin and to a much lesser extent concentrations of catecholamines (Lin et al., 1969), supporting the concept that the synthesis of catecholamines but not serotonin is subject to feedback inhibition.

There are many methods for estimating turnover rates of brain catecholamines (Anton-Tay and Wurtman, 1971; Wurtman, 1972) and serotonin (Morot-Gaudry et al., 1974) and each has inherent virtues and drawbacks (Wurtman, 1972; Weiner, 1974). Moreover, estimating changes in brain neurotransmitter turnover by current methods can only be assumed to reflect changes in synaptic activity. Catecholamines and probably serotonin exist in two or more intraneuronal pools; release and storage pools, and each may differ in their respective rates of turnover. Release pools are of primary interest with respect to transsynaptic communication; however, turnover measurements represent a composite of amine activity within multicompartmentalized pools. Thus, turnover studies provide estimates for comparative overall turnover rates under different experimental conditions (Wurtman, 1972; Weiner, 1974). Three of the most widely used methods for estimating monoamine turnover rates are: 1) measuring the rate of decline of monoamine concentrations or histofluorescence following drug treatments which inhibit their synthesis,

2) measuring the rate at which tritium labeled tyrosine and tryptophan accumulate into catecholamines and serotonin, respectively, and 3) measuring the accumulation of serotonin following drug treatments (MAO inhibitors) which block its metabolism. The third method was used in some of the experiments presented in this thesis.

III. Hypothalamic Control of Prolactin

A. General

Prolactin is best known for promoting mammary growth and lactation (Lyons et al., 1958; Meites, 1966). In addition, it may be important for development and growth of mammary tumors in humans (Jick et al., 1974; Frantz et al., 1972, 1973) and has been shown to stimulate growth and development of mammary tumors in rats (Meites, 1972, 1973). There also is evidence that prolactin is involved in development (Negro-Vilar et al., 1973) and function (Hafez et al., 1972; Bartke et al., 1975) of the male reproductive system and may have a role in cancer of the prostate (Farnsworth, 1972). In rats prolactin maintains luteal function during pregnancy and pseudopregnancy (Neill, 1974), and during the estrous cycle causes luteolysis of old corpora lutea prior to ovulation (Wuttke and Meites, 1971). In addition prolactin was reported to have a role in the control of adrenal, kidney and liver functions (Nicoll and Bern, 1972).

B. Hypothalamic Hormones

Prolactin Release-Inhibiting Factor

Under physiological conditions the mammalian hypothalamus exerts a predominantly inhibitory influence over prolactin secretion and a predominantly stimulatory influence over other anterior pituitary hormones (Meites, 1973). Everett (1954, 1956) first proposed that prolactin release in the rat is inhibited by the hypothalamus although prior observations had suggested this (Desclin, 1950). Following hypophysectomy and autotransplantation of the pituitary under the kidney capsule, Everett (1954, 1956) observed that these autografts maintained luteal function for several months as determined by ovarian histology and the formation of deciduomata induced by uterine traumatization. Similarly, pituitary stalk section was observed to induce luteal function and pseudopregnancy whereas other pituitary hormone-target tissues (ovarian follicles, adrenal cortex, thyroid) atrophied (Everett and Nikitovitch-Winer, 1963). Pituitary stalk section also was reported to enhance prolactin secretion in humans (Turkington, 1972a). Transplantation of a pituitary under the renal capsule resulted in prolonged elevation of blood prolactin concentrations in rats as measured by radioimmunoassay (Chen et al., 1970; Lu and Meites, 1972).

Electrolytic lesions of the median eminence or basal hypothalamus stimulated prolactin secretion as measured by enhanced mammary development (Sud et al., 1970), onset of lactation (McCann and Friedman, 1960; Nikitovitch-Winer, 1960) and high concentrations of circulating prolactin (Chen et al., 1970, Welsch et al., 1971). When placed in vitro

rat anterior pituitaries spontaneously secreted large amounts of prolactin (Meites et al., 1961; Talwalker et al., 1962; Meites and Nicoll, 1966). All of these observations indicate that removing a pituitary from hypothalamic control results in a marked increase in prolactin secretion.

Based on his early findings, Everett (1956) suggested that tonic inhibition of prolactin release may be mediated by a hypothalamic inhibitory hormone. This hormone was later named prolactin release-inhibiting factor (PIF) (Talwalker et al., 1961). Subsequent studies demonstrated that crude acid extracts of rat hypothalami inhibited the in vitro release of prolactin by rat pituitaries (Talwalker et al., 1961, 1963; Pasteels, 1961). Similarly, extracts of sheep, bovine and porcine hypothalami were observed to inhibit prolactin release in vitro (Schally et al., 1965). Injections of hypothalamic extracts into rats were found to inhibit prolactin based on measurements of pituitary prolactin content (Grosvenor et al., 1964, 1965) and reduced serum concentrations of prolactin (Amenomori and Meites, 1970; Watson et al., 1971; Kuhn et al., 1974). Infusion of hypothalamic extract directly into hypophyseal portal vessels of rats inhibited prolactin release in a dose related fashion (Kamberi et al., 1971c; Schally et al., 1974; Takahara et al., 1974a). There is widespread agreement on the existence of PIF; however, the structure of this substance has not been elucidated.

Prolactin-Releasing Factor and Thyrotropin-Releasing Hormone

Prolactin-releasing factor (PRF) activity was first reported in extracts of hypothalamus and cerebral cortex from both estrogen-primed

and lactating rats. Injection of these extracts, but not those of liver or kidney initiated lactation in estrogen-primed female rats (Meites et al., 1960; Mischkinsky et al., 1968). However, these workers could not exclude the possibility that agents other than PRF may have been involved since initiation of lactation is not a specific measure of prolactin release (Meites et al., 1963). Subsequently PRF activity was found in hypothalamic extracts of several avian species (Assenmacher and Texier-Vidal, 1964; Kragt and Meites, 1965; Meites and Nicoll, 1966; Chen et al., 1968).

Based on the observation that rat hypothalamic extracts initially inhibited and later stimulated in vitro release of prolactin by rat pituitaries, Nicoll et al. (1970) suggested the presence of both PIF and PRF in hypothalamic extracts. Valverde et al. (1972) reported chromatographic separation and partial purification of separate PIF and PRF fractions from bovine hypothalamus. By a similar method Dular et al. (1974) demonstrated PRF activity in porcine hypothalamus. In both cases the PRF activity was reported to be free of TRH. Recently the in vitro synthesis of PRF by rat hypothalamus was reported by Mitnick et al. (1973).

The ability of TRH to stimulate prolactin as well as TSH release is well established; however, under most conditions, TSH and prolactin are not released together. Cold temperature, the classical stimulus for TSH secretion, inhibited prolactin release in rats (Mueller et al., 1974) and bovine (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976); whereas, warm temperature increased serum prolactin concentrations and

decreased serum TSH concentrations. Blake (1974) observed in rats that on the afternoon of proestrus when serum prolactin concentrations were dramatically elevated, serum TSH concentrations were unchanged as compared to values on the morning of proestrus. In humans, nursing stimulated prolactin but not TSH release (Gautvik et al., 1973); whereas, in rats, suckling stimulated the release of both hormones (Blake, 1974). However, the rise in serum prolactin preceded that of TSH by 5 min indicating that separate stimulatory mechanisms were operating. Administration of physical stress or L-tryptophan elevated serum prolactin concentrations and inhibited TSH release in normal male rats (Mueller et al., 1976a). Pituitary TSH secretion is inversely related to circulating concentrations of thyroid hormones (Reichlin et al., 1972); whereas, serum prolactin concentrations were little affected by thyroid status in rats (Lu et al., 1972) and humans (Jacobs et al., 1971; Bowers et al., 1971; Refetoff et al., 1974). High doses of estrogen were also reported to inhibit TSH release (D'Angelo, 1968) and to stimulate prolactin release (Chen and Meites, 1970) in rats.

Taken together these observations that release of prolactin and TSH do not occur together strongly indicate that TRH is not involved in the physiological release of prolactin. Little is known about how the above conditions effect the synthesis and release of TRH. Reichlin et al. (1972) reported that in vivo cold exposure or administration of thyroxine enhanced the in vitro synthesis of TRH by rat hypothalami. Serum prolactin concentrations were decreased (Mueller et al., 1974; Wettemann and Tucker, 1974; Tucker and Wettemann, 1976) or unchanged

(Lu et al., 1972) by these treatments. Hypothyroidism reduced TRH synthesis (Reichlin et al., 1972) but had no effect on serum prolactin levels (Lu et al., 1972) in the rat. Under certain experimental conditions, e.g., following the administration of TRH, dopaminergic drugs (Mueller et al., 1976b) or serotonergic drugs (Chen and Meites, 1975a) changes in prolactin and TSH release occurred in parallel. However, there are no physiological data which directly suggest that TRH is PRF.

In pituitary incubations somatostatin was observed to inhibit the TRH induced release of TSH but not prolactin (Vale et al., 1975) and dopamine blocked TRH induced prolactin but not TSH release (Dibbett et al., 1974; Takahara et al., 1974c). These findings present the possibility that TRH, somatostatin and dopamine may act together in regulating pituitary TSH, prolactin, and growth hormone secretion. At present, this relationship must be considered when evaluating hypothalamic control of these three hormones.

C. Effects of Catecholamines on Prolactin Secretion

General

Central acting drugs have long been known to influence pituitary hormone secretion. Adrenergic and cholinergic drugs were reported to induce ovulation; whereas, respective blocking agents inhibited ovulation in rabbits (Sawyer et al., 1947; Markee et al., 1948; Sawyer et al., 1949). Similar observations also were made in the rat (Everett et al., 1949; Markee et al., 1952).

Chlorpromazine, a catecholamine receptor blocker (Carlson and Lindquist, 1963; Janssen et al., 1968) and reserpine, a drug which depletes neurotransmitters (Holzbauer and Vogt, 1956; Sheppard and Zimmerman, 1960) were reported to initiate lactation in humans (Sulman and Winnik, 1956; Rabinowits and Freedman, 1961) and laboratory animals (Meites, 1957; Kanematsu et al., 1962; Meites et al., 1963) indicating a possible involvement of neurotransmitters in the control of prolactin. Development of sensitive and specific radioimmunoassays for prolactin has made possible the evaluation of pituitary secretion rate based on changes in serum prolactin concentrations.

Catecholamines and PIF

The role of catecholamines in the control of prolactin secretion has been the subject of extensive investigation and many reviews (Meites et al., 1972; Meites and Clemens, 1972; McCann et al., 1972; Meites, 1973; MacLeod, 1974; Neill, 1974). Dopamine and to a lesser extent norepinephrine and epinephrine acted directly on the rat pituitary to inhibit prolactin release in vitro (MacLeod, 1969; Koch et al., 1970; MacLeod et al., 1970; Birge et al., 1970; MacLeod and Leymeyer, 1974; Quijada et al., 1973/74; Shaar and Clemens, 1974). Contrariwise other early studies indicated that catecholamines either had no effect (Talwalker et al., 1963) or (depending on dose) stimulated (Gala and Reece, 1965; Kock et al., 1970) pituitary prolactin release in vitro. Recently Shaar and Clemens (1974) clearly demonstrated that both dopamine and norepinephrine in concentrations at or below those found in rat hypothalamus significantly reduced the in vitro release of prolactin by rat

pituitaries. Dopamine was found to be much more effective than norepinephrine. Apomorphine, a potent dopamine receptor stimulating drug (Andén et al., 1967) also was observed to inhibit prolactin release in vitro (MacLeod and Leymeyer, 1974; Smalstig and Clemens, 1974; Smalstig et al., 1974) and this effect was antagonized by pimozide (Smalstig and Clemens, 1974; Smalstig et al., 1974), a dopamine receptor blocking drug (Janssen et al., 1968).

Kamberi et al. (1971a) reported that saline solutions of dopamine, norepinephrine and epinephrine had no effect on serum prolactin concentrations when infused directly into the anterior pituitary of male rats via a hypophyseal portal vessel. More recently both dopamine and norepinephrine dissolved in 5% glucose were observed to inhibit prolactin release in the same experimental model (Takahara et al., 1974a; Schally et al., 1974). This discrepancy has been explained on the basis of vehicles used, the latter workers finding that saline solutions of catecholamines quickly lost PIF activity as compared to 5% glucose solutions. Intravenous infusions (Shaar, 1975; Blake, 1976) but not single injections (Lu et al., 1970) of either dopamine, norepinephrine or epinephrine reduced basal serum concentrations and blocked the proestrous surge of prolactin in female rats. Dopamine is more effective than either norepinephrine or epinephrine (Blake, 1976).

Removal of catecholamines from rat hypothalamic extracts by aluminum oxide absorption or treatment with monoamine oxidase resulted in a complete loss of PIF activity in vitro, indicating that endogenous catecholamines accounted for all the PIF activity in crude hypothalamic

extracts (Shaar and Clemens, 1974). The possibility that catecholamines, particularly dopamine, may be PIF is under investigation. However, initial attempts to measure naturally occurring dopamine in hypophyseal portal blood were unsuccessful (Eskay et al., 1974; Ben-Jonathan et al., 1975b). In 1975 Porter and co-workers reported the presence of norepinephrine but not dopamine in hypophyseal portal blood of urethane anesthetized proestrous female rats (Ben-Jonathan et al., 1975a). Contrariwise, the same group subsequently reported detectable amounts of dopamine but not norepinephrine in portal blood (Ben-Jonathan et al., 1976). The difference between the two reports had not been explained and suggests that additional evidence is required before dopamine can be considered to be a natural and varying constituent of hypophyseal portal blood. Several laboratories have reported the isolation and partial purification of hypothalamic peptides with PIF activity (Takahara et al., 1974a; Schally et al., 1974; Dular et al., 1974; Greibrokk et al., 1974). The possibility that PIF may be a catechol-peptide complex does exist, although failure of proteolytic enzymes to abolish the in vitro PIF activity of rat hypothalamic extracts (Shaar and Clemens, 1974) argues against a peptide PIF. However, it should be noted that small peptides are not always cleaved by proteolytic enzymes (Lehninger, 1970). Quijada et al. (1973/74) reported that pharmacological blockade of pituitary dopamine receptors only partially counteracted the inhibitory influence of hypothalamic tissue on prolactin release in pituitary-hypothalamus co-incubations. These results suggest that action of one or more factors other than dopamine. Their experimental design did not,

however, preclude the possible involvement of norepinephrine. The ability of hypothalamic extracts to inhibit prolactin release *in vivo* cannot be explained on the basis of catecholamines present in the extracts. Shaar and Clemens (1974) reported a single rat hypothalamus contains about 0.03 μg of dopamine and 0.05 μg norepinephrine. Extracts of a single rat hypothalamus significantly reduced serum prolactin concentrations in male rats by 8 min after injection (Watson *et al.*, 1971) and also prevented the suckling and stress-induced decrease in pituitary prolactin content (Grosvenor *et al.*, 1965). Amenomori and Meites (1970) reported that extracts of eight rat hypothalami (which presumably contained about 0.24 μg of dopamine and 0.40 μg of norepinephrine) depressed serum prolactin concentrations by 1 and 4 hours after injection into normal male and female rats. By contrast, Lu *et al.* (1970) found that injections of 5 μg and 10 μg of either dopamine or norepinephrine had no effect on serum prolactin levels in female rats by 30, 60 and 120 min after injection. However, continuous infusions of very large amounts of dopamine (80 μg/hr) were reported to reduce basal prolactin concentrations by 1 hour and to prevent the spontaneous rise in serum prolactin on the afternoon of proestrus in female rats (Blake, 1976). These findings indicate that the relative *in vivo* PIF activity of crude rat hypothalamic extract is greater than that of pure dopamine or norepinephrine. This relationship is in direct opposition to the *in vitro* findings of Shaar and Clemens (1974) showing that catecholamines accounted for all of the PIF activity of rat hypothalamic extract. The difference between the *in vivo* and *in vitro* PIF activities of hypothalamic extracts

suggests that catecholamines are not PIF; however, the biochemical nature of PIF may be closely related to that of catecholamines. The chemical structure of PIF(s) remain to be determined although present evidence indicates that dopamine and dopamine-like compounds must be carefully considered.

Drugs which specifically stimulated dopamine activity inhibit prolactin; whereas, dopamine antagonists enhance prolactin release in rats and humans (see Meites, 1973). Both apomorphine and piribedil (ET 495) which act to stimulate dopamine receptors (Andén et al., 1967; Corrodi et al., 1971a) decreased serum prolactin concentrations in rats (Smalstig et al., 1974; Ojeda et al., 1974b; Lawson and Gala, 1975; Mueller et al., 1976b). Apomorphine also was reported to inhibit prolactin release in humans (Martin et al., 1974). Conversely, pimozide, haloperidol and chlorpromazine which act to block dopamine receptors (Janssen et al., 1968) dramatically stimulated prolactin release in rats (Meites and Clemens, 1972; Clemens et al., 1974; Dickerman et al., 1974; Ojeda and McCann, 1974; Ojeda et al., 1974a; Lawson and Gala, 1975). Haloperidol and chlorpromazine also stimulated prolactin release in humans (Apostokalis et al., 1972; Turkington, 1972a,b). Haloperidol was reported to decrease hypothalamic PIF activity in rats (Dickerman et al., 1974) and ewes (Bass et al., 1974). Pretreatment with haloperidol (Mueller et al., 1976b) or chlorpromazine (Smalstig et al., 1974) blocked the inhibitory effect of apomorphine on prolactin release in rats and similarly, haloperidol counteracted the inhibitory effect of piribedil (Mueller et al., 1976b). The interaction between these

dopaminergic drugs on prolactin release indicates that dopamine receptors probably have a role in the physiological control of prolactin secretion.

Like dopamine (Shaar, 1975; Blake, 1976), apomorphine was reported to block the suckling-induced and proestrous rise in serum prolactin concentrations in rats (Smalstig et al., 1974). Injection of either dopamine (Kamberi et al., 1971a) or apomorphine (Ojeda et al., 1974b) into the third ventricle of the brain depressed serum concentrations of prolactin and similar injections of dopamine were reported to elevate PIF activity in hypophyseal portal blood (Kamberi et al., 1970a, 1971b). Dopamine (Ben-Jonathan et al., 1975a) and other compounds (Ondo et al., 1972; Oliver et al., 1975) were observed to pass from the third ventricle into the hypophyseal portal blood, suggesting that the inhibitory effects of centrally administered dopamine and apomorphine on prolactin release may be due in part to a direct action on the pituitary. The observation that median eminence implants of pimozide were much more effective in elevating serum prolactin concentrations as compared with pituitary implants (Ojeda et al., 1974a) indicates that central dopamine receptors are the principle site at which dopaminergic drugs act to influence prolactin release.

Administration of L-dihydroxyphenylalanine (L-Dopa) dramatically elevated brain dopamine concentrations in rats (Everett and Borcherding, 1970; Hyyppä et al., 1971) and inhibited prolactin release in rats (Lu and Meites, 1971; Donoso et al., 1971; Smythe and Lazarus, 1974a; Chen et al., 1974; Chen and Meites, 1975a) and humans (Malarkey et al., 1971; Friesen et al., 1972; Frantz et al., 1972, 1973). L-Dopa was reported

to block the suckling-induced release of prolactin (Chen et al., 1974) and to depress elevated serum prolactin concentrations produced by estrogen (Chen and Meites, 1975a) median eminence lesions (Donoso et al., 1974; Shaar, 1975), pituitary transplants (Lu and Meites, 1972) and transplants of pituitary tumor tissue (Malarky and Daughaday, 1972) in rats. A single intraperitoneal injection of L-Dopa increased PIF activity in hypothalami and serum of intact and hypophysectomized rats (Lu and Meites, 1972). L-Dopa was reported to inhibit mammary tumor growth in rats (Quadri et al., 1973) and humans (Frantz et al., 1973), presumably by decreasing blood prolactin concentrations.

Ergot alkaloids and their derivatives are well-known for their ability to inhibit the in vivo (Lu et al., 1971; Malven and Hoge, 1971; Shaar and Clemens, 1972; Smith et al., 1974) in in vitro (Lu et al., 1971; MacLeod and Leymeyer, 1974; Clemens et al., 1975) release of prolactin, and these agents have been successfully used to reduce mammary cancer growth in rats (Cassell et al., 1971; Quadri et al., 1971). Recently ergocornine, ergocryptine and 2 bromo-alpha ergocryptine were shown to stimulate central dopamine receptors (Corrodi et al., 1973; Stone, 1974; Fuxe et al., 1974a), and ergot drugs were reported to elevate hypothalamic PIF activity in rats (Wuttke et al., 1971). In vitro inhibition of prolactin release by lergotrile mesylate, an ergot derivative, was found to be antagonized by pimozide but not by adrenergic blocking drugs, indicating that pituitary as well as central dopamine receptors may mediate the inhibitory effects of ergot drugs on prolactin release (Clemens et al., 1975; Shaar, 1975).

Together, the above reports demonstrate that under experimental conditions dopamine inhibits prolactin secretion by a central mechanism involving PIF release and by a direct action on the pituitary. Most of the recent evidence indicates, but does not prove, that dopamine is the PIF. The natural occurrence of dopamine in hypophyseal portal blood has not been clearly demonstrated and hypothalamic peptides, presumably free of catecholamines, were reported to have PIF activity.

There is no agreement on the role of norepinephrine in the control of prolactin. As mentioned earlier, norepinephrine acted directly on the pituitary to inhibit the *in vivo* (Takahara *et al.*, 1974a; Schally *et al.*, 1974; Blake, 1976) and *in vitro* (MacLeod, 1969; Birge *et al.*, 1970; MacLeod and Leymeyer, 1974; Shaar and Clemens, 1974) release of prolactin in rats. Kamberi *et al.* (1971a) reported that only very high doses of norepinephrine (100 µg) significantly lowered serum prolactin concentrations when injected into the third ventricle of the rat brain. Similarly Ojeda *et al.* (1974b) found that intraventricular injection of 2 µg norepinephrine had no effect on prolactin release. Quijada *et al.* (1973/74) concluded that norepinephrine was without effect on the *in vitro* release of PIF by rat hypothalami based on results obtained from pituitary-hypothalamus co-incubations.

Relatively high doses of clonidine (0.2-5.0 mg/kg, I.P.), an alpha adrenergic stimulating drug (Andén *et al.*, 1970; Haeusler, 1974) were reported to elevate prolactin levels in ovariectomized estrogen-primed rats (Lawson and Gala, 1975). By contrast we observed that lower doses of clonidine (0.3-0.30 mg/kg, I.P.) inhibited prolactin release in

normal male rats; whereas, higher doses (1-3 mg/kg) tended to elevate serum prolactin and appeared to cause physical stress (Mueller, Simpkins, Meites, Moore, unpublished). Stresses were reported to stimulate prolactin release in rats (Krulich et al., 1974) suggesting that high doses of clonidine may induce prolactin release by a nonspecific stress effect.

L-Dihydroxyphenyl serine (L-DOPS) which increases brain norepinephrine but not dopamine content (Fuxe and Hökfelt, 1969) was shown to stimulate prolactin release in rats (Donoso et al., 1971). Conversely, blockade of norepinephrine synthesis by disulfiram which acts to inhibit dopamine beta hydroxylase (Turner et al., 1974), was reported to depress serum concentrations of prolactin in rats (Meites and Clemens, 1972). These findings suggest that norepinephrine may stimulate prolactin release.

D. Serotonin and Prolactin Release

There is general agreement that serotonin stimulates prolactin release. Kamberi et al. (1971e) reported that injection of serotonin into the third ventricle of male rats evoked a prompt rise in serum prolactin concentrations. Systemic administration of serotonin precursors, tryptophan or 5-hydroxytryptophan, stimulated prolactin release in rats (Lu et al., 1972; Chen and Meites, 1975; Mueller et al., 1976a) and humans (MacIndoe and Turkington, 1973; Kato et al., 1974). Para-chlorophenylalanine, a drug which inhibits serotonin synthesis by blocking tryptophan hydroxylase (Miller et al., 1970), lowered serum prolactin concentrations in estrogen-primed rats (Chen and Meites, 1975) and blocked the suckling-induced release of prolactin (Kordon et al., 1974).

Caligaris and Taleisnik (1974) reported that para-chloroamphetamine, another tryptophan hydroxylase inhibitor (Miller et al., 1970), blocked the estrogen-induced release of prolactin in rats. Methysergide, an ergot derivative which is reported to block serotonin receptors (Douglas, 1971), inhibited the stress--(Koj and Krulich, 1975) and estrogen (Caligaris and Taleisnik, 1974)--induced release of prolactin in rats. Blockade of serotonin re-uptake by Lilly 110140 (3-[p-triflouromethyl-phenoxy]-3-phenyl-N-methyl-propylamine hydrochloride), which presumably prolongs the action of serotonin on its post synaptic receptor (Wong et al., 1974), potentiated the stimulatory effects of 5-hydroxytryptophan and restraint stress on prolactin release in male rats (Krulich, 1975).

Serotonin was reported to have no effect on the in vitro release of prolactin (Smalstig and Clemens, 1974) and did not alter serum prolactin levels in rats following infusion into the pituitary gland by way of a cannulated portal vessel (Kamberi et al., 1971e). These findings indicate that serotonin has no direct influence on pituitary prolactin secretion and that the actions of serotonergic drugs on prolactin release are mediated by a hypothalamic PIF and/or PRF mechanism.

Systemic administration of serotonin was reported to either have no effect (Lu et al., 1970) or to stimulate prolactin release in rats (Lawson and Gala, 1975). Entry of serotonin into the brain is blocked by the blood-brain barrier (Douglas, 1970) indicating that the stimulation of prolactin release produced by intravenous administration of serotonin (Lawson and Gala, 1975) may be due to a nonspecific stress effect of the drug.

E. Putative Brain Neurotransmitters and Prolactin Release

The influence of acetylcholine on prolactin release is unclear. Grandison et al. (1974) reported that lateral ventricle injections of acetylcholine or systemic injections of pilocarpine and physostigmine reduced serum prolactin concentrations in rats. Pilocarpine stimulates cholinergic receptors and physostigmine prevents acetylcholine metabolism by blocking choline esterase (Koelle, 1970). Similarly Kuhn and Lens (1974) and Libertun and McCann (1974a) reported that cholinergic drugs acutely inhibited prolactin release in rats. Recently Grandison et al. (unpublished) found that cholinergic agonists blocked the stress-induced release of prolactin and that pre-treatment with atropine had no effect on basal serum prolactin concentrations but blocked the pilocarpine induced-inhibition of prolactin release in male rats. Furthermore, pimozide also was observed to prevent the cholinergic-induced suppression of prolactin release indicating that acetylcholine may act to inhibit prolactin by stimulating a dopamine-PIF system.

On the other hand, Libertun and McCann (1973) reported that subcutaneous or third ventricle injections of large doses of atropine (1/4 to 1/2 LD50) blocked the proestrous rise in serum prolactin and gonadotropin concentrations in rats. Atropine was also reported to prevent the nocturnal rise in serum prolactin in pseudopregnant rats and this effect was reversed by physostigmine (McLean and Nikitovitch-Winer, 1975). Lawson and Gala (1975) found that both cholinergic and anticholinergic drugs generally had no effect on serum prolactin concentrations in estrogen-primed rats.

These apparently contradictory findings indicate that acetylcholine inhibits, stimulates or has no effect on prolactin release, and these differences may be due in part to drug doses and experimental animals used and possibly because of a dual function of cholinergic neurons on both PIF and PRF release mechanisms. It is too early to assign a specific role to cholinergic receptors in the physiological control of prolactin secretion.

Gamma-aminobutyric acid (Mioduszewski, 1976; Ondo, in press), melatonin (Kamberi et al., 1971e), cyclic adenosine monophosphate (Ojeda et al., 1974), and histamine (Libertum and McCann, 1974b; Donoso et al., 1976) all were reported to stimulate prolactin release in rats when administered directly into the brain. Methyl-histidine which blocks the formation of histamine, lowered serum prolactin concentrations and diphenhydramine, an antihistiminic drug, blocked the stress-induced release of prolactin (McCann and Mass, 1975). Some prostaglandins also were reported to stimulate prolactin release in rats (Harms et al., 1973; Ojeda et al., 1974c) and to block the inhibitory effects of third ventricle injections of dopamine and apomorphine (Ojeda et al., 1974c). The physiological significance of these observations remain to be determined.

F. Inhibitory Feedback of Prolactin on Pituitary Prolactin Secretion

General

Physiological control of prolactin secretion may involve a hypothalamic autoregulatory mechanism (Sgouris and Meites, 1953; Meites and

Clemens, 1972). Under normal conditions prolactin target tissues (mammary gland, corpora lutea, etc.) do not appear to exert hormonal feedback control on the secretion of prolactin (Meites and Clemens, 1972). High serum prolactin concentrations produced by transplants of mammotrophic pituitary tumor tissue were associated with decreased prolactin content in the *in situ* pituitary (MacLeod *et al.*, 1966; Chen *et al.*, 1967) and elevated PIF activity in the hypothalamus of rats (Chen *et al.*, 1967). Daily injections of purified prolactin or pituitary transplants under the kidney capsule also were reported to reduce the prolactin content of the *in situ* pituitary (Sinha and Tucker, 1968). Hypothalamic grafts of pituitary tissue inhibited prolactin secretion as determined by mammary gland regression in estrogen-primed rats (Averill, 1969). Prolactin implants into the median eminence of rats were found to depress the concentration of prolactin in the pituitary and blood (Clemens and Meites, 1968; Mishkinsky *et al.*, 1969; Voogt *et al.*, 1971, 1973), increase hypothalamic PIF activity (Clemens and Meites, 1968), shorten the duration of pregnancy and pseudopregnancy (Clemens and Meites, 1969a; Clemens *et al.*, 1968), and inhibit lactation (Clemens *et al.*, 1969b). Voogt and Meites (1973) reported that median eminence implants of prolactin reduced basal serum levels and blocked the proestrous and suckling induced release of prolactin in rats. Recently, Advis, Hodson and Meites (unpublished) found that pre-treatment with ovine prolactin inhibited the stress-induced release of prolactin in male rats. By contrast, prolactin did not act directly on the pituitary to alter the *in vitro* release of prolactin in rat pituitaries (Nicoll, 1971; Voogt and Ganong, 1974) indicating a brain site of action.

Tubero-Infundibular Dopamine Neurons and PIF

The inhibitory effect of prolactin on its own secretion appears to be mediated by a hypothalamic PIF system. Fuxe and co-workers (Fuxe and Hökfelt, 1969; Ahrén et al., 1971; Hökfelt and Fuxe, 1972a,b) first proposed that the tubero-infundibular dopamine neurons operate as a component in this mechanism. These workers observed that the rate of median eminence dopamine turnover as measured by histofluorescence techniques was directly related to circulating concentrations of prolactin. Under conditions when serum prolactin or lactogenic activity was known to be elevated (e.g., during pregnancy, lactation or after pituitary transplantation or the administration of exogenous prolactin) median eminence dopamine turnover was enhanced whereas in states associated with low serum prolactin concentrations (e.g., after hypophysectomy or treatment with ergot drugs), dopamine turnover was reduced (Ahrén et al., 1971; Hökfelt and Fuxe, 1972a,b; Fuxe et al., 1974b). During the rat estrous cycle, median eminence dopamine activity was reduced on proestrous afternoon at the time when surges of prolactin, LH and FSH normally occur. Following this period, dopamine turnover was enhanced and this may explain the low concentrations of serum prolactin seen during diestrus (Ahrén et al., 1971; Hökfelt and Fuxe, 1972a,b; Fuxe et al., 1974b). Administration of LH, FSH, TSH, ACTH or vasopressin had no effect on the activity of the tubero-infundibular neurons. Furthermore, prolactin stimulated dopamine turnover only in median eminence neurons; whereas, other dopaminergic systems (nigro-striatal and mesolimbic) were not affected (Hökfelt and Fuxe, 1972a, 1972b; Gudelski et al., in

preparation). Thus, only the activity of tubero-infundibular dopamine neurons are selectively sensitive to the influence of prolactin.

Dopamine injections into the third ventricle of rats were reported to elevate PIF activity in hypophyseal portal blood (Kamberi et al., 1970a, 1971b) and to inhibit prolactin release (Kamberi et al., 1971a; Ojeda et al., 1974b). On the basis of these observations it appears that prolactin acts on the hypothalamus to stimulate median eminence dopamine activity, resulting in increased PIF release and decreased prolactin release.

In support of this hypothesis, Clemens and Sawyer (1974) reported the natural occurrence of prolactin in the cerebral spinal fluid of rats and Gelato and Wuttke (unpublished) have observed the presence of specific prolactin binding activity in hypothalamic membrane preparations. Prolactin also was found to influence the firing rate of hypothalamic neurons in the rabbit (Clemens et al., 1971) and rat (Yamada, 1975). On the other hand, a prolactin-dopamine-PIF short loop feedback model does not explain the control of prolactin secretion under all conditions. Dramatic increases in serum prolactin concentrations regularly occurred twice daily during early pregnancy, and suckling induced a prompt release of prolactin in lactating rats (see Neill, 1974). In each state (pregnancy and lactation) median eminence dopamine turnover and presumably PIF release was enhanced (Hökfelt and Fuxe, 1972a, 1972b), indicating that a separate stimulatory mechanism may be operating to release prolactin under these conditions. The physiological significance of prolactin regulating its own secretion remains to be determined

although present experimental evidence indicates that this phenomenon may be involved in the normal control of prolactin secretion.

IV. Current Views of the Hypothalamic Control of Thyroid Stimulating Hormone (TSH), Growth Hormone and Luteinizing Hormone (LH)

A. TSH

General

Pituitary TSH secretion is primarily controlled by two opposing mechanisms: stimulation by TRH and inhibition by thyroid hormones. This relationship represents a classical neuroendocrine control system containing both neurogenic and target gland hormone-feedback regulation. The pituitary is the principal site for feedback inhibition by thyroid hormones; whereas, TRH secretion is under neural control by the brain (Reineke and Soliman, 1953; D'Angelo, 1963; Reichlin, 1966; Reichlin et al., 1972; Florsheim, 1974). Circulating concentrations of TSH control the synthesis and release of thyroid hormones which, in turn, have a profound influence on many metabolic systems. Thyroid hormones function in the control of oxygen consumption and heat production, growth and development, nerve function, the metabolism of lipids, carbohydrates, proteins, nucleic acids, vitamins, and inorganic ions, as well as the metabolism and effects of other hormones (Hoch, 1974).

Effects of Neurotransmitters on TRH-TSH Release

Relatively little is known about how specific neurotransmitters influence the release of TRH-TSH. Hypothalamic deafferentation (Hefco

et al., 1975a,b) and reserpine treatment (Reichlin et al., 1972; Tuomisto et al., 1973; Chen and Meites, 1975) were reported to induce basal serum concentrations and to block the cold-induced release of TSH in rats. In vivo administration of reserpine depressed the in vitro synthesis of TRH by isolated rat hypothalamic fragments (Reichlin et al., 1972). Both norepinephrine and dopamine stimulated the in vitro release of pulse labeled TRH by mouse hypothalamus. However, dopamine was not effective when its conversion to norepinephrine was blocked pharmacologically (Grimm and Reichlin, 1973) indicating that norepinephrine but not dopamine acts directly on the hypothalamus to stimulate TRH release. In agreement with these in vitro results, disulfiram and phentolamine which act to block norepinephrine synthesis and receptors, respectively (Turner et al., 1974; Nickerson, 1970), were reported to inhibit TSH release in rats (Tuomisto et al., 1973).

Administration of L-dopa consistently depressed elevated serum TSH levels in patients with long standing primary hypothyroidism (Rapoport et al., 1973; Refetoff et al., 1974; Minozzi et al., 1975) but had little effect on basal serum TSH concentrations in euthyroid humans (Eddy et al., 1971; Simonin et al., 1972; Minozzi et al., 1975) or rats (Chen and Meites, 1975). Recently we observed that apomorphine and piribedil (dopamine receptor stimulators) inhibited TSH release; whereas, blockade of dopamine receptors by pimozide markedly elevated TSH release. Further, pre-treatment with haloperidol blocked the inhibitory effect of apomorphine on TSH secretion in male rats (Mueller et al., 1976b, Thesis). These findings indicate that dopamine receptors have an inhibitory influence on TRH-TSH release.

Serotonin appears to inhibit TSH release. Grimm and Reichlin (1973) reported that serotonin inhibited the *in vitro* release of pulse labeled TRH by mouse hypothalamus. Administration of either tryptophan, 5-hydroxytryptophan or restraint stress to male rats elevated hypothalamic serotonin and reduced serum TSH concentrations in dose-related manners (Mueller *et al.*, 1976a). Similarly, hypothalamic implants of serotonin depressed pituitary-thyroid activity and reduced the content of TRH in the hypothalamus of rats (Mess and Peter, 1975). The activity of brain serotonin neurons was directly related to environmental temperature (Corrodi *et al.*, 1967; Aghajanian and Weiss, 1968; Reid *et al.*, 1968; Weiss and Aghajanian, 1971; Squires, 1974); whereas, TSH secretion was inversely related to temperature (see Reichlin, 1966; Reichlin *et al.*, 1972; Florsheim, 1974). Associated with reduced brain serotonin turnover, cold exposure was reported to enhance hypothalamic TRH synthesis (Reichlin *et al.*, 1972; Reichlin and Mitnick, 1973; Hefco *et al.*, 1975c) and release (Montoya *et al.*, 1975) in the rat. Furthermore, the stimulatory effects of thyroid hormones on brain serotonin turnover (Engstrom *et al.*, 1974, 1975; Rastogi *et al.*, 1974; Jacoby *et al.*, 1975) is just opposite to their inhibitory effects on TSH secretion. This relationship suggests a possible brain mechanism for feedback inhibition of thyroid hormones on TSH release. Together these reports indicate that increased activity of serotonin neurons reduces pituitary TSH release by inhibiting hypothalamic TRH secretion.

In apparent disagreement with these observations, Chen and Meites (1975a) reported that high doses of 5-hydroxytryptophan (30 µg/rat)

stimulated; whereas, blockade of serotonin synthesis with para chloroamphetamine (3 mg/rat, given 16 hr. prior to blood collection), inhibited TSH release in ovariectomized estrogen-primed rats. Similarly, Shopsin et al. (1974) found that inhibition of serotonin synthesis by para chlorophenyalanine (150 mg/kg daily for 5 days) depressed serum TSH and prolactin concentrations in male rats. These differences may be due to an effect of estrogen on the action of the serotonergic drugs used by Chen and Meites (1975a) and to the relatively high drug doses used in both studies. High doses of 5-hydroxytrptophan were found to reduce brain catecholamine concentrations and to induce the formation of serotonin in neurons which normally do not contain this transmitter (Butcher et al., 1972). The acute actions of para chloramphetamine and para chlorophenylalanine are not selective to inhibition of tryptophan hydroxylase in serotonin neurons. Both drugs were reported to alter the metabolism of catecholamines for the first 24 to 48 hours after their administration (Miller et al., 1970; Strada et al., 1970; Sanders-Bush et al., 1974). In both studies (Chen and Meites, 1975a; Shopsin et al., 1974), the tryptophan hydroxylase inhibitors were administered less than 24 hours before blood collection.

On the whole, the present data suggest that control of TRH-TSH release is under the stimulatory influence of norepinephrine and the inhibitory influence of dopamine and serotonin. Virtually nothing is known about the influence of other neurotransmitters (e.g., acetylcholine, epinephrine, histamine, gamma aminobutyric acid) on TRH-TSH release.

B. LH

General

Pituitary gonadotropin secretion is stimulated by the hypothalamic hormone LRH and is subject to the feedback influence of gonadal steroids. Thus, control of LH and FSH secretion involves both neurogenic and hormonal-feedback mechanisms. In the adult female, cyclic release of gonadotropins regulates estrous and menstrual cycles. There is general agreement that FSH stimulates the initial growth of the ovarian follicle beyond the stage of early antrum formation and then facilitates the action of LH to promote follicular maturation and estrogen production. The ovulatory surge of LH induces ovulation and formation of the corpus luteum. In the male, LH stimulates androgen secretion and FSH is required for spermatogenesis (see McCann, 1974).

Central acting drugs have long been known to effect gonadotropin secretion (Sawyer et al., 1947, 1949; Everett et al., 1949); however, the influence of neurotransmitters on LRH-gonadotropin release remains unclear and highly controversial.

Effects of Catecholamines on the Release of Gonadotropins

Dopamine injections into the third ventricle of rats were reported to elevate LRH activity in hypophyseal portal blood (Kamberi et al., 1969, 1971b) and stimulate the release of LH and FSH (Kamberi et al., 1970b, 1971d; Schneider and McCann, 1970a,b). Injections of norepinephrine and epinephrine also stimulated the release of gonadotropins but to a much lesser extent as compared with dopamine (Kamberi et al., 1970b, 1971d). In hypophysectomized female rats,

intraventricular injections of dopamine elevated LRH activity in the general circulation and this effect was blocked by the prior administration of estradiol (Schneider and McCann, 1970b). Ojeda and co-workers (Ojeda and McCann, 1973; Ojeda et al., 1974a) observed that pimozide (a dopamine receptor blocker) tended to reduce serum gonadotropin concentrations in rats. Catecholamines had no direct effect on pituitary gonadotropin secretion in vitro; however, dopamine stimulated the release of LRH by rat hypothalami as determined in pituitary-hypothalamus co-incubations (Schneider and McCann, 1969; Kamberi et al., 1970c). Subsequently, Schneider and McCann (1970c) showed that estradiol also counteracted the in vitro stimulatory effect of dopamine on LRH release. Although these earlier reports indicated that dopamine stimulates LRH-gonadotropin release, more recent work suggests that the action of norepinephrine may be more important in this respect.

Recently Quijada et al. (1973/74) found that dopamine had no effect and Miyachi et al. (1973) reported that dopamine inhibited the in vitro release of LRH by rat hypothalamus. Similarly, Cramer and Porter (1973) reported that they were unable to consistently stimulate LRH-gonadotropin release with intraventricular injections of dopamine. The apparent disagreement between these reports and earlier work showing that dopamine stimulated LRH-LH release (Kamberi et al., 1969, 1970b, 1970c, 1971b, 1971d; Schneider and McCann, 1969, 1970a,b,c) has not been adequately explained.

Drugs which stimulate dopamine receptors (apomorphine and piribedil) did not evoke LH release in normal male rats (Mueller et al.,

1976b). Rubinstein and Sawyer (1970) found that norepinephrine but not dopamine induced ovulation in proestrous rats anesthetized with pentobarbital. Recently Sawyer's group (Sawyer et al., 1974; Sawyer, 1975) reported that intraventricular injections of norepinephrine but not dopamine stimulated LH release in the rabbit, and that dopamine given in combination with norepinephrine completely inhibited stimulation of LH release by norepinephrine. Administration of dopamine beta hydroxylase inhibitors to block norepinephrine synthesis, reduced basal serum LH concentrations and blocked the proestrous, post-castration and steroid-induced rises in gonadotropins. Reinitiation of norepinephrine synthesis by dihydroxyphenyl serine generally restored LH release under these conditions (Donoso et al., 1971; Kalra et al., 1972; Kalra and McCann, 1973, 1974; Ojeda and McCann, 1973; Terasawa et al., 1975). Interestingly, alpha receptor blockers (phentolamine and phenoxybenzamine) were reported to inhibit the in vitro (Schneider and McCann, 1969; Kamberi et al., 1970c) and in vivo (Schneider and McCann, 1970a) stimulation of LRH release by dopamine, suggesting that dopamine may be acting through a noradrenergic mechanism. Phentolamine, an alpha receptor blocker, was reported to inhibit the post-castration rise in gonadotropins in rats (Ojeda and McCann, 1973) and to block the pulsatile release of LH which occurs in the ovariectomized monkey (Bhattacharya et al., 1972). Alpha-methyl-para-tyrosine, which inhibits the synthesis of catecholamines also was reported to block the post-castration rise in gonadotropins. This effect was partially counteracted by reinitiating norepinephrine synthesis with dihydroxyphenyl serine (Kalra et al., 1972; Kalra and McCann, 1973, 1974).

Fuxe and co-workers (Fuxe and Hökfelt, 1969; Ahrén et al., 1971; Hökfelt and Fuxe, 1972a, 1972b) have proposed that the tubero-infundibular dopamine neurons inhibit LRH release. These neurons showed reduced dopamine turnover on the afternoon of proestrus when the ovulatory surge of gonadotropins was known to occur. By contrast, estrogen treatment was associated with enhanced median eminence dopamine activity and reduced gonadotropin secretion (see Hökfelt and Fuxe, 1972b). Donoso and Moyano (1970) observed that in contrast to median eminence dopamine, content and turnover of hypothalamic norepinephrine was elevated on the afternoon of proestrus. Similarly Zachaeck and Wurtman (1973) reported that the rate whole brain catecholamine synthesis was markedly enhanced on the afternoon of proestrus as compared with the synthesis rates observed on estrus or diestrus. Thus, present data indicates that the ovulatory release of gonadotropins is associated with enhanced synthesis of hypothalamic norepinephrine and reduced turnover of median eminence dopamine, indicating that norepinephrine may stimulate LRH release under physiological conditions.

Effects of Serotonin and Other Agents on the Release of Gonadotropins

There is general agreement that serotonin and melatonin inhibit the release of gonadotropins. Both agents were found to reduce serum gonadotropin concentrations after injection into the third ventricle of rats (Kamberi et al., 1970b, 1971e; Schneider and McCann, 1970a). By contrast, serotonin and melatonin had no effect on pituitary gonadotropin release when infused directly into a hypophyseal portal vessel indicating a hypothalamic site of action (Kamberi et al., 1970b, 1971c).

Hypothalamic implants of serotonin (Wilson, 1974) or electrical stimulation of the midbrain raphae (serotonin nuclei) (Carrer and Taleisnik, 1970, 1972) both were reported to inhibit ovulation in rats. Systemic injections of 5-hydroxytryptophan inhibited; whereas, blockade of serotonin synthesis by para chlorophenylalanine facilitated ovulation in rats (Kordon and Glowinski, 1972). However, para chlorophenylalanine had no effect on serum LH and FSH concentrations in normal or castrated male rats, indicating that serotonin probably does not exert a tonic inhibitory influence on gonadotropin secretion in the male rat (Donoso et al., 1971).

Acetylcholine, gamma aminobutyric acid, histamine and some prostaglandins may stimulate the release of LRH. Subcutaneous and intraventricular injections of atropine were reported to inhibit the release of gonadotropins in rats (Markee et al., 1952; Libertun et al., 1974a) and acetylcholine stimulated the release of LRH when added to hypothalamus-pituitary co-incubations (Simonovic et al., 1973; Kamberi and Bacleon, 1973; Fiorindo and Martini, 1975). Consistent with these findings, Kamberi and Bacleon (1973) reported that atropine blocked the proestrous surge of gonadotropins and inhibited ovulation in rats. Ovulation was restored by the administration of either LH or crude hypothalamic extract indicating that atropine was acting through a brain mechanism. Intraventricular injections of carbachol (McCann and Moss, 1975), a colinergic receptor agonist (Koelle, 1970), or systemic administration of two other cholinergic agonists, physostigmine and pilocarpine (Libertun and McCann, 1974a), initially inhibited but later

stimulated the release of gonadotropins in ovariectomized estrogen-primed rats. Intraventricular injection of gamma aminobutyric acid (Ondo, 1974), histamine (Libertun and McCann, 1974b; Donoso et al., 1976) and some prostaglandins (Harms et al., 1974) also were reported to stimulate LH release in rats.

Presently it is too early to assign specific roles to cholinergic, histaminergic and gabaergic receptors in the control of gonadotropin secretion. However, these preliminary reports indicate that each of these receptors may be involved in the physiological control of the anterior pituitary. Under many conditions it appears that serotonin and probably dopamine inhibit; whereas, norepinephrine stimulates the release of LH and FSH.

C. Growth Hormone

General

The secretion of growth hormone is under the control of hypothalamic somatotropin release-inhibiting hormone (somatostatin) and growth hormone releasing-factor (GRF). In addition, a "short loop" feedback mechanism may operate (as with prolactin) since a classic target tissue-feedback relationship appears to be lacking. Preliminary evidence indicates that elevated concentrations of plasma growth hormone may stimulate the secretion of somatostatin (Root et al., 1973; Reichlin, 1974). Growth hormone release in humans also is stimulated by hypoglycemia and some amino acids, especially arginine. These effects are thought to be mediated through GRF and somatostatin and are not considered to be of primary importance in the physiological control of growth hormone

secretion (see Reichlin, 1974; Martin, 1976). Growth hormone is essential for normal growth and development, and functions in the control of protein, carbohydrate and fat metabolism.

Effects of Neurotransmitters on the Release of Growth Hormone

Dopamine, norepinephrine and serotonin each have been implicated in the control of growth hormone secretion. Stimulation of dopamine receptors by apomorphine elevated plasma growth hormone concentrations in rats (Mueller et al., 1976b) and humans (Lal et al., 1972; Brown et al., 1973; Maany et al., 1975). Piribedil, another dopamine receptor agonist, also stimulated the release of growth hormone; whereas, blockade of dopamine receptors with pimozide or haloperidol generally reduced plasma growth hormone concentrations in male rats. Furthermore, pretreatment with haloperidol blocked the stimulatory effect of apomorphine on growth hormone (Mueller et al., 1976b, Thesis) indicating that dopamine receptors function in the stimulatory control of growth hormone. The direct effects of these drugs on growth hormone release from the pituitary can probably be excluded since catecholamines and blockers of catecholamine action were shown to have no effect on the in vitro release of growth hormone by rat pituitaries (MacLeod, 1969; MacLeod et al., 1970).

Simon and George (1975) observed that diurnal variation in plasma growth hormone concentrations paralleled changes in hypothalamic dopamine content. Intraventricular injections of dopamine were reported to induce depletion of pituitary growth hormone content indicating stimulation of growth hormone release (Müller et al., 1968). Systemic

administration of L-dopa stimulated growth hormone release in the rat (Chen et al., 1974; Smythe et al., 1975), dog (Lovinger et al., 1974) and human (Boyd et al., 1970; Boden et al., 1972; Silver et al., 1974). By contrast, Collu et al. (1972) reported a fall in plasma growth hormone concentrations following intraventricular injection of dopamine into urethane-anesthetized rats. Other workers found that L-dopa either inhibited (Müller et al., 1973) or had no effect (Kato et al., 1974) on blood growth hormone concentrations in rats. These differences from the findings of others (Müller et al., 1968; Chen and Meites, 1975) may be due to the urethane-anesthesis and drug doses used (see Martin, 1976). On the whole, reports generally indicate that dopamine stimulates growth hormone release.

Efforts to determine the effect of norepinephrine on growth hormone secretion have produced several conflicting reports. Using pharmacological agents which stimulate or block alpha (phenylephrine and phentolamine) and beta (isoproterenol and propanolal) adrenergic receptors Kato et al. (1973) proposed that beta adrenergic receptors stimulate; whereas, alpha adrenergic receptors inhibit growth hormone secretion in the urethane-anesthetized rat. It should be noted there is substantial evidence indicating that the opposite situation exists in humans (see Reichlin, 1974; Wilson, 1974; Martin, 1976). In a single experiment we observed that clonidine, a central acting alpha adrenergic stimulator, evoked the release of growth hormone in male rats. Intraventricular injections of norepinephrine were reported to either stimulate (Müller et al., 1968), or to have no effect (Collu et al., 1972) on

the release of growth hormone in rats. Blockade of norepinephrine synthesis (by inhibiting dopamine beta hydroxylase with FLA-63) produced a fall in plasma growth hormone concentrations in male rats (Müller et al., 1973). Although these reports generally show norepinephrine to stimulate growth hormone release, the mechanism of its action is unclear.

There is some evidence that serotonin may be involved in the physiological control of growth hormone. Administration of tryptophan (Müller et al., 1974) or 5-hydroxytryptophan (Imura et al., 1973; stimulated growth hormone release in humans. Similarly, 5-hydroxytryptophan elevated plasma growth hormone concentrations in rats (Smythe and Lazarus, 1973b; Smythe et al., 1975). Pre-treatment with either cyproheptadine or methysergide, proposed serotonin antagonists, tended to reduce basal serum concentrations and partially blunted the insulin-induced release of growth hormone in humans (Bivens et al., 1973). Cyproheptadine also was reported to block the 5-hydroxytryptophan-induced release of growth hormone in rats (Smythe et al., 1975). Collu et al. (1972) found that large doses of serotonin (1 µg/rat) stimulated growth hormone release when injected into the lateral ventricle of urethane anesthetized rats. By contrast, Müller et al. (1973) reported that intra-ventricular administration of serotonin (1 µg/rat) or intraperitoneal injections of 5-hydroxytryptophan both elevated hypothalamic serotonin content but had no consistent affect on plasma concentrations of growth hormone. However, para chloroamphetamine reduced brain serotonin and slightly increased blood growth hormone concentrations in rats (Müller et al., 1973) indicating that serotonin may inhibit growth hormone.

Also, Martin (1976) observed a fall in growth hormone following electrical stimulation of the midbrain raphae (serotonin nuclei), and we found that a single injection of L-tryptophan reduced plasma growth hormone concentrations in male rats (Thesis).

Thus, the roles of serotonin and norepinephrine in the control of growth hormone remain to be established; whereas, there is general agreement that dopamine stimulates growth hormone release. The possible involvement of other neurotransmitters in growth hormone control has not been investigated. Just recently Dunn et al. (1974/74) and Martin (1976) observed that under normal conditions growth hormone is released from the rat pituitary in dramatic pulsatile bursts. These events occurred regularly, about every three hours within a given animal. The possibility that this normal oscillation of plasma growth hormone may have been interpreted as a drug effect in earlier studies is apparent. This may be the basis for many of the contradictions between reports on the control of growth hormone.

V. Effects of Environmental Temperature and Physical Stress on Pituitary Hormone Secretion and Brain Biogenic Amines

A. General

Environmental temperature and physical stress can have a profound influence on pituitary hormone secretion. Although ambient temperature is an easily definable and reproducible condition, the term "stress" encompasses a wide variety of noxious stimuli ranging from animal handling to severe physical and psychological trauma. Extreme

temperature conditions in this respect can be stressful. Temperature is best known for its effect on TSH-thyroid activity; whereas, stresses are the classical stimuli for ACTH release. However, both stress and temperature have direct and/or indirect affects on the secretion of other anterior pituitary hormones. Temperature and stresses are thought to evoke changes in pituitary function primarily by neuroendocrine reflex mechanisms involving sensory perception and central integration which lead to alterations in the release of hypothalamic hormones. Little is yet known about the specific roles neurotransmitters play in this sequence of events.

B. Effects of High ahd Low Temperature and Physical Stress on Anterior Pituitary Hormone Secretion

The rate of TSH secretion is inversely related to environmental temperature (see Reichlin et al., 1972); whereas, both high and low temperature stimulate ACTH release (see Harris, 1955). It should be noted that very severe and prolonged cold was observed to inhibit TSH-thyroid activity (Williams et al., 1949; Brown-Grant et al., 1954a) presumably because of a nonspecific stress effect on TSH release. Stresses were consistently associated with inhibition of TSH release in laboratory animals and humans (Brown-Grant et al., 1954b; Reichlin, 1966; Ducommun et al., 1966; Florsheim, 1974; Mueller et al., 1976a). Exposure of rats to low environmental temperature was reported to stimulate hypothalamic TRH synthesis (Reichlin et al., 1972; Hefco et al., 1975c) and release (Montoya et al., 1975) although Jobin et al. (1975) were unable to demonstrate an effect of low temperature on the content

of TRH in the rat hypothalamus. Virtually nothing is known about the effects of stress on TRH release or of temperature and stress on the secretion of other hypothalamic hormones.

In contrast to TSH, pituitary prolactin release was stimulated by warm temperature; whereas, cold temperatures inhibited prolactin in the rat (Mueller et al., 1974; Chen and Meites, 1975b; Simpkins et al., in preparation and bovine Wettemann and Tucker, 1974; Tucker and Wettemann, 1976). Stresses, however, profoundly elevated serum prolactin concentrations in rats (Krulich and Illner, 1973; Krulich et al., 1974; Euker et al., 1975; Mueller et al., 1976a). Jobin et al. (1975) reported a brief elevation in serum prolactin concentrations by 5 and 15 minutes (but not later) after placing rats in 5°C. This transient prolactin response to cold exposure is probably due to the accompanying stress associated with the novel environment (Krulich et al., 1974). Nicoll et al. (1960) observed that chronic cold exposure (0° for 5 days) initiated lactation in estrogen-primed female rats suggesting that cold temperature may stimulate prolactin release. However, cold-induced initiation of lactation may be due to activation of the ACTH-adrenal system since injections of glucocorticoids were reported to initiate lactation in estrogen-primed female rats (Meites et al., 1963). Consistent with this view Wettemann and Tucker (1974) found that chronic cold (4.5°C for up to 9 days) resulted in a sustained reduction in serum prolactin concentrations in the bovine. At present, all reports show warm temperature to stimulate prolactin release (Mueller et al., 1974; Chen et al., 1975b; Wettemann and Tucker, 1974; Tucker and Wettemann,

1976). However, it remains to be determined if this effect is due solely to temperature, or is part of a nonspecific stress response.

Many exteroceptive stimuli influence the secretion of growth hormone in mammals (see Reichlin et al., 1974; Martin, 1976). Prolonged exposure to low ambient temperature (4.5°C) reduced; whereas, warm temperature (32°C) slightly elevated serum growth hormone levels in the bovine (Tucker and Wetteman, 1976). Both warm and cold temperatures were reported by several laboratories to inhibit growth hormone release in rats (Schalch and Reichlin, 1968; Collu et al., 1974; Strosser et al., 1974). By contrast, we reported that acute changes in environmental temperature had no significant effect on growth hormone release in male rats (Mueller et al., 1974). The conditions of our study were different and may not have been as stressful as in the experiments cited above. There is widespread agreement that stresses inhibit growth hormone release in rats (Schalch and Reichlin, 1968; Collu et al., 1973, 1974; Dunn et al., 1973/74; Krulich et al., 1974; Thesis) and many other non-primate mammals (see Reichlin et al., 1974; Martin, 1976). Interestingly, stresses stimulated growth hormone release in monkeys (Brown et al., 1971) and humans (Baylis et al., 1968; Noel et al., 1972), indicating an important species difference in hypothalamic control of growth hormone secretion.

The effects of stress and temperature on gonadotropin secretion have not been extensively studied. Acute stresses were reported to stimulate gonadotropin release in rats (Ajika et al., 1972; Euker et al., 1975). Krulich et al. (1974) observed a biphasic response in which LH

release was initially stimulated and later inhibited by prolonged stresses. In agreement with these findings, Nicoll et al. (1960) observed that chronic stresses reduced ovarian and uterine weights in female rats, indicating a suppression of gonadotropin secretion. Although Neill (1970) reported that surgical stress had no effect on LH release in rats, this difference may be due to the time at which blood samples were collected following onset of the stress. There also is some evidence that temperature affects pituitary-gonadal function in rats. Female rats maintained at low ambient temperature exhibited lengthened estrous cycles (Lee, 1926; Bohanan, 1939) and tended to have reduced ovarian and uterine weights (Nicoll et al., 1960). Both of these conditions suggest decreased gonadotropin secretion.

The adrenal glands appear to have little, if any, role in mediating the acute effects of stress and temperature on the release of pituitary hormones other than ACTH. The inhibitory effect of stress on the release of thyroid hormones in rabbits was not altered by bilateral adrenalectomy in combination with constant cortisone replacement (Brown-Grant et al., 1954b). Similarly, Krulich (personal communication) found that adrenalectomy had no affect on the stress-induced inhibition of TSH release in rats. Pre-treatment with high doses of dexamethasone (50 µg/100 mg for 8 days), a synthetic glucocorticoid, only slightly reduced the release of prolactin and LH, but completely blocked the adrenal response associated with acute stress in rats (Euker et al., 1975). Both high and low temperatures stimulate adrenal cortex function but had opposite effects on the secretion of TSH and prolactin

(see above). Thus, acute changes in TSH and prolactin release produced by temperature occur independently from, and appear not to be directly related to, stimulation of ACTH-adrenal hormone release. Likewise, adrenal hormones probably have no major influence on changes in TSH, prolactin, growth hormone and gonadotropin release produced by acute stresses.

C. Effects of High and Low Temperature and Physical Stress on Brain Catecholamines and Serotonin

Brain neurotransmitters are thought to mediate the neuroendocrine responses to temperature and stress by specifically influencing the release of hypothalamic hypophysiotropic hormones. The effects of stress and temperature on central catecholamines and serotonin has just recently come under investigation.

Various forms of stresses were reported to decrease brain norepinephrine concentrations (Bliss et al., 1968; Carr and Moore, 1968; Stone, E. A., 1973) and increase norepinephrine turnover (Gordon et al., 1966; Thierry et al., 1968a; Corrodi et al., 1971b; Librink et al., 1972) in rats. Thus, there is general agreement that stresses enhance the activity of brain noradrenergic neurons. By contrast, the effect of stressful conditions on dopamine is not clearly understood. Stresses in rats were found to have no effect on brain concentrations of dopamine (Gordon et al., 1966; Bliss et al., 1968; Carr and Moore, 1968; Corrodi et al., 1971b). Dopamine turnover was reported to be increased (Bliss et al., 1968), decreased (Corrodi et al., 1971b; Lidbrink et al., 1972) or unchanged (Gordon et al., 1966; Thierry et al., 1968a) by various

stresses. Recently, Palkovits et al. (1975) reported that stresses reduced dopamine and norepinephrine concentrations, and increased tyrosine hydroxylase activity in the arcuate nucleus of rats; whereas, catecholamines and tyrosine hydroxylase activity in other hypothalamic nuclei and brain regions were little affected by stress. These findings suggest that catecholamines in the medial basal hypothalamus are probably involved in the neuroendocrine changes associated with stress.

There is some agreement that stresses stimulate brain serotonin turnover and lead to elevated concentrations of brain serotonin and 5-HIAA in rats (Thierry et al., 1968b; Bliss et al., 1972; Ladish, 1975). Recently we found that acute restraint stress enhanced the accumulation of serotonin in the hypothalamus of rats following MAO inhibition (Mueller et al., 1976a). These findings indicate that stresses stimulate hypothalamic serotonin turnover and this effect may be involved in stress-induced changes in pituitary hormone secretion.

Variations in environmental temperature also influence the synthesis and release of brain neurotransmitters. High temperature (32°C) was reported to produce a threefold increase in hypothalamic norepinephrine turnover while it had no effect on norepinephrine in the rest of the rat brain (Iversen and Simonds, 1969). Cold temperature (4°C) stimulated median eminence dopamine turnover (Lichtensteiger, 1969) but had no effect on hypothalamic norepinephrine metabolism (Iversen and Simonds, 1969). Brain serotonin turnover is directly related to ambient temperature. High temperatures stimulated (Corrodi et al., 1967; Aghajanian et al., 1968; Reid et al., 1968; Weiss and Aghajanian, 1971;

Squires, 1974); whereas, low temperatures reduced (Corrodi et al., 1967; Mueller, unpublished) brain serotonin turnover in rats.

Taken together these findings demonstrate that both temperature and stress influence the activity of central catecholamine and serotonin neurons. The relationship of these changes to the secretion of specific hypothalamic hormones remains to be determined.

MATERIALS AND METHODS

I. Animals, Treatment and Blood Collection

Mature male and female Sprague-Dawley rats (Spartan Research Animals, Haslett, Mich.) and male hypophysectomized rats (Hormone Assay Labs, Chicago, Ill.) were housed in a temperature (24°C) and light controlled environment (lights on from 6:00 AM to 8:00 PM) for at least 4 days prior to each experiment. Rats were provided with Purina Rat Chow (Ralston Purina Company, St. Louis, Mo.) and tap water ad libitum. Hypophysectomized rats received orange slices and sugar cubes as a daily food supplement. Thyroidectomy (THX) and anterior pituitary (AP) transplantation were performed under deep ether anesthesia. All hypophysectomized rats were given a single AP graft from a male donor rat under the left renal capsule. Surgically treated rats were given 0.2 ml Longicil (60,000 units of penicillin G; Fort Dodge Laboratories, Fort Dodge, IA) post-operatively to prevent infection and THX rats were given 0.1% calcium lactate solution in their drinking water for 5 days after surgery. All experiments with the exception of Experiment I were carried out using male rats.

Synthetic tryrotropin-releasing hormone (TRH; provided by Dr. K. Folkers, Institute for Biomedical Research, University of Texas, Austin, Tx.), pargyline hydrochloride (Sigma Chemical Co., St. Louis, Mo.),

piribedil mesylate (ET 495; provided by Dr. M. Derome-Tremblay, Les Laboratoires Servier, Neuilly, France) and DL-5-hydroxytryptophan, ethyl ester, hydrochloride (5-HTP; Sigma Chemical Co., Morton Grove, Ill.) were dissolved in 0.9% NaCl. Pimozide (obtained from Dr. P. A. J. Janssen, Janssen Pharmaceutical Research Laboratories, Beerse, Belgium) and haloperidol (obtained from Dr. Kleis, McNeil Laboratories, Inc., Fort Washington, Pa.) were dissolved in 0.3% tartaric acid. Apomorphine hydrochloride (Eli Lilly and Co., Indianapolis, Inc.) was dissolved in 0.1% sodium metabisulfite. D- and L-tryptophan (Sigma Chemical Co., St. Louis, Mo.) was suspended in 0.9% NaCl containing 1% carboxymethylcellulose. Estradiol benzoate (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in corn oil.

Temperature and restraint stress experiments were carried out using mature male rats. Control rats were maintained at room temperature (24 $\pm$ 1°C). For high temperature the rats were placed in a ventilated drying oven at 40 $\pm$ 2°C and for cold temperature the rats were placed in individual cages in a room at 4 $\pm$ 1°C. Restraint stress was administered by taping rats to wire test tube racks and then placing the rats on their backs. Route, dose and time of treatments (drugs or appropriate vehicles, temperature and restraint stress) are given in the section entitled "Experimental".

Blood samples were collected either by decapitation or orbital sinus cannulation under light ether anesthesia (see Experimental). Plasma samples for growth hormone assay were obtained by mixing 0.1 ml of 100 mg% sodium heparin (Sigma Chemical Co., St. Louis, Mo.) with 1.0

ml blood prior to clotting. Plasma and serum were separated by centrifugation and stored at -20°C until assayed for hormone content.

II. Radioimmunoassays of Blood Hormones

Determination of blood hormone concentrations were made by standard radioimmunoassay procedures. Serum prolactin was measured by the method of Niswender et al. (1969), serum TSH by the method of Dickerman et al. (1972). Serum LH was measured by a sensitive micro-radioimmunoassay (Marshall, Bruni, Campbell and Meites, in press) developed as a minor modification of the method of Niswender et al. (1968). Values are expressed in terms of NIAMDD-rat prolactin-RP-1, NIAMDD-rat TSH-RP-1, NIAMDD-rat GH-RP-1 and NIAMDD-rat LH-RP-1, respectively. All blood samples from an individual experiment were assayed in the same radioimmunoassay.

III. Brain Tryptophan, Serotonin and 5-Hydroxyindole-acetic Acid (5-HIAA) Assays

After decapitation the brain was immediately removed and the pineal gland discarded. The cerebellum was separated from the brain stem by sectioning the cerebellar peduncles. The hypothalamic area removed constituted the region lying between the rostral borders of the optic chiasm and mammillary bodies and medial from the optic tracts which was removed to a depth of about 3 mm. These two structures and remaining tissue (brain) were frozen on Dry Ice subsequent to weighing (tissues were weighed while still frozen) and biochemical analysis. Average

tissue weights (mean ± 1 standard error) based on 50 samples were cerebellum = 234.1 ± 2.7 mg, hypothalamus = 47.3 ± 0.6 mg and brain = 1.454 ± 0.007 gm.

Serotonin and 5-HIAA in the brain and serotonin in the hypothalamus were assayed by the solvent extraction methods of Cruzon and Green (1968) as modified by Hyyppä et al., (1973). A detailed description of reagents used and assay procedures is presented in Appendix A. In brief, this method involved homogenization of brain tissue in acidified butanol. Following centrifugation an aliquot of the supernatant was transferred to a test tube containing heptane and 0.1 N HCl. Organic and inorganic phases were mixed by shaking and separated by centrifugation. The inorganic phase containing serotonin was mixed with O-phthaldehyde (OPT)-hydrochloric acid solution and then heated to 100°C to form a highly fluorescent serotonin-OPT complex. The 5-HIAA present in the organic phase was extracted into 0.5 M phosphate buffer (pH 7.5) by shaking and centrifugation and the aqueous phase was then mixed with OPT and heated to 100°C. After cooling to room temperature sample fluorescence was read in an Aminco-Bowman spectrophotofluorimeter (American Optical Comp., Silver Spring, Ma.) at 355 nm excitation and 480 nm emission wave lengths. Recoveries of pure 5-HT and 5-HIAA standards by this method averaged 95% for serotonin and 70-80% for 5-HIAA. Cross extraction of serotonin into the 5-HIAA fraction and visa versa were less than 1%.

Tryptophan in the cerebellum was assayed by the method of Denckla and Dewey (1967) within 24 hours after the samples were

collected. Because the extraction procedure for tryptophan is not compatible with that of serotonin and 5-HIAA, the effects of various treatments on concentrations of cerebellum tryptophan were determined presuming that these would reflect the relative changes in tryptophan concentrations occurring in other regions of the brain. Changes in cerebellum tryptophan concentrations as affected by diet were found to parallel changes in tryptophan content which occurred in other regions of the brain (Colmanares and Wurtman, unpublished). A detailed description of the tryptophan assay is presented in Appendix B. This method involved tissue homogenization and tricholoroacetic acid protein precipitation. After centrifugation tryptophan in the supernatant was converted to the fluorophore norharman by condensation with formaldehyde and oxidation with ferric chloride ($FeCl_3$) at 100°C. Sample fluorescence was read at 371 nm excitation and 443 nm emission wavelengths. Recovery of pure tryptophan standard was 65%.

IV. Methods of Statistical Analysis

All statistical analysis was carried out as described by Sokal and Rohlf (1969). The specific tests used are indicated under Experimental.

EXPERIMENTAL

I. Effects of Thyrotropin-Releasing Hormone (TRH) on the In Vivo Release of Prolactin and TSH in Proestrous Female, Male and Estrogen-Primed Male Rats

A. Objectives

Synthetic TRH, pyroglutamylhistidylproline amide, stimulates release of TSH in several species in vivo and in vitro (see Reichlin et al., 1972; Vale et al., 1973b; Schally et al., 1973; Florsheim, 1974). Tashjian et al. (1971) observed that TRH increased prolactin release when added to incubations of clonal cells from pituitary tumor. However, prior to the present study TRH had not been clearly shown to stimulate in vitro release of prolactin from normal rat hemi-pituitaries (Bowers, 1971; Lu et al., 1972) or from incubated bovine pituitary tissue (LaBella and Vivian, 1971; Convey et al., 1973). Similarly TRH had not been demonstrated to stimulate in vivo release of prolactin in rats (Lu et al., 1972) although it was active in the human (Jacobs et al., 1971; Bowers et al., 1971) and bovine (Convey et al., 1973). This study was undertaken to further explore the possibility that TRH may induce in vivo release of prolactin in the rat, and to examine the relation of estrogen to TRH induced prolactin release. In addition the effect of TRH on TSH release under these conditions was examined.

B. Materials and Methods

Mature Sprague-Dawley virgin female and male rats were used in this experiment. Daily vaginal smears were taken from female rats for at least two recurrent 4- or 5-day estrous cycles to insure they were undergoing regular cycles. On the morning of proestrus, a pre-treatment blood sample was collected from the orbital sinus under light ether anesthesia at about 11:00 AM, and immediately thereafter the animals were injected intravenously with 0.2, 0.5, 1, 5 or 25 μg TRH. In all cases the injection volume was 0.2 ml of 0.8% NaCl and the controls were given NaCl alone. Post-treatment samples were collected 10 and 60 min after injection. The male rats were given subcutaneous injections of 10 μg estradiol benzoate dissolved in 0.2 ml corn oil or corn oil alone for 5 days. On the sixth day a pre-treatment blood sample was collected at about 11:00 AM and the animals were immediately injected intravenously with either 0.2 ml NaCl or 1 μg TRH dissolved in 0.2 ml NaCl. Post-treatment samples were collected after 10 and 60 min. Student's t test was used to determine significance of differences between mean prolactin or TSH values of any 2 groups. Pre-treatment serum samples from the non-estrogen-primed male rats were pooled in order to obtain enough serum for the assay of TSH (see Table 1).

C. Results

All doses of TRH evoked a 2 to 3.5 fold increase in serum prolactin by 10 min after injection into proestrous rats as compared with control rats (Table 1). The 1 μg dose produced the greatest increase in serum prolactin 10 min after injection when compared to control values,

Table 1. Effects of TRH on Prolactin Release in Proestrous Female and in Untreated and Estrogen-Primed Male Rats

Treatment (no. rats)	Serum prolactin (ng/ml ± SE of mean)		
	Pretreatment	10 min	60 min
Proestrous female rats			
Controls (14)	44 ± 9	68 ± 12	72 ± 12
200 ng TRH (11)	42 ± 11	127 ± 24$^{\alpha}$	73 ± 16
500 ng TRH (10)	63 ± 15	187 ± 25$^{\alpha}$	107 ± 22
1 µg TRH (9)	61 ± 17	222 ± 26$^{\alpha}$	73 ± 12
5 µg TRH (9)	42 ± 13	132 ± 18$^{\alpha}$	68 ± 16
25 µg TRH (10)	51 ± 12	142 ± 23$^{\alpha}$	127 ± 24$^{\alpha}$
Male rats			
Controls, no estrogen (9)	15 ± 2	45 ± 8	32 ± 4
1 µg TRH, no estrogen (9)	20 ± 4	73 ± 8$^{\alpha}$	46 ± 7
Controls, estrogen (9)	106 ± 9	113 ± 5	146 ± 11
1 µg TRH, estrogen (10)	120 ± 9	262 ± 11$^{\alpha}$	216 ± 20$^{\alpha}$

Controls vs experimental group at same time period; $^{\alpha}p < 0.05$.

and was slightly more effective than the other doses of TRH when compared with pre-treatment values. By 60 min after TRH injection, serum prolactin fell from peak concentrations but still tended to be higher than pre-treatment values. There appeared to be no definite dose-response relationship observed between the doses of TRH given and increases in serum prolactin.

In both untreated and estrogen-primed male rats, significant increases in serum prolactin were observed after injection of 1 μg TRH. The pre-treatment control, non-estrogen-primed male rats had less serum prolactin than the control female rats ($p < 0.05$) and estrogen markedly increased serum prolactin concentration ($p < 0.05$).

All doses of TRH used dramatically increased serum TSH by 10 min after injection into proestrous female rats as compared with pre-treatment control values (Table 2). A clear dose-response relationship was observed over a range of doses from 200 ng to 5 μg TRH; whereas, the highest dose (25 μg) was less effective than the 5 μg dose in elevating serum TSH concentration by 10 min after injection. By 60 min after injection serum TSH concentrations fell but were still higher than pre-treatment values.

Injection of 1 μg TRH profoundly elevated serum TSH concentrations in both normal and estrogen-primed male rats by 10 min after injection as compared with control values. Estrogen appeared to have no affect on TSH release induced by this dose of TRH. Sequential blood sampling at 10 and 60 min was associated with significant reductions in serum TSH concentrations in both normal and estrogen-primed,

Table 2. Effects of TRH on TSH Release in Proestrous Female and in Untreated and Estrogen-Primed Male Rats

Treatment (no. rats)	Serum TSH *(ng/ml ± SE of mean) Pretreatment	10 min	60 min
Proestrous female rats			
Controls (8)	< 30	< 30	< 30
200 ng TRH (10)	30	734 ± 168$^{\alpha}$	98 ± 13$^{\alpha}$
500 ng TRH (9)	30	1459 ± 156$^{\alpha}$	139 ± 34$^{\alpha}$
1 µg TRH (9)	30	1598 ± 260$^{\alpha}$	254 ± 92$^{\alpha}$
5 µg TRH (9)	30	2186 ± 379$^{\alpha}$	820 ± 326$^{\alpha}$
25 µg TRH (9)	30	1173 ± 146$^{\alpha}$	517 ± 92$^{\alpha}$
Male rats			
Controls, no estrogen (3)	308 ± 59	216 ± 50	35 ± 5$^{\omega}$
1 µg TRH, no estrogen (4)		7194 ± 1122$^{\alpha}$	153 ± 30$^{\alpha\omega}$
Controls, estrogen (6)	184 ± 43$^{\alpha}$	86 ± 9$^{\omega}$	82 ± 28$^{\omega}$
1 µg THR, estrogen (7)	125 ± 30$^{\alpha}$	7085 ± 656$^{\alpha}$	1240 ± 85$^{\alpha}$

Controls vs experimental group at same time period; $^{\alpha}p < 0.05$.

10 min and 60 min values significantly reduced from pre-treatment control values; $^{\omega}p < 0.05$.

saline-injected male rats as compared with pre-treatment control values. Pre-treatment serum TSH concentrations were highest in normal male rats, lowest in proestrous female rats, and estrogen-priming significantly reduced serum TSH concentrations in male rats.

D. Discussion

This study shows that a single injection of synthetic TRH can stimulate a rapid increase in serum prolactin and TSH in proestrous female rats and in normal and estrogen-primed male rats. Previously our laboratory had reported that a single intravenous injection of 7.5 μg TRH or intracarotid infusion of 5 μg TRH failed to increase serum prolactin by 15 or 30 min after administration (Lu et al., 1972). This difference may be due to the different preparation (obtained from Abbott Labs, Chicago, Ill. and Merck, Sharp and Dohme Research Labs, Rahway, N.J.) of TRH used. Also, Valverde et al. (1972) observed no increase in serum prolactin in estrogen-primed male rats after injection of 100 ng TRH, but this dose may have been insufficient to cause prolactin release. The TRH dose employed in the present study in male rats was 10 times as great, and was effective in both estrogen-primed and normal male rats.

These observations that TRH stimulated TSH release are consistent with many earlier reports (see Schally et al., 1973). However, the finding that 5 μg TRH was more effective than 25 μg TRH in stimulating TSH release in proestrous female rats cannot presently be explained. Estrogen-priming reduced serum TSH concentrations in male rats, in agreement with an earlier report by D'Angelo (1968) but

appeared to have no affect on TRH induced TSH release indicating that estrogen does not change the sensitivity of the pituitary to 1 μg TRH. The reduction in serum TSH and increase in serum prolactin in the NaCl injected control rats with time is believed to reflect the stress associated with multiple blood collections and anesthesia (Mueller et al., 1976a). These observations indicate that in the rat, synthetic TRH stimulates the in vivo release of prolactin as well as TSH.

II. Effects of Heat and Cold on the Release of TSH, Growth Hormone and Prolactin in Male Rats

A. Objectives

Pronounced alterations in temperature evoke rapid changes in release of TSH in rats, with cold producing an increase and heat a decrease in TSH release. Cold was reported to decrease plasma growth hormone concentrations in rats (Schalch and Reichlin, 1968; Collu et al., 1974 and an inverse relationship between environmental temperature and circulating prolactin and growth hormone concentrations was reported in the bovine (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976). The effects of warm temperature on growth hormone and of warm or cold temperature on prolactin release in rats had not been previously reported. The present investigation was undertaken to determine effects of warm and cold temperatures on blood concentrations of TSH, growth hormone and prolactin in normal and hypophysectomized-AP transplanted male rats. Also, the possible roles of thyroid hormones and dopamine receptors in cold induced changes in blood TSH, growth hormone and prolactin concentrations were investigated.

B. Materials and Methods

Mature Sprague-Dawley intact and hypophysectomized male rats were used in this study. Thyroidectomy (THX) was performed ten days, and AP transplantation into hypophysectomized rats seven days prior to the temperature experiments. Pimozide (2.0 mg/kg) or 1% tartaric acid vehicle (0.2 ml/100 gm B.W.) were injected subcutaneously 2 hours before decapitation. Warm (40°C for 30 min) or cold (4°C for 120 min) were administered as discussed under the general Material and Methods section. All blood samples were collected by decapitation. Student's t test was used to determine significance of difference between control and experimental serum hormone concentrations: $p < 0.05$ was chosen as the level of significance. The term "blood concentrations" used in tables and figures refers to the concentrations of prolactin and TSH in the serum and of growth hormone in the plasma.

C. Results

A temperature of 40°C for 30 min decreased serum TSH to 27% of control values and plasma GH to 45% of control values, and produced about a five-fold increase in serum prolactin (Table 3). The growth hormone difference was not significant due to a large variation in the control group. A temperature of 4°C for 120 min evoked almost a two-fold increase in serum TSH, and a significant fall in serum prolactin from 25 ng/ml to 6 ng/ml. Plasma growth hormone concentrations were not altered by cold exposure.

Table 4 shows the effects of heat and cold on blood TSH, growth hormone and prolactin concentrations in male hypophysectomized-AP

Table 3. Effects of Heat and Cold on Blood Concentrations of TSH, GH and PRL in Male Rats

Treatment and no. of rats	Serum TSH µg/ml	Plasma GH ng/ml	Serum PRL ng/ml
Controls, 24°C (8)	0.46 ± 0.08	152 ± 55	25 ± 3
Heat, 40°C (8)	0.12 ± 0.02$^{\alpha}$	69 ± 4	123 ± 8$^{\alpha}$
Cold 4°C (7)	0.84 ± 0.16$^{\alpha}$	192 ± 55	6 ± 1$^{\alpha}$

Heat was for 30 min and cold was for 120 min.

Values $^{\alpha}$significantly different from room temperature (24°C) control value $^{\alpha}p < 0.05$.

Table 4. Effects of Heat and Cold on Blood Concentrations of TSH, GH and PRL in Male Hypophysectomized-AP Transplanted Rats

Treatment and no. of rats	Serum TSH ng/ml	Plasma GH ng/ml	Serum PRL ng/ml
Controls, 24°C (7)	< 30	74 ± 4	19 ± 6
Heat, 40°C (7)	< 30	66 ± 7	32 ± 6
Cold 4°C (7)	< 30	59 ± 7	23 ± 4

Heat was for 30 min and cold was for 120 min.

transplanted rats. Neither heat nor cold had a significant effect on blood concentrations of any of these three hormones.

Both cold and to a much greater extent thyroidectomy (THX) increased serum TSH concentrations as compared with room temperature intact control (Veh) values (Figure 1). Cold in combination with THX did not further elevate serum TSH above the value produced by THX alone. Neither THX nor cold had a significant effect on plasma growth hormone concentrations under these conditions. Serum prolactin concentrations at room temperature were significantly elevated by ten days THX as compared with intact control values (14 $\pm$ 2 ng/ml vs. 23 $\pm$ 1 ng/ml). Cold exposure significantly reduced serum prolactin in both intact and THX groups as compared with room temperature control values.

Pre-treatment with pimozide (2 mg/kg, 2 hr.) at room temperature produced almost a three-fold elevation in serum TSH content as compared with vehicle injected (Veh) control values (Figure 2). Cold significantly elevated blood TSH in Veh control animals but cold in combination with pimozide did not produce a further elevation in serum TSH above that evoked by pimozide alone. Growth hormone tended to be reduced by both cold and pimozide; however, neither treatment given alone or in combination had a significant effect on plasma GH concentrations. Serum prolactin values were reduced by cold and markedly elevated by pimozide. Cold was ineffective in reducing serum prolactin in animals pre-treated with pimozide.

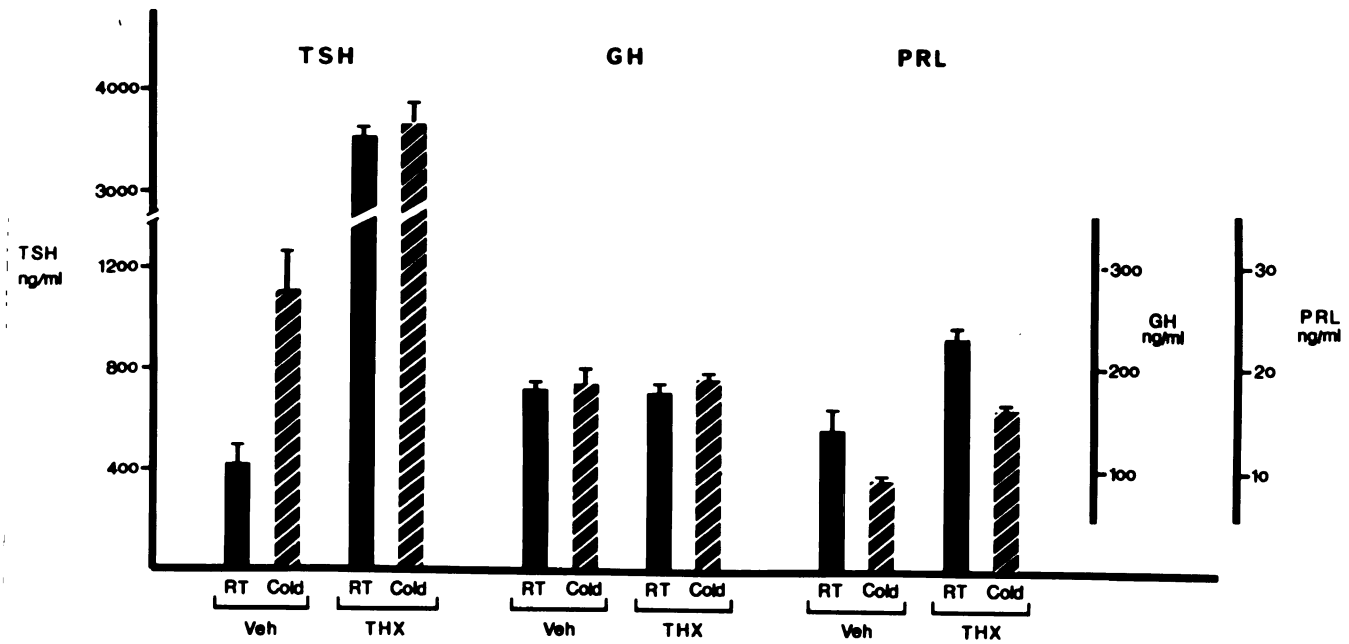

Figure 1. Effects of thyroidectomy on cold induced changes in blood concentrations of TSH, growth hormone (GH) and prolactin (PRL) in male rats.

Rats were either left intact (Veh) or thyroidectomized (THX) 10 days prior to temperature exposure. Each bar represents the mean of 8 determinations. Verticle lines projected on each bar represent $\pm$ 1 standard error. Room temperature (RT) = 24°C; cold = 4°C for 120 min.

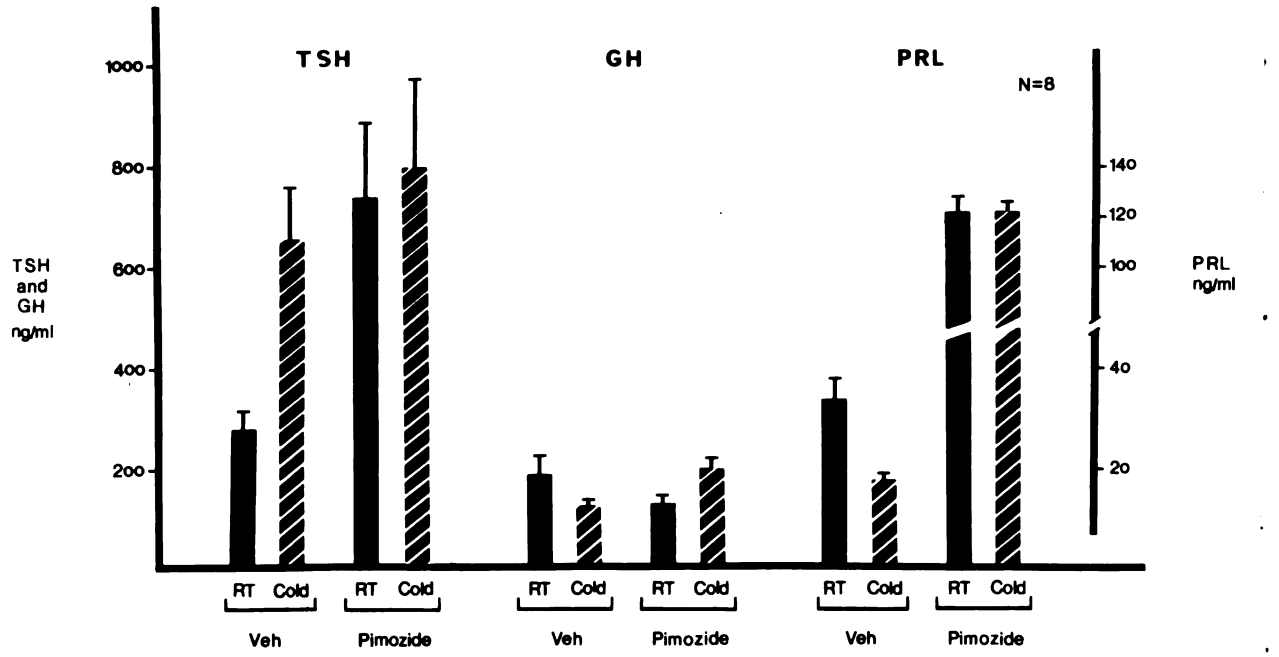

Figure 2. Effects of pimozide on cold-induced changes in blood concentrations of TSH, growth hormone (GH) and prolactin (PRL) in male rats.

Rats were injected subcutaneously with pimozide (2.0 mg/kg) or vehicle (Veh) 2 hours prior to sacrifice. Room temperature = 24°C; cold = 4°C for 120 min. Each bar represents the mean of 8 determinations. The verticle lines projected on each bar represent $\pm$ 1 standard error.

D. Conclusions

A warm temperature of 40°C produced a rapid decrease in circulating TSH and a marked increase in prolactin. Conversely, cold temperature (4°C) stimulated TSH and inhibited prolactin release. Neither warm nor cold temperature had a significant effect on plasma growth hormone concentrations under these conditions. Whether these results are due solely to changes in temperature or also to the possible stress experienced by these animals is unknown at present. Stresses have been reported to inhibit TSH and to stimulate prolactin release in rats (see Florsheim, 1974; Neill, 1974; Mueller, 1976a). In the present study, cold temperature was observed to have the opposite effect on the release of these two hormones indicating that changes in pituitary hormone secretion associated with acute cold exposure (4°C for 120 min) are primarily due to the affect of temperature and can not be explained on the basis of a stress response. Further, stresses were reported to inhibit growth hormone release in the rat (Schalch and Reichlin, 1968; Collu et al., 1973; Krulich et al., 1974; Mueller et al., 1976a). In the present study neither warm nor cold temperature had an affect on growth hormone secretion.

Removal of the pituitary from hypothalamic influence blocked both heat and cold induced changes in pituitary hormone secretion (Table 4) indicating that changes in TSH and prolactin release associated with variations in ambient temperature are mediated by hypothalamic control mechanisms which are not effective by the general circulation.

Serum TSH concentrations were elevated more than nine-fold by 10 days THX and cold in combination with THX failed to further elevate serum TSH as compared with room temperature THX values (Figure 1). These findings indicate that THX is a maximal stimulus for the secretion of TSH. Cold exposure reduced serum prolactin concentrations to a similar extent in both intact and THX animals as compared with room temperature control values. Thus, inhibition of prolactin secretion by two hours cold exposure is not mediated by the cold induced stimulation of TSH-throid activity.

Pimozide alone dramatically elevated serum TSH and prolactin concentrations (Figure 2) indicating that TSH as well as prolactin may normally be under a dopamine-mediated tonic inhibitory influence by the hypothalamus. Cold temperature in combination with pimozide failed to further elevate serum TSH or to reduce prolactin as compared room temperature pimozide control values. These findings suggest that dopamine is involved in the physiological regulation of TSH and prolactin; however, the mechanism is unclear. Cold was reported to enhance the fluorescence of median eminence dopamine neurons (Lichtensteiger, 1969) and to stimulate tyrosine hydroxylase activity in the arcuate nucleus (Palkovits et al., 1975). These findings suggest that enhanced tuberoinfundibular dopamine activity may be responsible for the cold induced inhibition of prolactin release. This model does not, however, explain cold stimulation of TSH release since the effect of pimozide and other dopaminergic drugs on TSH (Mueller et al., 1976b, Thesis) indicate that enhanced dopamine activity inhibits TSH release. Most likely another

neurotransmitter, probably serotonin, is involved in the cold-induced stimulation of TSH. Cold temperature has been reported to reduce brain serotonin turnover (Corrodi et al., 1967; and unpublished findings); whereas, high temperature stimulated brain serotonin turnover (Corrodi et al., 1967; Aghajanian et al., 1968; Reid et al., 1968; Weiss and Aghajanian, 1971; Squires, 1974), and inhibits TSH release. Under experimental conditions serotonin inhibited TRH-TSH release in rats (Grimm and Reichlin, 1973; Mess and Peter, 1975; Mueller et al., 1976a). Together these findings suggest that the rise in serum TSH produced by cold may be due to reduced brain serotonergic activity; whereas, the opposite situation may exist in the case of warm temperature. Roles that other neurotransmitters may play in mediating the effects of temperature on pituitary hormone secretion remain to be determined.

Little is known about the effects of temperature on the hypothalamic hypophysiotrophic hormones. TRH induces prolactin as well as TSH release in many species, and cold was observed to increase TRH synthesis (Reichlin et al., 1972; Hefco et al., 1975c) and release (Montoya et al., 1975) by the rat hypothalamus. However, the observation that cold increased TSH but decreased serum prolactin; whereas, heat produced the opposite effects on the release of these two hormones, indicates that TRH is not responsible for the changes observed on prolactin release.

III. Effects of Dopaminergic Drugs on the Release of Prolactin, TSH, Growth Hormone, and LH in Male Rats

A. Objectives

It is well-established that conditions which elevate brain dopaminergic activity also inhibit prolactin secretion, presumably because release of PIF is increased or by a direct action of dopamine on the pituitary. By contrast, the influence of dopaminergic neurons on release of TSH, growth hormone and LH is unclear (see Literature Review). Development of specific and highly potent dopamine receptor agonists has made it possible to more carefully evaluate the influence of dopamine receptor stimulation on secretion of pituitary hormones. Apomorphine, the prototype dopamine agonist (Andén et al., 1967), piribedil, a long-acting dopamine agonist (Corrodi et al., 1971) and haloperidol, a dopamine receptor blocker were used in this study to determine the effects of dopamine receptor activity on secretion of prolactin, TSH, growth hormone and LH in male rats.

B. Materials and Methods

Male Sprague-Dawley rats weighing 200-225 gm were used in this study. All drugs or appropriate vehicles (see general Materials and Methods section) were given as single subcutaneous injections in a volume of 0.2 ml/100 gm body weight at the dose and time schedules indicated in Results. All blood samples were collected by decapitation between 10:00 AM-1:00 PM. Effects of drug treatments on behavioral activity were determined by Dr. K. E. Moore. Student's t test was used

to test significance between control and experimental blood hormone concentrations; the level of significance was chosen as $p < 0.05$. Abbreviations on figures are prolactin (PRL) and growth hormone (GH) and the term "blood concentrations" refers to serum concentrations of prolactin, TSH and LH, and plasma concentrations of growth hormone.

C. Results

Time Course Effects of Apomorphine on Blood Concentrations of Prolactin, TSH, Growth Hormone and LH

An initial experiment was carried out to determine the time course of the effects of a large dose of apomorphine on blood content of four pituitary hormones. Groups of 8 rats each were injected subcutaneously with 1 mg/kg apomorphine and killed at various times thereafter (Figure 3). Both serum prolactin and TSH concentrations were reduced at 15, 30 and 60 min after injection. By 120 min prolactin values were beginning to return toward normal but remained significantly lower than the control mean; whereas, TSH concentrations had returned to control values. The time course of these effects are consistent with the short duration of action of apomorphine. Since maximal effects were observed 30 min after the administration of apomorphine, subsequent dose-response effects were examined at this time interval.

Plasma growth hormone rose progressively throughout the later time intervals to 355 $\pm$ 41 ng/ml at 120 min as compared with 71 $\pm$ 2 ng/ml in the control group. The reason for this unexpected response to apomorphine was not understood at the time of the experiment, but became clear when the dose-response relationships of this hormone were

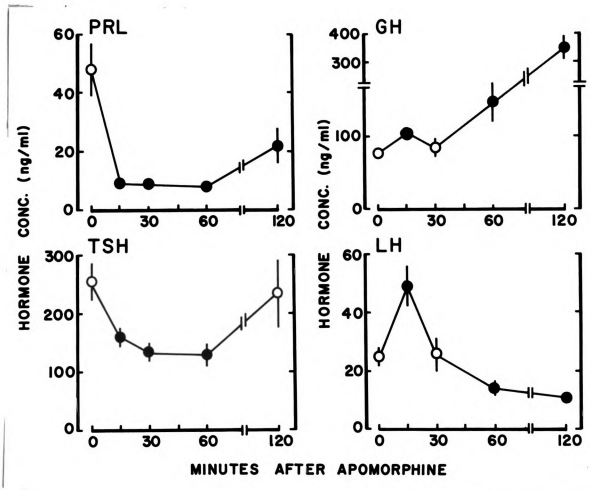

Figure 3. Time course of the effects of apomorphine on blood content of pituitary hormones in male rats.

Rats were injected subcutaneously with apomorphine (1 mg/kg) and sacrificed at various times thereafter. Each symbol represents the mean of 8 determinations. Vertical lines projected on each symbol represent $\pm$ 1 standard error; where not shown the standard error is less than the radius of the symbol. Solid symbols indicate values that are significantly different ($p < 0.05$) from vehicle (0.5% sodium metabisulfide) treatment (zero-time value).

determined. Serum LH was elevated at 15 min but was slightly reduced at 60 and 120 min after apomorphine administration.

Effects of Graduated Doses of Apomorphine on Blood Concentrations of Prolactin, TSH, Growth Hormone and LH

Apomorphine reduced both serum prolactin and TSH concentrations by 30 min after injection, the lowest dose tested (0.03 mg/kg) producing a near maximal depression of serum prolactin (Figure 4). Increasing doses of apomorphine caused serum TSH values to fall in a dose-related manner with the 0.3 mg/kg dose producing the first significant reduction. Plasma growth hormone was elevated by lower doses (0.03, 0.1 and 0.3 mg/kg) but was unchanged by the higher doses (1, 3 and 10 mg/kg) of apomorphine. No clear pattern in LH response was observed.

In order to determine the threshold of the prolactin response to apomorphine and to repeat, in part, the observations on TSH, growth hormone and LH, a second dose-response experiment was done using a lower range of doses (Figure 5). Serum prolactin was not altered by 0.003 mg/kg but was maximally reduced by 0.01 mg/kg apomorphine. Serum TSH values were not significantly altered by the lower doses of apomorphine used in this experiment. Plasma growth hormone concentrations were markedly increased by the 0.03 mg/kg dose, as in Figure 4, but was not altered by the two lower doses of apomorphine. Again, no consistent LH response was observed during this time interval.

Effects of Apomorphine on Serum TSH and Prolactin Concentrations in Hypothyroid Rats

In light of the pronounced inhibitory effects of apomorphine on TSH and prolactin secretion, it was of interest to test this drug in rats with elevated TSH values 10 days after thyroidectomy. The effects

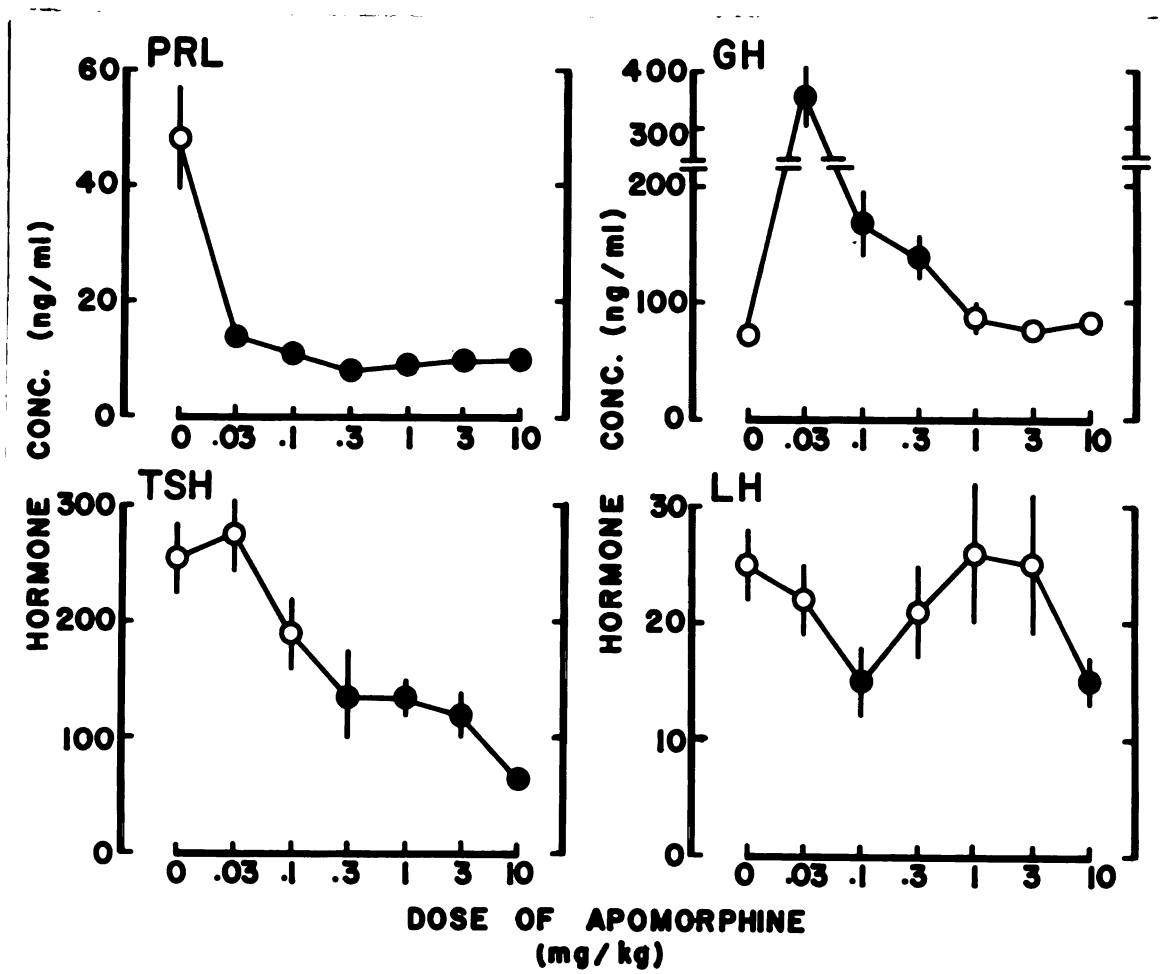

Figure 4. Dose-related effects of high doses of apomorphine on blood content of pituitary hormones in male rats.

Rats were injected subcutaneously with various doses of apomorphine or vehicle and sacrificed 30 min. later. Each symbol represents the mean of 8 determinations. Vertical lines projected on each symbol represent $\pm$ 1 standard error; where not shown the standard error is less than the radius of the symbol. Solid symbols indicate values that are significantly different ($P < 0.05$) from vehicle treatment (zero-dose of drug).

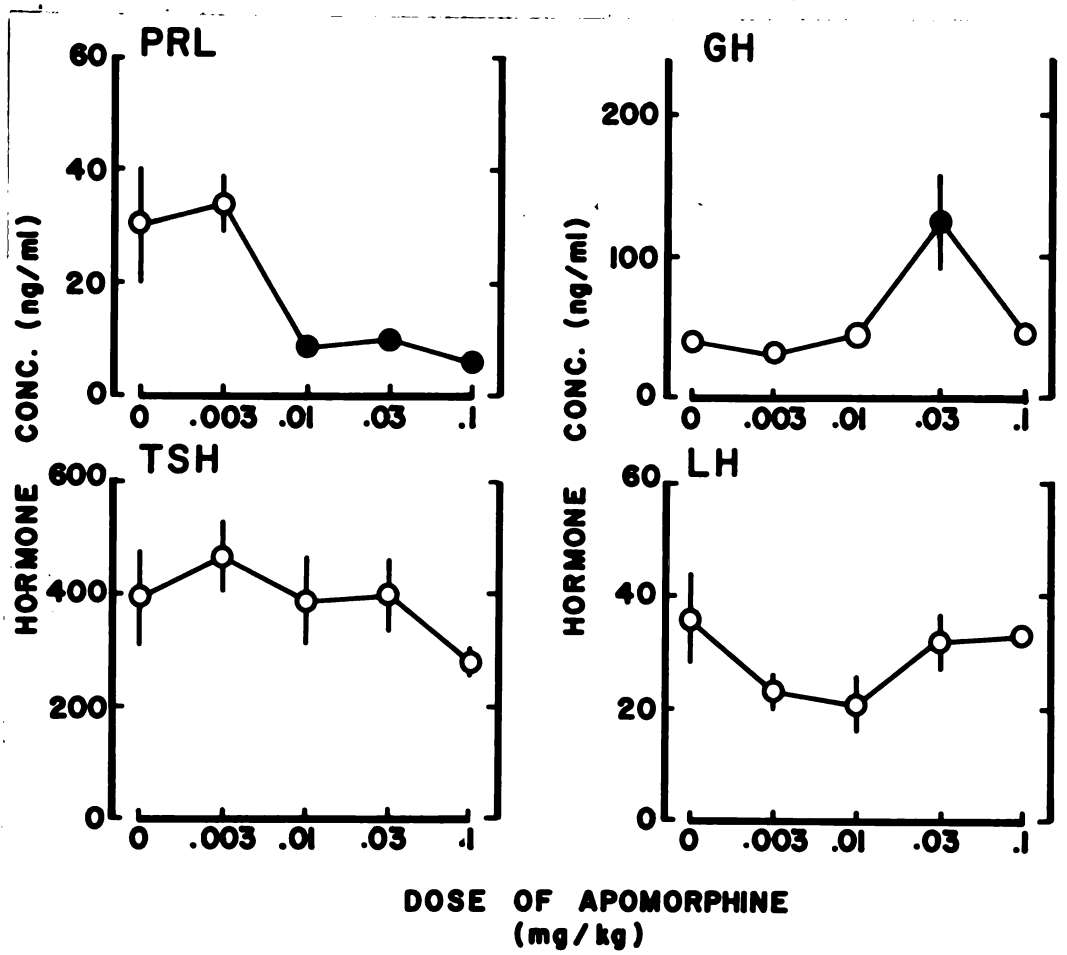

Figure 5. Effects of low doses of apomorphine on blood content of pituitary hormones in male rats.

Rats were injected subcutaneously with various doses of apomorphine or vehicle and sacrificed 30 min. later. Each symbol represents the mean of 8 determinations. Vertical lines projected on each symbol represent $\pm$ 1 standard error; where not shown the standard error is less than the radius of the symbol. Solid symbols indicate values that are significantly different ($P < 0.05$) from vehicle treatment (zero-dose of drug).

of a single dose of apomorphine on blood TSH and prolactin concentrations in intact and thyroidectomized rats by 30 min after injection are given in Table 5. Thyroidectomy produced a greater than 8-fold rise in serum TSH and had no significant effect on blood prolactin. A dose of 0.1 mg/kg apomorphine significantly reduced serum prolactin values by 30 min both in the intact and thyroidectomized animals. The 0.1 mg/kg dose also reduced serum TSH in the intact rats, but neither the 0.1 mg/kg nor 0.3 mg/kg significantly altered TSH in the thyroidectomized rats.

Dose-Response Effects of Piribedil on Blood Prolactin, TSH, Growth Hormone and LH Concentrations

Like apomorphine, piribedil stimulates dopamine receptors in the central nervous system but the latter drug has a much longer duration of action (Corrodi et al., 1971; Thornburg and Moore, 1973). Since these two drugs share similar actions on the brain their effects on pituitary hormone release also would be expected to be similar. Graded doses of piribedil were injected subcutaneously and blood samples were collected 1 hr later (Figure 6). Piribedil caused dose-related reductions both in serum prolactin and TSH. Similar to apomorphine (Figure 4) the dose of piribedil required to reduce serum TSH was approximately 30 times the dose needed to reduce blood prolactin concentrations. Consistent with the effect of apomorphine on growth hormone release, the lower doses of piribedil (0.3, 1, 3 and 10 mg/kg) increased; whereas, the highest dose (30 mg/kg) was without effect on plasma growth hormone concentrations. Serum LH was significantly reduced to about 60% of control values by the 1, 3, 10 and 30 mg/kg doses; however, due to the low basal LH concentrations no clear dose-response pattern was observed.

Table 5. Effects of Apomorphine on Serum Prolactin and TSH Concentrations in Intact and Thyroidectomized Rats

Treatment	Prolactin ng/ml	TSH µg/ml
Intact		
Vehicle	28 ± 9	0.50 ± 0.08
0.1 mg/kg Apomorphine	6 ± 1*	0.33 ± 0.02*
Thyroidectomized		
Vehicle	30 ± 7	3.77 ± 0.12
0.1 mg/kg Apomorphine	7 ± 1**	3.93 ± 0.82
0.3 mg/kg Apomorphine	6 ± 1**	3.71 ± 0.21

Intact or thyroidectomized (10 days) rats were injected with vehicle or apomorphine and sacrificed 30 min later. Each value represents the mean of 8 determinations ± 1 standard error.

*Values significantly reduced from intact vehicle controls, $p < 0.05$.

**Values significantly different from thyroidectomized vehicle controls, $p < 0.05$.

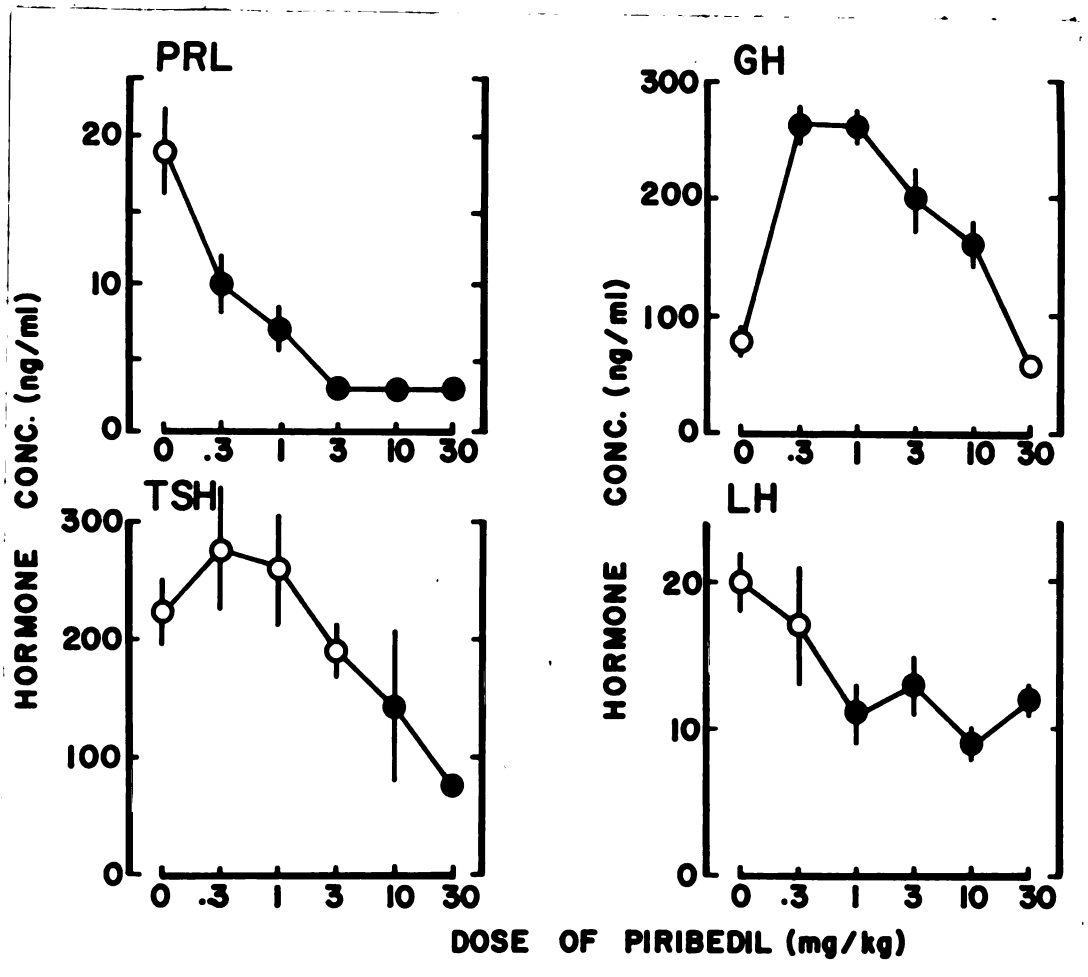

Figure 6. Dose-related effects of piribedil on blood content of pituitary hormones in male rats.

Rats were injected sc with various doses of piribedil mesylate or vehicle and sacrificed 1 hr. later. Each symbol represents the mean of 8 determinations. Verticle lines projected on each symbol represent $\pm$ 1 standard error; where not shown the standard error is less than the radius of the symbol. Solid symbols indicate values that are significantly different ($p < 0.05$) from vehicle treatment (zero-dose of drug).

Effects of Haloperidol Pre-treatment on Apomorphine Induced Changes in Blood Hormone Concentrations

To demonstrate that the effects of apomorphine on pituitary secretion were due to the dopamine agonist properties of this drug, apomorphine was administered to animals pre-treated with haloperidol, a dopamine receptor blocker (Janssen et al., 1968). The effects of a standard dose of apomorphine (0.3 mg/kg) on the content of blood hormones in rats pre-treated with increasing doses of haloperidol are shown in Figure 7. Haloperidol was administered 3 hr and apomorphine 30 min before blood collection. The larger doses of haloperidol produced a dose-related increase in serum prolactin; whereas, apomorphine significantly reduced serum prolactin to about 50% of vehicle injected control values. The ability of apomorphine to reduce serum prolactin was progressively diminished by increasing doses of haloperidol and completely inhibited by the highest dose of this drug (1.0 mg/kg).

Haloperidol produced only a modest reduction of serum TSH values. Apomorphine alone depressed serum TSH to about 30% of control levels and the higher doses of haloperidol (0.1-1 mg/kg) blocked this apomorphine-induced reduction in serum TSH.

Plasma growth hormone was dramatically reduced by haloperidol, the two highest doses (0.3 and 1 mg/kg) causing concentrations to fall to less than 20% of control values. Apomorphine alone (0.3 mg/kg) did not alter resting values of plasma growth hormone. This 0.3 mg/kg dose of apomorphine previously was shown (Figure 4) to be 10 times greater than the dose which maximally elevated plasma growth hormone.

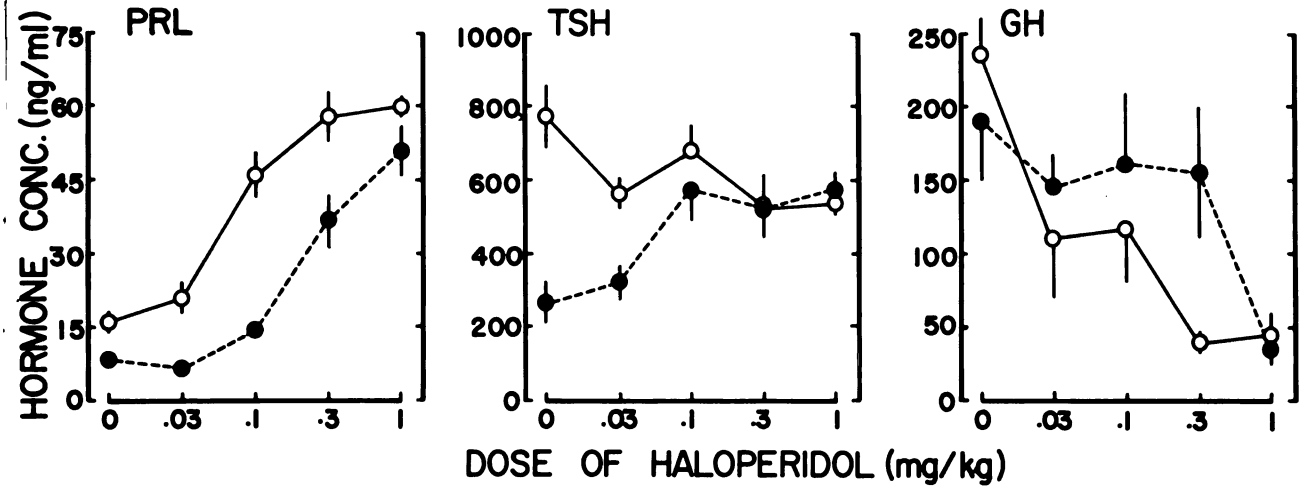

Figure 7. Effects of increasing doses of haloperidol alone or in combination with apomorphine on blood content of pituitary hormones in male rats.

Various doses of haloperidol or vehicle were injected sc 3 hours prior to sacrifice. Thirty minutes prior to sacrifice the same animals received sc injections of apomorphine (0.3 mg/kg) (●-----●) or its vehicle (o———o). Each symbol represents the mean of 8 determinations and the vertical line represents ± 1 standard error.

When given in combination with an intermediate dose of haloperidol (0.3 mg/kg), apomorphine elevated plasma growth hormone. Presumably this dose of haloperidol reduces the effective concentration of apomorphine at dopamine receptors into a range that stimulates growth hormone release. However, in combination with the highest dose of haloperidol (1.0 mg/kg), apomorphine had no effect on plasma growth hormone. Serum LH was not altered by either haloperidol, apomorphine or by treatment with the two drugs in combination (not shown).

Drug-induced Behavioral Responses

Dopaminergic agonists cause a variety of motor responses which are collectively referred to as stereotyped behaviors. In rats these behaviors have been described as a series of dose-related events characterized at low doses by sniffing, licking and rearing, and at higher doses by gnawing, biting and restricted locomotor activity. The behaviors of drug-treated animals in the foregoing experiments were recorded and the minally effective doses of apomorphine and piribedil necessary to cause stereotypes and to alter blood concentrations of hormones are summarized in Table 6. The reduction of prolactin values and the increase in growth hormone values occurred at doses of apomorphine and piribedil that were 1/10 to 1/30 of those necessary to produce stereotyped sniffing. The doses of both dopaminergic agonists that reduced TSH levels also caused stereotypes. Thus, the latter, but not the former drug-induced hormonal responses may be influenced by or associated with the stereotyped behaviors. The failure of the higher doses of apomorphine (1-10 mg/kg) and piribedil (30 mg/kg) to elevate

Table 6. Minimally Effective Doses (mg/kg, sc) of Dopaminergic Agonists Required to Alter the Blood Concentration of Pituitary Hormones and to Cause Stereotyped Sniffing in Male Rats

	Reduce Prolactin	Increase Growth Hormone	Reduce TSH	Stereotyped Sniffing
Apomorphine	0.01	0.03	0.3	0.3
Piribedil	0.30	0.30	10.0	3.0

blood content of growth hormone may have resulted indirectly from stress associated with the stereotyped behavior, which then counteracted the direct ability of the drug to release growth hormone.

At low doses neuroleptics selectively block the behavioral effects of dopaminergic aponists and at higher doses these drugs cause catalepsy. In the experiment depicted in Figure 7, the stereotyped sniffing caused by 0.3 mg/kg of apomorphine was blocked by 0.1 but not by 0.03 mg/kg haloperidol; only at the highest dose (1 mg/kg) did haloperidol cause the characteristic cataleptic response.

D. Conclusions

The results of this study demonstrate that pharmacological stimulation of brain dopamine receptors produce differential effects on release of prolactin, TSH, growth hormone and LH. Both apomorphine and piribedil evoked dramatic reductions in serum prolactin and TSH which are consistent with the relative dopaminergic properties (potencies and durations of action) of these two drugs. The sensitivity of the prolactin response to these agents was about 30-fold greater than that observed for TSH. Blockade of dopamine receptors by haloperidol resulted in dose-related increases in serum prolactin concentrations and tended to reduce serum TSH but blocked the ability of apomorphine to inhibit the release of either hormone. These observations support the view that dopamine mediates on inhibitory influence over prolactin release (Hökfelt and Fuxe, 1972a,b; Meites, 1973; Ojeda et al., 1974; Clemens et al., 1974), and also demonstrate that dopamine exerts an inhibitory action on release of TSH. The ability of apomorphine (at doses tested)

to suppress TSH release in euthyroid animals was eliminated when TSH was highly elevated by thyroidectomy, but the capacity of apomorphine to reduce serum prolactin was not affected by thyroidectomy. These observations suggest that the lack of feedback inhibition by thyroid hormones is such a powerful stimulus for TSH release that it cannot be overcome by a dopaminergic inhibitory mechanism.

Release of growth hormone was stimulated both by apomorphine and piribedil, although this effect was observed only over a lower range of doses for both drugs. The observation that plasma growth hormone was markedly increased at the latest time interval (2 hr, Figure 3) after administration of a relatively large dose of apomorphine (1.0 mg/kg) indicates again that small doses of dopamine agonists stimulate growth hormone release. The ability of haloperidol to reduce plasma growth hormone concentrations suggest that growth hormone may be tonically released by a dopaminergic stimulatory system. The specificity of the growth hormone response to dopamine receptor stimulation is demonstrated by the blockade of this response by the highest dose of haloperidol; whereas, apomorphine prevented the inhibitory action of intermediate doses of haloperidol on growth hormone release. L-dopa, a dopamine precursor, also has been reported to increase blood growth hormone concentrations in rats (Chen et al., 1974; Smythe et al., 1975) and humans (Boyd et al., 1970; Boden et al., 1972; Silver et al., 1973). These results do not agree with the findings of Collu et al. (1972) who injected dopamine (1 μg) intraventricularly and found that this agent reduced plasma growth hormone concentrations in urethane-anesthetized

male rats, nor with the observations of Müller et al. (1973) who reported that L-dopa lowered plasma growth hormone values in rats pretreated with alpha-methyl-para-tyrosine. However, Martin et al. (1975) has shown that the episodic release of growth hormone is inhibited by alpha-methyl-para-tyrosine, a drug which inhibits the synthesis of catecholamines. Possible explanations for these differences are that the drug combinations they employed modified the effect of dopamine or that the doses used were stressful. Stresses were reported to inhibit growth hormone secretion (Schlach and Reichlin, 1968; Collu et al., 1973, 1974; Dunn et al., 1973/74; Krulich et al., 1974; Thesis).

Serum LH concentrations were generally reduced by apomorphine and piribedil. These observations are in agreement with the reports that dopamine does not stimulate and may inhibit gonadotropin release. Fuxe and co-workers (Fuxe and Hökfelt, 1969; Ahrén et al., 1971; Hökfelt and Fuxe, 1972a,b) proposed that median eminence dopamine neurons inhibit LH release in rats. Recently Sawyer (1975) observed that intraventricular injections of norepinephrine but not dopamine stimulated LH release in the rabbit, and that dopamine given in combination with norepinephrine completely inhibited stimulation of LH release by norepinephrine. By contrast, Kamberi et al. (1969, 1970b) reported that injections of dopamine into the third ventricle of rats markedly elevated blood concentrations of LH and increased the concentration of LRH in hypophyseal portal blood. In vitro release of LRH by rat hypothalamic fragments also was observed to be stimulated by dopamine (Schneider and McCann, 1970c). Our results on LH are not in agreement with those of

Kamberi et al. (1969, 1970b) or of Schneider and McCann (1970c), although their experimental designs did not preclude the possible conversion of dopamine to norepinephrine, the latter acting to stimulate LRH-LH release (Sawyer et al., 1974; Sawyer, 1975).

Last year our laboratory reported (Chen and Meites, 1975a) that drugs which modify catecholamines had little or no effect on TSH secretion in the rat. However, the drugs used did not differentiate dopamine from norepinephrine activity. The present results show that stimulation of dopamine receptors inhibited TSH release in the male rat. Grimm and Reichlin (1973) reported that norepinephrine stimulated TRH release from the mouse hypothalamus. These observations suggest that TSH release may be inhibited by dopamine but stimulated by norepinephrine. It has been reported that L-dopa reduces serum TSH in human subjects (Rapoport et al., 1973; Refetoff et al., 1974; Minozzi et al., 1975). The tendency for haloperidol to reduce serum TSH in the present study may be due to the weak noradrenergic blocking properties of this drug (Janssen et al., 1968).

The role of the hypothalamic hypophysiotropic hormones in mediating the actions of the dopamine agonists and haloperidol on prolactin, TSH, growth hormone and LH release remain to be elucidated. Stimulation of dopaminergic activity is believed to inhibit prolactin release by increasing PIF activity (Meites, 1973) and possibly by a direct action of dopamine on the pituitary (Koch et al., 1970; Shaar and Clemens, 1974). Apomorphine also has been shown to act directly on the pituitary to inhibit the in vitro release of prolactin (Smalstig et al., 1974;

Smalstig and Clemens, 1974). Thus, the observed difference in response to prolactin and TSH to both dopamine agonists used in the present study may be due to a direct action of these drugs on the pituitary to inhibit prolactin release; whereas, inhibition of TSH release may be mediated by neuronal mechanisms which require higher doses. TRH has been shown to induce prolactin release in animals (Mueller et al., 1973; Convey et al., 1973) and man (Jacobs et al., 1971). Our findings demonstrate parallel reductions in both prolactin and TSH by the dopamine agonists, but do not prove that these are mediated via reduction of TRH release. The dose-dependent stimulation of growth hormone secretion by apomorphine and piribedil may reflect an alteration in the balance of hypothalamic GRF and somatostatin release. Low doses of dopamine agonists may stimulate GRF and/or inhibit somatostatin release; whereas, higher doses may not disturb the balance of release between these two hypothalamic hormones. The dopaminergic agonists produced either no change or minimal decreases in serum LH, suggesting that dopamine does not stimulate LRH release in male rats.

The decrease in prolactin and increase in growth hormone produced by dopaminergic agonists are probably not due to the behavioral effects of these drugs. The minimally effective dose of these drugs for reducing prolactin and increasing growth hormone is 1/10 to 1/30 of that required to induce stereotyped sniffing. However, the minimally effective dose of apomorphine needed to reduce TSH was the same as that which induced stereotyped sniffing, and, therefore, a relationship between the drug induced behavior and the TSH reduction is possible.

IV. Effects of Tryptophan, 5-HTP and Restraint Stress on Hypothalamic and Brain Serotonin Turnover and Pituitary Hormone Release

A. Objectives

Little is yet known about how alterations in brain serotonin metabolism affect release of TSH and prolactin. Recently Grimm and Reichlin (1973) found that serotonin inhibited *in vitro* release of pulse labeled TRH from mouse hypothalamic tissue, suggesting that serotonin may inhibit TSH release *in vivo*. However, Chen and Meites (1975a) reported that large doses of 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin, stimulated TSH release in ovariectomized, estrogen-primed rats. Reports from other laboratories indicated that serotonin may either stimulate (Shopsin *et al.*, 1974) or inhibit (Mess and Peter, 1975; Mueller *et al.*, 1976a, Thesis) TRH-TSH release. There is general agreement that serotonin stimulates release of prolactin (see Meites, 1973). Stresses were reported to either inhibit (Ducommun *et al.*, 1966) or stimulate (Krulich and Illner, 1973) release of TSH; whereas, acute stress profoundly increased prolactin release (Krulich *et al.*, 1974; Euker *et al.*, 1975). Serotonin metabolism was reported to be enhanced by stress (Thierry *et al.*, 1968; Ladisich, 1975).

The purpose of this study was to attempt to correlate alterations in brain serotonin metabolism produced by administration of tryptophan, 5-HTP, pargyline and restraint stress with the release of pituitary TSH and prolactin in rats.

B. Materials and Methods

Male Sprague-Dawley rats weighing 225-250 gm each were used in this study. D- and L-tryptophan, 5-HTP, pargyline or appropriate vehicle were given as single intraperitoneal injections in a volume of 0.2 ml/100 gm at dose and treatment times indicated under Results. Animals were killed by decapitation and biochemical and hormone assays were performed as described in the general Materials and Methods section. Data were analyzed statistically using analysis of variance and the Least Significant Difference test (Sokal and Rohlf, 1969); the level of significance was chosen as $p < 0.05$. The time course effects of pargyline on accumulation of central serotonin (Figure 8) were analyzed by a least squares regression analysis (Sokal and Rohlf, 1969). The slope of the regression line calculated for data points at 0, 15, 30 and 60 minutes post-treatment was used to determine the turnover rate of brain and hypothalamic serotonin in nanomoles/gram/hour. When possible, serum concentrations of growth hormone were measured following a minor modification in assay reagents which eliminated the need to use plasma samples for growth hormone determinations. Abbreviations used in figures and tables are prolactin (PRL), growth hormone (GH) and 5-hydroxytryptophan (5-HTP).

C. Results

Time Course Effects of Pargyline on Concentrations of Hypothalamic and Brain Serotonin

The time course effects of a single injection of pargyline (75 mg/kg, i.p.) on the accumulation of hypothalamic and brain serotonin are shown in Figure 8. Pargyline at a dose of 75 mg/kg was found in our

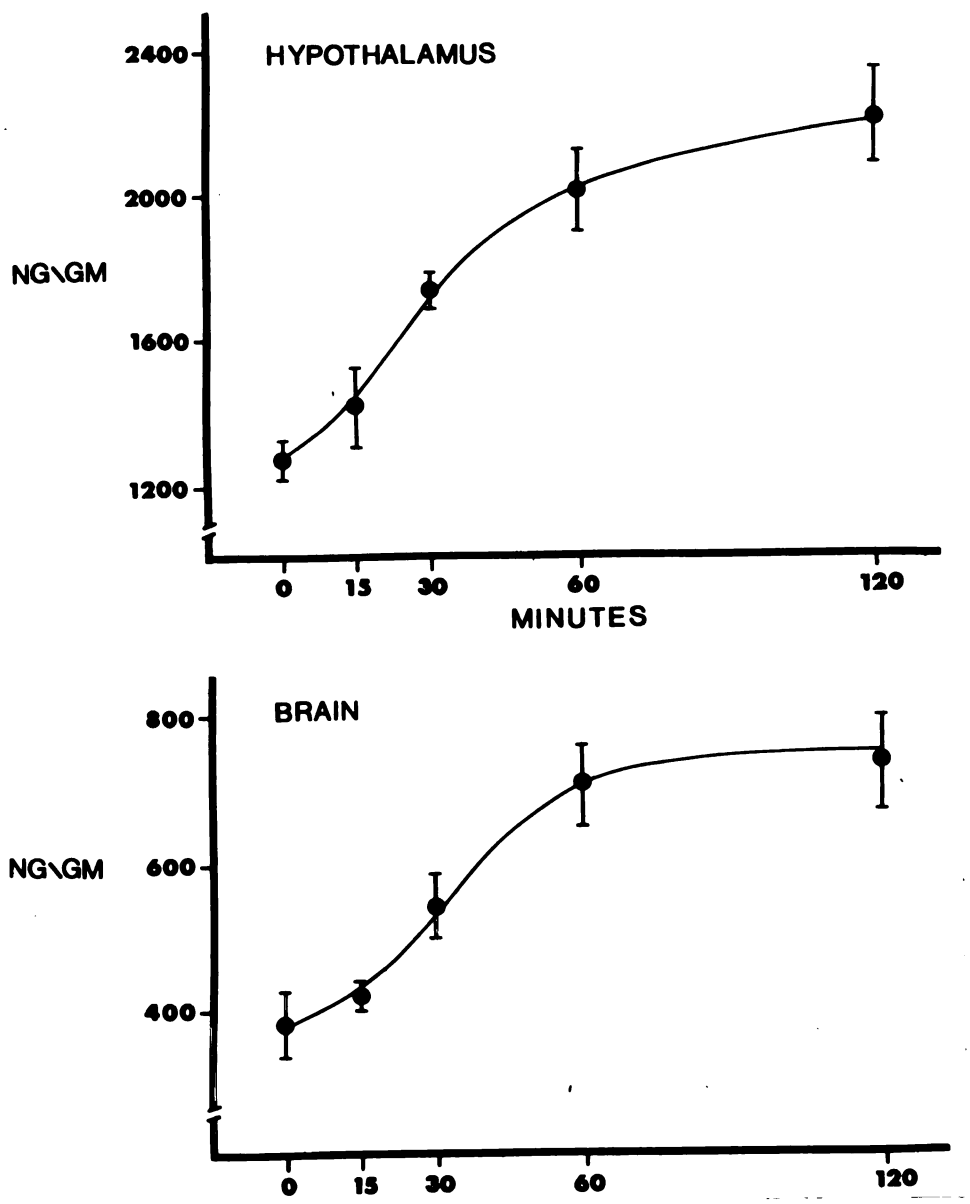

Figure 8. Time course of the effects of pargyline on hypothalamic and brain concentrations of serotonin in male rats.

Rats were injected i.p. with pargyline (75 mg/kg) and killed at various times thereafter. Each symbol represents the mean of 6-7 determinations. Verticle lines projected on each symbol represent ± 1 standard error. Zero-time control values represent the serotonin levels of animals treated with vehicle 60 min. prior to decapitation.

laboratory (W. Chen, Mueller and Meites, unpublished) and reported by others (Tozer et al., 1966; Lin et al., 1969; Morot-Gaudry et al., 1974) to effectively inhibit brain monoamine oxidase (MAO) activity in rats. A higher dose of pargyline (250 mg/kg) produced a slightly greater inhibition of MAO (as determined by the accumulation of serotonin after injection) as compared with a dose of 75 mg/kg, but also produced marked behavioral effects and appeared to be toxic to the animals (Chen, Mueller and Meites, unpublished). Pargyline (75 mg/kg, i.p.) caused hypothalamic and brain serotonin concentrations to rise at linear rates for 60 minutes after injection. The turnover of serotonin in the hypothalamus and brain was calculated to be 4.4 nm/gm/hr and 1.7 nm/gm/hr, respectively.

Time Course Effects of L-tryptophan on Concentrations of Cerebellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Serum TSH and Prolactin

A single injection of L-tryptophan (200 mg/kg, i.p.) produced rapid and prolonged increases in concentrations of cerebellum tryptophan, hypothalamic and brain serotonin, and brain 5-HIAA in male rats. Serum concentrations of TSH were decreased by 30, 60 and 120 min after injection as compared with vehicle injected control values. Serum prolactin concentrations were significantly increased by 30 min but returned to control values by 60 and 120 min after L-tryptophan injection. Changes in hypothalamic and brain serotonin, brain 5-HIAA and serum TSH concentrations were maximal by 60 min after injection; whereas, concentrations of cerebellum tryptophan rose progressively throughout the 2-hour treatment period.

Effects of D- and L-tryptophan on Concentrations of Cerebellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA and Blood Hormones

A single injection of D-tryptophan (200 mg/kg, i.p.) increased concentrations of cerebellum tryptophan, hypothalamic and brain serotonin and brain 5-HIAA in a time related manner (Table 8). These changes were not as great as those produced by the same dose (200 mg/kg) of L-tryptophan by 120 min after injection. Both D- and L-tryptophan tended to reduce serum TSH and growth hormone concentrations, the L-isomer being more effective than D-tryptophan by 120 min after injection. Serum prolactin was slightly elevated by 30 min after injection of D-tryptophan.

Effects of L-tryptophan in Combination With Pargyline on Concentrations of Hypothalamic and Brain Serotonin, and Serum TSH and Prolactin

The effects of increasing doses of L-tryptophan given in combination with pargyline (75 mg/kg) on hypothalamic and brain concentrations of serotonin and serum concentrations of TSH and prolactin are shown in Table 9. In addition the effects of 5-HTP (30 mg/kg) alone on the above parameters was tested. All treatments were given as single i.p. injections 30 min prior to decapitation and control rats received appropriate vehicle injections. Pargyline alone significantly increased hypothalamic and brain concentrations of serotonin, reduced serum TSH and had no significant effect on serum prolactin concentrations. Increasing doses of L-tryptophan given in combination with pargyline produced dose-related increases in hypothalamic and brain concentrations of serotonin but did not significantly reduce serum TSH values below

Table 7. Effects of L-tryptophan on Concentrations of Cerebellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Serum TSH and Prolactin in Male Rats

Treatment	Cerebellum Tryptophan µg/gm	Hypothal. Serotonin ng/gm	Brain Serotonin ng/gm	Brain 5-HIAA ng/gm	Serum TSH ng/ml	Serum PRL ng/ml
Vehicle Controls						
60 min	2.70 ± 0.13	1283 ± 72	305 ± 32	258 ± 16	685 ± 143	6 ± 1
L-tryptophan 200 mg/kg						
30 min	56.39 ± 3.72*	1824 ± 68*	515 ± 53*	314 ± 18*	214 ± 36*	14 ± 3*
60 min	72.79 ± 3.18*	2144 ± 79*	682 ± 31*	337 ± 24*	153 ± 21*	8 ± 1
120 min	87.49 ± 8.10*	2148 ± 68*	681 ± 19*	324 ± 34*	164 ± 40*	6 ± 1

Rats were injected with vehicle or L-tryptophan (200 mg/kg, i.p.) and decapitated at specified times thereafter. Each value represents the mean of 6-7 determination ± 1 standard error.

*Values significantly different from vehicle injected controls, $p < 0.05$).

Table 8. Effects of D- and L-tryptophan on Concentrations of Cerbellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Blood Hormones in Male Rats

Treatment	Cerebellum Tryptophan µg/gm	Hypothal. Serotonin ng/gm	Brain Serotonin ng/gm	Brain 5-HIAA ng/gm	Serum TSH ng/ml	Serum PRL ng/ml	Serum GH ng/ml
Vehicle 60 min	4.7 ± 0.2	1563 ± 23	563 ± 13	484 ± 20	712 ± 102	10 ± 1	308 ± 59
D-Tryptophan							
30 min	20.5 ± 3.8*	1641 ± 49*	647 ± 21*	565 ± 19*	776 ± 205	13 ± 1*	215 ± 35
60 min	37.8 ± 1.1*	1967 ± 42*	706 ± 13*	776 ± 11*	496 ± 78	12 ± 1	233 ± 28
120 min	23.1 ± 1.3*	1996 ± 30*	770 ± 14*	937 ± 23*	625 ± 57	11 ± 1	238 ± 52
L-Tryptophan							
120 min	56.7 ± 6.9*	2270 ± 51*	862 ± 14*	1245 ± 40*	453 ± 80*	14 ± 3	201 ± 26*

Rats were injected with vehicle or D- or L-tryptophan (200 mg/kg, i.p.) and decapitated at specified times thereafter. Each value represents the mean of 6-7 determinations ± standard error.

*Values significantly different from vehicle injected controls, $p < 0.05$.

Table 9. Effects of Pargyline, Pargyline Plus L-tryptophan, and 5-HTP on Concentrations of Hypothalamic and Brain Serotonin and Serum TSH and Prolactin in male Rats

Treatment	Hypothal. Serotonin ng/gm	Brain Serotonin ng/gm	Serum TSH ng/ml	Serum PRL ng/ml
Vehicle Controls	1532 ± 48	510 ± 16	421 ± 51	6 ± 1
Pargyline (75 mg/kg) Controls	1743 ± 70*	692 ± 18*	247 ± 94*	6 ± 1
Pargyline + L-tryptophan				
25 mg/kg	2056 ± 48**	761 ± 22*	259 ± 94*	9 ± 1
50 mg/kg	2196 ± 57**	864 ± 29**	193 ± 39*	11 ± 1**
100 mg/kg	2341 ± 119**	899 ± 15**	165 ± 24*	14 ± 1**
5-HTP (30 mg/kg)	3449 ± 143**	1863 ± 48**	434 ± 58	16 ± 1**

All drugs and/or vehicles were given as i.p. injections 30 min before decapitation. Each value represents the mean of 6-7 determinations ± 1 standard error.

*Values significantly different from vehicle injected controls, $p < 0.05$.
**Values significantly different from both vehicle and pargyline injected controls, $p < 0.05$.

those produced by pargyline treatment alone. Interestingly, 5-HTP markedly increased concentrations of serotonin in the hypothalamus and brain but had no effect on serum TSH as compared to the vehicle injected control mean. This dose of 5-HTP produced about a two-fold increase in serum prolactin concentrations.

Dose-response Effects of 5-HTP on Concentrations of Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Blood Hormones

Graduated doses of 5-HTP dramatically increased concentrations of hypothalamic and brain serotonin and brain 5-HIAA by 30 min in a manner which was exponentially related to the log of the 5-HTP dose (Figure 9). Serum prolactin concentrations were increased by the 30 mg/kg and 100 mg/kg doses of 5-HTP but not by lower doses. All doses of 5-HTP significantly reduced serum concentrations of TSH. Serum growth hormone concentrations were not consistently affected by increasing doses of 5-HTP.

Effects of Restraint Stress on Concentrations of Cerebellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Blood Hormones

To further investigate the relationship between brain serotonin metabolism and pituitary TSH, prolactin and growth hormone secretion, effects of stress on these processes was examined. Restraint stress for periods from 5 to 150 min increased concentrations of hypothalamic and brain serotonin, brain 5-HIAA and reduced serum TSH and growth hormone concentrations in a time related fashion (Table 10). Serum prolactin was maximally elevated (11-fold) by 5 and 15 min of restraint but returned towards but not to non-stress control values by 45 and 150 min.

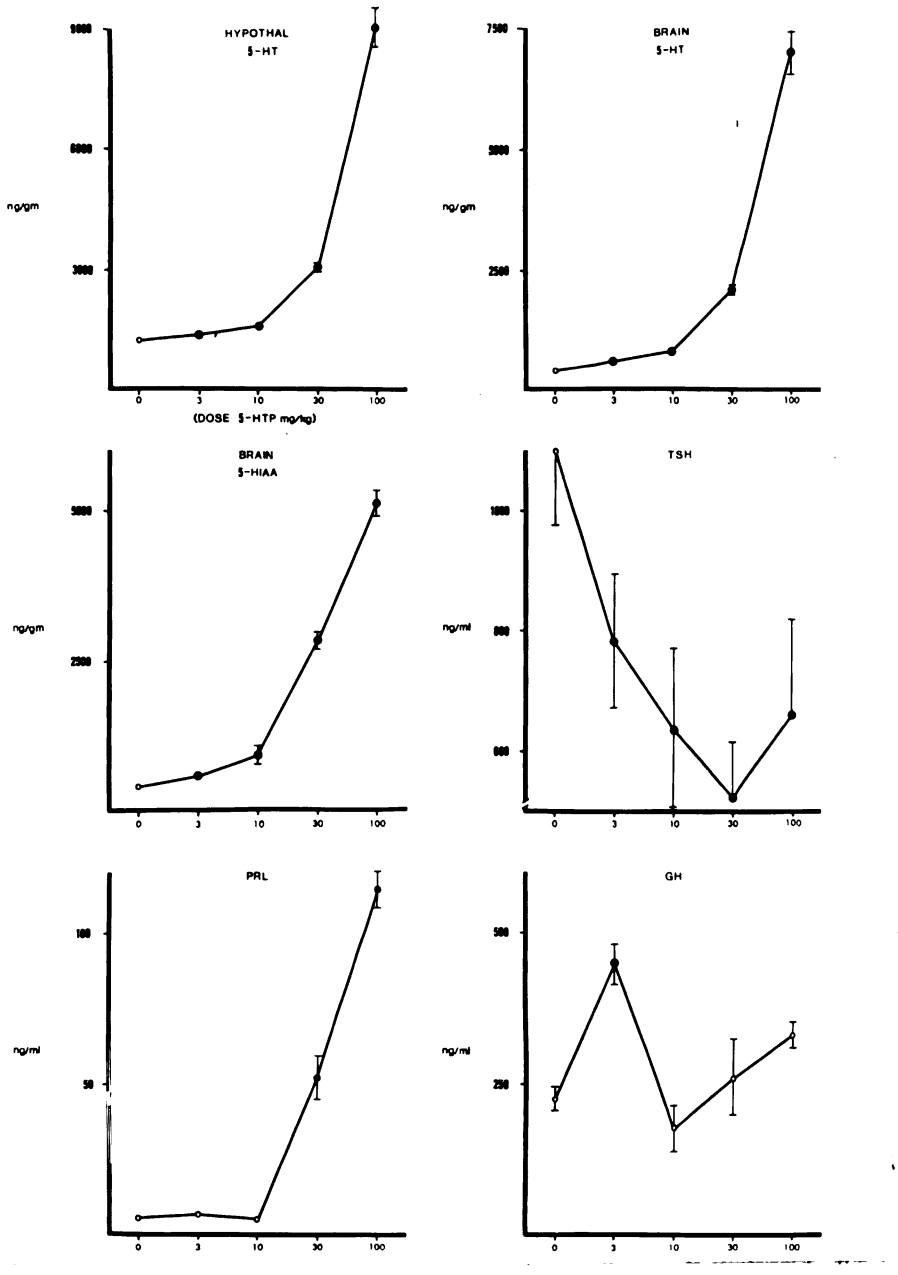

Figure 9. Dose-related effects of 5-HTP on concentrations of hypothalamic and brain serotonin, brain 5-HIAA, and blood hormones in male rats.

Rats were injected i.p. with various doses of 5-HTP or vehicle and killed 30 min. thereafter. Each symbol represents the mean of 7-8 determinations. Verticle lines projected on each symbol represent $\pm$ 1 standard error; where not shown standard error is less than the radius of the symbol. Solid symbols indicate values that are significantly different ($P < 0.05$) from vehicle treatment (zero-dose of drug).

Table 10. Effects of Restraint Stress on Concentrations of Cerebellum Tryptophan, Hypothalamic and Brain Serotonin, Brain 5-HIAA, and Blood Hormones in Male Rats

Treatment	Cerebellum Tryptophan µg/gm	Hypothal. Serotonin ng/gm	Brain Serotonin ng/gm	Brain 5-HIAA ng/gm	Serum TSH ng/ml	Serum PRL ng/ml	Serum GH ng/ml
Non-stress Controls	4.06 ± 0.25	1027 ± 34	483 ± 9	227 ± 7	571 ± 79	8 ± 1	604 ± 55
Stress							
5 min	4.56 ± 0.13	1111 ± 31	528 ± 16*	267 ± 9*	310 ± 90*	90 ± 12*	225 ± 26*
15 min	3.94 ± 0.10	1125 ± 56	504 ± 12	256 ± 12*	199 ± 42*	93 ± 7*	174 ± 9*
45 min	4.42 ± 0.19	1194 ± 53*	528 ± 16*	294 ± 8*	113 ± 19*	67 ± 5*	151 ± 12*
150 min	4.42 ± 0.17	1250 ± 73*	538 ± 16*	336 ± 14*	76 ± 35*	26 ± 3*	134 ± 5*

Rats were restrained for 0, 5, 15, 45 or 150 min before decapitation. Each value represents the mean of 6-7 determinations ± 1 standard error.

*Values significantly different from non-stress controls, $p < 0.05$.

Tryptophan concentrations in the cerebellum were not significantly altered by restraint stress.

Effect of Restraint Stress on the Accumulation of Central Serotonin in Rats Pre-treated with Pargyline

The effects of restraint stress on the accumulation of serotonin in the hypothalamus and brains of pargyline treated animals is shown in Figure 10. All animals received pargyline (75 mg/kg) 15 min prior to decapitation. Restraint was administered for the last 5 min or for the entire 15 min pargyline treatment period. Restraint stress produced rapid and time related increases in hypothalamus and brain concentrations of serotonin which were significant by 15 min as compared to non-stress control values.

D. Conclusions

The results of this study demonstrate that stimulation of serotonin turnover by administration of either tryptophan or physical restraint is associated with inhibition of TSH and growth hormone, and stimulation of prolactin release. A single injection of L-tryptophan (200 mg/kg) increased concentrations of hypothalamic and brain serotonin and brain 5-HIAA indicating stimulation of serotonin synthesis. These findings are in agreement with the earlier work of Grahme-Smith (1971), Carlsson and Lindquist (1972) and Colmenares et al. (1976). Corresponding with enhanced serotonin metabolism in the hypothalamus and brain, serum TSH and growth hormone were reduced and serum prolactin was increased. D-tryptophan tended to produce similar changes in concentrations of hypothalamic and brain serotonin and blood hormones but to

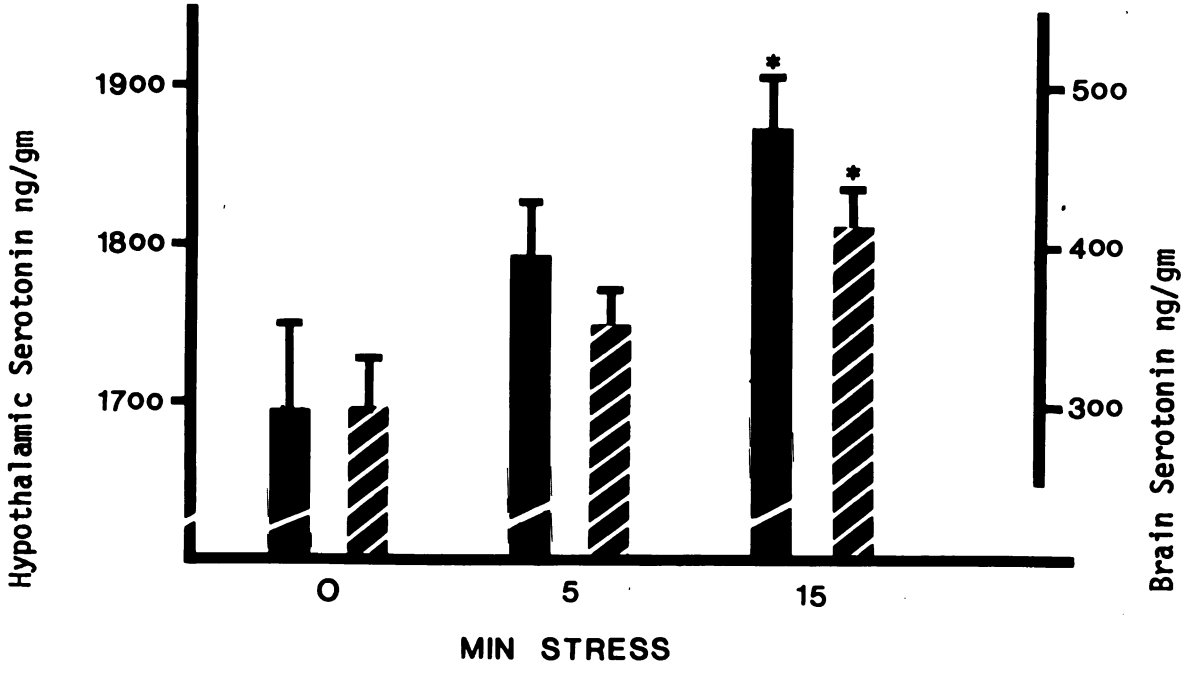

Figure 10. Time course of the effects of restraint stress on the accumulation of serotonin in hypothalamus (solid bars) and brain (striped bars) of pargyline treated male rats.

All animals received pargyline (75 mg/kg, i.p.)15 min prior to decapitation. Restraint was administered for the last 5 min or for the entire 15 min of pargyline treatment. Control animals received pargyline alone. Values are the means of 6 determinations $\pm$ 1 standard error.

*Values significantly different from non-stressed controls; $p < 0.05$.

a much lesser extent as compared to an equal dose of L-tryptophan. This difference is probably due to the required conversion of D- to L-tryptophan in the liver prior to incorporation into brain 5-hydroxy-indoles (Yuwiler, 1973). The biosynthetic pathway allows for enhanced metabolism of D-tryptophan as compared to the L-isomer.

The turnover rates of hypothalamic and brain serotonin were calculated to be 4.4 nm/gm/hr and 1.7 nm/gm/hr, respectively. The difference in rates is probably due to the relatively high content of serotonin nerve terminals located in the hypothalamus as compared with most other brain regions (see Fuxe and Jonsson, 1974). Presumably terminal regions on neurons turnover neurotransmitters faster than any other portion. The turnover rates reported here are in close agreement with those determined by many others (Tozer et al., 1966; Lin et al., 1969; Hery et al., 1972; Millard et al., 1972; Carlsson et al., 1972), although lower than the rate reported by Morot-Gaudry et al. (1975).

In combination with MAO inhibition by pargyline, L-tryptophan administration produced dose related increases in concentrations of hypothalamic and brain serotonin and in serum prolactin. Failure of L-tryptophan to reduce serum TSH concentrations below those produced by MAO inhibition alone suggest that pargyline causes a maximum inhibition of pituitary TSH release. L-tryptophan was reported to increase brain tryptamine concentrations (Saavedra and Axelrod, 1973) and produce symptoms of stress (Grahme-Smith, 1971) in rats pre-treated with MAO inhibitors. The possibility that tryptamine or a nonspecific stress effect may be involved in the hormone changes observed following

tryptophan or combined pargyline-tryptophan treatment cannot be excluded. However, neither of these treatments produce behavioral responses indicating they were stressful to the rats.

Injection of 5-HTP, the immediate precursor to serotonin, increased concentrations of hypothalamic and brain serotonin in a manner which appears to be exponentially related to the log dose of 5-HTP. Serum prolactin concentrations were generally elevated by 5-HTP. However, in one experiment a single dose of 5-HTP (30 mg/kg) had no effect on serum TSH (Table 9); whereas, in a subsequent dose-response experiment, this dose and others reduced serum TSH (Figure 9). Serum concentrations of growth hormone also were not consistently affected by increasing doses of 5-HTP. These findings on TSH and growth hormone may be explained by the effect of exogenous 5-HTP on catecholamine neurons. Dopamine was reported to inhibit (Mueller et al., 1976b, Thesis) and norepinephrine to stimulate (Grimm and Reichlin, 1973) TRH-TSH release. Butcher et al. (1972) reported that administration of DL-5-HTP resulted in the appearance of indoleamine fluorescence in catecholaminergic nerve cells correlated biochemically with increases in central serotonin and reductions in brain dopamine and norepinephrine concentrations. By contrast, administration of L-tryptophan selectively elevated serotonin only in serotonin containing neurons (Aghajanian and Asher, 1971). This suggests the possibility that 5-HTP but not L-tryptophan alters the function of both catecholamine and serotonin neurons, thus producing changes in pituitary secretion which may not be specific to the initial activation of serotonin neurons. This may explain the earlier report of Chen and Meites (1975a) who found that large doses of 5-HTP stimulated TSH

release in estrogen-primed ovariectomized rats. A similar situation may exist in the case of growth hormone since serum growth hormone concentrations were not consistently altered by 5-HTP but were reduced by administration of L-tryptophan (Table 8).

Restraint stress produced time related increases in concentrations of hypothalamic and brain serotonin and brain 5-HIAA (Table 10). In combination with pargyline, restraint significantly increased hypothalamic and brain serotonin concentrations by 15 min (Figure 10) indicating that stimulation of serotonin turnover is associated with physical restraint was very rapid. Equally rapid were the changes in pituitary TSH, prolactin and growth hormone release. Serum TSH and growth hormone fell to 35% and 29% of control values respectively by 15 min of restraint. Prolactin was maximally elevated 11-fold by 5 and 15 min of restraint but returned towards non-stress control values by 45 and 150 min restraint. The decline in pituitary prolactin release at the longer restraint periods (45 and 150 min) as compared to the shorter periods (5 and 15 min) may be due to the negative feedback of prolactin on its own secretion. Elevated levels of circulating prolactin were reported to enhance median eminence dopamine turnover (Hökfelt and Fuxe, 1972a,b; Fuxe et al., 1974b) and stimulation of dopamine activity is believed to inhibit prolactin release by increasing PIF activity (see Meites, 1973) and possibly by a direct action on the pituitary (Shaar and Clemens, 1974). Another possible explanation for the decline in serum prolactin concentrations at the longer restraint periods as compared to the shorter periods is that the clearance of this hormone from the serum may be

gradually increased to a rate above that at which prolactin is being released from the anterior pituitary.

The probable involvement of noradrenergic and other types of neurons in mediating the neuroendocrine changes observed in this study cannot be overlooked. Althouth L-tryptophan appears to be specific for the activation of serotonin neurons (Aghajanian and Asher, 1971), stresses were reported to alter turnover of both catecholamines (Corrodi et al., 1971; Palkovits et al., 1975) and serotonin (Thierry et al., 1968; Ladisich, 1975).

Cerebellum tryptophan concentrations were not significantly altered by stress suggesting that restraint induced stimulation of serotonin metabolism was not due to an increase in precursor availability. This presumes that changes in cerebellum tryptophan reflect the availability of tryptophan within serotonin neurons. Changes in cerebellum tryptophan concentration as affected by diet were reported to generally parallel the changes in tryptophan concentration which occurred in other regions of the brain (Colmenares et al., 1976).

The role of the hypothalamic hypophysiotrophic hormones in mediating the effects of stress on TSH prolactin and growth hormone remain to be elucidated. These findings demonstrate an inverse relationship between central serotonin turnover and the release of TSH and growth hormone. These TSH results are in agreement with the findings of Grimm and Reichlin (1973) who reported serotonin inhibited the in vitro release of TRH from mouse hypothalamus. Exogenous TRH has been

reported to induce prolactin release in animals (Convey et al., 1973; Mueller et al., 1973) and man (Jacobs et al., 1973). However, the observation that L-tryptophan and stress have opposite effects on TSH and PRL do not support a physiological role for TRH induced prolactin release. Serotonin may inhibit GRF release and/or stimulate the release of somatostatin. Conversely, serotonin may inhibit PIF and/or stimulate the release of prolactin releasing factor.

GENERAL DISCUSSION

The data presented in this thesis show that both exteroceptive and pharmacological stimuli have differential effects on the secretion of anterior pituitary hormones. Thyrotropin-releasing hormone (TRH) rapidly stimulated the *in vivo* release of prolactin as well as TSH, suggesting that TRH may release prolactin under physiological conditions in the rat. Consistent with this view, Noel *et al.* (1974) reported that the smallest dose of TRH required to evoke TSH release in humans also produced an increase in serum prolactin concentrations. However, in rats, the pronounced rise in serum prolactin as a result of being placed in a warm temperature, and fall in serum prolactin produced by cold were just opposite to the TSH responses to these temperature changes. The findings presented here and reports of others indicate that the effects of temperature on TSH and prolactin secretion are mediated by the hypothalamus and occur independently from associated changes in thyroid and adrenal function. There is considerable evidence that cold-induced TSH release is mediated by enhanced TRH secretion (Reichlin *et al.*, 1972; Hefco *et al.*, 1975c; Montoya *et al.*, 1975). The observation that cold decreased serum prolactin indicates that TRH is not responsible for the temperature-induced changes in prolactin release. This suggests that different mechanisms in the hypothalamus are activated by temperature changes to alter the secretion of TSH and prolactin. Thus, temperature changes provide an interesting approach for studying the differential control of these two hormones.

Physical restraint stress increased serum prolactin and decreased serum TSH and growth hormone concentrations. This also suggests that prolactin release is not dependent on TRH release during physical restraint, but may be due to a mechanism involving increased serotonin turnover as shown here. Enhanced rates of hypothalamic serotonin turnover as produced by administration of L-tryptophan and physical restraint were associated with stimulation of prolactin release and inhibition of TSH and growth hormone release. These results provide evidence that serotonin neurons are involved in the neuroendocrine responses to physical restraint and may act to inhibit the release of prolactin inhibiting factor (PIF) and TRH, and to stimulate prolactin releasing factor (PRF) and somatostatin. The decrease in both TSH and growth hormone as a result of physical restraint may be due in part to increased release of hypothalamic somatostatin, since somatostatin can inhibit growth hormone release and also can depress TRH-induced release of TSH (Vale et al., 1975). Serotonergic mechanisms also may be involved in the neuroendocrine responses to temperature changes. Brain serotonin turnover was reported to be increased by high ambient temperature (Corrodi et al., 1968; Aghajanian et al., 1968; Reid et al., 1968; Weiss and Aghajanian, 1971; Squires, 1974) and reduced by low temperature (Corrodi et al., 1967). These findings taken together with hormone results presented here show that brain serotonin turnover and pituitary prolactin release are directly related to ambient temperature; whereas, TSH release is inversely related to ambient temperature and serotonin turnover. This comparison is in agreement with the view that serotonin stimulates

prolactin and inhibits TSH and suggests that serotonin neurons mediate certain neuroendocrine responses to temperature changes.

The probable involvement of other neurotransmitters in mediating temperature and stress-induced changes in pituitary hormone release cannot be overlooked. Blockade of dopamine receptors by pimozide prevented the fall in prolactin release normally produced by cold exposure. Lichtensteiger (1969) reported that low ambient temperature stimulated median eminence dopamine activity as determined by histofluorescence techniques. Together these findings suggest that dopamine neurons also are involved in the cold-induced inhibition of prolactin release. However, this dopaminergic activity must be isolated to a specific prolactin control system since low temperatures enhanced TSH secretion; whereas, general pharmacological stimulation of brain dopamine receptors inhibited TSH release. Median eminence (tubero-infundibular) dopamine neurons are believed to function mainly in the inhibitory control of prolactin secretion (Fuxe and Hökfelt, 1969; Ahrén et al., 1971; Hökfelt and Fuxe, 1972a,b); whereas, virtually nothing is known about function of other hypothalamic dopamine neurons in the control of pituitary hormone release. The major portion of hypothalamic dopamine (about 80%) appears to be located outside the arcuate nucleus-median eminence complex and recently the presence of a new hypothalamic dopamine system, the incerto-hypothalamic was described (see Björklund et al., 1975). The possibility exists that the incerto-hypothalamic system is involved in the inhibition of TRH-TSH release. It would be interesting if dopamine turnover in the anterior hypothalamus, the terminal projection

area of the incerto-hypothalamic neurons, correlated with physiological changes in TRH-TSH release. Presumably cold temperature would reduce dopamine turnover in this area; whereas, warm temperature and stresses may stimulate dopamine turnover. Similar relationships between the function of incerto-hypothalamic dopamine neurons and the release of other pituitary hormones also may exist and these too await further investigation.

Hypothalamic norepinephrine turnover was reported to be stimulated by high ambient temperature (Iversen and Simonds, 1969) and physical stress (Lidbrink et al., 1972). Similar conditions of temperature and stress stimulated prolactin release and inhibited TSH release (Thesis). Although these few findings suggest that noradrenergic neurons stimulate prolactin and inhibit TSH, other reports indicate that the opposite situation may exist. Grimm and Reichlin (1973) found that norepinephrine stimulated the in vitro release of TRH from mouse hypothalamus, suggesting that norepinephrine stimulates TSH release in vivo. Disulfiram and phentolamine, which block norepinephrine synthesis and receptors, respectively, were reported to inhibit TSH release in rats (Tuomisto et al., 1973). We observed that low doses of clonidine (an alpha receptor agonist) stimulated TSH release and inhibited prolactin release (Mueller, Simpkins, Meites and Moore, unpublished). Others found that injections of norepinephrine into the third ventricle had little effect on prolactin release in rats (Kamberi et al., 1971c; Ojeda et al., 1974b); whereas, other reports showed that norepinephrine may stimulate prolactin release (Donoso et al., 1971; Meites and Clemens,

1972; Lawson and Gala, 1975). At present the physiological role(s) of noradrenergic neurons in the control of prolactin and TSH is unclear. Apparent differences between conclusions made in various reports probably arise from the use of nonspecific drugs, different experimental designs and possibly to multiple functions of noradrenergic neurons on the control of a single pituitary hormone. Development and application of specific agonists and antagonists to central norepinephrine receptors, measurement of norepinephrine turnover in individual hypothalamic nuclei, and careful electrical stimulation and lesioning of the ascending noradrenergic fibers which innervate the hypothalamus used in conjunction with endocrine measurements should further clarify the role of norepinephrine in the control of prolactin and TSH secretion. In contrast to the effects of norepinephrine on these two hormones, there is accumulating evidence that norepinephrine stimulates LH release and that dopamine can inhibit the stimulatory effect of norepinephrine (Sawyer et al., 1974; Sawyer, 1975). This may explain why in the present study, the two dopamine agonists used, apomorphine and piribedil, either reduced or had no effect on LH release. The effect of norepinephrine on growth hormone release is not clear at present, in contrast to the considerable evidence that dopamine stimulates growth hormone release, as also shown in the present study.

Several of the experiments presented in this thesis were designed to investigate the influence of serotonergic and dopaminergic neurons on the release of TSH, prolactin and growth hormone. In the case of prolactin and growth hormone, the action of these two neurotransmitters were

found to be antagonistic. Dopamine inhibited prolactin and stimulated growth hormone; whereas, serotonin had the opposite effects on the secretion of these two hormones. By contrast, both dopamine and serotonin inhibited TSH release in male rats. These findings are consistent with current understanding of neurotransmitter control of prolactin release (see Meites, 1973) and provide new evidence on the regulation of TSH and growth hormone.

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APPENDICES

APPENDIX A

Serotonin and 5-HIAA Assay Procedures

Reagents:

Acid Butanol	1 Liter butanol plus 0.85 ml concentrated hydrochloric acid (HCl)
1% Cysteine	0.1 gm L-cysteine (Sigma Chemical Comp.) dissolved in 10 ml 0.1 N HCl
OPT solution	4.0 mg O-phthaldehyde (A grade, Calbiochem) dissolved in 100 ml concentrated HCl
0.5 M Phosphate Buffer	Make separate 0.5 M solutions mono and dibasic Na phosphate Monobasic = 6.0 gm HaH_2PO_4-H_2O in 100 ml H_2O Dibasic = 7.1 gm Na_2HOP_4 in 100 ml H_2O Titrate dibasic with monobasic to pH 7.5
Stock serotonin standard	Dissolve 43.97 mg serotonin creatinine sulfate (Sigma Chemical Comp.) in 100 ml of 0.1 N HCl (20 mg free base/100 ml), store at 4°C for up to three weeks
Stock 5-HIAA Standard	Dissolve 20 mg 5-HIAA (Sigma Chemical Comp.) in 100 ml of 0.1 N HCl and store at 4°C for up to three weeks

Procedure for Brain Samples:

1. Homogenize brain (about 1.45 gm tissue) in 7.0 ml acid butanol.
2. Centrifuge at 15,000 RPM (27,000 x G) for 15 min. at 4°C. (supernatant volume = 7.75 ml for volume correction)

3. Transfer 3.0 ml of supernatant to a glass screw cap test tube containing 2.5 ml of 0.1 N HCl and 6.0 ml heptane.

4. Shake for 10 min.

5. Centrifuge at maximum speed in an International Clinical Centrifuge (table top model) for 5 min.

6. Transfer 5.0 ml or organic phase (containing 5-HIAA) to a glass screw test tube containing 1.5 ml of 0.5 M phosphate buffer pH 7.5. Continue at step 8.

7. Draw off remaining organic phase by suction and transfer 1.0 ml aqueous phase (containing serotonin) to a glass screw cap test tube containing 100 ul of 1% cysteine. Continue at step 10.

8. Shake for 10 min. and centrifuge for 5 min.

9. Draw off organic phase and transfer 1.0 ml aqueous phase (containing 5-HIAA) to a glass screw cap test tube containing 100 ul of 1% cysteine.

10. Add 2.0 ml OPT solution to serotonin and 5-HIAA fractions, mix and cap.

11. Heat in 100°C water bath for 10 min.

12. Cool to room temperature and read sample fluorescence at 355 nm excitation and 470 nm emission.

Procedure for Hypothalamus Samples:

1. Homogenize hypothalamus in 2.0 ml acid butanol.

2. Rinse with 2.0 ml acid butanol and combine with initial homogenate.

3. Centrifuge 10,000 RPM (12,350 x G) for 15 min at 4°C.

4. Transfer 3.0 ml supernatant to a glass screw cap test tube and proceed at step 4 in the procedure for brain samples.

Procedure for Standards:

1. Separately dilute 100 ul aliquots of serotonin and 5-HIAA stock standards 1 to 100 with 0.1 N HCl (100 ul Stb. + 9.9 ml 0.1 N HCl). Final concentration of working standards is 2 ng/ul.

2. Place 100 ul of both serotonin and 5-HIAA standards together in 9.8 ml 0.1 N HCl to make a "combined" working standard.

3. To determine % recovery for brain extraction procedure, place in duplicate: 0 ul, 100 ul and 200 ul of combined working standard (0 ng/200 ng and 400 ng) in glass screw cap test tubes containing 3.0 ml acid butanol, 6.0 ml heotane and 2.5 ml, 2.4 ml and 2.3 ml 0.1 N HCl respectively. Begin at step 4 in the procedure for brain samples.

4. To determine % recovery for hypothalamus extraction procedure, place in duplicate: 0 ul, 50 ul and 100 ul working serotonin standard (0 ng, 100 ng and 200 ng) in glass screw cap test tubes containing 3.0 ml acid butanol, 6.0 ml heptane and 1.50 ml, 1.45 ml and 1.40 ml 0.1 N HCl respectively. Begin at step 4 in the procedure for brain samples. (Note, hypothalamic levels of 5-HIAA are below the limits of detection by this assay.)

5. Serotonin standard curves can be run at 0 ng, 50 ng, 100 ng and 200 ng in duplicate. Place 0 ul, 25 ul, 50 ul and 100 ul of working standard in glass screw cap test tubes containing 0.1 ml 1% cysteine, and 1.0 ml, 0.975 ml, 0.950 ml and 0.900 ml 0.1 N HCl. Begin at step 10 in the procedure for brain samples.

6. 5-HIAA standard curves are made up similar to those for serotonin with the exception that the glass screw cap tubes contain 0.5 M phosphate buffer pH 7.5 in place of 0.1 N HCl.

APPENDIX B

Tryptophan Assay Procedure

Reagents:

10^{-4} N HCl

75% Trichloroacetic Acid (TCA)

10^{-4} N HCl/75% TCA solution	23 ml 10^{-4} HCl + 3 ml 75% TCA
HCHO solution	1.0 ml 37% Formaldehyde + 19.5 ml H_2O
HCHO/$FeCl_3$	48.6 mg $FeCl_3$ in 100 ml HCHO solution
0.1 N NH_4OH	6.75 ml NH_4OH diluted up to 1 liter with H_2O
Stock Tryptophan Standard	20 mg of L-tryptophan (Sigma Chemical Comp.) dissolved in 100 ml 0.1 N NH_4OH (200 ng/ul). Store at 4°C for up to three weeks.
Working Tryptophan Standard	Dilute 1.0 ul Stock 9.9 ml 0.1 N NH_4OH (2 ng/ul).

Procedure for Samples:

1. Homogenize tissue in 5 to 20 volumes of 10^{-4} HCl. (Use a convenient volume based on the tissue weight.)
2. Transfer 300 ul of homogenate to a plastic centrifuge tube containing 2.0 ml 10^{-4} N HCl.
3. Add 300 ul 75% TCA and vortex.
4. Centrifuge at 10,000 RPM (12,350 x G) for 15 min at 4°C.
5. Transfer 2.0 ml supernatant to a glass screw cap test tube.
6. Add 200 ul HCHO/$FeCl_3$ reagent and vortex.

7. Cap and heat at 100°C for 60 min.

8. Cool to room temperature and read sample fluorescence at 371 nm excitation, 443 nm emission.

Procedure for Standards:

1. To determine % recovery for extraction procedure place in duplicate: 0 ul, 5 ul and 15 ul stock standard (0, 1 ug and 3 ug) in a volume of 10^{-4} HCl comparable to the volumes used for tissue homogenization. Begin at step two in the procedure for samples.

2. Tryptophan standard curves can be run at 0 ng, 50 ng, 100 ng and 200 ng. Place 0 ul, 25 ul, 50 ul and 100 ul of working standard in glass screw cap test tubes containing 2.0 ml, 1.975 ml, 1.950 ml and 1.900 ml of 10^{-4} HCl/75% TCA solution respectively. Begin at step 6 in the procedure for samples.

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TALKS PRESENTED AT SCIENTIFIC MEETINGS:

Meeting	Date	Topic
58th Annual FASEB Meeting Atlantic City, New Jersey	1974	Effects of estrogen, T_4 and TRH on Serum Prolactin and TSH in Rats
60th Annual FASEB Meeting Anaheim, California	1976	Effect of L-tryptophan and Restraint Stress on Serotonin Turnover and TSH and Prolactin (PRL) Release in Rats

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