NEUROTRANSMITTER REGULATION OF GROWTH HORMONE SECRETION

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ABSTRACT

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1. Sequential plasma samples collected throughout the day indicated that plasma growth hormone (GH) was released in large episodes every 3 - 3 1/2 hours. Basal GH concentrations were approximately 20 - 30 ng/ml plasma, and during an episode of GH release, plasma concentrations reached upwards of 200 - 300 ng/ml. These major episodes occured at 0930 - 1030, 1200 - 1300, and 1500 - 1600 hours.

2. The effects of specific adrenergic drugs on GH release were studied *in vivo* and *in vitro*. Clonidine, an alpha adrenergic agonist, stimulated GH release *in vivo*. This increase was prevented by concurrent injections of phentolamine, an α-adrenergic receptor blocker, but not by propranalol, a β-adrenergic receptor blocker. Chlorpromazine, a catecholamine blocker, also reduced GH release. These observations were confirmed *in vitro* using a pituitary-hypothalamus co-incubation system. In such a system, the hypothalamus inhibited GH release by the pituitary. Norepinephrine (NE) did not act directly on the pituitary to stimulate GH release, but removed the inhibitory influence of the hypothalamus on

GH release. The effects of NE *in vitro* were reversed by concurrent treatment with phentolamine, but not by propranalol or pimozide, the latter a dopamine receptor blocker. This suggests that GH is regulated at least in part by the α-adrenergic receptor for NE.

3. Para-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, also significantly decreased serum GH concentrations 2 - 8 days after injection. Another serotonin antagonist, methsergide, significantly decreased serum GH concentrations 10, 30 and 60 minutes after injection in a dose related manner. A specific neurotoxin for serotonergic neutons, 5, 7-dihydroxytryptamine injected intraventricularly significantly reduced serum GH within two days after injection. Serum GH was maximally depleted ten days after the initial injection. When 5, 7-dihydroxytryptamine was injected 45 - 60 min after desmethylimipramine, serum GH concentrations were significantly reduced by 6 days and maximally reduced by 12 days after injections. *In vitro* co-incubation of anterior pituitary halves with hypothalamic fragments released less GH than anterior pituitary halves alone. When varying doses of serotonin were added to the incubation medium, serotonin removed the hypothalamic inhibition of GH release. Methysergide added to the incubation medium reversed the effects of serotonin in a dose related manner, indicating

that serotonin stimulated GH release.

4. Acetylcholine and the cholinergic agonists, pilocarpine and physostigmine, increased GH release in vivo. The increase in GH release by pilocarpine was reversed by concurrent administration of the cholinergic receptor blocker, atropine, whereas atropine alone decreased basal GH concentrations. Cholinergic stimulation of GH release appears to be partially mediated through a catecholaminergic system since the response was partially inhibited by pimozide, a dopamine receptor blocker, or by phentolamine, an α-adrenergic receptor blocker. The increase in GH release produced by pilocarpine, was also prevented by α-methyl-paratyrosine, an inhibitor of catecholamine synthesis. Atropine similarly prevented GH release induced by pilocarpine or acetylcholine in a co-incubation system. Monoaminergic blocking drugs such as para-chloroamphetamine, haloperidol, pimozide and methysergide, did not alter GH release induced by pilocarpine, indicating that this action is a specific function of acetylcholine.

5. Intraventricular injections of gamma-aminobutyric acid (GABA) or parenteral injection of amino-oxyacetic acid, a GABA agonist significantly decreased GH release. Conversely, intraventricular injection of bicuculline methyliodide or systemic injections of bicuculline or picrotoxin,

GABA antagonists, significantly increased serum GH concentrations and reversed the effects of GABA injections. The effects of bicuculline appear to be mediated through the catecholamines, since alpha-methyl-para-tyrosine prevented the increase in GH produced by bicuculline injections. Similarly, bicuculline injections increased the hypothalamic NE turnover index. GABA appears to decrease GH via a neuronal mechanism since GABA does not act directly on the pituitary *in vivo* or *in vitro*. When GABA was co-incubated with anterior pituitary and hypothalamus, GABA further reduced GH release produced by the presence of the hypothalamus.

6. Systemic injections of morphine or the morphinomimetic peptide, methionine enkephalin, increased GH release. Injections of naloxone, a specific opioid antagonist, decreased GH release. Concurrent injections of naloxone with either morphone or methionine enkephalin, partially prevented the increase in serum GH produced by either drug. It is possible that the endogenous opioid peptides participate in the stress induced decrease in GH release, since morphine injection prevented the stress induced decrease in GH release.

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INTRODUCTION

The importance of the hypothalamus to the functional integrity of the pituitary gland is well established. The synthesis and release of anterior pituitary (AP) hormones are regulated in part by hypophysiotropic hormones (releasing factors) produced by peptidergic neurons contained within the hypothalamus. These peptidergic neurons participate as a transducer neuron. The peptidergic neurons receive afferent signals from the autonomic nervous system and external environment. The signal is then transferred (transduced) from a neuronal response to an endocrine response by the release of an AP hormone.

Since there is no direct neural connection between the AP and the hypothalamus, these releasing factors are carried to the AP through the portal vessels which connect the AP with the hypothalamus. Recent observations indicate that the hypothalamus not only sends its releasing factors to the pituitary, but the hormones released by the pituitary also are transported in small amounts, retrograde, to the hypothalamus. This supports the concept that the hypothalamus not only governs pituitary secretion, but the pituitary also influences the secretion of hypothalamic hormones ("short-loop" feedback).

In addition to the hypothalamic hormones which alter AP hormone secretion, the hypothalamus contains high concentrations of neurotransmitters. These neurotransmitter substances mediate the transmission of nerve impulses between neurons. Among the neurotransmitters which have been shown to be in high concentrations in the hypothalamus are dopamine, norepinephrine, serotonin, acetylcholine, gamma-aminobutyric acid and histamine. Methionine and leucine enkephalin have been isolated from the hypothalamus, but the role in neural transmission is not clearly understood. Most of these neurotransmitters were previously shown to alter AP hormone secretion, either by a direct action on the pituitary or through altering the release of hypothalamic releasing factors (for review see Meites et al., 1977).

It is not within the scope of this thesis to consider the effects of neurotransmitters on all AP hormones. I shall consider primarily the neurotransmitter control of growth hormone (GH) secretion. Generally, GH is primarily controlled by neural mechanisms in the rat. Thus hypoglycemia, glucose infusion, insulin and carbohydrates do not alter GH release in rats as in man and several other species. Stress and sleep are among the primary neural stimuli for GH release in human subjects but not in rats. Dopamine appears to be a potent stimulus for GH release both in man and rats. However, the role of serotonin and norepinephrine in the control of GH release are not clearly understood. A portion of this thesis is devoted to determining the role of serotonin and

norepinephrine on GH secretion *in vivo* and *in vitro*.

Gamma-aminobutyric acid (GABA) has been shown to alter prolactin secretion, and to stimulate leutinizing hormone (LH) release, and to inhibit adrenocorticotropic hormone (ACTH) and melanocyte stimulating hormone (MSH) release. A portion of this thesis is devoted to determining the effects of GABA on GH release *in vivo* and *in vitro*. Also, several mechanisms whereby GABA alters GH release are reported.

Acetylcholine (Ach) has been shown to increase LH release and to inhibit prolactin release. Two preliminary reports have suggested that Ach may increase GH release. One report by Cehovic *et al*. (1972) claimed that paraoxon, a cholinesterase inhibitor, increased pituitary GH concentrations in the rat. However there was no evidence that paraoxon increased or decreased GH release into the blood. In human subjects, β-methylcholine, a cholinergic agonist, was reported to stimulate GH release, but inadequate controls were used. (Soulairac *et al*., 1968). In this thesis the effects of Ach, a cholinergic agonist and a cholinergic antagonist on GH release *in vivo* and *in vitro* were studied. Also, the mechanism whereby these drugs altered GH release was investigated.

Lastly, the effects of morphine, an endogenous opioid peptide, methionine-enkephalin, and naloxone, an opioid antagonist, were investigated for their effects on GH release and their possible role in regulating GH secretion.

LITERATURE REVIEW

I. Hypothalamic Control of Anterior Pituitary Hormone Secretion

Early Observations

The earliest observations suggesting that the central nervous system (CNS) participated in pituitary function were observed by the 2nd century Greek physician and medical scientist, Claudius Galenasi (Galen). He theorized that "Spirits" from the brain were conveyed to the body, but the wastes were conveyed down the pituitary stalk, through the pituitary and voided to the exterior via the nose. This philosophy persisted for nearly 1500 years when Lower in 1670 disproved the original hypothesis. He surmised that these substances were reabsorbed into the vasculature and voided elsewhere (see Harris, 1972).

Early in the 20th century the pituitary was recognized to have an important role in physiology. Among the earliest observations that the pituitary was necessary for maintainence of homeostasis were those by Cushing and co-workers (Crowe et al., 1910). The pituitary was found to produce substances necessary for growth (Evans and Long 1921, 1922), thyroid function (Allen 1919; Smith et al., 1922), adrenal

cortical function (Allen, 1920; Smith, 1926a), gonadal function (Zondeck and Aschheim, 1926; Smith 1926b), and milk production (Stricker and Grueter, 1928).

One of the first recorded observations that the CNS influenced anterior pituitary function was made by Haighton (1792), who observed that several hours after coitus, the female rabbit ovulated and formed corpora lutea. The precise mechanism was inexplicit, but appeared to be of neurological origin rather than due to the presence of sperm near the ovary. This phenomenon was later referred to as "a neuroendocrine reflex"; sensory impulses from the genito-urital tract are relayed to the brain and cause the release of luteinizing hormone (LH), and subsequently, ovulation. Today, many additional exteroceptive stimuli are known to alter pituitary hormone secretion (eg. light, temperature, smell, taste, and touch) by action through the CNS (for reviews see Marshall, 1936; 1942; Harris, 1955). Similar studies on the structural and functional relationship of the pituitary with the brain have been expanded into the field of neuroendocrinology.

Early observations suggesting that the pituitary is regulated by the hypothalamus include those of Aschner (1912). He showed that lesions in the anterior hypothalamus resulted in gonadal atrophy in dogs. These observations were confirmed in the dog (Westman and Jacobson, 1940), in rats (Camus and Roussy, 1920),

and in guinea pigs (Dey, 1943). Hypothalamic lesions also were reported to result in atrophy of the adrenal cortex (deGroot and Harris, 1950), and the thyroid gland (Cahane and Cahane, 1938; Greer, 1952; Bogdanove and Halmi 1953; Harris and Woods, 1958). Cahane and Cahane (1938) also observed reduced growth rates of rats bearing hypothalamic lesions.

In human patients, (Armstrong and Durh (1922) and Frazier, (1936) observed that tumors of the infundibulum and hypophyseal stalk resulted in growth retardation.

Further information on hypothalamic control of pituitary function came from experiments in which various hypothalamic regions were stimulated. Stimulation of the anterior hypothalamus and the median eminence induced ovulation in the rabbit (Harris, 1937; Haterius and Derbyshire, 1937), whereas stimulation of the medial basal hypothalamus enhanced thyroid function (Harris, 1948a). Stimulation of the posterior hypothalamus inhibited adrenal cortical function (deGroot and Harris, 1950). By contrast, stimulation of the anterior pituitary failed to induce physiological changes in these target organs (Markee et al., 1946; Harris, 1948b).

In addition to the observations that manipulation of the hypothalamus had an effect on target organ physiology, the indirect hypothalamic control of target organ physiology also was indicated after removal of the pituitary gland (Harris, 1948a; 1955; Everett, 1954; 1956). Removal of the pituitary or stalk section yielded very profound

effects on the body and target organs (Dott, 1923; Smith 1926a; Harris, 1937). Harris (1948a) demonstrated that the hypophysial stalk must be intact for the hypothalamus to regulate pituitary function, and following stalk section rapid regeneration of the portal vessels occurred (Harris, 1948a; Harris and Jacobson, 1950). Insertion of a small wax plate between the pituitary and hypothalamus prevented vascular regeneration, and inhibited pituitary function. Removal of the pituitary from the sella turcica and transplantation to an ectopic location resulted in maintenance of corpora lutea (Harris, 1948a; 1955; Everett, 1954; 1956), and the mammary gland (Meites, 1967). Atrophy of the gonads, thyroid and adrenals also was observed in animals bearing ectopic pituitary transplants (Harris, 1937; 1948; and 1950). When the pituitary was transplanted to its in situ location, normal endocrine physiology resumed (Nikitovich-Weiner and Everett, 1958). These observations demonstrated that the hypothalamus has both a stimulatory and inhibitory influence on pituitary function.

Hypothalamic Anatomy

Before one can understand the hormonal and humoral influence of the brain or more specifically, the hypothalamus, on endocrine function, it is necessary to have an understanding of the anatomical relationship of these structures. The hypothalamus is the most ventral portion of the diencephalon (for general anatomical reviews consult

DeGroot, 1959; Netter, 1968; Szentagothai, 1968; Jenkins, 1972; Knigge, 1974: C. Martin, 1976).

Viewing the ventral side of the brain, rostral, medial and caudal landmarks of the hypothalamus can be ovserved: the optic chiasma, tubercinereum, and mammillary bodies, respectively. The rostral border of the hypothalamus extends from the optic chiasm dorsally, following the lamina terminalis to the anterior commisure. Dorsally, the hypothalamus is separated from the thalamus by the hypothalamic sulcus. The caudal border follows the interpenduncular fossa to the hypothalamic sulcus. The lateral border which lies between the hypothalamus and subthalamus is less discernable. However, the hypothalamus is separated from the subthalamus in part by the internal capsule, the optic tracts and the subthalamic nuclei.

Histologically, one may observe 3 major gray regions in a rostral-caudal sequence. These are the anterior (surpaoptic), intermediate (tuberal), and posterior (mammilary) hypothalamic regions. Except for the arcuate nucleus and the median eminence, specific hypothalamic nuclei which are distributed bilaterally on each side of the third ventrical include these three hypothalamic regions. The anterior and tuberal regions of the hypothalamus appear to be more important in pituitary function than the posterior hypothalamic nuclei (Mayer, 1953; Halasz and Pupp, 1965; Gorski, 1966; Krey, 1975; C. Martin, 1976). The hypothalamic nuclei in a rostral-caudal direction are as follows: The preoptic nucleus

lies anterior to the supraoptic nucleus which lies dorsal to the optic chiasm. Also contained within the supraoptic region is the paraventricular nucleus which is responsible for antidiuretic hormone (ADH) synthesis (Bargman and Sharrer, 1951). The tuberal region consists of the arcuate nucleus (AN), the ventromedial nucleus (VMN), the dorsal medial nucleus (DMN), the lateral hypothalamus (LHA) and the dorsal hypothalamic nuclei (DHN). Finally, the caudal hypothalamus is composed primarily of mammillary nuclei and the posterior hypothalamic nucleus.

The hypothalamus receives its major afferent nerve tracts from the median forebrain bundle (MFB) which conveys impulses from the hippocampus and amygdaloid formations to the anterior and ventromedial hypothalamus. The stria terminals sends its major imputs into the medial hypothalamus and is named the medial cortical hypothalamic tract. Additionally, the hypothalamus receives afferent fibers from the fornix, the thalamus and the mammillary peduncle.

Three major efferent systems originate in the hypothalamus. These are (1) an ascending tract to the basal forebrain area, (2) a descending tract to the brain stem which controls autonomic functions, and (3) the hypothalamo-hypophysical tract which transports the neural peptides of the posterior pituitary from their site of production in the hypothalamus to the posterior pituitary where they are stored and released.

Pituitary Anatomy

The pituitary gland is attached to the most ventral portion of the diencephalon, the hypothalamus, by the infundibular or hypopohysial stalk. The hypophysial stalk consists primarily of (1) blood vessels which communicate between the hypothalamus and pituitary gland (2) nerve tracts which transport oxytocin and antidiuretic hormone from their sites of production in the paraventricular nucleus and supraoptic nucleus, respectively, to the posterior pituitary via the hypothalamo-hypophyseal tract, and (3) structural elements consisting of pituicytes and connective tissue.

The pituitary gland (hypophysis) is composed of primarily two types of tissue. The anterior pituitary (adenohypophysis; pars distalis; AP), and the intermediate lobes (pars intermedia) are composed of glandular epithelial tissue. The posterior pituitary (neural hypophysis; pars nervosa, PP) is composed of axons, terminal buttons, and glial cells (pituicytes) (Bucy, 1932). Both the AP and the PP are enclosed in a fossa within the sphenoid bone, the sella turcica, (Atwell, 1926) and are covered by a specialized extension of the dura mater, the diaphragma sella.

The AP is formed by an evagination of the buccal ectoderm, Rathke's pouch, whereas the PP is formed by a diverticulum of the third cerebral ventricle. As these structures converge the AP envelops the PP near the hypothalamus forming the tuber cinereum (see Netter, 1974).

Histological examination of the AP discloses 3 types of epithelial tissue (1) 35% acidophils (2) 15% basophils, and (3) 50% chromophobes (see Purves and Griesbach, 1956; Netter, 1974; Baker, 1974; and Rodin, 1974). The acidophils secrete the protein hormones; growth hormone (GH), and prolactin (PRL). The glycoproteins which consist of luteinizing hormone (LH), thyrotropin (TSH), and follicle stimulating hormone (FSH) are produced by the basophils. Additionally, the polypeptide hormone adrenocorticotropin (ACTH) from the AP and β melanocyte stimulating hormone (MSH) from the intermediate lobe are believed to be produced by the basophilis (see Netter, 1974; Rodin, 1974; and Baker, 1974). Recently these cells have been characterized by immunohistochemical staining techniques (see Nakame, 1970).

The AP is perfused by the inferior hypophyseal artery which branches from the posterior communicating artery of the Circle of Willis, whereas the PP is perfused by the superior hypophysial artery which arises from the internal carotid artery. Popa and Fielding (1930, 1933) first observed the hypophysial vasculature to be a true portal system which connected the capillary bed in the median eminence with the sinusoids of the AP. However, Popa and Fielding surmised that the blood flowed from the pituitary to the hypothalamus. Similar observations were made in living amphibians by Houssay et al. (1935), except they reported that the blood flowed from the hypothalamus to the pituitary. One year later, Wislocki and co-workers (1936, 1937, 1938)

using systemic injected dyes, confirmed the observations of Houssay in mammalian animals. Furthermore, these results were confirmed by Green and Harris (1947, 1949) in the rat, by Torok (1954) in the dog, and by Worthington (1955) in mice. Recently, Page et al.(1976) and Oliver et al.(1977) observed that a small percentage of the portal blood ascends from the subependymal plexus through the pituitary and perfuses the medial basal hypothalamus, supporting at least part of the early observation of Popa and Fielding (1930, 1933) that the blood flowed up the pitaitary stalk.

Closer examination of the hypophysial portal system reveals two distinct types of portal vessels; long portal vessels which arise from the capillary plexus of the median eminence which traverses the anterio-lateral portion of the infundibular stalk, and short portal vessels which arise toward the caudal infundibular region (see Netter, 1974; Adams et al. 1963, 1965; Daniel et al., 1956, 1966).

II. Hypothalamic Hormones and Neurotransmitters

Hypothalamic Releasing Factors

In the late 1930's several investigators postulated that the neurons of the central nervous system secreted substances into the hypophysial portal circulation (Hensey, 1937) or directly into the general circulation (Scharrer and Scharrer, 1940). This hypothesis was further examined by Bargman and Scharrer (1951) and Scharrer and Scharrer (1954) who demonstrated that the cell bodies in the paraventricular and supraoptic nuclei synthesized oxytocin and antidiurectic hormone, respectively. These hormones are transported down the axons of the hypothalamo-hypophysial tract and are stored in the terminal buttons located in the posterior pituitary. This neurosecretory hypothesis was confirmed and widely accepted.

Harris (1947) suggested that the AP was controlled by endocrine-like substances produced in the hypothalamus, secreted into the hypophysial portal circulation and transported to the AP where they controlled the release of AP hormones. This "chemotransmitter hypothesis" was formulated entirely on the anatomical relationships observed between the pituitary and hypothalamus.

Inasmuch as the "chemotransmitter hypothesis" was formulated to explain the control of AP function, many questions remained unanswered. First, investigators had to determine whether the hypothalamus produced hormone-like

substances to modulate pituitary function. Working independently, Saffran and Schally (1955), and Guillemin and Rosenberg (1955) demonstrated that hypothalamic extracts in vitro caused adrenocorticotropin (ACTH) release from the AP as measured by the adrenal ascorbic acid depletion assay. Further in vitro experiments revealed that there were other active factors in the hypothalamus which released thyroid stimulating hormone (TSH) (Shibusawa et al., 1956), luteinizing hormone (LH) (McCann et al., 1960), prolactin (PRL) (Meites et al., 1960), growth hormone (GH) (Franz et al., 1962, Deuben and Meites, 1964, 1965; Krulich et al., 1965), and follicle stimulating hormone (FSH) (Mittler and Meites, 1964, Igarashi and McCann, 1964). Additionally, 2 hypothalamic release inhibiting factors were demonstrated for PRL (Pasteel, 1961: Talwalker et al., 1961, 1963) and GH (Krulich et al., 1968).

Many attempts have been made to isolate and characterize the releasing and inhibiting factors. However the relative success in this area has been limited. Corticotrophin releasing factor (CRF) was first hypothalamic hypophysiotropic hormone located in hypothalamic tissue (Saffran and Schally, 1955; Saffran et al., 1955; Guillemin et al., 1957). Saffran and Schally named this active principle a "releasing factor" because the compound was a chemically uncharacterized hypophysiotropic agent that released ACTH. The term "releasing hormone" applies only to those agents which have been fully characterized. Although this was the

first hypothalamic hormone to be isolated, and physiological significance has been attributed to this "factor", the chemical structure of the hormone has not been determined to date. Royce and Sayers (1958) partially purified CRF and in 1964, Schally and Bowers proposed that the structure of CRF was a tridecapeptide. Additionally an extrahypothalamic or tissue CRF was identified and was believed to function under severe stress (Witorsch and Brodish, 1972; Lymangrover and Brodish, 1973.)

The second hypothalamic factor, luteinizing hormone releasing factor (LHRH), was first identified by McCann et al. (1960). Eleven years later, Schally and colleqgues reported the structure of LRF (Matso et al., 1971a,b; and Schally et al., 1971a). The structure of luteininzing hormone releasing hormone (LHRH) was determined to be a decapeptide with the following amino acid sequence: Pyro-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. LHRH not only stimulated the release of LH from the pituitary but also FSH in vivo and in vitro (Schally et al., 1971b). Therefore this releasing hormone also was named gonadotropin releasing hormone (GnRH) by some. However, the existence of a single GnRH does ont preclude the possibility of an individual LHRH and FSHRH which remain to be identified.

Also in 1960, a prolactin releasing factor (PRF) was identified by Meites et al. PRF appears to be a peptide in nature and is separate from thyrotropin releasing hormone which has been reported to release PRL in addition to TSH (Boyd et al., 1976).

TRF was first reported to be present in many tissues and urine in 1956 by Shibushawa et al. and later was claimed to be confirmed by Schreiber et al. (1961) who used a non-specific TSH assay. Schally et al. (1966) first reported that TRF from porcine hypothalami contained three amino acids: glutamic acid, proline and histidine in equimolar concentration. In 1969, Burgus et. al., Folkers et. al., and Boler et. al. reported the sequence of these amino acids in the tripeptide hormone, which appears to be identical in all species tested. Even though releasing hormones were originally thought to release only one AP hormone, TRH has been shown to release PRL in vivo and in vitro (Jacobs et al., 1971; Bowers et. al., 1971; Convey et. al., 1972, 1973; Mueller et. al., 1973; Takahara et. al., 1974a,b; Dibbet et. al., 1974; Smith and Convey, 1975). TRH also has been demonstrated to release GH in acromegalic human subjects (Faglia et. al., 1973; Liuzzi et. al., 1974) and in male and female rats (Panerai et. al., 1977; and Ojeda et. al., 1977).

The existence of GH inhibiting factor (GIF) or somatostatin was first reported by Krulich et. al. in 1968. In 1973, Brazeau and Guillemin isolated and characterized GIF which they renamed somatostatin. Somatostatin is a tetradecapeptide containing the following amino acid sequence: H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thy-Phe-Thr-Sey-Cys-OH, either in a cyclic or linear conformation. Somatostatin decreased GH secretion in vivo and in vitro (for review see Vale, 1975 and Martin, 1976). However, somatostatin not only

decreased GH but also decreased TRH induced TSH release (Hall et. al., 1973; Yen et. al., 1974).

In addition to its high concentrations in the hypothalamus, somatostatin also is located in the D-cells of the pancreas (Dubois, 1975). The functional significance of somatostatin in the pancreas is not clear. Somatostatin inhibits both glucagon from the α-cells and insulin from the β-cells of the pancreas (Gerich et. al., 1974; Efendic et. al., 1976). These observations offer the possible use of somatostatin as a potential therapeutic agent in controlling diabetes mellitus, since somatostatin is approximately 20 times as effective in suppressing glucagon as compared to insulin (Gerich et. al., 1971a, b). Somatostatin also inhibited gastrin production by the human stomach (Bloom et. al., 1974). (Further discussion under Somatostatin.)

Aside from TRH, GnRH and somatostatin, the remaining releasing or inhibiting factors have not been chemically characterized. These include the prolactin inhibiting and releasing factors, growth hormone releasing factor, melancyte stimulating hormone releasing and inhibiting factors, or separate releasing factors for LH and FSH.

Putative Hypothalamic Neurotransmitters

Chemically active substances which mediate the transmission of nerve impulses across synapses, are neurotransmitters. I will refer to these chemical substances as putative neurotransmitters (PN) because all criteria necessary

for a substance to be classified as a neurotransmitter have not been fulfilled. These criteria are:

1) The chemical must be released by the presynaptic nerve ending upon appropriate stimulation. After release, the PN must be chemically identified.

2) The transmitter must be localized to the synapse and be present in the synaptic cleft after stimulation.

3) The PN when applied microiontophoretically must mimic the post-synaptic response to stimulation of the pre-synaptic neuron.

4) The post-synaptic membrane potential should elicite similar responses when agonists to the PN are applied.

5) The postsynaptic membrane should be inhibited when blocking agents are applied (Cooper et al., 1974).

The putative neurotransmitters to which I will limit my discussion are: the catecholamines, dopamine (DA) and norepinephrine (NE); the indolamine, serotonin (5HT); the neurally active amino acid γ-amino-butyric acid (GABA); the neurally active peptides, β-endorphin and methionine-enkephalin; and acetylcholine.

Norepinephrine (NE)

NE was first demonstrated to be present in the hypothalamus of dogs and cats (Bogt, 1954). Later, using a histofluorescent technique (Falck et. al., 1962), NE was found in high concentrations in the anterior hypothalamus and the internal layer of the median eminence of rats (Carlsson et. al.,

1962; Dahlstrom and Fuxe, 1964, 1965). Additionally, small quantities of NE are found in the posterior hypothalamus (Hökfelt et. al., 1978). Employing the same technique, Ungerstedt (1971) showed that the noradrenergic cell bodies were localized in the locus coeruleus (also Anden et al., 1966a; Kobayashi et al., 1974), and mesencephalic reticular formation. These nuclei send their axons into the medial forebrain bundle which innervates the paraventricular nucleus, pre- and supra-optic nuclei and the ventromedial-arcuate complex (Ungerstedt, 1971).

Two additional techniques which provided valuable knowledge regarding the localization of hypothalamic NE were: the micro-punch technique (Palkovits, 1973) and a sensitive radioenzymatic assay for DA and NE (Cuello et al., 1973; Coyle and Henry, 1973). Palkovits et al. (1974a) found NE unevenly distributed throughout the hypothalamus. The anterior and medial basal hypothalamus contained the highest concentrations of NE.

Hypothalamic NE appears to be derived entirely from extra hypothalamic noradrenergic cell bodies. Hypothalamic deafferentation results in a dramatic reduction in hypothalamic NE and total loss of dopamine - β -hydroxylase, the enzyme which converts DA to NE (Brownstein et al., 1976). However, Iversen (1974) found the hypothalamus to contain small concentrations of NE in neuroglia which are refractory to extrahypothalamic lesions.

Dopamine

The central dopamine stores are different from those of norepinephrine (Carlsson, 1959). The dopaminergic system is divided into three separate distinct systems. First the nigro-striatal dopaminergic system which arises from the midbrain and terminates in the basal ganglia (Andén et. al., 1964, 1965; Hökfelt and Ungerstedt, 1969). The second dopaminergic system also originates in the midbrain and terminates in the olfactory tubercles and nucleus acumbens (Andén, 1966b; Ungerstedt, 1971). The final dopaminergic system is confined almost entirely to the hypothalamus and is referred to as the tuberoinfundibular DA system (Fuxe and Hökfelt, 1966; Weiner et. al., 1972).

The hypothalamic dopaminergic cell bodies consist of two major gourps: a dorsal gourp, and a ventral group (Björklund et. al., 1975a; Hökfelt et. al., 1978). Both nuclei lie lateral to the third ventricle. However, the dorsal nuclear group has not been implicated in endocrine function. Total deafferentation did not result in any detectable change in the hypothalamic DA content (Weiner et. al., 1972) indicating this DA system was confined to the hypothalamus. The ventral tuberoinfundibular system arises from the arcuate nucleus (A12) and terminates primarily in the external layer of the median eminence (Fuxe, 1963; Palkovits et. al., 1974b, Browstein et. al., 1974; Hökfelt et. al., 1978).

Serotonin (5-HT)

Regional distribution of 5-HT was first reported in the dog (Amin et. al., 1954). The hypothalamus, mid-rain, cerebellum and limbic system contained varying concentrations of 5-HT (Welsch, 1969). Histofluorescence of the hypothalamus disclosed that the suprachiasmatic nucleus contained the highest concentration of 5-HT (Fuxe, 1965; Loizou, 1972). More recently, Saavedra et. al. (1974) employing the microdisection method (Palkovits, 1973) confirmed this observation. In addition to the suprachiasmatic nucleus, they found high concentrations of 5-HT in the median eminence and arcuate nucleus. Several other hypothalamic nuclei contained minuscule concentrations of 5-HT. The hypothalamus receives its major 5-HT input from the pontine raphae nucleus in the brainstem. These axons unite with the medial forebrain bundle to supply 5-HT to the hypothalamus and median eminence (Dahlstrom et. al., 1964, Ungerstedt, 1971; Baumgarten, 1972). Baumgarten et. al. (1974) also observed 5-HT terminals in the median eminence using electron microscope and histofluorescence methods.

γ-Aminobutyric acid (GABA)

Like the monoamines, GABA is widely and unevenly distributed in the mammalian central nervous system. In the rat, GABA is present in high concentrations in the substantia nigra, basal ganglia, hypothalamus and brainstem (10-15 μmoles/g brain tissue, Okada et. al., 1971). Similarly, high

concentrations were found in these structures in humans (Perry et. al., 1971) and the rhesus monkey (Fahn et. al., 1968; Cote et. al., 1969). The relative neural concentrations of GABA correlate well with the enzyme glutamate decarboxylase (GAD), the major anabolic enzyme in GABA synthesis (Müller et. al., 1962; McGeer et. al., 1971) (See Figure 1). Likewise, GABA-transaminase (GABA-T) has been shown to be present in the hypothalamus (Salvador et. al., 1959; Sheridan et. al., 1967).

GABA was detected in high concentrations in the lateral hypothalamus and ventral medial nucleus of the hypothalamus (Kimura and Kuriyama, 1975). GAD was shown to be present in the anterior hypothalamus, suprachiasmatic nucleus, paraventricular nucleus and dorsomedial nucleus (Tappaz et. al., 1976). Positive GAD terminals also are found in the internal and external layers of the median eminence which appear to have their origin within the hypothalamus (Hökfelt et. al., 1978). Nonetheless, this does not account for all the GABA contained within the hypothalamus. Therefore the hypothalamus must receive extra-hypothalamic GABA-ergic innervation.

Endorphins and Enkephalins

Recently, two classes of morphinomimetic peptides have been isolated from the pituitary and brain tissue. The first of these were methionine and leucine enkephalin (Hughes et. al., 1975). Methionine-enkephalin (Met-Enk) is in much higher concentrations in the rat brain than leucine enkephalin

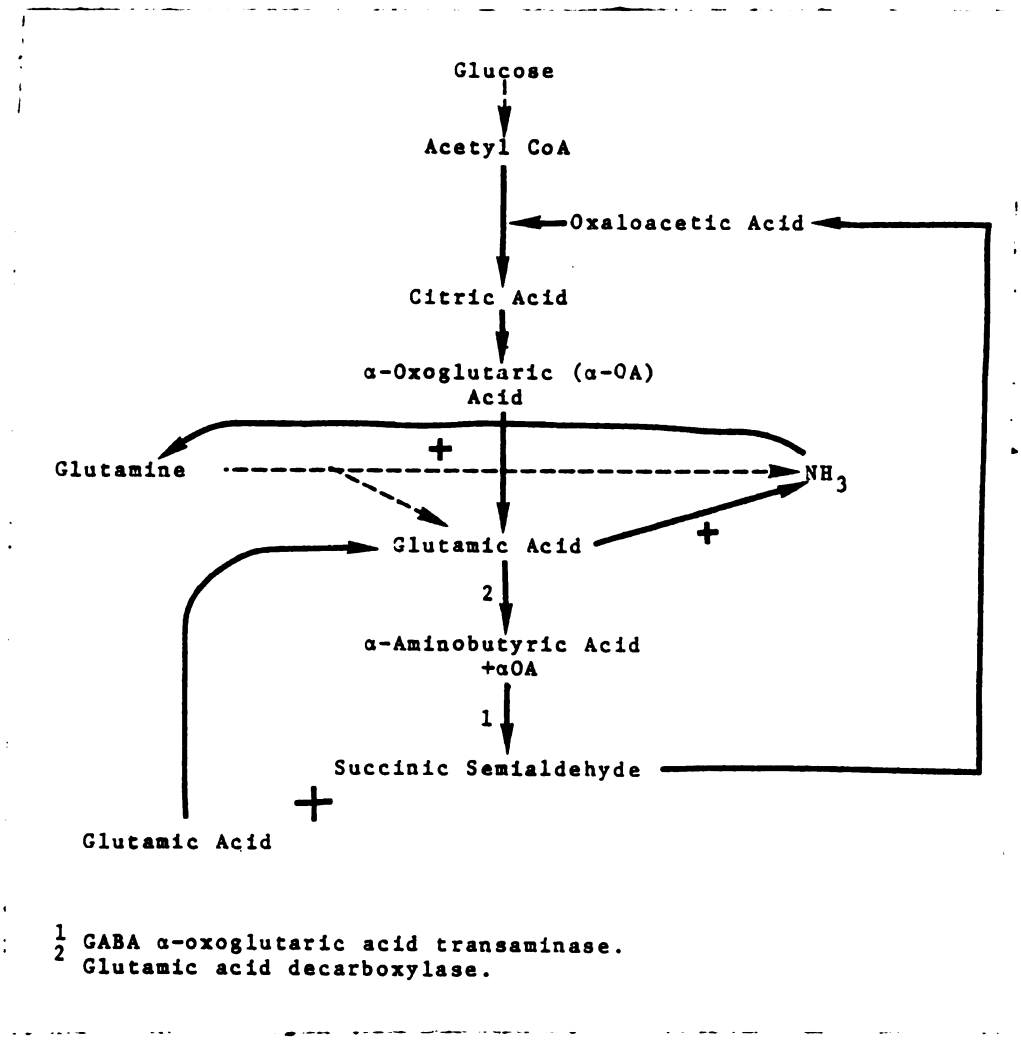

Figure 1. Metabolic pathway of gamma-aminobutyric acid.

(Leu-Enk) (Yang et. al., 1977). Using autoradiography, immunohistochemistry and the radioreceptor assay, the distribution of the enkephalins in the CNS paralleled the relative distribution of the opiate receptors. (see Pert et. al., 1976; Simantov et. al., 1976, 1977; Johansson et. al., 1978). The enkephalins are contained in several hypothalamic nuclei involved in neuroendocrine regulation. Among these are: the preoptic, paraventricular, ventromedial, and arcuate nuclei (see Elde and Hökfelt, 1978). Many enkephalin positive terminals were also found in other areas of the brain (Pert et. al., 1976; Simantov et. al., 1977; Atweh et. al., 1977a,b).

The second class of morphinomimetic peptides was isolated from the camel pituitary (Cox et. al., 1976). This compound was referred to as β-endorphin or C-fragment. Closer examination of the sequence of β-endorphin revealed that it contained the same amino acid sequence, 61 to 91, of β-lipotropin. This raised the question whether β-lipotropin was a prohormone for β-endorphin. Concurrently, Lazarus et. al. (1976) isolated α-, β - and γ-endorphin which also possess morphinomimetic properties, all which were contained in the β-lipotropin molecule. High concentrations of β-endorphin was also reported to be present in the hypothalamus (490 ng/g), septum (234 ng/g), and the midbrain (207 ng/g) (see Bloom et. al., 1978). To my knowledge, there have been no reports regarding the precise location of β-endorphin in the hypothalamus.

Acetylcholine (Ach)

Inasmuch as Ach was one of the first neurotransmitter discovered, the distribution of cholinergic neurons has not been well documented. Early observations suggested that neural concentrations of Ach should parallel the concentrations of acetylcholinesterases (ACE), the major catabolic enzyme for Ach (Shute and Lewis, 1961, 1967, 1969). Some of the cholinergic nerve tracts, like those of DA, appear to be contained entirely within the hypothalamus (Brownstein et. al., 1976), since hypothalamic deafferentation does not alter choline acetyltransferase in the median eminence. Intrahypothalamic cholinergic nerve tracts arise from the preoptic area, and amygdala and extend to the supraoptic nucleus (Shute and Lewis, 1967).

Since acetylcholine cannot be measured at the cellular level (McGeer et. al., 1974), the relative distribution of Ach has been correlated with the distribution of the major catabolic enzyme, acetylcholinesterase (ACE) or the major anabolic enzyme choline acetyltransferase (CAT) (see Figure 2). CAT has been measured in the external and internal layers of the median eminence (Kizer et. al., 1976 a,b; Brownstein et. al., 1975). Similarly, ACE has been reported to be present in high concentrations in the posterior hypothalamic nucleus, (Uchimura et. al., 1975), the supraoptic nucleus, the pre-optic area, and the medial forebrain bundle (Palkovits and Jacobowitcz, 1974). Criteria for the cholinergic system in the hypothalamus have been delineated by Hebb et. al., (1970).

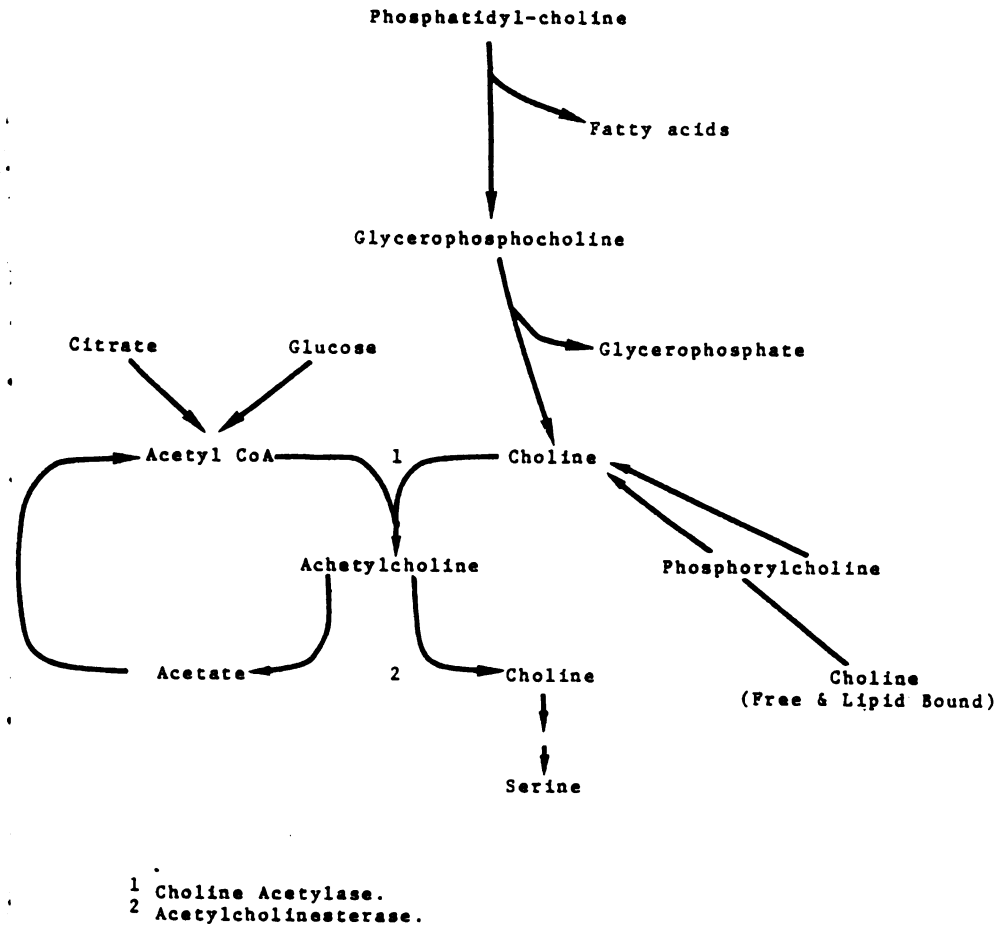

Figure 2. Metabolic pathway for acetylcholine.

III. Control of GH Secretion

Growth Hormone Releasing Factor

Armstrong and Durh (1922) observed that infundibular and hypophysial tumors resulted in growth cessation in human patients. Subsequently other investigators tried different approaches to prove that pituitary growth hormone was controlled by the hypothalamus. Cahane and Cahane (1938) lesioned the hypothalamus; Uotila (1939) sectioned the infundibular stalk and pituitaries were transplanted from their *in situ* location to an ectopic site (Greep, 1936; Goldberg and Knobil, 1957) to assess whether the hypothalamus controlled growth hormone (GH) secretion. In all of these experiments and others, there was either a cessation or reduction in body growth in rats. In view of these results and the observations of the portal circulation (Wistocki *et. al.*, 1937, 1938), and the chemotransmitter hypothesis (Green and Harris, 1949) the presumption was made that the hypothalamus must contain a growth hormone releasing factor (GHRF).

The first conclusive evidence that the hypothalamus contained a GHRF was reported by Deuben and Meites (1964, 1965). Using neutralized acid extracts of rat hypothalamus in a pituitary incubation system, they reported an approximate 5-fold increase in GH release from a 6-day pituitary culture. Cerebral cortical extracts failed to alter GH release from pituitary culture. Thus the hypothalamus was believed to contain GHRF. These *in vitro* results were confirmed *in vitro* and *in vivo* (Dhariwal *et. al.*, 1965, 1965/66; Ishida *et. al.*,

et. al., 1977), the cow (Convey et. al., 1973; Tucker et. al., 1975), and in human patients with acromegaly (Irie et. al., 1972), renal failure (Gonzalez-Barcena et. al., 1973), and depression (Maeda et. al., 1975). These effects apparently resulted from a direct action of TRH on the AP (Underschini et. al., 1976; Pamerai et. al., 1977). TRH may have mediated its effects through cyclic adenosine monophosphate (C-AMP) (Dannies et. al., 1976) or some other mechanism (Hinkle et. al., 1977).

Growth Hormone Release Inhibiting Hormone - Somatostatin

While trying to isolate GHRF, Krulich et. al. with the use of chromatography, (1972) partially purified a peptide (GIF) that inhibited pituitary GH release in vitro. One year later, Brazeau and co-workers (1973) isolated and characterized somatostatin (GIF) from ovine hypothalami as a tetradecapeptide. After the isolation and characterization of GIF, the hypothalamic localization of the substance became of interest. In 1975, Arimura et. al. developed a radioimmunoassay for GIF which would enable investigators to measure somatostatin by immunohistochemistry and radio-immunoassay in various hypothalamic regions. Radioimmuno-assay of somatostatin revealed that the median eminence contained the highest concentration of GIF in the hypothalamus (Brownstein et. al., 1975). High concentrations of GIF also were found in the ventromedial nucleus, arcuate nucleus, paraventricular nucleus, and to a lessor extent, in other hypothalamic areas (Brownstein et. al., 1975b; Palkovits

1965; Krulich et. al., 1965; Schally et. al., 1968; Dickerman et. al., 1969b; Sawano et. al., 1968; as measured by tibia test for GH (Greenspan et. al., 1949). However, when crude hypothalamic extract was injected into animals or incubated with pituitaries there was no change in radioimmunoassayable GH (Daughaday et. al., 1968; Schalach and Reichlin, 1966). These discrepancies in radioimmunoassayable and bioassayable GH remain unclear to date. GHRF was purified from ovine hypothalami (Krulich, et. al., 1965), porcine and bovine hypothalami (Ishida et. al., 1965; Schally et. al., 1965, 1966, 1969). A GHRF was decapeptide was isolated which stimulated bioassayable, but not radioimmunoassayable GH. Later it was found to be a portion of the β-chain of hemoglobin, which was considered to be an artifact of extraction procedures (see Martin et. al., 1977).

Vasopressin also has been demonstrated to release GH in the rat (Arimura et. al., 1967; Malacara et. al., 1972; Undeschini et. al., 1976), in the monkey (Meyer and Knobil, 1966, 1967; Krey et. al., 1975), and in the human (Greenwood and Landon, 1966; Müller et. al., 1967; Heidingsfelder and Blackard, 1968). The effects of vasopressin (antidiuretic hormone - ADH) may be mediated directly on the pituitary since rats bearing an ectopic pituitary under the kidney capsule released more GH when injected with ADH than their hypophysectomized controls (Undeschini et. al., 1976). Additionally TRH was reported to stimulate GH in the rat (Takahara et. al., 1974b; Kato et. al., 1975; Chihara et. al., 1976a, b; Undeschini et. al., 1976; Ojeda et. al., 1977; Panerai

et. al., 1976).

Immunohistochemical examination of the rat hypothalamus revealed that GIF was distributed in neuronal synaptsomes (Pelletier et. al., 1976; Styne et. al., 1977), and cell bodies (Elde et. al., 1975; Hökfelt et. al., 1976). GIF was located in high concentrations in the anterior hypothalamus; more specifically, the preoptic, anterior hypothalamus, and anterior ventromedial nucleus (Alpert et. al., 1976). Additionally, GIF was localized in very high concentrations in the median eminence and tuberoinfundibular stalk (Pelletier et. al., 1974, 1975a; Setalo et. al., 1975). In the guinea pig, no somatostatin was present in the hippocampus or parietal cortex (Hökfelt et. al., 1974), but was measured in high concentrations in the hypothalamus.

GIF is not confined to the limits of the CNS. GIF was reported to be present in the fetal (Dubois et. al., 1975a,b, 1976) and adult pancreas of man (Polak et. al., 1975; Dubois 1975 a,b; Pelletier et. al., 1975b; Orci et. al., 1976), and dog (Rufener et. al., 1975). The cells of the pancreas which contained GIF are different from the and cells which secrete glucagon and insulin. These cells were designated as D cells. Immunohistochemical examination of the thyroid gland (Parsons et. al., 1976) and gastrointestinal tract (Polak et. al., 1975; Rufener et. al., 1975, and Dubois et. al., 1976) showed GIF containing cells in these organs also.

The first reported physiological action of GIF was

depression of GH *in vivo* and *in vitro* (Brazeau *et. al.*, 1973; Belanger *et. al.*, 1974; Stachura, 1976). Treatment of rats with an anti-somatostatin elevated serum GH concentrations without changing the intervals of episodic release (Ferland *et. al.*, 1976; Arimura and Schally, 1976). Antisera to GIF also prevented the stress induced decrease in serum GH concentrations in rats (Arimura *et. al.*, 1976). GIF inhibited the insulin induced hypoglycemia stimulation of serum GH in human patients (Hall *et. al.*, 1973). These results suggest that GIF is a physiological regulator of GH release and synthesis.

Electrical stimulation of the ventromedial nucleus and the basolateral amygdala resulted in an increase in serum GH concentrations. This increase was prevented by prior injections of GIF in the rat (Martin, 1974). The effects of morphine on GH release were also prevented by concurrent injections of GIF (Martin *et. al.*, 1975). Similarly GIF prevented the increase in serum GH concentrations due to pentobarbital anesthesia in rats (Brazeau *et. al.*, 1974). The insulin (Hall *et. al.*, 1973), and TRH (Carlson *et. al.*, 1974) induced increase in serum GH concentrations was also attenuated by prior administration of GIF. Other effects of GIF are well documented (see Martin *et. al.*, 1977).

The effects of GIF on the pituitary are not limited to the regulation of GH secretion. GIF inhibited ACTH in human subjects with hypersecretion of ACTH (Nelson's syndrome, Tyrrell *et. al.*, 1975). GIF also inhibited TRH induced TSH secretion without altering basal serum concentrations of TSH

or prolactin concentrations (see Martin et al., 1977).

Inasmuch as the highest concentrations of GIF are located in the hypothalamus and gastrointestinal tract, many investigators believed that GIF may participate in the regulation of blood glucose. GIF was reported to inhibit glucagon and insulin secretion from the pancreas of cats (Koerker et al., 1974), rats (Koerker et al., 1974; Orci et al., 1976; Brown et al., 1976), and human subjects (Yen et al., 1974; Gerich et al., 1975 a,b). Presumably, GIF inhibited the actions of glucagon on liver by inhibiting C-AMP accumulation (Oliver et al., 1976; Vinicor et al., 1977), which was similar to the effects of GIF on pituitary cyclic nucleotides (Kaneko et al., 1973; or Boss et al., 1975). These studies revealed a potential application of somatostatin as an adjunct treatment with insulin in control of diabetis mellitus in juvenile and maturity onset diabetes.

Noradrenergic Control of GH

The most widely accepted hypothesis for the control of GH regulation is that which has been adopted through manipulation of brain neurotransmitters. Generally, neurotransmitter substances which enhance GH release, either stimulate the release of GRF or inhibit the secretion of GIF. However, GRF has not been isolated, and to my knowledge, measurement of GIF concentrations or turnover under various neuropharmacological manipulation has not been pursued to date.

GH appears to be under direct stimulatory action

of the central noradrenergic pathways. Several methods have been employed to study the role of NE in the regulation of GH in primate and sub-primate species. L-dopa, the immediate precursor to DA which is converted to NE by DA-β-hydroxylase, stimulates GH release in rats (Chen et. al., 1974) and in human patients (Martin, 1972). The effects of l-dopa appeared to be mediated through the α-adrenergic receptor because the effects of l-dopa were partially prevented by the α-adrenergic blocking agent, phentolamine (in rats, Martin et. al., 1977, Kato et. al, 1973; in humans, Liuzz et. al., 1971; Heidingsfelder et. al., 1968) in baboons (Toivala et. al., 1971). Sheep responded opposite compared to rats to α-receptor blocker phenoxybenzamine with an increase of serum PRL and GH concentrations (Davis and Borger, 1973).

NE has opposite effects in urethane anesthetized rats (Kato et. al., 1973 Collu et. al., 1972). NE decreased serum GH and this decrease was inhibited by prior administration of the β-receptor blocker propranalol. There has long been this decrepency between urethane anesthetized rats and rats handled under ether, pentobarbital or merely through sampling without anesthesia (Martin, 1976).

The β-adrenergic receptor appears to have little or no effect on GH secretion (Bruni, Ph.D. thesis). The secretion of GH via adrenergic agents appears to correlate well with the diurnal episodes of GH release. A good correlation appears to exist between central adrenergic and serotonergic activity and other neural transmitters modulating this effect

(J.B. Martin, personal communication). The direct effect of NE on GH release may be excuded since NE does not stimulate the pituitary directly (MacLeod, 1969; MacLeod et. al., 1970, (Bruni, Ph.D. thesis) but requires the presence of the hypothalamus (Bruni, Ph.D. thesis).

Dopaminergic Control of GH

The effects of DA and DA precursors on serum GH in human subjects and in rats had been somewhat of a mystery until the past several years. Several methods have been used to determine the effects of DA on GH release in humans and in animals. One method of administration of monoamine precursors in humans to treat depression (Carrol, 1971). After injections of L-dopa or tyrosine there is a marked increase in DA and NE in the brain. Another method is injection of apomorphine, a DA receptor stimulator (Anden et. al., 1967), or piribedil, another DA agonist.

In general, DA agonists and precursors stimulate GH release in normal human patients. L-dopa the immediate precursor to DA stimulated GH release in normal human patients (Boyd et. al., 1970; Parlow et. al., 1972; Kansal et. al., 1972; Millar et. al., 1973; Silver et. al., 1974), and Parkinsonian patients (Parlow et. al., 1972). However, in patients with pituitary insufficiency, l-dopa stimulated GH to a lesser degree (Laron et. al., 1973). Apomorphine, a DA agonist, similarly elevated serum GH in human patients (Lal et. al., 1972; Brown et. al., 1973; Maany et. al., 1975;

Nilsson, 1975). However, in patients with acromegaly (Cryer and Daughaday, 1974; Ghiodini et. al., 1974), Huntington's Chorea (Podolsky and Leopold, 1974) or obesity (Fingerhat and Krieger, 1974), l-dopa decreased serum GH concentrations. The reason for this pathological discrepency is not known. DA could possible be affected by other neurotransmitters like GABA or Ach (Perry et. al., 1973; McGeer et. al., 1973; Bird and Iverson, 1974).

Injections of DA into the lateral ventricle of rats induced a depletion in pituitary GH content indicating that DA stimulated GH release in the rat (Müller et. al., 1968). Similarly, systemic injection of l-dopa (Chen et. al., 1974; Smythe et. al., 1975; Bruni, Ph.D. thesis) increased serum GH in rats, dogs (Lovinger et. al., 1974), and monkeys (Chambers and Brown, 1976). Other workers have reported either no change (Kato et. al., 1973) or inhibition (Müller et. al., 1973) of GH release in urethane anethetized rats. Martin (1976) attributes these differences to the methods of anesthesia.

Two other methods have been used to clarify these discrepencies. Mueller et. al. (1976), showed that injections of apomorphine or peribedil increased serum GH in unanesthetized rats. This increase was prevented by prior administration of haloperidol, a DA receptor blocker. Similarly, Willoughby et. al. (1977) partially prevented the episodic release of GH by butaclamol, another DA receptor blocker. Simon and George (1975) observed that the diurnal variation in brain dopamine concentrations correlated with changes in

serum GH concentrations.

From these results one may conclude that DA stimulates GH release in rats, dogs, monkeys and humans. The mechanism whereby DA stimulated GH release appears to lie within the hypothalamic since DA does not act directly on the pituitary to stimulate GH release or synthesis (MacLeod, 1969; MacLeod et. al., 1970).

Serotonergic Control of GH

The role of the indoleamine, 5-HT, in the control of GH secretion is less conclusive than that of DA and NE. Several experimental models have been used to determine the role of 5-HT in control of GH Collu et. al. (1972) reported that intraventricular injections of 5HT into urethane anesthetized rats dramatically increased serum GH concentrations. This increase was completely prevented by prior injection of phenoxybenzamine, which blocks the α-adrenergic receptor, as well as the serotonergic receptor. Similarly, systemic injections of 5-hydroxytryptophan (5HTP), the immediate precursor to 5-HT, increased serum GH concentrations in unanesthetized rats, (Smythe and Lazarus, 1973). The release of GH due to 5HTP injections was inhibited by prior injection of cyproheptadine, a proposed 5-HT antagonist (Smythe et. al., 1975) or methysergide (Meites et. al., 1977, Bruni, Ph.D. thesis).

In contrast to these results, Müller et. al. (1973) showed that intraventricular injections of 5-HT significantly decreased serum GH concentrations in rats. Systemic injections of 5HTP

which elevated hypothalamic 5-HT did not alter serum GH concentrations (Müller et al., 1973). However, systemic injections of para-chloroamphetamine, a drug which decreases brain serotonin, increased serum GH, indicating that the serotonergic system inhibits GH in the rat.

In the monkey (Chambers and Brown, 1976), parenteral injections of 5HTP increased serum GH concentrations. Tryptophan (Müller et al., 1974) or 5HTP (Imura et al., 1973) increased serum GH concentrations in human subjects.

The discrepencies in the above mentioned results may be due to the lack of specificity of the drugs used and the time when blood samples were taken. Butcher et al. (1972) and Wurtman and Fernström (1972) showed that systemic injections of 5HTP resulted in an increase in hypothalamic serotonin. This increase, however, was not confined to serotonergic neurons. These investigators demonstrated that any neurons which contain l-aromatic amino acid decarboxylase (dopa decarboxylase) are capable of decarboxylating dopa to DA or 5HTP to 5-HT. 5-HT in a dopaminergic or adrenergic neuron will displace the endogenous neurotransmitter initially and later act as a false transmitter.

After specific neurotoxins were isolated, the effects of 5,7-dihydroxytryptamine (Baumgarten et al., 1972a, b; Baumgarten et al., 1974) on brain and hypothalamic 5HT content were studied. 5,7-dihydroxytryptamine when injected together with desmethylimipramine, resulted in a significant depletion of brain and hypothalamic 5HT. These drugs also inhibited the morning episodes of GH release without altering the

periodicity of GH release (Bruni, Ph.D. thesis). Also, methysergide attenuated the episode release of GH from the pituitary (Martin et al., 1978; Bruni, Ph.D. thesis).

Collectively, these results suggest that 5-HT is a positive modulator of GH secretion. Despite some of the earlier disagreement as to whether 5-HT is stimulatory or inhibitory to GH release, ablation of the serotonergic system results in reduced GH release. Therefore, one amay conclude that 5-HT is a stimulator of GH secretion.

Other Putative Neurotransmitters

Acetylcholine, GABA, met-enkephalin and β-endorphin all have been implicated in the control of pituitary function (see Müller et al., 1977). However, little information on the effects of these agents on the secretion of GH is yet available.

Paraoxon, an anti-cholinesterase, was reported to increase pituitary GH concentrations in rats (Cehovic et al., 1972). However Cehovic et al., (1972) did not mention whether paraoxon increased the release or synthesis of GH. Additionally, Soulairac et al. (1968) reported that injections of β-methylcholine, a cholinergic agonist, increased serum GH concentrations in humans. This study was not conclusive since he did not have adequate controls throughout his experiment. Ach pilocarpine, and physostigmine, all cholinergic agonists increased serum GH in rats, as will be shown by this thesis. These effects were inhibited by the muscarinic

receptor blocker, atropine. Atropine also inhibited the morning episodic release of GH (Bruni, Ph.D. thesis). These results suggest that the cholinergic system may participate in the physiological control of GH secretion. The effects of Ach appear to be mediated through a neural mechanism since Ach did not stimulate GH release directly from the piruitary but required the presence of hypothalamic tissue (Bruni, Ph.D. thesis).

GABA appears to inhibit GH secretion (Bruni et al., 1977a). Similarly the GABA agonist, aminooxyacetic acid, inhibited GH release. The GABA antagonists, bicuculline and picrotoxin, increased serum GH concentrations in vivo (Bruni et al. 1977a, Bruni, Ph.D. thesis). GABA did not act directly on the pituitary to inhibit GH secretion in vivo and in vitro, as will be shown.

The endogenous opioid peptides also appear to modulate pituitary hormone secretion (Bruni et al., 1977b). β-endorphin was reported to increase serum GH concentration in vivo (Rivier et al., 1977). Similarly met-enkephalin increased serum GH in vivo (Bruni et al. 1977b; Shaar et al., 1977). These effects probably require a neural mechanism since met-enkephalin did not act directly on the pituitary to alter GH release in vitro (Bruni, unpublished).

Hypothalamic and Extrahypothalamic Centers for Control of GH

The hypothalamic sites which primarily control GH secretion appear to be located in the medial basal hypothalamus. Isolation of the medial basal hypothalamus increased serum GH concentrations (Rice et al., 1976; Mitchell et al., 1973). The primary loci within the medial basal hypothalamus which control GH are the median eminence and ventromedial-arcuate complex. Stimulation of the median eminence in rats increased radioimmunoassayable serum GH concentrations in rats (Bernardis and Frohman, 1971) and inhibited GH release in sheep (Malven, 1975). The inhibition in sheep was attributed to an increase in secretion of GIF even though GIF concentrations were not measured. Stimulation of the ventromedial nucleus also resulted in elevated serum GH concentrations (Bernardis and Frohman, 1971; Martin, 1972). Lesions of the ventromedial nucleus decreased GH concentrations as measured by the tibia test (Bernardis et al. 1963; Frohman and Bernardis 1968) and body growth.

The hypothalamus receives afferent neurons from the amygdala, hippocampus lucus coeruleus; and raphae nucleus (see section I). The ventromedial nucleus receives afferent neurons from both the amygdala and hippocampal formations. The corticomedial and basolateral amygdala send efferent fibers to the ventromedial nucleus, whereas the hippocampus sends it efferent fibers to both the ventromedial nucleus and arcuate nucleus.

Lesions of the amygdala increased radioimmunoasayable GH in the rat (Newman et al., 1967). These observations were confirmed by Martin (1972, 1974). However, Martin reported that stimulation of the basolateral amygdala and mesencephalic interpeduncular nucleus increased plasma GH concentrations whereas stimulation of the cortiomedial amygdala inhibited plasma GH concentrations in the rat. The difference in corticomedial amygdala stimulation and basolateral amygdala stimulations are not clearly understood since both of these areas send efferent fibers to the ventromedial nucleus. Stimulation of the hippocampus also resulted in elevated plasma GH concentrations (Martin, 1972). These effects from hippocampal stimulation may have resulted from the hippocompal efferents which innervate the amygdala (Rosene and VanHoesen, 1977).

The extrahypothalamic structures which send efferent neurons to the hypothalamus may participate in the stress induced increase in serum GH in humans (Greenwood and Landon, 1966) and monkeys (Mason et al., 1974) and the decrease in serum GH in rats (Collu et al., 1973). Also these structures may be partially responsible for the episodic release of GH in all species thus far examined (see Martin, 1976). Before one may conclude that these structures are involved in the physiological control of GH, one must first examine the neuronal activity of these structures throughout the day and during stress and correlate the activity of these neurons with serum GH concentrations.

Shortloop Feedback of GH

Since GH does not have a specific target organ which produced another hormone to control its secretion, a "short-loop" negative feedback mechanism for GH was proposed by Voogt et al. (1971). Implantation of human GH pellets into the median eminence decreased anterior pituitary weight, and decreased serum and pituitary GH concentrations. These observations were confirmed by implanting GH into the hypothalamus or GH secreting tumors systemically (Brown and Reichlin, 1972). In human beings and monkeys, administration of GH, prior to arginine infusion or insulin induced hypoglycemia, prevented the increase in plasma GH due to these stimuli (in humans, Abrams et al. 1971; in monkeys, Sakuma and Knobil, 1970). The recent observation of Oliver et al., (1977) that pituitary hormones can be collected from the descending portal vessels in very high concentrations (2000-3000 ng/ml) supports the concept of a "short loop" feedback control of AP secretion. If GH is secreted by the AP and transported back into the hypothalamus to stimulate GIF and inhibit GHRF secretion, this may explain in part the episodic release of GH from the AP.

Hormone Effects on GH

GH is controlled in part by hormones from other endocrine organs. Among these are oxytocin, antidiuretic hormone (ADH), thyroid hormone, adrenal hormones, insulin, and gonadal steroids.

Oxytocin (Malacara et al., 1972) stimulated GH in estrogen-progesterone primed male rats. The other posterior pituitary hormone ADH was once thought to be GRF (see Growth Hormone Releasing Factor). Pitressin and lysine-vasopressin stimulated GH release in the rhesus monkey (Meyer and Knobil, 1966,1967). In monkeys which have had their medial basal hypothalamus deafferentated, ADH elicited a larger increase in plasma GH concentrations (Krey et al., 1975). Similarly in human patients (Greenwood and Landon, 1966; Heidingsfelder and Blackard, 1968) and in rats (Arimura et al., 1967), ADH increased serum GH concentrations.

Thyroidectomy in rats resulted in growth retardation (Koneff et al., 1949) and a decrease in pituitary GH concentrations (Knigge 1958; Soloman and Greep 1950; Meites and Fiel 1967). This decrease in pituitary GH may have resulted from several effects of thyroid hormone. Purves and Griesbach (1956) observed that thyroidectomy caused degranulation of pituitary acidophils. This degranulation may be reversed by either thyroxine or cortisol (Meyer and Evans, 1964). Meites and Fiel (1967) demonstrated that thyroidectomy resulted in a decreased concentration of hypothalamic GHRF which could be replenished by thyroxine therapy. Presumably, pituitary GH concentration was decreased after thyroidectomy (Lewis et al., 1968; Hervas et al., 1975) or propylthiouracil treatment (Daughady et al., 1968; Peake et al., 1973). In vitro experiments revealed that pituitaries from thyroidectomized rats synthesized smaller amounts of GH as revealed by H^3

leucine incorporation into GH (Augustine and MacLeod, 1975). In urethane anesthetized rats, serum GH concentrations are increased after propylthiouracil treatment (Chihara et al., 1976b). These contradictory results were probably a function of the method of anesthesia.

The effects of the adrenal steroids on pituitary and serum GH are not well understood. Frantz and Rabkin (1964) observed that glucocorticoid therapy in human patients prevented the insulin induced hypoglycemia discharge of pituitary GH. These observations led investigators to believe that corticoid induced dwarfism was a result of suppression of pituitary GH secretion. However, if one considers some of the earlier work, (Marx et al., 1943), there appears to be a direct antagonism between the glucocorticoids and GH on the growth of the epiphysis in hypophysectomized rats. These results were later believed to be the result of competition of cortisol with the somatomedins (Mosier and Jansons, 1976), but cortisol did not alter somatomedin production from the liver. Therefore the decrease in body growth was attributed to a decrease in food intake in rats. Reichlin and Brown (1960) observed that adrenalectomy resulted in impaired growth but pituitary GH concentrations were unaltered and food intake was reduced.

Other investigators thought that cortisol stimulated GH synthesis by the pituitary (Meyer and Evans 1964). Cortisol injected into propylthiouracil treated rats caused acidophil regranulation which was believed to indicate an increase in

pituitary GH concentration. However, quantitative measurements of GH failed to confirm this observation (Daughaday et al., 1968).

The effects of the adrenal steroids on GH secretion have not been well documented. Several experiments which need to be done include: 1) the effects of adrenalectomy on the pulsatile release of GH, 2) the effects of glucocorticoid replacement on this pulsatile secretion, immediately following thearapy and several hours after replacement therapy 3) the diurnal rhythm of GH release in patients with Cushing's disease and Addison's disease. The study by Grimm et al. (1974) is instructive. These investigators reported on the effects of high serum cortisol in renin hypertensive patients. Plasma GH concentrations or GH secretory patterns were found to be unaltered in these patients.

The first evidence that estrogen effected GH secretion in human subjects was reported by Frantz and Rabkin in 1964 (see Reichlin, 1974). They randomly sampled blood from women and men and found no change in basal GH concentrations. However in patients requiring bed rest, GH concentrations were higher in women. They also observed a decrease in serum GH concentrations in post-menopausal women indicating that estrogens must stimulate GH secretion. However, estrogen treatment was known to ameliorate symptoms of acromegaly (see Reichlin, 1974) indicating that estrogens inhibit GH release in humans. These discrepencies could be explained if estrogen in some way inhibits systemic utilization of GH.

Estrogens in rats appears to inhibit GH release *in vivo* (Gaarestrom and Levie, 1939) via inhibition of body growth. Daughaday *et al.* (1969) reported similar results in ovariectomized rats given estrogen and observed an increase in serum GH as measured by the tibia test. These results were confirmed in the mouse by radioimmunoassay (Sinha *et al.*, 1972).

The mechanism whereby estrogen inhibits GH release appears to be by acting directly on the pituitary (MacLeod and Lehmeyer, 1974; Dannies *et al.*, 1977). Pituitaries, incubated in the presence of estradiol, decreased the synthesis of GH as measured by incorporation of tritiated leucine. (MacLeod and Lehmeyer, 1974). Dannies *et al.* (1977) observed that pituitary cells incubated with several antiestrogens synthesised more GH and that these effects were reversed by estradiol. However, GH release *in vitro* is not affected by the stage of the estrous cycle and therefore estrogens probably sensitize the pituitary to the action of GIF or desensitize the pituitary to GRF. These experiments have not been performed.

Progestational compounds appear to be inhibitory to GH secretion in human subjects (Lawrence and Kirsteins, 1970). Medroxyprogesterone decreased serum GH in normal and acromegalic patients. These results were exemplified by Yen *et al.* (1970), who showed that plasma GH concentrations were lower in pregnant than in non-pregnant control women after insulin induced hypoglycemia.

The male gonadal steroid, testosterone appears to increase plasma GH concentration in castrated male rats (Daughaday *et al.*,

1968). Also in human patients, testosterone enhanced the GH response to insulin (Deller et al., 1966).

The most dynamic hormonal effects on the regulation of GH secretion in humans are elicited by insulin. In 1963, Roth et al. first demonstrated that insulin induced hypoglycemia greatly increased GH secretion. These observations were subsequently confirmed by many other investigators (Glick et al., 1963; Frantz et al., 1964; Tchobroutsky et al., 1966; Millar et al., 1973; Hampshire et al., 1975).

In search for a mechanism which may be responsible for the glucose depression of GH in monkeys (Blanco et al. 1966) or insulin induced hypoglycemia stimulations of GH in humans (see above), Oomura et al. (1969) reported the existence of glucose receptors in the ventromedial nucleus. Mayer (1953) showed that the neurons in the ventromedial nucleus increased their firing rate following increases in blood glucose. Injections of gold thioglucose into mice specifically lesioned the ventromedial nucleus and decreased serum GH concentrations (Sinha et al., 1975). However, the ventromedial nucleus is not the only hypothalamic center responsive to changes in glucose concentrations. Himsworth et al. (1972) microinjected 2-deoxy-D-glucose, a compound which interferes with the metabolism of glucose into several hypothalamic loci in monkeys. He reported that injections into the ventromedial nucleus resulted in decreased plasma concentrations of GH. GH hypersecretion was produced in those monkeys in which 2-deoxy-D-glucose was infused into the lateral hypothalamic area. One may surmise that there is a reciprocal relationship between

the lateral hypothalamic and the ventromedial hypothalamus in the control of GH secretion (see Reichlin, 1974).

Insulin induced hypoglycemia does not stimulate GH release in rats (see Martin et al., 1977; Reichlin, 1974, and Brown and Reichlin 1972). There was no correlation between the episodic release of GH and that of insulin in the rat (Tannenbaun et al., 1976). Therefore glucose does not appear to be a major regulator of GH in the rat.

Age, Nutrition and Stress Effects on GH

The relation of serum GH concentrations to the rate at which an animal grows is very poor. In the human subject, fetal GH was detectable early in gestation and reached a peak at about midgestation, and declined until delivery (Kaplan et al., 1972). However, the relative growth of the fetus was independent of plasma concentrations of GH (Reichlin, 1973). Following partuition, GH concentrations were relatively high and declined soon thereafter (Cornblath et al., 1965). GH concentrations remained relatively constant throughout childhood and adulthood (Reichlin, 1974).

In the rat, pituitary GH was detected late in gestation and increased dramatically until the first week of neonatal life (Birge et al., 1967). Serum GH concentrations decreased steadily from gestation until day 12 of neonatal life (Walker et al., 1977). From day 12 until day 20 serum GH concentrations increased steadily then another peak was observed on day 20 (Blazquez et al., 1974; Walker et al., 1977). Serum GH concentrations again declined, exhibiting several peaks, until

immediately after puberty when serum GH concentrations increased to another zenith at about 52 days of age (Walker et al., 1977). These changes in serum GH concentrations from birth to puberty negatively correlated with the hypothalamic concentrations of somatostatin (Walker et al., 1977). Subsequently, serum GH concentrations remained relatively unchanged until old age, at which time a slight, but insignificant decrease in serum GH concentrations occurred (Bruni, unpublished).

Serum GH concentrations are also effected by diet (for review see Reichlin, 1974). A decline in blood glucose concentrations resulted in an increase in blood GH concentrations in human patients (Luft et al., 1966) and in monkeys and dogs (Tsushima et al., 1971). Opposite effects of insulin induced hypoglycemia were observed in the rat (see Martin et al., 1977; and Brown and Reichlin, 1972). Amino acids, particularly arginine, induced GH release in primates and inhibited GH release in the rat. Similarly, free fatty acids stimulated GH release in humans (Lucke et al., 1972).

Starvation in rats decreased serum GH concentrations (Dickerman et al, 1969a; Trenkle, 1970). Undernourishment during neonatal development similarly decreased plasma and pituitary GH concentrations in the rat (Sinha et al., 1973), and in mice (Sinha et al., 1975). These decreases resulted in a decrease in body growth. However, the decrease in serum GH concentrations did not alter the ability of the liver to produce somatomedin (Phillips and Young, 1976). Nevertheless,

a proper diet is needed for GH and somatomedin production.

The effects of nutrition on GH regulation are probably mediated through the ability of the pituitary to synthesize GH and the hypothalamic activity during various nutritive states. The ventromedial nucleus and the lateral hypothalamus have been shown to effect appetite and feeding behavior (Ellison, 1968; Sorensen and Ellison, 1970).

Stress in human subjects and monkeys has been reported to increase GH secretion (Greenwood and Landon, 1966; Meyer and Knobil, 1967; Mason et al., 1974; Reichlin, 1974). In sub-primate species (i.e., dog, pig, rat, moose), stresses have been shown to decrease GH secretion (Bellinger and Mendel, 1975; Martin, 1976). The mechanisms whereby stresses alters GH release are not clearly understood, but appear to be mediated through extrahypothalamic structures (Collu et al., 1973). Complete and incomplete hypothalamic deafferentation attenuated the inhibition of GH release by auditory and ether stress in rats. Ether stress was partially inhibited by α-methyl-para-tyrosine, indicating that the catecholamines mediate the effects of ether stress. Other PN may participate in stress induced changes in GH release since heat and cold stress had opposite effects on GH release (Mueller et al., 1974). These neuronal mechanisms remain to be investigated.

IV. Metabolic Effects of GH

GH affects carbohydrate, protein, lipid, and calcium metabolism. These effects have been reviewed in most textbooks of endocringology (see Knobil and Greep, 1959; Cheek and Hill, 1974; Kostyo and Nutting, 1974; Goodman and Schwartz, 1974; Altszuler, 1974; Williams, 1974; C. Martin, 1976; Turner and Bagnara, 1976; Pecile and Müller, 1976).

Evans and Long (1921) first reported that alkaline pituitary extracts injected into rats produced a profound increase in body mass. These observations led other investigators to believe that this pituitary extract promoted body growth through altering general body metabolism. This pituitary factor which is known as GH has a wide range of effects on protein metabolism. GH has been demonstrated to decrease urinary nitrogen excretions. These observations were confirmed by other observations that GH increased amino acid uptake into skeletal muscle, kidneys, livers and a variety of other tissues. Moreover, GH has been shown to counteract the gluconeogenic effects of the glucocorticoids.

However these observations did not explain the mechanism whereby GH promoted a positive nitrogen balance. In addition to promoting amino acid uptake, GH appears to stimulate protein anabolism by increasing the cellular synthetic mechanisms for protein synthesis.

GH also has been shown to have both agonistic and antagonistic properties to that of insulin. Initially after GH injections there was a rapid uptake of sugars into skeletal

muscle and adipose tissue. Later GH prevented this active uptake of sugars into these tissues, demonstrating the diabetogenic properties of GH. This action of GH is probably a protective mechanism to prevent the loss of plasma glucose to the brain while concurrently preventing a negative nitrogen balance by retaining amino acids and stimulating protein synthesis.

The effects of GH on fats was first demonstrated in the early 1900's. Pituitary extracts were shown to decrease total body fats and to increase total body proteins. GH stimulates lipolysis which leads to an increase in serum free fatty acids. However GH appears to have a permissive role in this process because thyroxine and glucocorticoids are also necessary for GH to promote lypolysis. These effects can probably be mediated through a cyclic adenosine monophosphate (C-AMP) dependent lipase (C. Martin, 1976).

Normal bone growth is also dependent on the presence of GH. However, GH does not stimulate the bone directly, but requires the presence of a factor produced in the liver. This factor was initially called sulfation factor because it promoted the uptake of sulfate into cartilage. Later the sulfation factor was renamed somatomedin. It mediates the effects of GH on bone growth. For normal growth to occur, one not only requires GH, but also thyroxine and the glucocorticoids. While trying to isolate a single somatomedin, three somatomedins were isolated. Somatomedin A was found to be more potent as a "sulfation factor" than the other

somatomedins. On the other hand, somatomedin B exerted its effects primarily on thymidine incorporation into DNA. Finally, somatomedin C appears to bind to the placenta and act as a fetal growth factor. The precise role of the somatomedins is not fully understood and requires more investigation. GH also stimulated erythropoietin from the kidney (Crafts, 1953; Peschle et al., 1972). GH was found to be necessary for lactation in cattle (Shaw et al., 1955), but not in rats (Meites, 1957).

The major metabolic effects of GH have been discussed. However the biochemical intermediate and changes in all cells have not been totally defined (for a more comprehensive review, see references cited above).

MATERIALS AND METHODS

I. Animals and Blood Collection

Mature male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) and male Wistar rats (Harlan Ind., Indianapolis, IN), or hypophysectomized male Sprague Dawley rats (Hormone Assay Labs, Chicago, IL) were housed in temperature (25°±1°C) and light controlled (14 h on 10 h off) rooms. Rats were provided with Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum. Hypophysectomized (HYPOX) rats and Wistar rats received a dietary supplement of fresh whole wheat bread and oranges ad libitum. Surgically treated animals received 0.2 ml Longicil-S (60,000 units of penicillin G (Fort Dodge Labs., Fort Dodge, IA).

Blood samples were collected either by decapitation, via an indwelling atrial cannula or orbital sinus cannulation under light ether anesthesia (for details, see Experiments). Blood samples were allowed to clot for 24 h at 4°C and centrifuged 3,000 x g for 20 min. The serum was separated and stored at -20°C until assayed for GH.

II. Cannulation of the Right Atria

The right atrium was cannulated to remove serial blood samples in unanesthetized, unrestrained rats. Cannulae were prepared using Silastic Medical Grade Tubing (Dow Corning Co., Midland, MI). First a 6 in. segment of Silastic Tubing 0.030 in. inside diameter (ID), 0.065 in. outside diameter (OD), was dilated using a fine pair of hemostats. A smaller segment of tubing, 1.5 in. length, 0.020 in. ID, and 0.037 in. OD, was inserted into the dilated end, and then trimmed to 3 cm in length.

Rats were anesthetized with Nembutal (Abbot Laboratories, North Chicago, IL; 35 mg/kg). After reaching a surgical level of anesthesia, a ventral sagittal incision, 0.75 in. long, was made rostral to the clavical midway between the sternum and the right shoulder. The jugular vein was isolated using blunt dissecting techniques to avoid injury to the vessel. After the jugular vein was isolated, the cephalic portion was ligated with silk suture, and a small nick was made in the vein 0.5 cm above the pectoralis major. The hole was held open, and the Silastic tubing was inserted into the jugular vein until the end reached the right atria (3 cm). The vessel was ligated around the tubing caudal to where the cannulae entered the vessel and anchored to the pectoralis major.

The cannula was then routed subsutaneously to the dorsal side of the rat where it was exposed through a small incision placed in the skin in the cervicle region of the rat. The efficiency of the cannula was examined and the animal received an intravenous injection of 1600 units/kg Sodium heparin (Sigma Chemical Co., St. Louis, MO) prepared in .87% NaCl (saline). The cannula was sealed with a plug made of wire and the incisions were sutured.

The animals were allowed to recover for several days postoperatively before experimentation began. On the night prior to the experiment, the animals were removed from the animal rooms and transported to the surgery room and housed in individual plexiglass cages. On the morning of the experiment a longer cannula made of the larger tubing was attached to the indwelling cannula for injections and serial blood sampling, after initially injecting the animals intravenously with 500 IU of heparinized saline. Blood samples were withdrawn with a 1 ml tuberculine syringe at designated time intervals and volumes described in the Experimental Section.

III. Cannulation of the Lateral Cerebral Ventricle of the Rat

For injections of GABA, acetylcholine, bicuculline methyliodine, and 5,7-dihydroxytrptamine, cannulae were implanted chronically into the skull. (Verster et al., 1971). Cannulae were constructed from polyethylene tubing #10 (Clay Adams, Parsippany, N.J.). A segment of wire 0.009" in diameter was threaded through the lumen of the tubing before heating

over an electric soldering iron. The tubing was allowed to soften and the ends were pushed toward the middle forming a bulb. The tubing was allowed to cool, and trimmed to the following specification: the distance from the bulb to one end was 4 mm with a 1 mm bevel on the end, and the distance from the bulb to the other was 5-6 cm.

Prior to cannulation, rats were anesthetized with ether or Nembutal (Abbott Laboratoreis, North Chicago, IL; 35 mg/kg). After reaching a surgical level of anesthesia, animals were placed in a stellar stereotaxic instrument (C.H. Stoelling, East Chicago, IL). The scalp was shaved, a longitudinal incision was made from the middle of the frontal bone to the beginning of the occipital bone following the sagittal suture. The skin and the underlying facia were retracted. One hole, 0.03 inch in diameter was drilled 2 mm lateral and 1 mm caudal to the intersection of the coronal and sagittal sutures, bregma. The dura mater was pierced with a hypodermic needle and the 4 mm end of the cannula was inserted through the hole into the lateral ventricle. The other end was sealed with heat. Two additional holes were drilled 3-5 mm caudal to the coronal suture. The skull was dried and metal screws (bowline anchor screws, Shuron/Continental, Rochester, N.Y.) were inserted into the later 2 holes to allow for support of the cannula. Dental cement (NuWeld Caulk, L.D., Caulk Co., Milford, DL) was used to anchor the cannulae to the anchoring screws. After the cement hardened completely, the incision was sutured. The animals were allowed to recover for three days postoperatively.

Two days prior to the experiments, the animals were gentled by handling and cannulae were checked by injecting 10 ul of 0.87% NaCl with a 10 ul microsyringe (Glenco Scientific, Ind., Houston, TX).

Following injections of experimental drugs and blood collection, 20 ul of an aqueous solution of methylene blue dye was injected into the cannulae, and the brain was removed and sectioned along the coronal plane through the hypothalamus. Data was only used from rats showing stain in the medican eminence and third ventricle.

IV. Radioimmunoassay for Serum GH

Serum concentrations of GH were determined using a standard double antibody ratioimmunoassay procedures as described in the NIAMDD-kit for rat GH. The second antibody was harvested from our goat and diluted to the appropriate antibody concentration where maximal binding was observed. Serum hormone concentrations are expressed in terms of the standard reference preparation, NIAMDD-rat-GH-RP-1. All serum and incubation samples were assayed in duplicate or triplicate. Hormone concentrations were determined only from volumes which resulted in hormone values on the linear portion of the standard curve. All samples from an individual experiment were tested in the same assay to avoid interassay variations. Also, a standard serum sample was used with each assay to monitor interassay variations.

V. Assay of Norepinephrine and Dopamine in Hypothalamic Tissue

Immediately following decapitation, brains were removed and placed dorsal side down in a pool of ice cold 0.89% NaCl. The hypothalamus was removed with find iris scissors using the following landmarks: anterior hypothalamus, immediately rostral to the optic chiasm following the lamina terminals 30° rostral to a perpendicular line of the horizontal axis; caudal hypothalamus, middle of the mammilary bodies perpendicular to the horizontal axis; lateral hypothalamus following the hypothalamic sulci; and dorsal hypothalamus 2-3 mm to the dorsal hypothalamic surface.

The median eminence (ME) was dissected from the hypothalamus using a dissecting microscope and fine iris scissors and the following landmarks: posterior border of the infundibular stalk, anterior border of the infundibular stalk and along the lateral aspects of the tubercinereum at an angle of 20° from the ventral hypothalamic horizontal surface yielding a piece of tissue containing 10±2 mg protein (N=24) as assayed by the method of Lowry et al., 1951. Medial basal hypothalamus (MBH) was dissected using similar boundaries as the ME except cuts were made 45° from the horizontal plane following the ventral hypothalamic surface. These tissues contained 35±3mg protein (N = 24).

Whole hypothalami were homogenized in 0.4 N perchloric acid containing 10% EDTA (1 mg tissue/ 10 ul homogenate) using microhomoginizers and centrifuged in a microcentrifuge (Coleman International, Oak Brook, IL) to separate the particulate

matter from the supernatant. ME were homogenized in 25 ul and MBH in 40 ul of the PCA solution. Dopamine (DA) and norephinephrine (NE) tissue concentrations were determined in 10 ul aliquote of the supernatant assayed by the method of Coyle and Henry (1973).

VI. In Vitro Co-Incubation

Anterior pituitaries and hypothalami were removed immediately after decapitation and placed in 10 ml scintillation vials containing 2 ml DIFCO medium 199 (DIFCO Labs, Detroit, MI), prepared as described in DIFCO supplimentary literature. The vials were placed in a Dubnoff metabolic shaker, 60 cpm at 37° $\pm$ 1°C and incubated under constant gassing 95% 0_2 and 5% CO_2 for 1 h. After 1 h pre-incubation, the medium was discarded and 2 ml of fresh medium was added with or without drug treatment (see Experimental section for treatments), and incubation was resumed for 2 h. After 2 h incubation, the medium was removed and placed into 12 x 75 mm culture tubes, capped and frozen until assayed. Two ml of fresh treatment medium was added to the pituitaries and hypothalami and incubation resumed. Upon completion of the incubation, the pituitary halves were weighed to the nearest tenth of a milligram and discarded.

Later the medium was thawed, diluted 1:50 with 0.1% gelatin PBS and assayed in triplicate for GH. Results are expressed as ug GH/ml/h/mg AP.

VII. Statistical Methods

Unless otherwise stated all *in vivo* experiments were analysed by analysis of variance and students - Newman-Keuls test for multiple comparisons between groups.

In vitro incubations were analysed using the paired-t-test.

EXPERIMENTAL DATA

I. Pulsatile Secretory Pattern of Plasma GH

Introduction

GH is released in episodes throughout the day and night in experimental animals and in man (Finkelstein et al., 1972; Tannenbaum and Martin, 1976; Tannenbaum et al., 1976). These secretory patterns were not effected by insulin, hyperglycemia or hypoglycemia (Tannenbaum et al., 1976). A series of experiments were performed to determine when these pulsatile secretions of GH occurred so that experiments could be designed to either potentiate or inhibit the episodic release of GH.

Materials and Methods

The right atria of male Sprague-Dawley rats were cannulated, as described in Materials and Methods, 2 hours prior to the experiment. 1600 units/kg of sodium heparin was injected intravenously to avoid coagulation of the blood in the cannula. 200 µl samples were withdrawn from the cannulae every 15 min for the duration of the experimental period. 200 µl of 0.87% NaCl was reinjected intravenously into each animal following the sampling procedure. At the end of the experimental period, blood samples were centrifuged, plasma was separated and stored for radioimmunoassay. These experiments

were performed at0900 to 1200, 1100 to 1400, and 1400 to 1600 hours (h).

Results

GH concentrations remained at approximately 30 ng/ml throughout the sampling period, except during episodes of GH release at 930-1030, 1200-1300, and 1500-1600 hours. Figure 3 represents the typical response of 4 animals sampled between 1100 and 1300 hours. Figure 4 represents the average response of 10 animals sampled at these times.

Conclusions

These observations confirm observations that GH is released in episodes every 3-3.5 hours throughout the day in the rat (Tannenbaum and Martin, 1976; Tannenbaum et al., 1976). These results helped to establish the times at which several of the experiments which follow were performed. If a change in GH concentration was expected, I investigated the effect of various drug treatments on plasma or serum GH concentrations during the morning or early afternoon espisodes of GH release.

II. Adrenergic Control of GH Release in vivo and in vitro

Introduction

The hypothalamus contains catecholaminergic neurons which appear to modulate the release of all anterior pituitary hormones (Meites et al., 1977). L-dopa, the immediate precursor to DA and NE increased serum GH concentration in rats

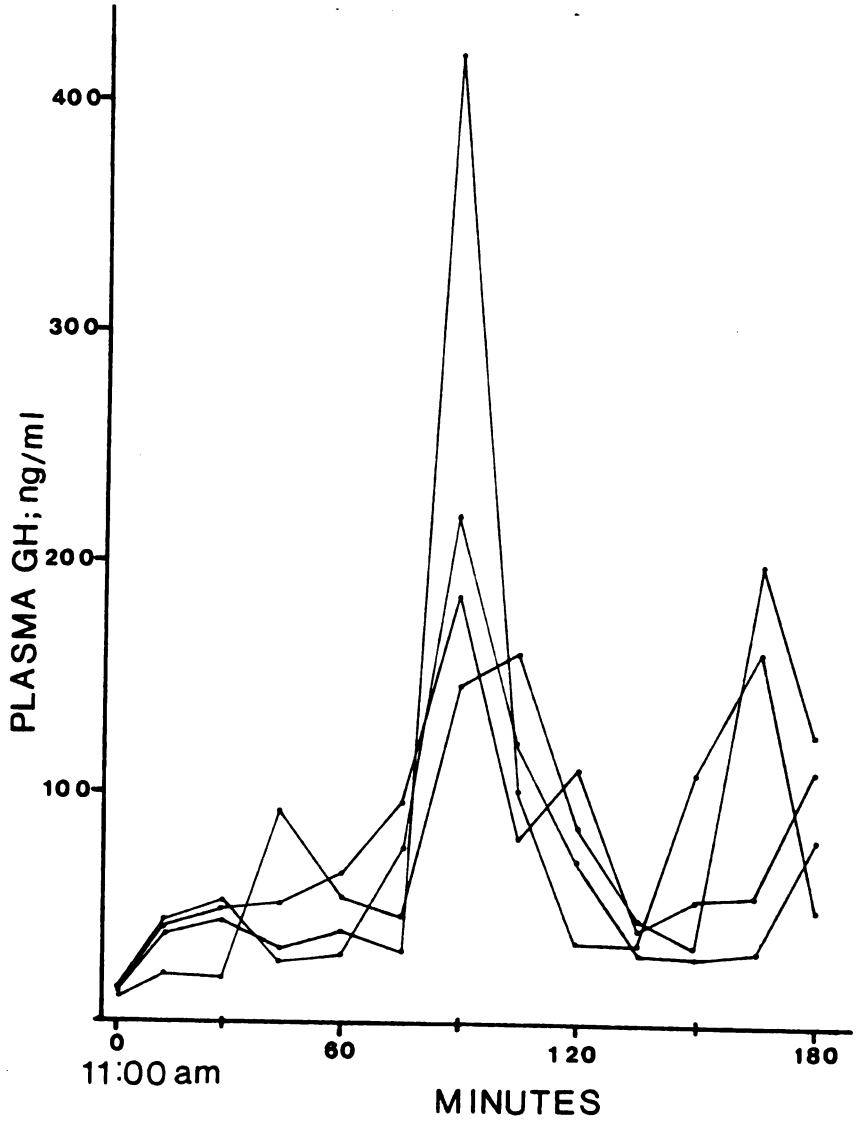

Figure 3. Normal secretory pattern of GH in four male rats.

(Chen et al., 1974) and in human patients (Martin, 1973). These effects apparently were mediated through the α-adrenergic receptor, since phentolamine, a α-adrenergic receptor blocker, partially inhibited this response (Liuzzi et al., 1971; Kato et al., 1973; Martin et al., 1977).

In rats anesthetized with urethane, the β-adrenergic receptor blocker, propranalol, was most effective in stimulating GH release (Collu et al., 1972; Kato et al., 1973). This stimulation may have resulted from the anesthesia used. The present study shows the effects of several noradrenergic drugs on release of GH in vivo and in vitro.

Materials and Methods

Male Sprague-Dawley rats (225-250g) were used in all of these studies. In the first experiment, chlorpromazine (CPZ) (Smith, Klein and French Labs, Philadelphia, Penn.) a catecholamine reuptake inhibitor, was injected intraperitoneally (i.p.) to rats. Animals were bled under light ether anesthesia at 30, 60 and 120 min intervals after injection.

The second experiment represents a dose response study on the effects of clonidine (Beohringer Ingelheim Ltd., Elmsford, N.Y.), an α-adrenergic receptor stimulator, on serum GH concentrations. Rats were injected with 0.01, 0.02, 0.05 and 1 mg/kg clonidine dissolved in 0.87% NaCl solution. Each rat received an injection of 0.2 ml/100 g B.W. Blood samples were obtained 30 min and 60 min, after injection during the second A.M. surge of GH. All rats were decapitated between 1200 and 1300 hours. Blood from the cervical wound was

collected and treated as described in Materials and Methods.

In the third experiment, rats bearing an indwelling carotid cannula were injected i.p. with clonidine. Blood samples of 0.5 ml each, were collected via the cannula at 0, 30, 60 and 120 min after the initial injection to determine the time-related effects of clonidine on serum GH concentrations.

In the fourth experiment, male rats were injected i.p. with propranalol (PROP), (Ayerst Labs., N.Y.), a β-adrenergic receptor blocker, phenoyxbenzamine (Smith, Klein and French Labs, Philadelphia, Penn.), and α-adrenergic receptor blocker, and several doses of phentolamine (PHEN), (CIBA Pharmaceutical Co., Summitt, N.J.), an α-adrenergic receptor blocker. After 1 h between 1200 and 1300 hours, animals were decapitated (Table 4).

In the fifth experiment, PROP and PHEN were injected alone and simultaneously with clonidine to investigate the interactions of the α and β receptor blockers with the α-adrenergic receptor stimulator. Blood samples were taken via cardiac puncture under light ether anesthesia between 900 and 1000 hours.

The final experiment was performed to determine whether the <u>in vivo</u> effects of the α and β adrenergic drugs required the presence of the hypothalamus. Pituitaries and hypothalami were co-incubated as described in Materials and Methods. NE (Sigma Chemical Co., St. Louis, MO.), PROP, PHEN, and pimozide (PIM), (McNeil Labs, Ft. Washington, Penn), were added to the incubation media to examine the effects

of the drugs directly on the pituitary and on the hypothalamus and pituitary together. For doses and times, see Figures 5 - 8.

Results

CPZ, the catecholamine receptor blocker, significantly reduced serum GH concentrations 30, 60 and 120 min after injection (Table 1). In a second trial similar observations were made. Rats injected with varying doses of clonidine (CLON) had significantly higher serum GH concentrations than control rats injected with 0.87% NaCl. However, the 1 mg/kg dose of CLON did not alter serum GH concentrations. CLON did not increase GH in a dose related manner (Table 2).

Using the most effective doses of CLON (0.02 and 0.05 mg/kg) CLON significantly increased serum GH concentrations maximally 60 min after injection (Table 3). However, there were no significant differences between the 30 and 60 min sampling periods. After 60 min, GH concentrations declined, but remained higher than control concentrations.

The effects of PROP, PHEN and phenoxybenzamine are shown in Table 4. PROP (5 mg/kg) did not alter serum GH 1 hr after injections. Phenoxybenzamine (0.5 mg/kg) slightly, but insignificantly, reduced serum GH concentrations 1 hr after injections. However, the other α-adrenergic receptor blocker, PHEN, at doses of 0.5, 1.0 and 5.0 mg/kg, significatnly decreased serum GH concentrations 1 hr after injections.

Table 1.

Effects of Chlorpromazine on Serum GH

	Minutes After Injection			
	0	30	60	120
		I		
Controls (8)	96.9 ± 8	100 ± 12	85 ± 14	80 ± 20
CPZ 3mg/rat (8)	100 ± 8	47.5 ± 7[b]	42 ± 6[b]	27 ± 4[b]
		II		
Controls (8)	100 ± 18	95 ± 20	89 ± 12	85 ± 18
CPZ 3mg/rat (8)	101 ± 20	43 ± 9[b]	39 ± 15[b]	48 ± 3[b]

[a] $\bar{x}$ ± S.E.M.; ng/ml.
[b] $P < 0.05$ compared to controls. (Each time period was on a different day.)

Table 2.

Dose Response Effects of Clonidine on Serum GH

n = 8	30 min	60 min
Controls (0.87% NaCl)	78 ± 19[a]	52 ± 20
0.01 mg/kg	202 ± 34[b]	268 ± 73[b]
0.02 mg/kg	416 ± 66[b]	498 ± 58[b]
0.05 mg/kg	462 ± 106[b]	565 ± 75[b]
1.0 mg/kg	156 ± 80	175 ± 68

[a] $\bar{x}$ ± S.E.M.; ng/serum.
[b] $P<0.05$ compared to controls at each blood sampling.

Table 3.

Time Related Effects of Clonidine on Serum GH

n = 8	T 0	T 30	T 60	T 210
Controls (0.87% NaCl)	98 ± 16[a]	125 ± 30	75 ± 38	115 ± 37
Clonidine (0.02 mg/kg)		387 ± 60[b]	439 ± 53[b]	256 ± 37[b]
Clonidine (0.05 mg/kg)		436 ± 76[b]	525 ± 88[b]	396 ± 63[b]

a $\bar{x}$ ± S.E.M.; ng/serum.
b $P < 0.05$ compared to controls at each time of blood sampling

T = time in minutes

Table 4.

Effects of α and β-Adrenergic Blocking Drugs on Serum GH

n = 10/group	I		II
Controls (0.87% NaCl)	125 ± 18[a]	Controls (0.87% NaCl)	106 ± 26
Propranalol (5.0 mg/kg)	136 ± 26	Propranalol (5.0 mg/kg)	98 ± 29
Phenoxybenzamine (0.5 mg/kg)	87 ± 16	Phenoxybenzamine (1 mg/kg)	76 ± 12
Phentolamine (0.5 mg/kg)	68 ± 12[b]	Phentolamine (0.5 mg/kg)	68 ± 15
Phentolamine (1 mg/kg)	52 ± 8[b]	Phentolamine (1 mg/kg)	62 ± 9[b]
Phentolamine (5 mg/kg)	48 ± 10[b]	Phentolamine (5.0 mg/kg)	33 ± 16[b]

[a] $\bar{x}$ ± S.E.M.; ng/serum.
[b] $P < 0.05$ compared with controls.

Injection of PROP (5 mg/kg) alone did not alter serum GH concentrations (Table 5). On the other hand, PHEN (5mg/kg) significantly decreased GH release; whereas CLON (0.02 mg/kg) significantly increased GH release. This increase was significantly attenuated by PHEN, but not PROP.

In vitro co-incubation of pituitary halves with and without a hypothalamic fragment revealed that the presence of the hypothalamus significantly reduced GH release from the AP (Figure 5). Addition of 1, 10, and 100 ng NE/ml to incubation medium had no effect on pituitary GH release. Moreover, addition of NE to tubes containing both an AP half and a hypothalamus significantly increased GH release, as compared to AP halves and hypothalami incubated alone. Removal of this medium after 2 hrs and replacement with fresh medium not containing NE in the presence of a hypothalamus significantly reduced GH release for the AP.

When PIM, a dopamine receptor blocker, was added to the incubation medium, GH release was not changed (Figure 6). Also, PIM did not alter the effects of 100 ng NE/ml media on hypothalamic stimulated GH release.

PHEN alone did not alter GH release directly from the pituitary or hypothalamic inhibited GH release from AP halves co-incubated with hypothalamus (Figure 7). However, PHEN decreased the NE stimulated increase in GH release from the co-incubated pituitary halves. PROP did not alter GH release or NE induced GH release from co-incubated pituitaries (Figure 8).

Table 5.

Effects of Clonidine and Adrenergic Blocking Agents on Serum GH

n = 8	0	60 min
Controls (0.87% NaCl)	112 ± 15[a]	98 ± 22
Propranalol (5 mg/kg)	98 ± 21	76 ± 31
Phentolamine (5 mg/kg)	95 ± 31	38 ± 6[b]
Clonidine (0.02 mg/kg)	113 ± 18	323 ± 36[b]
Propranalol + Clonidine	129 ± 18	296 ± 51[b]
Phentolamine + Clonidine	108 ± 12	126 ± 31

[a] $\bar{x}$ ± S.E.M.; ng/serum.
[b] $P < 0.05$ compared to controls.

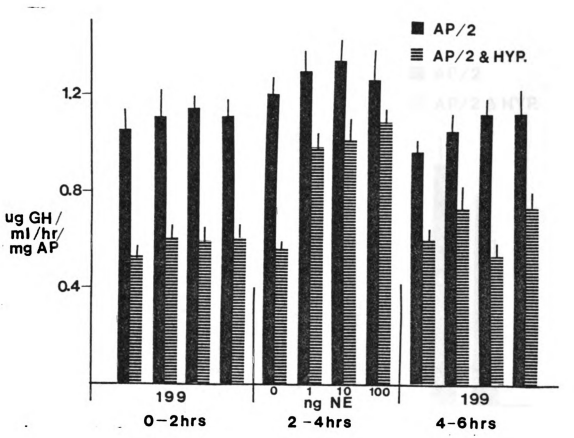

Figure 5. In vitro effects of NE on GH release. Vertical bars represent SEM. N=6/group. AP/2=Anterior Pituitary half; Hyp=Hypothalamus

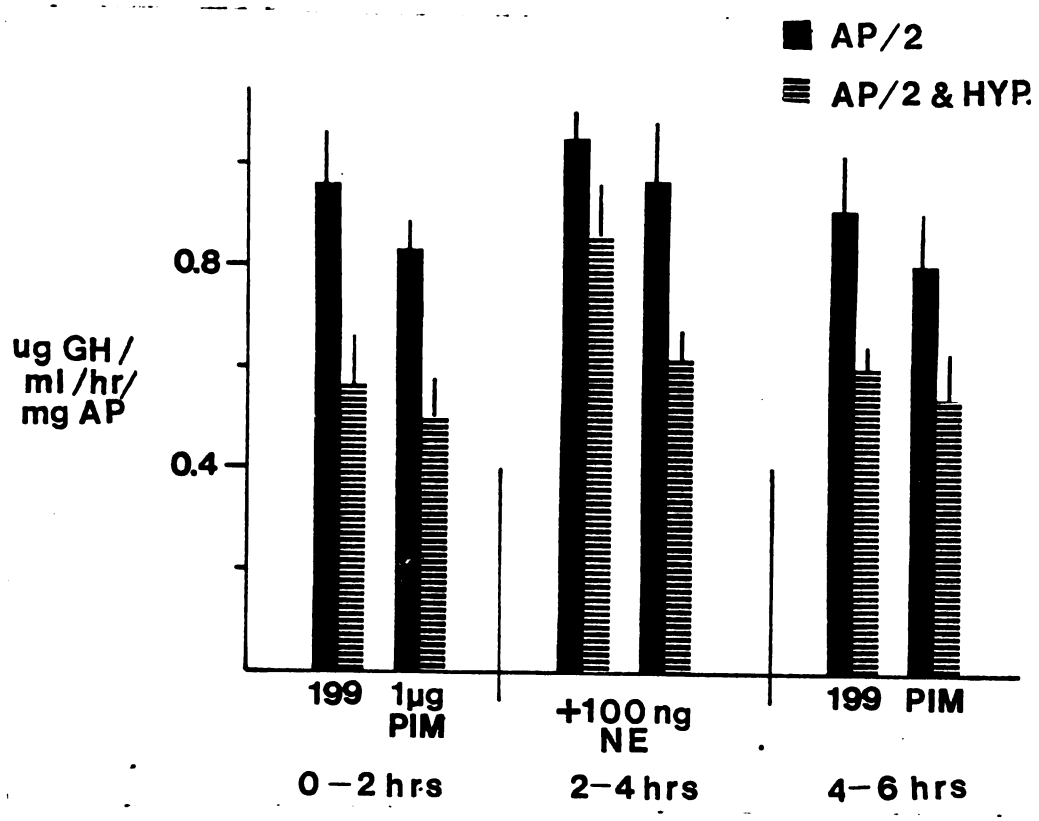

Figure 6. In vitro effects of PIM and NE on GH release. Vertical bars represent SEM. N=6/group. AP/2=Anterior Pituitary half; Hyp=Hypothalamus

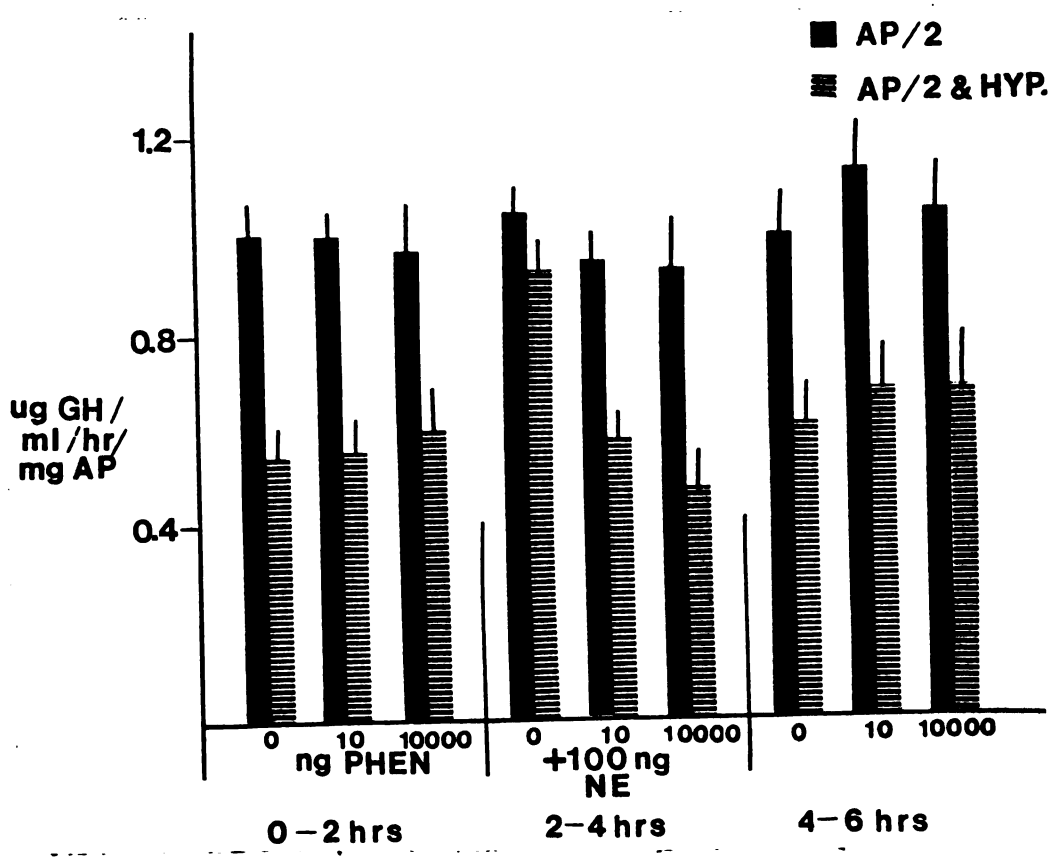

Figure 7. In vitro effects of phentolamine and NE on GH release. Vertical bars represent SEM. N=6/ group. AP/2=Anterior Pituitary half; Hyp= Hypothalamus.

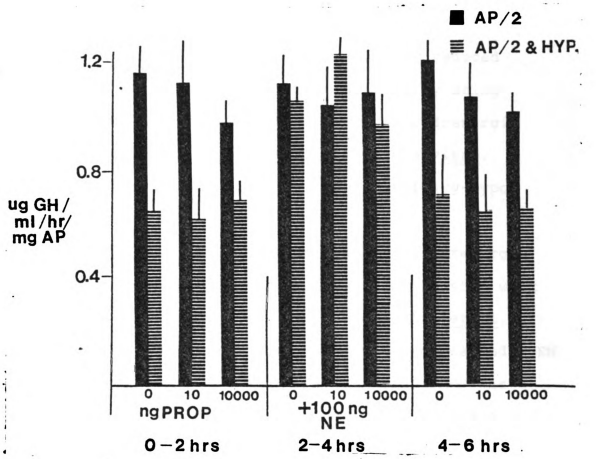

Figure 8. In vitro effects of propranolol and NE on GH release. Vertical bars represent SEM. N=6/group. AP /2=Anterior Pituitary half; Hyp=Hypothalamus.

Conclusions

GH release was blocked by the catecholamine receptor blocker, CPZ. These effects are believed to be the result of blocking both DA and NE. However, when a specific α-adrenergic receptor stimulator, CLON, was injected into male rats, there was a significant increase in serum GH concentrations. Phenoxybenzamine slightly reduced serum GH. These observations were clarified by using another αadrenergic blocker, PHEN. PROP, a βadrenergic receptor blocker, had no effect *in vivo* or *in vitro*. These results were confirmed by using a pituitary-hypothalamus co-incubation system.

I conclude from these results that the α-adrenergic receptors actively stimulate GH release. While this work was in progress, Durand *et al.*, 1977 and Martin *et al.*, 1978, published similar results. Phenoxybenzamine and PHEN both inhibited the pulsatile release of GH. PROP was without effect. CLON similarly enhanced the episodic release of GH. However, they did not examine the direct effects of these drugs on the pituitary and hypothalamus.

NE appears to stimulate GH release by removing inhibition of the hypothalamic influence on GH release *in vitro*. It is possible that NE through an α-adrenergic receptor, inhibits the release of GIF from the pituitary, thus increasing GH release *in vivo* and *in vitro*. However, GIF was not measured in these experiments due to the unavailability of somatostatin radioimmunoassay.

III. Serotonergic Control of GH *in vivo* and *in vitro*

Introduction

Several experimental models have been used to determine the role of 5-HT in the control of GH. However, the results from these experiments have been inconclusive. Injections of 5-HT into the lateral cerebral ventricle of urethane anesthetized rats increased serum GH concentrations (Collu *et al.*, 1975). Similarly, systemic injections of 5-HTP, the immediate precursor to 5-HT, increased serum GH concentrations in unanesthetized rats. Injections of cyproheptadine, a 5-HT receptor blocker, inhibited GH release in the neonatal rat (Stuart *et al.*, 1976).

Conversely, intraventricular injections of 5-HT significatnly decreased serum GH concentrations, and systemic injections of 5-HTP which elevated hypothalamic concentrations of 5-HT did not alter GH release. Injections of parachloramphetamine, a drug which inhibits tryptophan hydroxylase, increased serum GH concentrations (Müller *et al.*, 1973).

In view of these discrepencies, the effects of 5, 7-dihydroxytryptamine, a specific neurotoxin for 5-HT; methysergide, a serotonin receptor blocker; and parachlorophenylalanine, a tryptophan hydroxyalase inhibitor, were studied on GH release *in vivo*. Additionally, the direct effects of 5-HT and methysergide (METH) were studied in a pituitary-hypothalamus co-incubation system.

Materials and Methods

Thirty male Sprague-Dawley rats were injected i.p. on day 0 with 300 mg/kg of paraclorophenylalanine (PCPA), (Aldrich Chem. Co., Milwaukee, Wis.). Thirty additional rats were injected with 0.87% NaCl (0.2 ml/100 g B.W.) and served as controls. On days 2, 4, 6 and 8, following injections, 6 animals were decapitated and blood was collected for GH assays.

Twenty animals bearing an indwelling atrial cannula were used in this study. Beginning at 0930 hrs, 10 animals were injected with 1600 units of sodium heparin/kg. Immediately following heparin injections, 5 animals were injected intravenously with 10 mg/kg METH (Sandoz Pharmaceuticals, Hanover, N.J.). Five animals served as controls and were injected with equivalent volumes of 0.87% NaCl. Blood samples were obtained 10, 30, and 60 min after injections. On separate days during the same time interval, the experiment was repeated using 20 mg/kg and 40 mg/kg METH.

In a third study, rats were injected intraventricularly (lateral ventrical) with 5, 7-dihydroxytryptamine (5,7-DHT), Regis Chemical Co., Morton Grove, Ill). Another group of rats was pre-treated with desmethylimipramine (DMI), (U.S.V. Pharmaceuticals, Tuckahoe, N.Y.) 45 - 60 min before 5,7-DHT injections. One additional group of rats was injected with DMI; and another group which was injected with 0.87% NaCl served as controls. Rats injected with DMI

were killed on days 2 and 10 after injections. Rats given the other drug treatments were killed on days 2, 6, 10, and 14. Blood was collected from the cervical wound and stored for radioimmunoassay (RIA).

The final study was performed to determine the direct effects of 5-HT (Sigma Chemical Co., St. Louis, MO), and METH on GH release in a co-incubation system (see Materials and Methods, and Tables 9 and 10 for doses of drugs and times at which blood samples were taken).

Results

The 5-HT antagonist, PCPA, decreased serum GH concentrations 2 days after injections (Table 6). Serum GH concentrations were not maximally reduced until 4 days after injections. There was no further change in serum GH concentrations 6 and 8 days following the initial injections.

A dose of 10 mg/kg METH did not alter serum GH concentrations 10, 30, or 60 min after injections (Table 7). Rats injected i.v. with 20 mg/kg METH had lower serum GH concentrations 30 and 60 min after injections as compared to controls ($p < 0.05$). Rats given the 40 mg dose of METH showed reduced serum GH concentrations reduced throughout the entire experimental period ($p < 0.05$).

Rats injected with DMI, the catecholamine reuptake inhibitor, had serum GH concentrations which were not significantly different from control concentrations (Table 8). 5,7-DHT alone significantly decreased serum GH, 2, 6, 10 and 14 days after intraventricular injections.

Table 6.

Effects of PCPA on Serum GH

	Day 0	Day 2	Day 4	Day 6	Day 8
Controls (6)	67 ± 6	83 ± 5	91 ± 5	96 ± 8	87 ± 10
PCPA 300 mg/kg (6)	---	58 ± 9[b]	36 ± 9[b]	28 ± 6[b]	22 ± 12[b]

[a] $\bar{x}$ ± S.E.M.; ng/ml.
[b] $P<0.05$ compared to controls.
PCPA = parachlorophenylalanine.

Table 7.

Effects of Methysergide on GH Release in vivo

(n = 5 / group)

I	T = 0	10 min.	30 min.	60 min.
Controls (0.87% NaCl)	150 ± 19[a]	136 ± 40	116 ± 29	139 ± 12
METH (10 mg/kg)	164 ± 25	152 ± 18	132 ± 25	142 ± 21
II				
Controls (0.87% NaCl)	142 ± 21	165 ± 32	180 ± 26	152 ± 19
METH (20 mg/kg)	151 ± 18	126 ± 20	65 ± 10[b]	42 ± 9[b]
III				
Controls (0.87% NaCl)	107 ± 15	136 ± 20	127 ± 16	156 ± 27
METH (40 mg/kg)	142 ± 19	85 ± 11[b]	48 ± 15[b]	28 ± 6[b]

[a] $\bar{x}$ S.E.M.
[b] $P<0.05$ as compared to controls.

Table 8.

Effects of Desmethylimipramine and 5,7-Dihydroxytryptamine on Serum GH

	Day 2	Day 6	Day 10	Day 14
Controls	75 ± 4[a] (5)	79 ± 5 (5)	73 ± 5 (5)	76 ± 7 (5)
DMI (25 mg/kg)	89 ± 10 (8)	---	78 ± 9 (8)	---
5,7,DHT 200 µg	47 ± 6[b] (6)	18 ± 5[b] (6)	12 ± 8[b] (6)	15 ± 10[b] (6)
5,7,DHT + DMI	67 ± 3 (10)	29 ± 2[b] (10)	12 ± 2[b] (10)	16 ± 2[b] (10)

[a] $\bar{x}$ S.E.M.; ng/ml.
[b] $P<0.05$ compared to controls.

N = 8/group

When 5,7-DHT was injected 45-60 min after parenteral injections of DMI, there was a significant decrease in serum GH concentrations on days 6, 10, and 14 after the initial injections.

Co-incubated AP halves in the presence of a hypothalamus released less GH than AP halves incubated alone (Table 9). Addition of 10, 100, and 1000 ng 5-HT/ml of incubation media prevented the hypothalamic inhibition of GH release. However, all doses were equally effective in suppressing the hypothalamic inhibition of GH release *in vitro*. METH at doses of 1, 10, 100 and 1000 ng/ml of incubation media did not alter GH release directly on the AP nor on the AP halves incubated with a hypothalamic fragment (Table 10). The 10 and 100 ng doses of METH slightly inhibited the 5-HT stimulated increase in GH release from co-incubated AP halves. Only the 1µg dose completely inhibited the 5-HT stimulated GH release from co-incubated AP halves.

Conclusions

These results indicate that GH is in part at least controlled by central serotonergic activity. 5-HT does not act directly on the pituitary to stimulate GH release. However, 5-HT is capable of removing the inhibitory influence of the hypothalamus on GH release *in vitro*. Two 5-HT antagonists, METH and PCPA, both inhibited GH release *in vivo*. Additionally, 5,7-DHT, a specific

Table 9.

In Vitro Stimulation of GH by 5-HT

n = 6 per group

	µg GH/ml/mg AP/hr	
	0-2 hrs	2-4 hrs
	199	199
Pituitary	1.67 ± .32[a]	1.58 ± .21
Pituitary + Hypothalamus	1.01 ± .13[b]	.97 ± .32
	5-HT (10 ng/ml)	5-HT (10 ng/ml)
Pituitary	1.58 ± .21	1.67 ± .31
Pituitary + Hypothalamus	1.62 ± .31	1.49 ± .23
	5-HT (100 ng/ml)	5-HT (100 ng/ml)
Pituitary	1.49 ± .28	1.63 ± .22
Pituitary + Hypothalamus	1.72 ± .25	1.65 ± .29
	5-HT (1 µg/ml)	5-HT (1 µg/ml)
Pituitary	1.53 ± .21	1.48 ± .16
Pituitary + Hypothalamus	1.81 ± .17	1.76 ± .21

[a] Mean ± S.E.M.
[b] P<0.05 as compared to control.

Table 10.

In Vitro Effects of Methysergide on Serotonin Induced GH Secretion

n = 6 per group

	μg/GH/ml/mg AP/hr	
	0-2 hrs	2-4 hrs
	199	METH (1 ng/ml)
Pituitary	1.58 ± .21[a]	1.48 ± .18
Pituitary + Hypothalamus	.87 ± .31[b]	.93 ± .21[b]
	METH (1 ng/ml)	METH + 5-HT (1 ng/ml) (100 ng/ml)
Pituitary	1.47 ± .21	1.52 ± .21
Pituitary + Hypothalamus	.92 ± .13[b]	1.61 ± .17
	METH (10 ng/ml)	METH + 5-HT (10 ng/ml) (100 ng/ml)
Pituitary	1.62 ± .31	1.76 ± .32
Pituitary + Hypothalamus	1.06 ± .21[b]	1.45 ± .26
	METH (100 ng/ml)	METH + 5-HT (100 ng/ml) (100 ng/ml)
Pituitary	1.45 ± .18	1.63 ± .18
Pituitary + Hypothalamus	1.10 ± .07[b]	1.23 ± .21
	METH (1 μg/ml)	METH + 5-HT μg/ml) (100 ng/ml)
Pituitary	1.72 ± .27	1.55 ± .09
Pituitary + Hypothalamus	1.22 ± .11[b]	.97 ± .21[b]

[a] Mean ± S.E.M.
[b] $P<0.05$ as compared to control.

neurotoxin for 5-HT neurons, inhibited GH release when injected alone or in combination with DMI, the catecholamine reuptake inhibitor.

5,7-DHT, when injected alone, decreases serum GH concentrations 2 days following injections as opposed to 4 days after injection in combination with DMI. This difference is probably due to the effects of 5,7-DHT on hypothalamic catecholamines. 5,7-DHT, when injected alone, caused a 70% depletion of hypothalamic 5-HT and a 40% depletion of hypothalamic NE. When rats were pretreated with DMI, 5,7-DHT, injections resulted in an 80% depletion of hypothalamic 5-HT without altering hypothalamic DA or NE concentrations (Björklund et al., 1975).

Collectively these results suggest that 5-HT is a positive modulator in the control of GH release. These results are in agreement with the previous report that intraventricular injections of 5-HT increased serum GH concentrations (Collu et al., 1972). However, these results are not in agreement with the report that systemic injections of 5-HTP or intraventricular injections of 5-HT decreased GH release (Müller et al., 1973).

One explanation for the discrepency in these results may be due to the time at which blood samples were taken. Another possible explanation for this discrepency is the fact that 5-HTP is not specifically transported into serotonergic neurons (Butcher et al., 1972). 5-HTP may be actively transported into catecholamine containing neurons and be decarboxylated to 5-HT. However, 5-HT in a

catecholamine neuron can displace the endogenous transmitter and act as a false transmitter (Butcher et al., 1972; Wurtman and Fernström, 1972). These results further support the positive role of 5-HT in the control of GH secretion.

IV. Cholinergic Control of GH Release

Introduction

Secretion of anterior pituitary (AP) hormones has been shown to be influenced by hypothalamic catecholaminergic and serotonergic neurons (Martin, 1976; Meites et al., 1977). Administration of L-dopa, apomorphine, and peribidel, all dopaminergic drugs, were reported to induce release of GH in rats (Chen et al., 1974; Mueller et al., 1976), whereas serotonin either stimulated (Collu et al., 1972; Bruni, PhD. thesis) or inhibited GH release (Müller et al., 1968, 1973). Acetylcholine, present in high concentrations in the hypothalamus (Kostlow et al., 1974),has been implicated in the control of LH (Everett et al., 1949a,b; Sawyer et al., 1949; Libertun and McCann, 1971, 1973) and PRL secretion (Libertun and McCann, 1973; Grandison et al., 1974; Grandison and Meites, 1976). A cholinesterase inhibitor, paraoxon as reported to increase pituitary GH concentrations in rats (Cehovic et al., 1972), and a cholinergic agonist, β-methylcholine, increased serum GH concentrations in human subjects (Soulairac et al., 1968).

The following study was designed to determine the effects

acetylcholine and other cholinergic drugs of the release of GH from the rat pituitary *in vivo* and *in vitro*.

Materials and Methods

Rats were implanted with an indwelling polyethylene cannula in the lateral ventrical by the method of Verster *et al*. (1971). Rats were allowed to recover from surgery for 1 wk and injected with 10 µl 0.87% NaCl intraventricularly for 3 days prior to drug treatment. A dose of 25 or 50 µg acetylcholine bromide (K and K Labs, Plans View, N.Y.) was injected into the lateral ventrical. An additional control group of non-cannulated, uninjected rats was added to the experiment. All animals were decapitated 30 min after injection of acetylcholine bromide (Ach) between 1000 and 1100 hrs.

In a second experiment, male rats were injected i.p. with the cholinergic receptor stimulator, pilocarpine nitrate (Nutritional Biochemical Co., Cleveland, OH), (5 mg/kg) or the cholinesterase inhibitor, physostigmine sulfate (Merk and Co., Rahway, N.J.), (0.3 mg/kg). Six animals in each group were decapitated at 30, 60, and 120 min after injection. An additional 6 animals were killed at 0 time to provide the initial control GH concentration.

In a third experiment, pilocarpine nitrate, in doses of 0, 2, 5, or 10 mg/kg, and physostigmine sulfate, in doses of 0, 0.1, 0.3 and 0.5 mg/kg, were injected i.p. and the animals were decapitated 1 hr after injection.

Animals bearing an indwelling atrial cannula were injected i.v. with 2.5 and 10 mg/kg atropine sulfate (Sigma Chemical Co., St. Louis, MO). Blood samples (0.5 ml) were taken from the cannula 15, 30, 45, and 60 min after injection. An initial control blood sample was taken 5 min prior to injections. Each dose was tested on a different day with adequate control animals.

In a fifth experiment, male rats were injected i.p. with pilocarpine nitrate, physostigmine sulfate, or atropine sulfate alone or a combination of atropine and pilocarpine (for doses see Figure 12). Rats were decapitated 1 hr after injections.

Alpha-methylparatyrosine (250 mg/kg, Regis, Morton Grove, IL) was injected into control rats and rats injected with pilocarpine nitrate. One hr after injections, rats were decapitated and blood was collected for hormone assays. To determine whether the cholinergic drugs were eliciting their actions through DA, or noradrenergic receptor, rats were injected with pilocarpine (PIL), (5 mg/kg) alone and in combination with pimozide (PIM), (McNeil Labs, Ft. Washington, PA), a dopamine receptor blocker; PHEN (CIBA Pharmaceutical, Summitt, N.J.), an α-adrenergic receptor blocker and PROP (Ayerst Labs, N.Y.),an α-adrenergic receptor blocker. Rats were decapitated 1 hr after injections.

In a final <u>in vivo</u> experiment, male rats were injected with PIL. One half hr after injections, rats were immobilized for an additional 1/2 fr. After a total elapsed time of 1 hr, rats were decapitated to determine whether cholinergic agonists could prevent the stress induced decrease in serum GH concentrations.

The effects of Ach of GH release were examined in a co-incubation system (for procedure see Materials and Methods, for doses and times of Ach see Table 13). The effects of the serotonergic blocker, para-chloramphetamine (PCA), (Nutritional Biochemical Co., Cleveland, OH) and methysergide (METH), the DA receptor blocker, pimozide (PIM) and haloperiodol (HAL), (McNeal Labs., Ft. Washington, PA), and the cholinergic receptor blocker, atropine (ATR) were observed directly on the pituitary and on co-incubation systems (for doses and times see Tables 13 - 15).

Results

The effects of injections of acetylcholine into the lateral ventricle are shown in Figure 9. Intraventricular injections of 10 µl of 0.87% NaCl or 25µg Ach failed to significantly alter serum GH concentrations, whereas a 50 µg dose of Ach significantly increased serum GH concentrations by approximately 50%.

The effects of pilocarpine, a cholinergic receptor stimulator, and physostigmine, an anti-cholinesterase, are shown in Figure 10 and Table 11. Intraperitoneal injection of 0.3 mg/kg physostigmine, maximally increased serum GH concentrations 1 hr after injection (Figure 10). This appeared to be the optimal dose employed in the dose-response study (Table 11). Intraperitoneal injections of PIL (5 mg/kg) maximally elevated serum GH concentrations 1 - 2 hrs after injection, with no significant difference between the 1 and 2 hr blood samples. Injections of the 10 mg dose of PIL/kg

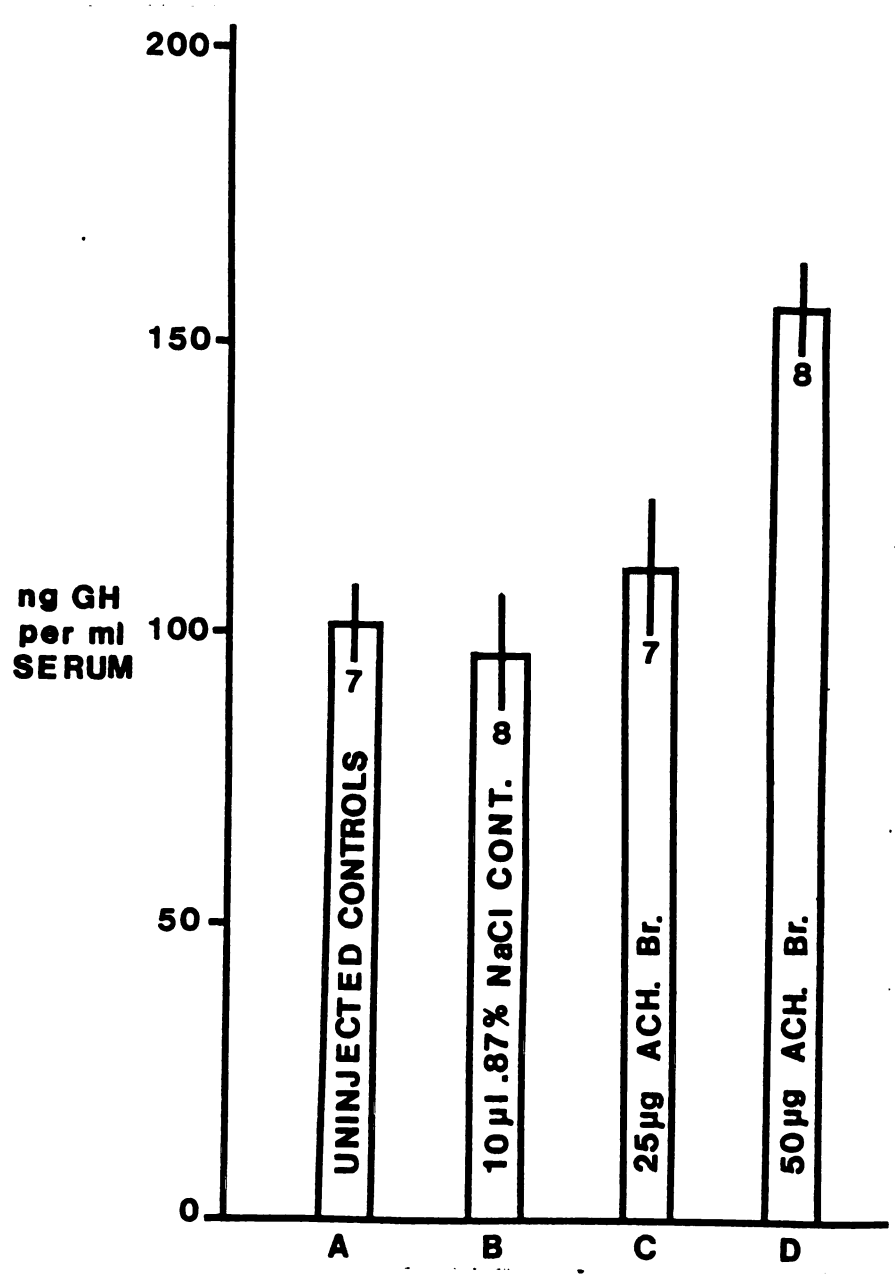

Figure 9. Effects of acetylcholine bromide on serum GH. Vertical bars represent SEM. N=8 or 8/group.

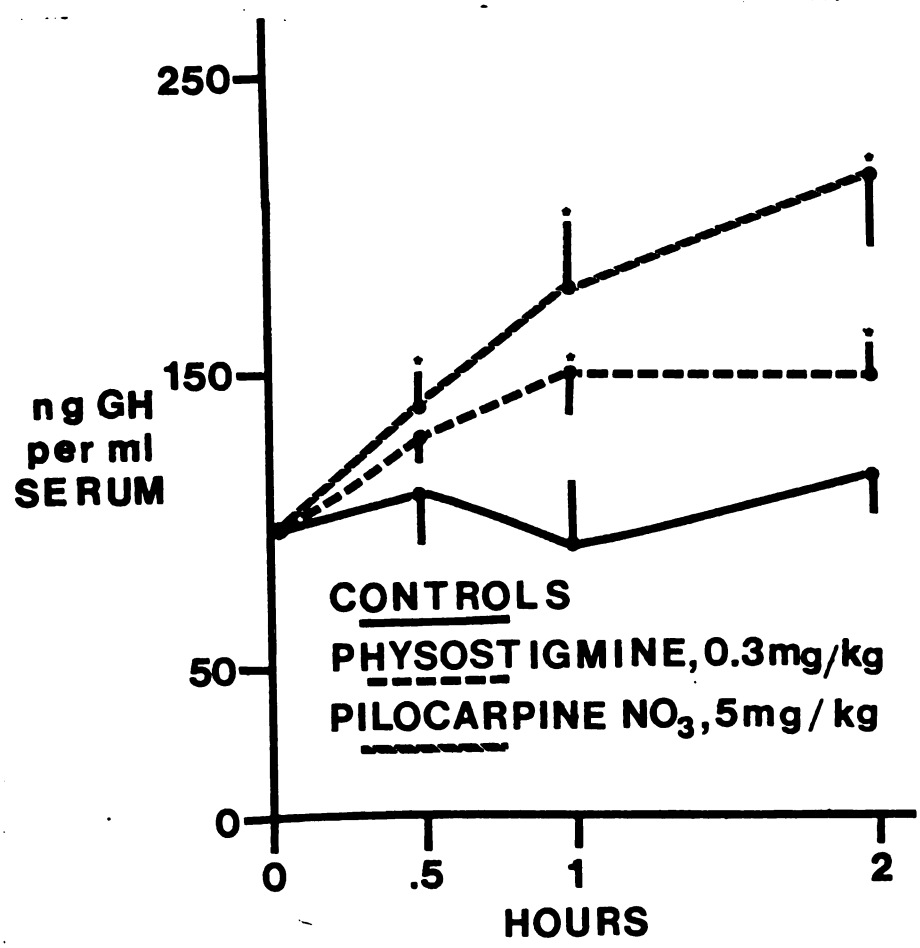

Figure 10. Time course effects of cholinergic drugs on serum GH. Vertical bars represent SEM. N=6/determination.

Table 11.

EFFECTS OF CHOLINERGIC DRUGS ON SERUM GH

ng/ml

PILOCARPINE NO_3	PHYSOSTIGMINE
CONTROLS - 98±14 [8]	CONTROLS - 125±8 [8]
2.0 mg/kg - 126±12 [8]	0.1 mg/kg - 143±10 [8]
5.0 mg/kg - 165±17 · [8]	0.3 mg/kg - 175±14 · [8]
10.0 mg/kg - 109±27 [8]	0.5 mg/kg - 168±17 · [8]

· $P < 0.05$ vs CONTROLS

stressed the animals as indicated by increased gastrointestinal motility and secretion of hematin from the eyes.

Intravenous injections of atropine sulfate at doses of 2, 5 and 10 mg/kg attenuated the morning episode of GH release (Figure 11). The 5 and 10 mg doses appear to be more effective in suppressing GH release than the 2 mg dose. These results suggest that Ach may participate in the episodic release of GH.

PHYSOS (0.3 mg/kg) and PIL (5 mg/kg) significantly elevated serum GH concentrations 1 hr after parenteral injections (Figure 12). Atropine sulfate (ATROP), a cholinergic receptor blocker, slightly decreased serum GH, when injected alone, and inhibited the increase produced by PIL injection.

Alpha-methylparatyrosine (α -MPT) decreased serum GH concentrations when injected alone and in combination with PIL (Figure 13). These results suggest that cholinergic neurons alter the release of GH through the catecholamines, DA and/or NE. When PIM or PHEN were injected into animals which also were given PIL, PIM and PHEN, the increase in serum GH concentration produced by PIL was prevented (Figure 14).

Ach and cholinergic agonists clearly increased GH release *in vivo*. However PIL did not prevent the stress induced decrease in serum GH concentrations (Table 12). Ach (100 ng/ml) does not act directly on the AP to increase GH release *in vitro* (Table 13). However, Ach prevents the hypothalamic inhibition of GH release *in vitro* using a

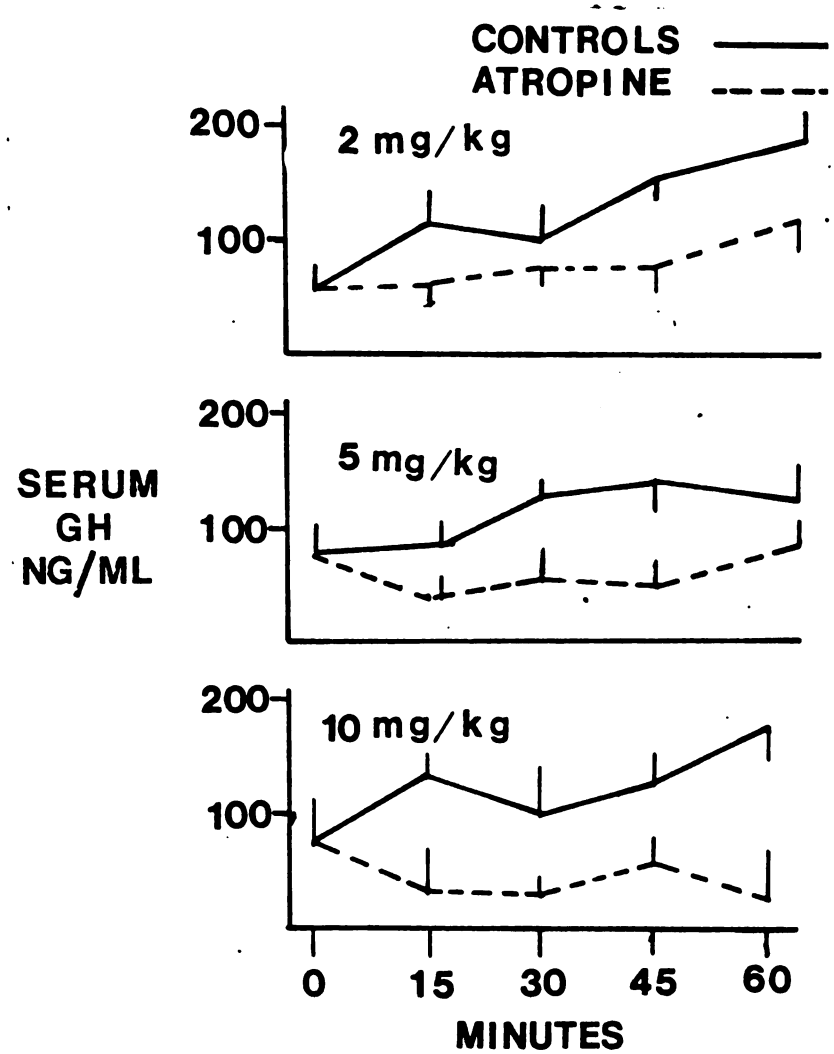

Figure 11. Effects of atropine sulfate on plasma GH. Vertical bars represent SEM. N=8/group.

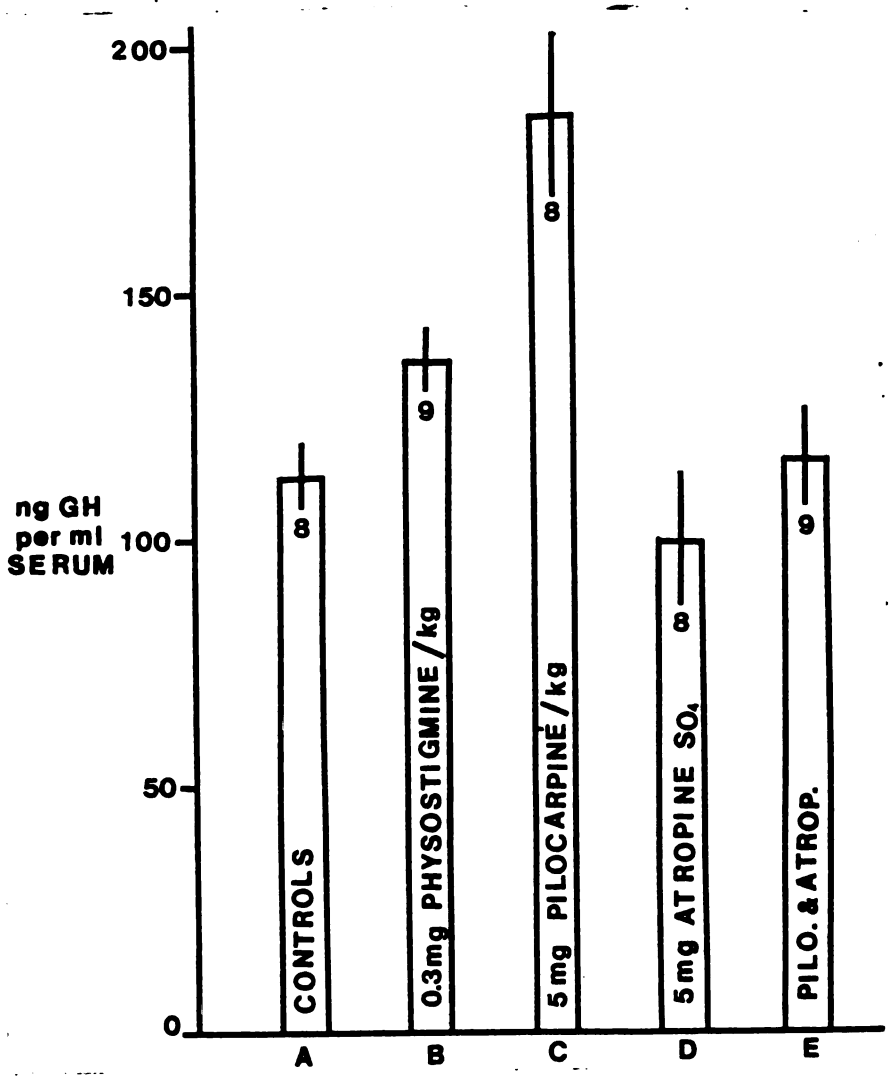

Figure 12. Effects of pilocarpine, physostigmine and atropine on serum GH. Vertical bars represent SEM. N=8 or 9/group.

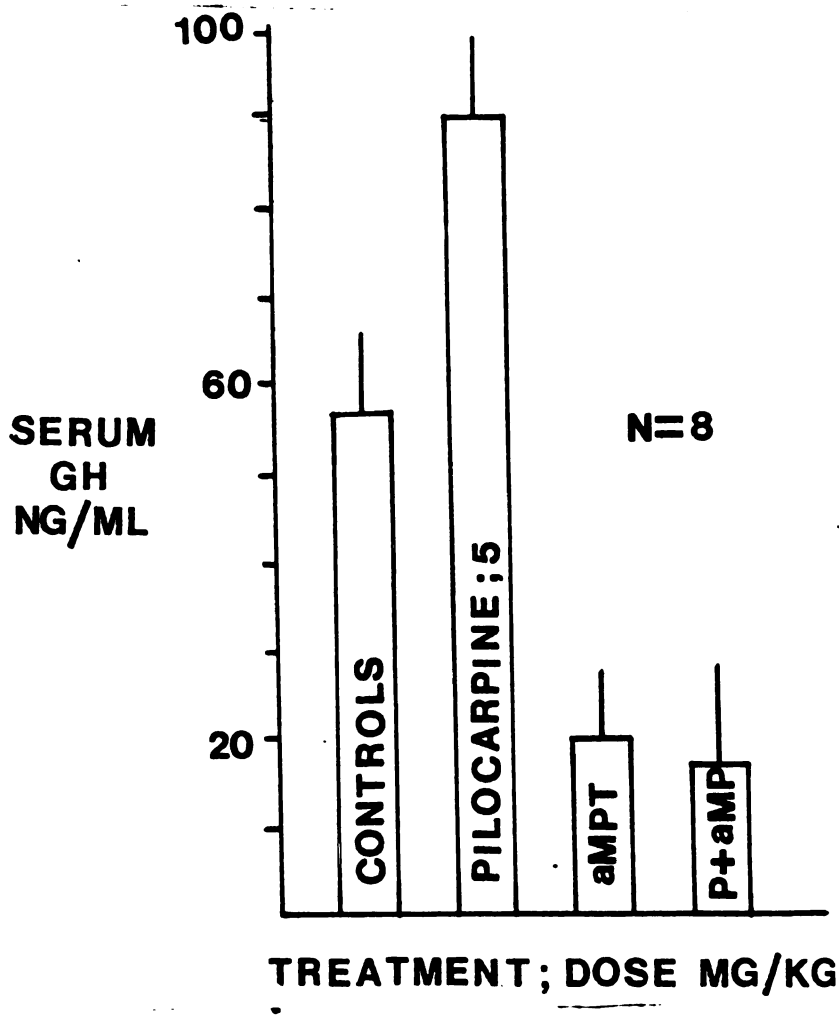

Figure 13. Effects of α-methyl-paratyrosine on pilocarpine induced GH release. Vertical bars represent SEM.

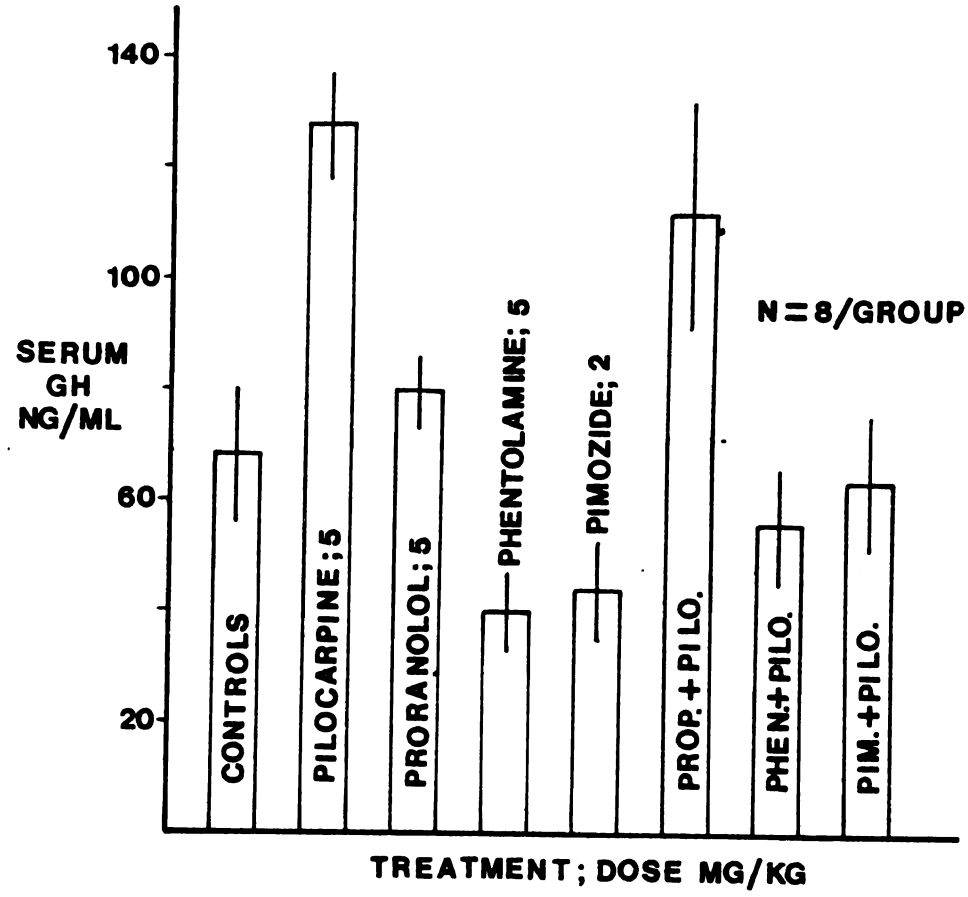

Figure 14. Effects of catecholamine blocking drugs on pilocarpine induced GH release. Vertical bars represent SEM.

Table 12.

Effects of Pilocarpine on Stress Induced Decrease in Serum GH

Controls (10)	I Stress (10)	Stress + 5 mg/kg Pilocarpine (10)
85 ± 8[a]	21 ± 15[b]	18 ± 9[b]
	II	
108 ± 9	17 ± 8[b]	25 ± 10[b]

a $\bar{X}$ ± S.E.M.; ng/ml.
b $P < 0.05$ compared to controls.

Table 13.

In Vitro Stimulation of GH by Acethycholine

n = 6 per group

	µg GH/ml/mg AP/hr		
	0-2 hrs	2-4 hrs	4-6 hrs
	199	ACh (100 ng/ml)	199
Pituitary	2.29 ± .18[a]	2.49 ± .26	2.18 ± .12
Pituitary + Hypothalamus	1.64 ± .15[b]	[c] →2.36 ± .25	[c] →1.55 ± .16[b]
	PCA (1 µg/ml)	PCA & ACh	PCA
Pituitary	1.94 ± .22	2.05 ± .16	2.00 ± .17
Pituitary + Hypothalamus	1.30 ± .06[b]	[c] →2.07 ± .13	[c] →1.42 ± .25
	PIM (100 ng/ml)	PIM & ACh	PIM
Pituitary	1.93 ± .26	1.83 ± .18	1.96 ± .14
Pituitary + Hypothalamus	1.13 ± .14[b]	1.95 ± .12	[c] →1.44 ± .09[b]
	ACh	ACh & ATR (100 ng/ml)	ACh
Pituitary	2.29 ± .16	2.17 ± .17	2.32 ± .31
Pituitary + Hypothalamus	2.18 ± .21	[c] →1.71 ± .21[b]	[c] →2.09 ± .18

[a] Mean ± S.E.M.
[b] $P<0.05$ compared to control.
[c] $P<0.05$ compared with preceeding 2 h period.
ACh = acetylcholine; PCA = parachloramphetamine;
PIM = pimozide; ATR = atropine.

co-incubation system. The 5-HT antagonist PCA or the DA receptor blocker PIM did not alter GH release directly on the AP or on the AP co-incubated with the hypothalamus. Also, these drugs did not prevent the Ach stimulated release of GH from co-incubated AP halves. ATR[illegible] completely prevented the cholinergic stimulated release of GH from co-incubated APs, without acting directly on the pituitary to alter GH release (Table 13).

PIL, at doses of 1, 10 and 100 ng/ml similarly prevented hypothalamic inhibition of GH release from co-incubated APs (Table 14). ATR[illegible] (100 ng/ml) completely prevented the increase in GH release from co-incubated APs without altering GH release directly in the pituitary (Table 14). Neither PIM, HAL, nor METH altered GH release from co-incubated AP halves which were treated with PIL (Table 15). Each of these drugs did not effect GH release directly from the AP or from co-incubated AP halves.

Conclusions

These studies provide evidence that cholinergic drugs can stimulate release of GH. Ach, PIL, and PHYSOS each stimulated GH release *in vivo*. Ach and PIL both stimulated GH release from co-incubated AP halves. Atropine, a muscarinic receptor blocker prevented the action of Ach on GH release *in vivo* and *in vitro*, indicating that this is a specific action of Ach. Since all the *in vivo* experiments were performed during an episodic surge of GH, these results suggest that Ach may potentiate the episodic release of GH

Table 14.

In Vitro Stimulation of GH by Pilocarpine

n = 6 per group

	µg GH/ml/mg AP/hr	
	0-2 hrs	2-4 hrs
	199	199
Pituitary	1.85 ± .21[a]	1.63 ± .11
Pituitary + Hypothalamus	1.27 ± .16[b]	1.31 ± .18
	Pilo. (1 ng/ml)	Pilo. (1 ng/ml) + Atropine (100 ng/ml)
Pituitary	2.01 ± .14	1.95 ± .22
Pituitary + Hypothalamus	1.87 ± .23	c → 1.36 ± .16[b]
	Pilo. (10 ng/ml)	Pilo. (10 ng/ml) + Atropine (100 ng/ml)
Pituitary	1.77 ± .23	1.85 ± .14
Pituitary + Hypothalamus	1.98 ± .31	1.41 ± .20[b]
	Pilo. (100 ng/ml)	Pilo. (100 ng/ml) + Atropine (100 ng/ml)
Pituitary	1.96 ± .21	1.81 ± .27
Pituitary + Hypothalamus	1.98 ± .32	1.53 ± .29[b]

[a] Mean ± S.E.M.
[b] $P<0.05$ compared to control AP half.
[c] $P<0.05$ compared with preceeding 2 h period.
Pilo.= pilocarpine.

Table 15.

In Vitro Effects of Monoaminergic Receptor Blockers on Pilocarpine Induced GH Secretion

n = 6 per group

	µg GH/ml/mg AP/hr	
	0-2 hrs	2-4 hrs
	PIM (1 ng/ml)	PIM (1 ng/ml) + Pilo. (100 ng/ml)
Pituitary	1.86 ± .32[a]	2.01 ± .16
Pituitary + Hypothalamus	1.35 ± .18[b]	c → 1.96 ± .25
	PIM (100 ng/ml)	PIM (100 ng/ml) + Pilo. (100 ng/ml)
Pituitary	1.75 ± .24	1.86 ± .21
Pituitary + Hypothalamus	1.25 ± .16[b]	c → 1.75 ± .19
	HAL (10 ng/ml)	HAL (10 ng/ml) + Pilo. (100 ng/ml)
Pituitary	1.98 ± .23	1.87 ± .22
Pituitary + Hypothalamus	1.38 ± .33	1.65 ± .09
	HAL (10 µg/ml)	HAL (1 µg/ml) + Pilo. (100 ng/ml)
Pituitary	1.97 ± .16	1.87 ± .23
Pituitary + Hypothalamus	1.26 ± .08[b]	c → 1.93 ± .31
	METH (10 ng/ml)	METH (10 ng/ml) + Pilo. (100 ng/ml)
Pituitary	1.87 ± .21	1.75 ± .31
Pituitary +	1.21 ± .10[b]	1.88 ± .21

[a] Mean ± S.E.M.
[b] $P < 0.05$ compared to control.
[c] $P < 0.05$ compared to previous 2 h period.
PIM = pimozide; HAL = haloperidol; METH = methysergide; Pilo. = polocarpine.

in vivo. The dose of Ach (50 μg) injected into the lateral ventricle, inducing GH release, may be pharmacological. However, the observation that PIL and PHYSOS also increased GH release, as well as the earlier observations on promotion of GH release by other cholinergic drugs (Cehovic et al., 1972; Soulairac et al., 1967) suggest that Ach may have a physiological role in regulating GH secretion. In addition, there is evidence that the hypothalamic cholinergic system may help regulate the secretion of LH, FSH (Everett et al., 1949a,b; Libertun and McCann, 1973) and PRL (Grandison et al., 1974; Grandison and Meites, 1976).

The observation that cholinergic stimulation of GH release can be blocked by a dopamine receptor blocker, PIM or PHEN, an α adrenergic receptor blocker, suggests that the action of Ach on GH release is mediated via the catecholaminergic system. Grandison and Meites (1976) reported that cholinergic inhibition of PRL release in rats is similarly mediated via the catecholaminergic system.

Since PIL did not alter the stress-induced decrease in serum GH, there appear to be other neuronal mechanisms which decrease GH release during stress, and probably also effect the diurnal variations in GH release.

V. A Possible Role of GABA in the Control of GH Release

Introduction

Neurotransmitters in the hypothalamus have been shown to alter the release of hormones from the pituitary (for reviews see Blackwell and Guillemin, 1973; Meites et al., 1977; Müller et al., 1978). The neurally active amino acid,GABA, also was found to be present in high concentrations in the hypothalamus (Kimura and Kuriyama, 1975; Tappaz et al., 1976). GABA was reported to inhibit ACTH (Burden et al., 1974; Markara and Stark, 1974) and MSH (Takeisnik et al., 1973/74), and to stimulate LH and PRL release (Ondo, 1974; Mioduszewski et al., 1976; Ondo and Pass, 1976). The present series of experiments were undertaken to examine the effects of GABA, and GABA agonists and antagonists on GH release.

Materials and Methods

GABA (Nutritional Biochemical Co., Cleveland, OH), and bicuculline(Pierce, Rockford, IL) and bicuculline methyliodide (Pierce, Rockford, IL), both GABA receptor blockers, were dissolved in 0.87% NaCl and the pH of these solutions was adjusted to pH-7 by addition of 0.1N NaOH. Picrotoxin (PIC) (Nutritional Biochemical Corp., Cleveland, OH), a GABA receptor blocker, amino-oxyacetic acid, a GABA agonist (AOAA, Aldrich Chemical Co., Milwaukee, WI), a α-methyl-paratyrosine, PIM, MET, PROP, and PHEN were dissolved in 0.87% NaCl.

In the first three experiments, male rats were implanted with a polyethylene cannula in the lateral cerebral ventricle (see Materials and Methods; Verster et al., 1971). GABA, bicuculline methyliodide(BIC MI) and combinations of the two drugs were injected into the lateral ventricle. Each drug was infused (60 sec) into the lateral ventricle in a volume of 8 µl. The cannulae were rinsed with 2 µl of 0.87% NaCl. Control rats were injected with 10 µl of 0.87% NaCl.

In the next two experiments, AOAA, a GABA agonist; picrotoxin, and bicuculline, both GABA receptor blockers were injected systemically. Rats injected with AOAA were bled via orbital sinus cannulation under light ether anesthesia 1.5 and 6 h after injection. Rats injected with BIC and PIC were decapitated 30 min after injections. The effects of BIC on hypothalamic and median eminence (ME) DA and NE concentrations, and turnover index were determined in a fifth experiment. Male rats were injected i.p. with BIC (2.5 mg/kg) alone and in combination with α-MPT (250 mg/kg). After 1 hr rats were decapitated, brains removed and the hypothalamus and median eminence were dissected away from the brain using a dissecting microscope. The tissue was rapidly frozen on dry ice and transferred to a -40°C freezer until assayed for DA and NE concentrations by the methods of Coyle and Henry, 1973, and Cuello et al., 1973 (see Materials and Methods). Blood was collected from the cervical wound for GH assays.

blood was collected from the cervical wound for GH assays.

In a sixth experiment, the direct effects of GABA on GH release in vivo were examined. Male hypophysectomized (HYPOX) rats (Hormone Assay Labs, Chicago, IL) were transplanted with a single AP under the kidney capsule. Ten HYPOX rats served as controls. Rats given AP transplants were injected daily with 10, and 100 mg/kg GABA beginning on the second day after the APs were transplanted under the kidney capsule. Blood samples were taken via cardiac puncture under light ether anesthesia 1 hr after injection on days 2, 3, and 5 after initial GABA injections.

GABA at doses of 1, 10, 100, and 1000 mg/ml were incubated with AP halves to determine the direct in vitro effects of GABA on GH release. Also GABA at several doses (Figure 23) was co-incubated with AP halves and a hypothalamus to determine whether GABA acted on the hypothalamus to alter GH release. GABA was also incubated with monoaminergic receptor blockers and PIC in medium containing hypothalamus and pituitary (for doses of drugs see Figure 24 and Methods and Materials).

Results

Intraventricular injections of GABA at doses of 0.5 µM or 1 µM per rat slightly reduced serum GH concentrations 20 min after injection (Figure 15). The 5 and 10 µM doses of GABA significantly reduced serum GH concentrations during the same time interval. Conversely, i.v. injections of BIC MI at doses of 0.2 and 0.4 ug slightly increased serum GH,

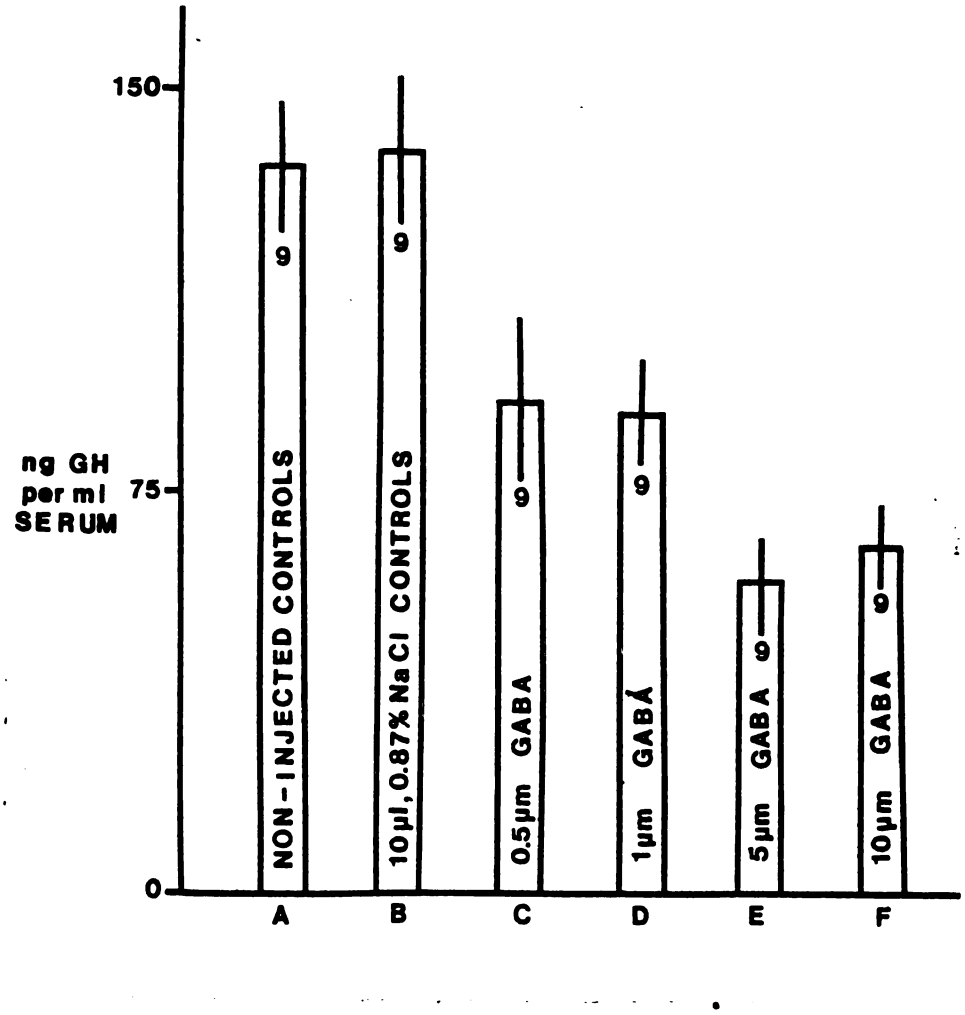

Figure 15. Dose response effects of GABA on serum GH. Vertical bars represent SEM. N=9/group.

concentrations whereas the 0.6 μg dose significantly increased serum GH concentrations 20 min after injections (Figure 16).

In another experiment 10 μM of GABA significantly reduced serum GH concentrations, whereas 0.7 μg BIC MI significantly increased serum GH concentrations 20 min after injection (Figure 17). These two drugs injected together did not alter serum GH concentrations as compared to control rats injected with 0.87% NaCl.

Intraperitoneal injections of AOAA, a GABA agonist, at a dose of 25 mg/kg significantly reduced serum GH concentrations 1.5 hr, but not 6 hr after injection (Figure 18). The 50 mg dose of AOAA significantly reduced serum GH concentrations 6 hr after injection, but not 1.5 hr after injection. This is a result of the large standard error observed 1.5 hr after injection.

Systemic injections of BIC (1.25 and 2.5 mg/kg) or PIC (0.5 mg/kg) significantly increased serum GH concentration above control values and those animals injected with 10 μM GABA (Figure 19). The larger dose of picrotoxin did not alter serum GH concentrations. BIC, (2.5 mg/kg) i.p., significantly increased GH release (Figure 20), and α-MPT alone decreased serum GH concentrations. When α-MPT was injected concurrently with BIC, α-MPT completely inhibited GH release produced by BIC. Measurement of the biogenic amines revealed that BIC significantly increased NE turnover index in the hypothalamus, as revealed by a greater NE depletion after α-MPT treatment

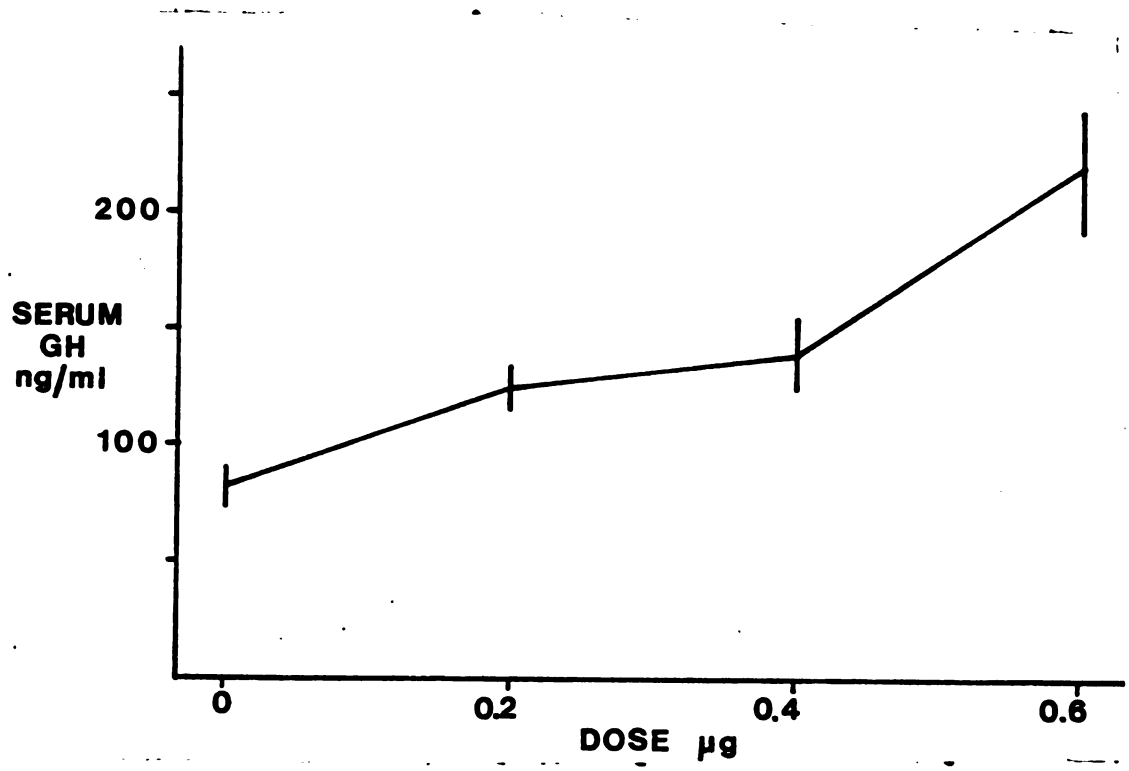

Figure 16. Effects of intraventricular injections of bicuculline methyliodide on serum GH. Vertical bars represent SEM. N=8/determination. R=0.84.

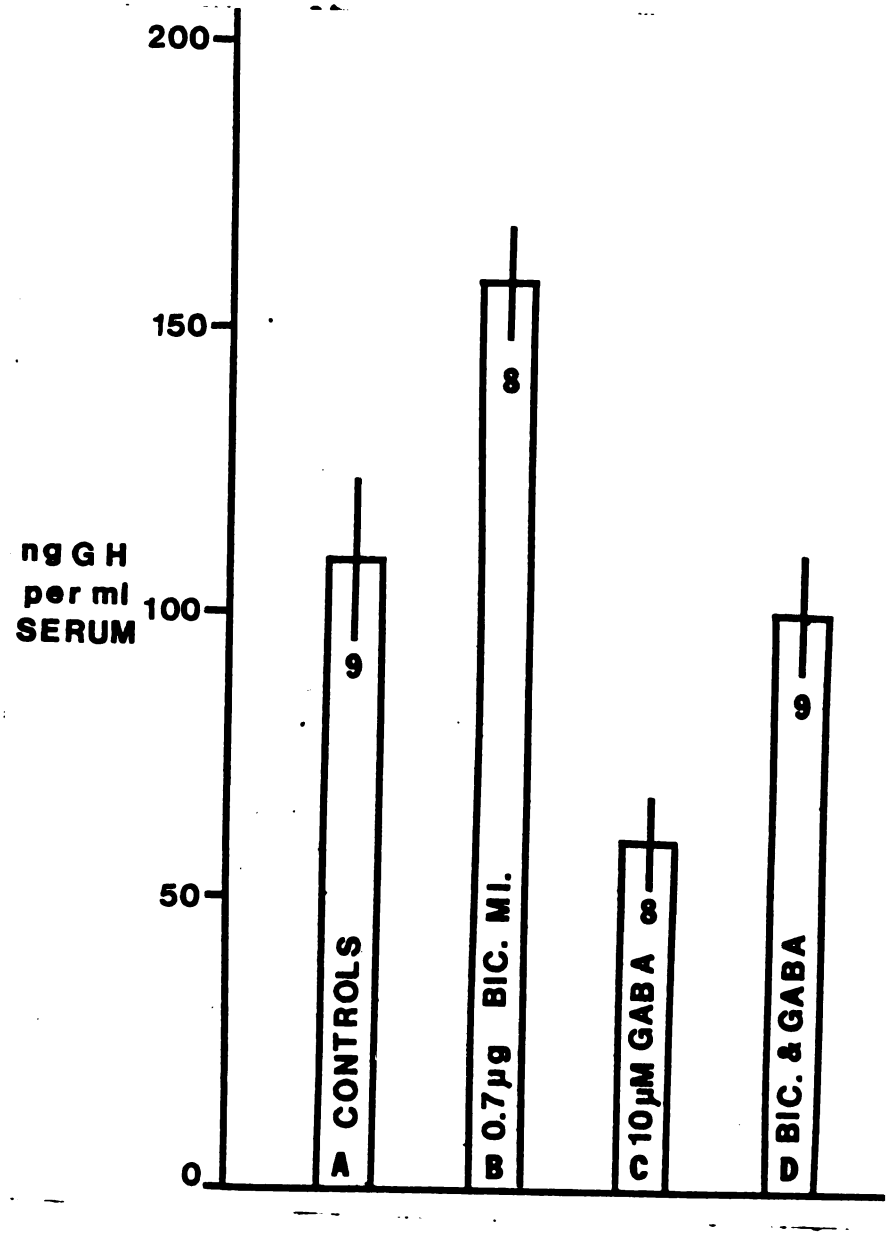

Figure 17. Effects of GABA and bicuculline methyliodide on serum GH. Vertical bars represent SEM. N=8 or 9/group.

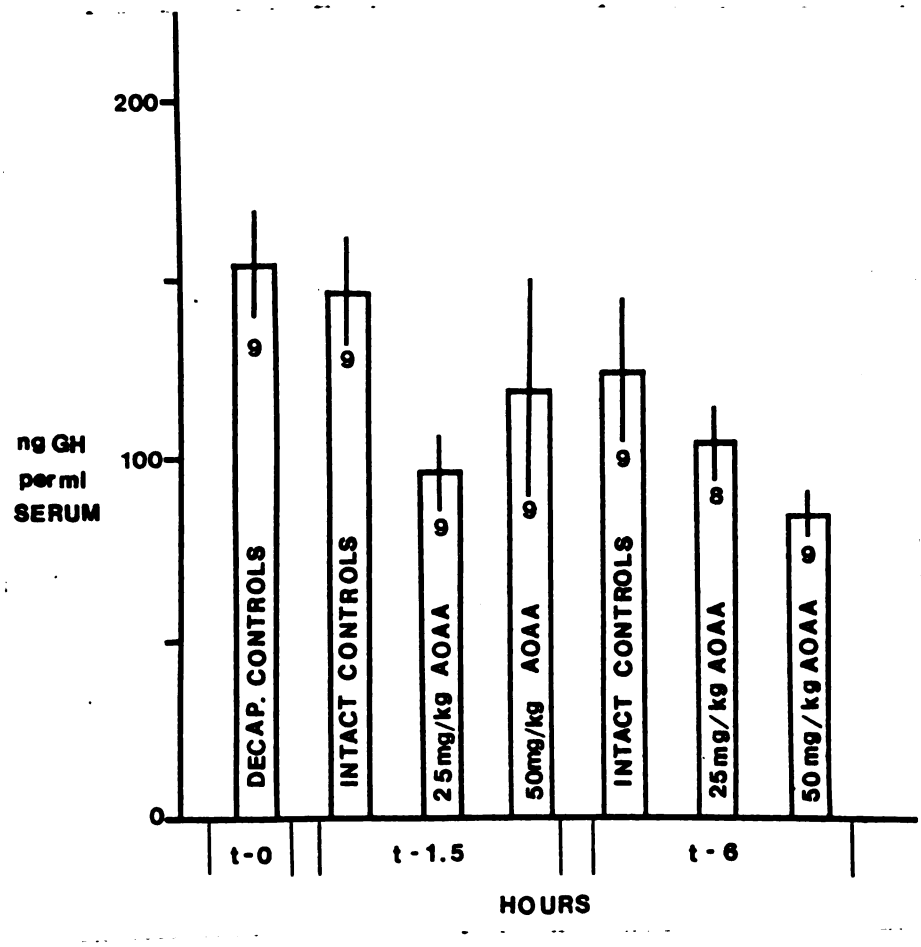

Figure 18. Time course effects of amino-oxyacetic acid (AOAA) on serum GH. Vertical bars represent SEM. N=8 or 9/group.

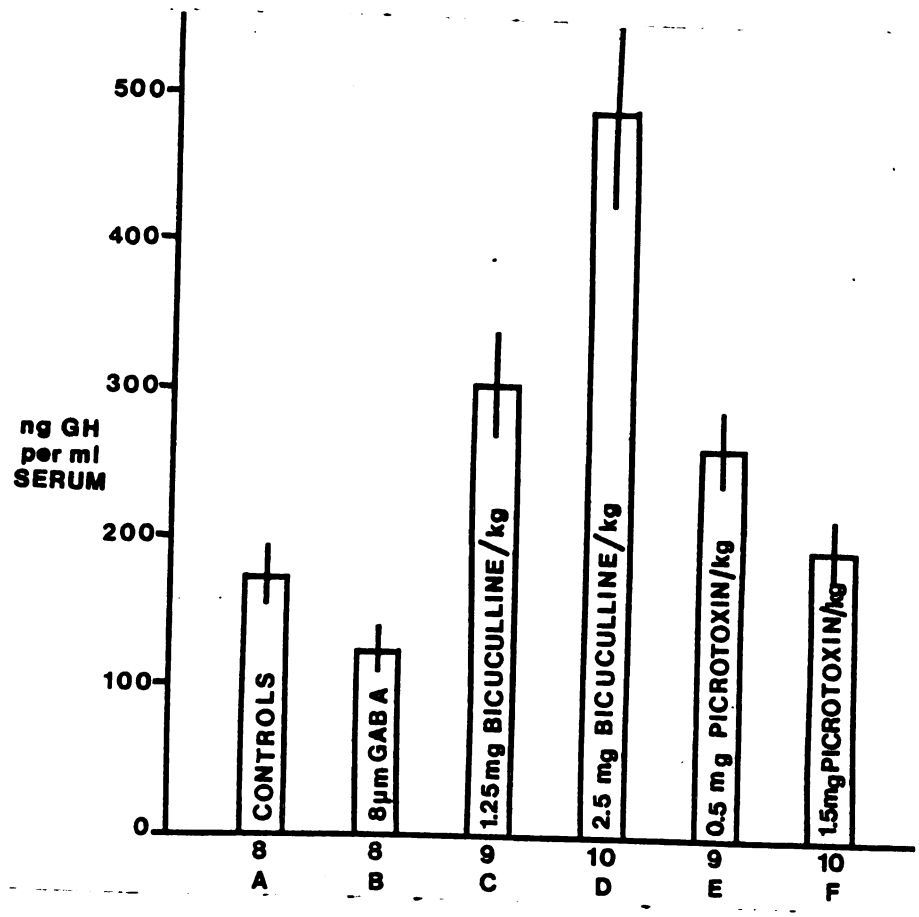

Figure 19. Effects of GABA antagonists on serum GH. Vertical bars represent SEM. N=8, 9 or 10/group.

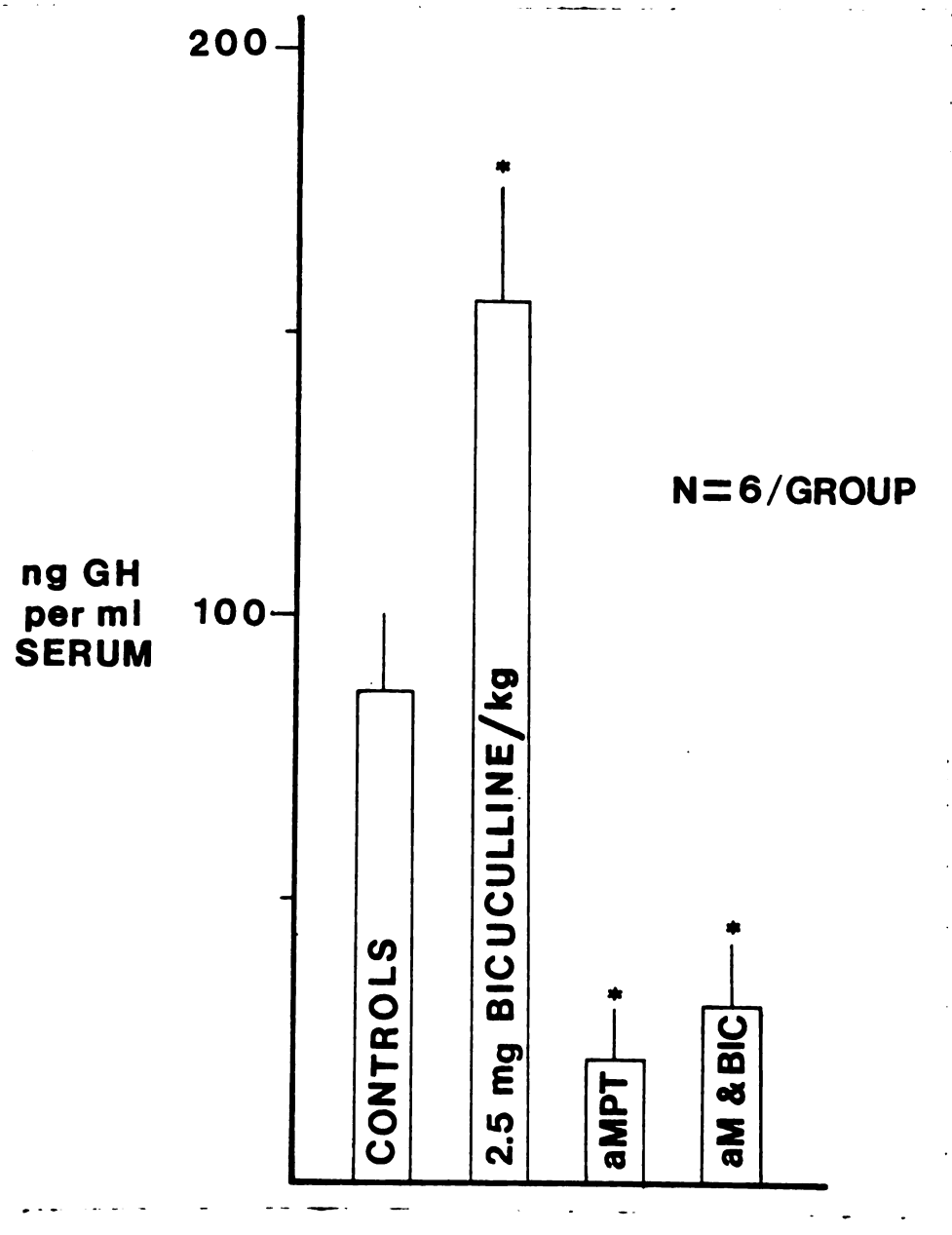

Figure 20. Effects of bicuculline and α-methyl-paratyrosine on serum GH. Vertical bars represent SEM. N=6/group. a MPT= α-methyl-paratyrosine.

(Figure 21). BIC did not alter steady state concentrations of DA or NE. Additionally, BIC did not alter ME steady state or turnover index concentrations of DA or NE. Intraperitoneal injections of GABA into HYPOX rats bearing a single AP transplanted under the kidney capsule did not alter GH release from transplanted pituitaries (Table 16).

In vitro incubation of GABA with AP halves did not alter GH release from the AP (Figure 22). Co-incubation of GABA at several doses with AP halves and hypothalami increased the inhibition of GH release produced by the hypothalamus alone (Figure 23). This inhibition was overcome by addition of PIC to the incubation media. The DA receptor blocker, PIM; the 5-HT receptor blocker, MET; the GABA receptor blocker, PIC; the β-adrenergic receptor blocker, PRO; and the α-adrenergic receptor blocker, PHE, did not alter GH release directly from AP alone or from co-incubated APs (Figure 24). Addition of 1 μg GABA/ml media did not effect GH release from these co-incubated AP halves.

Conclusions

An increase in central GABA, either by intraventricular injections of GABA or by parenteral injections of AOAA, resulted in decreased GH release. Even though AOAA elevates brain GABA for 6 hrs (Wallach, 1961; Perry et al., 1974), the effects on GH at this time were minimal. This discrepency could be explained if other neural mechanisms

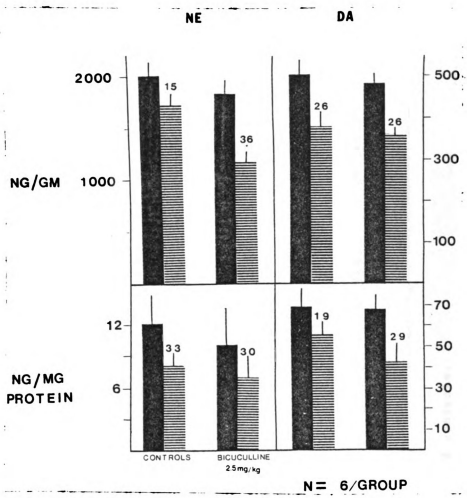

Figure 21. Effects of bicuculline on hypothalamic and median eminence NE and DA. Lower panel represents median eminence and the upper panel represents the remainder of the hypothalamus. Solid bars represent steady state concentrations; broken bars represent concentrations 1 h after αMPT. Vertical bars represent SEM. Numbers above broken bars represent % depletion.

Table 16.

Direct Effects of GABA on Serum GH In Vivo

	Day 2 Pretreatment	Day 2 1 hr Post-treatment	Day 3 1 hr Post-treatment	Day 5 1 hr Post-treatment
HYPOX Controls (10)	12.6±1.1[a]	13.5±2.0	14.6±5,0	18.2±3.6
HYPOX + 1 AP (KC)	29.7±2.0[b]	27.3±4.0[b]	30.1±1.8[b]	31.6±5.0[b]
HYPOX + 1AP (KC)+ 10mg/kg GABA	24.3±0.8[b]	26.5±2.1[b]	32.6±4.0[b]	28.5±0.8[b]
HYPOX + 1 AP (KC)+ 100 mg/kg GABA	31.6±3.0[b]	28.5±4.0[b]	29.5±6.0[b]	29.1±0.9[b]

a $\bar{x}$ ± S.E.M.; ng/ml.
b P<0.05 compared to HYPOX controls.
c P<0.05 compared to HYPOX rats with 1 AP under the kidney capsule (KC)

W=10/group.

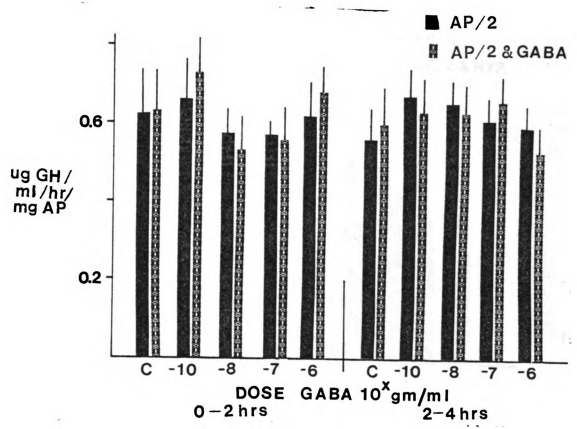

Figure 22. In vitro effects of GABA on GH release. Vertical bars represent SEM. N=8/group. AP/2=Anterior Pituitary half.

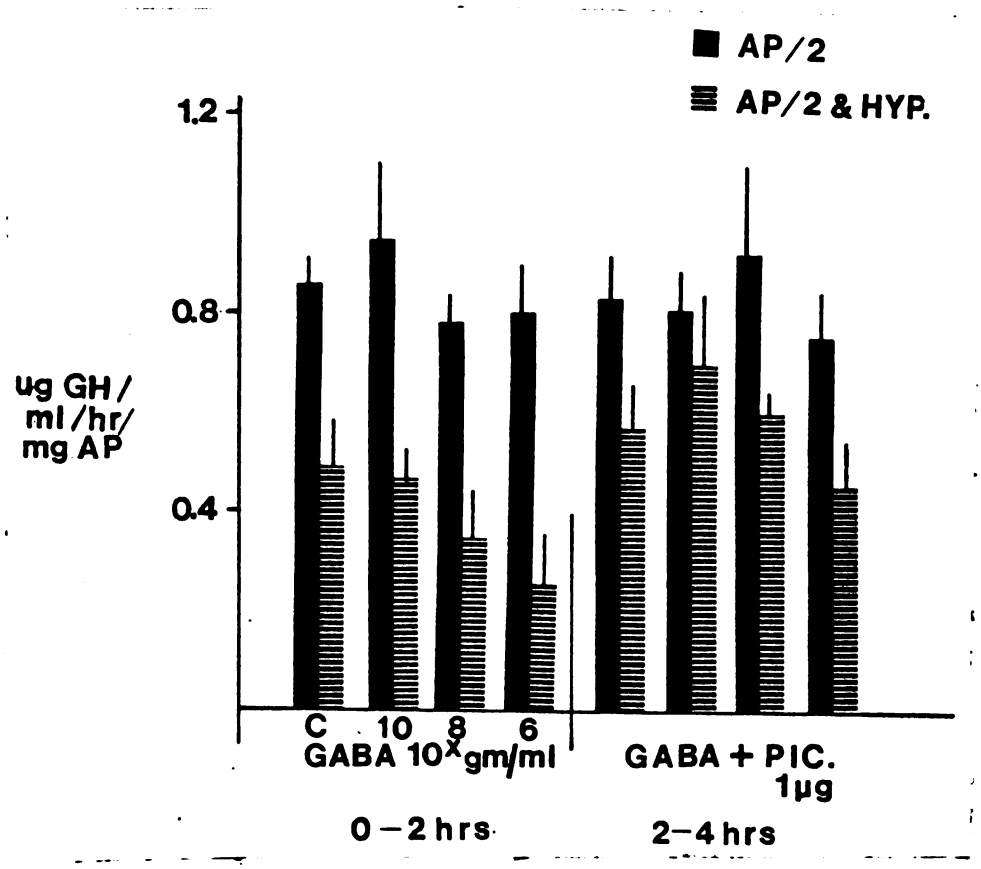

Figure 23. In vitro effects of GABA and picrotoxin on GH release in co-incubation. Vertical bars represent SEM. N=8/group. AP/2=Anterior Pituitary half; Hyp=Hypothalamus.

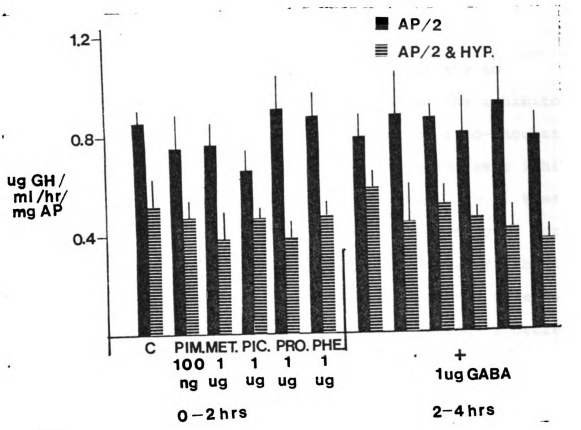

Figure 24. In vitro effects of central monoaminergic blocking drugs and GABA on GH release in co-incubation. Vertical bars represent SEM. N=8/group. AP/2=Anterior Pituitary half; Hyp=Hypothalamus.

changed to compensate for this increase in brain GABA. BIC, BIC MI, and PIC all GABA receptor blockers (Curtis et al., 1971; Pong and Graham, 1972; Nistri et al., 1974; Shank et al., 1974) significantly increased serum GH concentrations 20 and 60 min after injections. Additionally, BIC MI was capable of reversing the decrease in GH release produced by GABA.

GABA does not act directly on the pituitary to decrease GH release, but appears to increase the inhibitory effects of the hypothalamus on GH release in a co-incubation. NE was previously shown to decrease the hypothalamic inhibitory influence on GH release in vitro (Bruni, Ph.D. thesis). In view of these results, GABA could be decreasing NE activity in the hypothalamus, thus resulting in greater inhibition of GH release. GABA also may stimulate the release of somatostatin from the hypothalamus, resulting in decreased GH release observed in vitro. α-MPT decreased the stimulatory effects of BIC on GH release. These results suggest that BIC exerts its effects on GH release through the catecholamines. Examination of hypothalamic catecholamine activity showed that the NE turnover index (TI) was increased by treatment with BIC.

NE was previously shown to stimulate GH release in vivo (Luizzi et al., 1971; Durrand et al., 1977; Bruni, Ph.D. thesis) and in vitro in a co-incubation system (Bruni, Ph.D. thesis). Thus, GABA may inhibit NE turnover which can result in depression of GH release. Measurement of hypothalamic somatostatin concentrations following drug

treatment would help clarify the mechanism whereby these putative neurotransmitters affect GH release.

VI. Effects of Methionine-Enkephalin, Naloxone and Morphine on GH Release

Introduction

Recently several morphinomimetic peptides have been isolated from the mammalian central nervous system, including methionine- (MET-) and leucine- (LEU-) enkephalin (ENK) (Hughes et al., 1975), and β-endorphin (Cox et al., 1976). These peptides share common amino acid sequences with the β-lipotropin molecule, which may infer a role for β-lipotropin as a prohormone for endorphins (Lazarus et al., 1976). The pars intermedia and isolated clusters of cells in the pars distalis were shown to contain high concentrations of β-lipotropin (Pelletier et al., 1977). These same cells also contained abundant amounts of ACTH (Pelletier et al., 1977).

Several labs recently reported that morphinomimetic peptides released PRL and GH (Lein et al., 1976; Rivier et al., 1977b, Bruni et al., 1977b; Bruni, Ph.D. thesis). The stimulatory effects of β-endorphin and MET-ENK on GH and PRL release were shown to be reversed by concurrent treatment with the opiate antagonist, naloxone (NAL) (Rivier et al., 1977b, Bruni et al., 1977b, Bruni, Ph.D. thesis).

Similarly, morphine has been shown to increase GH

release in rats (Kokka et al., 1972; Kokka et al., 1973; Martin et al., 1975; Rivier et al., 1977a, Bruni et al., 1977b, Bruni, Ph.D. thesis). This study was performed to determine the effects of MET-ENK, morhpine (MS) and NAL on GH release in rats. Also the effects of MS on the stress induced decrease in serum GH concentrations were observed.

Materials and Methods

Male Sprague-Dawley rats (200-250g each) were injected with NAL (Endo Labs, Garden City, N.Y.), MS (Mallinkrodt Labs., St. Louis, MO) or MET-ENK (Bachem, Marina Del Ray, CA), each given individually, or combinations of these drugs injected simultaneously. The drugs were injected i.p. in 0.1 ml of 0.87% NaCl/100 g B.W. (for doses see Tables 17 and 18). All rats were decapitated 20 min after injections.

In a second experiment MS was injected i.v. 30 min after the initial blood sample was taken. Sequential blood samples (200 µl) were taken from an indwelling atrial cannula every 5 min for the next 30 min. Plasma was separated and assayed for GH.

Forty male Sprague-Dawley rats were used in a third experiment. Ten rats served as controls and were decapitated 30 min after i.p. injections of 0.87% NaCl. Ten of the remaining rats were injected with 0.87% NaCl and were restrained for 30 min. The remaining 2 groups of rats were injected i.p. with 2 mg/kg MS or 5 mg MS and were restrained for 30 min. After 30 min of restraint stress

rats were decapitated and blood was collected for GH radioimmunoassay.

Results

The effects of NAL, MS, and MET-ENK on serum GH concentrations are shown in Tables 17 and 18. NAL at a dose of 0.2 and 5 mg/kg significantly reduced serum GH concentrations 20 min after injections. The 2 mg dose was ineffective due to the large standard error. MS at doses of 2, 10 and 15 mg/kg significantly increased serum GH concentration 20 min after injection. Similarly, MET-ENK (5 mg/kg) significantly increased serum GH concentrations after injection. When MS or MET-ENK were injected concurrently with NAL, NAL attenuated the increase in serum GH concentrations produced by MS or MET-ENK.

Intravenous injections of MS (5 mg/kg) significantly increased serum GH concentrations 10 min after injections (Figure 25). Serum GH continued to increase until 20 min after injection and thereafter plateaued and remained higher than controls for the continuation of the experiments.

Rats placed under restraint stress had significantly lower serum GH concentrations than the non-restrained control rats (Table 19). MS (2 mg/kg) failed to increase serum GH concentrations 30 min after injections. However, MS (10 mg/kg) injected into restrained rats significantly increased serum GH concentrations above control values and prevented the decrease in serum GH induced by restraint stress.

Table 17.

EFFECTS OF NALOXONE, MORPHINE, AND METHIONINE ENKEPHALIN ON SERUM GH

n = 10/GROUP	ng/ml
CONTROLS 0.87% NaCl	148 ± 17[a]
NALOXONE 0.2 mg/kg	74 ± 24[b]
MORPHINE 10.0 mg/kg	1622 ± 129[b]
MET-ENK 5.0 mg/kg	258 ± 62[b]
NAL & ME 0.2 + 5.0	149 ± 16
NAL & MOR 0.2 + 10.0	1155 ± 56[b]

[a] $\bar{x}$ S.E.M. [b] $P<0.05$ compared with controls.

Table 18.

EFFECTS OF NALOXONE, MORPHINE, AND METHIONINE ENKEPHALIN ON SERUM GH

n = 10/GROUP	ng/ml
CONTROLS 0.87% NaCl	131 ± 23[a]
NALOXONE 0.2 mg/kg	77 ± 10[b]
NALOXONE 2.0 mg/kg	103 ± 40
NALOXONE 5.0 mg/kg	48 ± 6[b]
MORPHINE 2.0 mg/kg	839 ± 172[b]
MORPHINE 10.0 mg/kg	1211 ± 185[b]
MORPHINE 15.0 mg/kg	1775 ± 172[b]
MET-ENK 5.0 mg/kg	215 ± 27[b]
NAL & MOR 0.2 + 2.0	383 ± 71[b]
NAL & MOR 0.2 + 10.0	902 ± 168[b]
NAL & ME 0.2 + 5.0	138 ± 37

[a] $\bar{x}$ ± S.E.M. [b] P<0.05 compared with controls.

Table 19.

EFFECT OF MORPHINE SULFATE ON STRESS INDUCED DECREASE IN SERUM GH

1 CONTROLS N=10/GROUP	2 STRESS	3 STRESS + 2 mg/kg MS	4 STRESS + 10 mg/kg MS
53±9[a]	20±2	27±4	144±37
2,3,4[b]	1,4	1,4	1,2,3

a; X ± SEM

b; $p < 0.05$ COMPARED TO INDICATED GROUP

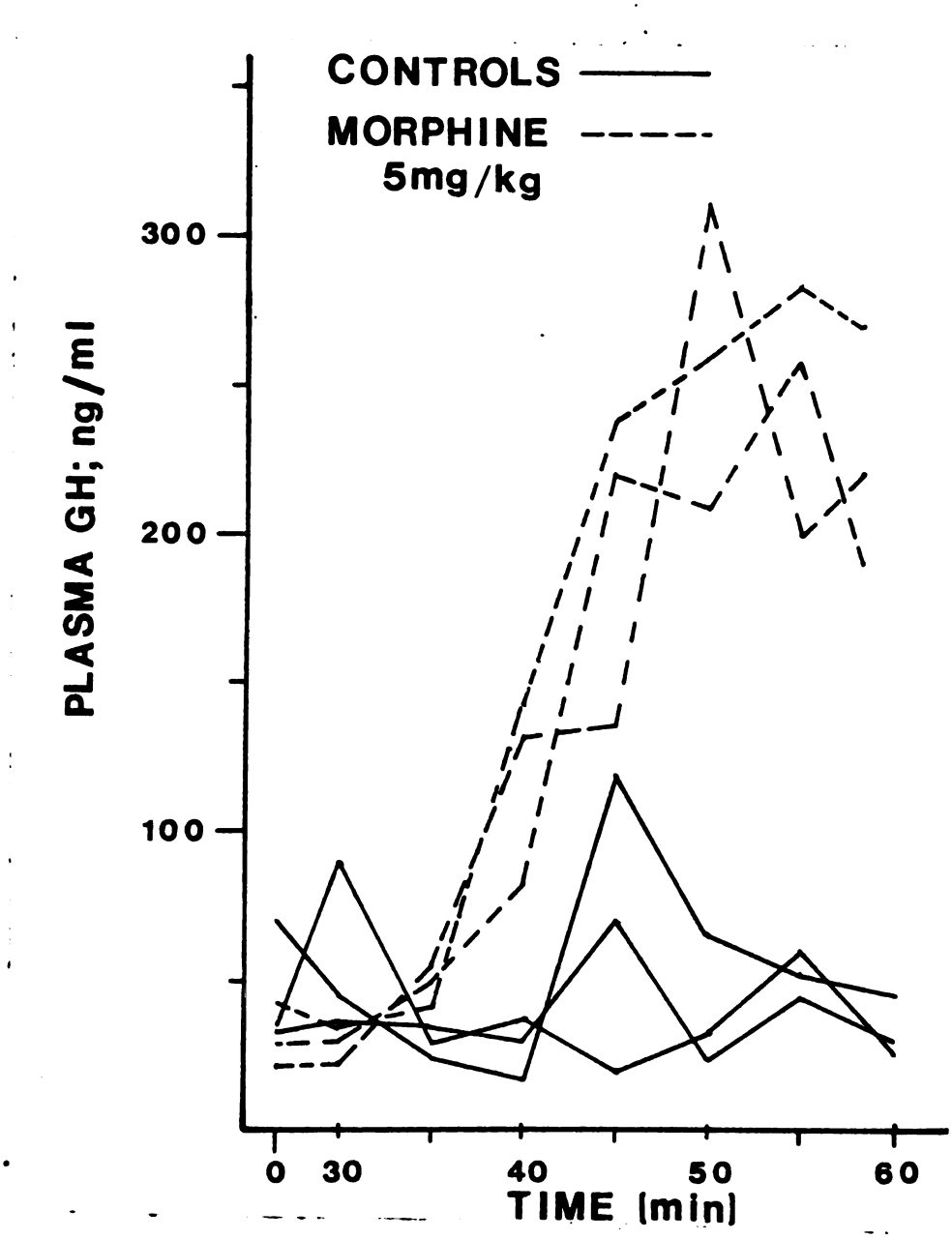

Figure 25. Effects of morphine on serum GH.

Conclusions

These results show that NAL, an opiate antagonist, decreases serum GH concentrations, and that MS and MET-ENK significantly increase serum GH concentrations. This suggests that the endogenous opiates may help maintain basal serum concentrations of GH. Concurrent injections of NAL with MS or MET-ENK reduced the ability of the latter 2 drugs to stimulate GH release. Since stress has been shown to increase endogenous opioid activity in the brain (Akil *et al*. 1976; Müller *et al*. 1977), which would then be expected to increase GH release, it is unlikely that MET-ENK or β-endorphin are involved in the stress-induced decrease in serum GH concentrations. The reduction in GH release by stress apparently involves other hypothalamic mechanisms.

GENERAL DISCUSSION

Interaction between neurotransmitters and hypothalamic hypophysiotropic hormones in the control of anterior pituitary function is widely accepted (for reviews see Meites et al., 1977, and Müller et al., 1977). The role of neurotransmitters in the control of GH secretion is still controversial (see Literature Review). Generally, GH is believed to be controlled by two hypophysiotropic hormones, GH-releasing factor (GRF) and GH-inhibiting hormone (somatostatin, GIF). The release of the hypothalamic hormones appears to be modulated by the catecholamines, indolamines, acetylcholine, gamma-aminobutyric acid and the endogenous opioid peptides, as indicated in this thesis.

The catecholamine, dopamine, appears to have a stimulatory effect on GH secretion in rats, and primates (Müller et al., 1976; Martin et al., 1977). The precise mechanism whereby dopamine stimulates GH release has not been determined. Dopamine may inhibit the release of GIF or stimulate the release of GRF. Dopamine does not act directly on the pituitary to prompte GH release (MacLeod et al., 1970). The possible interactions of the tuberoinfundibular dopaminergic system with other neurotransmitters also have not been investigated.

One of the major stimuli for GH secretion is norepinephrine. Norepinephrine appears to exert its effects through an α-adrenergic receptor, since GH release is inhibited by the α-adrenergic receptor blocker, phentolamine, and is stimulated by the α-adrenergic agonist, clonidine. These results appear to be in agreement with the observations of Durand et al. (1977) and Martin et al. (1978), who also reported that the α-adrenergic receptor was effective in released GH.

In vitro results further support the importance of the α-adrenergic receptor in the control of GH release. Addition of norepinephrine to pituitary halves co-incubated with hypothalamus, counteracted the inhibitory effects of the hypothalamus on GH release. These effects were blocked by the α-adrenergic receptor blocker, phentolamine, whereas propranalol did not alter GH release from a pituitary-hypothalamus co-incubation system. Pimozide, a dopamine receptor blocker, did not alter GH release by a direct on action the pituitary or from a hypothalamus-pituitary co-incubation system.

Since hypothalamic hormone release from incubated hypothalamus was not measured in any of the in vitro experiments, one must assume that norepinephrine either inhibits hypothalamic somatostatin or stimulated hypothalamic GRF release, or both. Unfortunately, the radioimmunoassay for somatostatin is not readily available and GRF cannot be measured, since its structure is unknown.

The role of serotonin in the control of GH is very controversial. Work by Müller *et al*. (1968) has indicated that serotonin inhibits GH release. The data presented in this thesis support previous work indicating that serotonin stimulated GH release (Collu *et al*., 1972). Using a specific neurotoxin for serotinin, 5, 7, dihydroxytryptamine, basal and episodic GH release were impaired. Methysergide, a serotonin receptor blocker, similarly decreased basal serum GH concentrations. It is unlikely that the effects of methysergide, like most other ergot drugs, increases central dopaminergic activity since methysergide is almost void of vasoconstrictor properties possessed by other ergot alkaloids. These results were supported further by employing the tryptophan hydroxylase inhibitor, parachlorophenylalanine. Thus decreased serotonin concentrations and GH secretion was reduced for several days after drug treatment.

In vitro results suggest that the effect of serotonin, on GH release, is mediated within the hypothalamus rather than directly on the pituitary. The mechanism responsible for release of GH appears to be mediated specifically via a serotonergic pathway, since the effects of serotonin on GH release in pituitary-hypothalamus co-incubation were only reversed by the serotonergic receptor blocker, methysergide. Moreover, methysergide did not alter GH release *in vitro*, induced by Ach or NE in a pituitary-hypothalamus co-incubation. However, the effects of methysergide on GH release

induced by dopamine were not studied *in vivo* or *in vitro*.

Acetylcholine appears to have a stimulatory role on GH release. However, the effect of acetylcholine appears to be mediated via catecholaminergic receptors since pimozide, a dopamine receptor blocker, phentolamine, an α-adrenergic receptor blocker, and α-methylparatyrosine, a tyrosine hydroxylase inhibitor, all were able to block the effects of the cholinergic agonist, pilocarpine, on GH release. The actions of cholinergic drugs on GH release appear to be specific since the muscarinic blocking drug, atropine, inhibited the effects of pilocarpine.

These data on the cholinergic effects on GH secretion appear to be in agreement with the earlier reports of Cehovic *et al*. (1972) and Soulairac *et al*. (1968) on the effects of cholinergic drugs on GH, although the former only measured pituitary GH concentrations after injections of the anti-cholinesterase, paraoxon, and the latter used inadequate controls. It is of interest that whereas the cholinergic system is stimulatory to GH secretion, it apparently is inhibitory to prolactin secretion (see Grandison *et al*., 1974; Grandison and Meites, 1976). This is one further indication that control of these two hormones is not necessarily exerted by similar systems.

Experiments need to be done to determine the effects of cholinergic drugs on dopamine and norepinephrine turnover in various hypothalamic nuclei. This additional information would further clarify the mechanisms controlling GH

secretion. It also is necessary to develop an assay method to measure Ach concentrations and turnover in the hypothalamus in order to determine changes in Ach in the hypothalamus in different physiological states such as stress, lactation, etc.

Gamma-aminobutyric acid (GABA), a neurally active amino acid, previously was shown to alter LH and prolactin release (for review see, Müller et al., 1977). The data presented in the thesis indicate that GABA decreased GH release. This decrease in GH release may be related to a decrease in hypothalamic NE activity, as indicated by the reduced NE turnover index after α-methyl-paratyrosine treatment. Alpha-methyl-paratyrosine also prevented the increase in serum GH concentration produced by bicuculline, a GABA antagonist.

GABA, like other neurotransmitter substances, does not act directly on the pituitary to decrease GH release. It is possible that GABA stimulates release of GIF which in turn inhibits GH release, as shown by in vitro experiments. These effects were not altered by other non-catecholaminergic blocking drugs.

Methionine-enkephalin (Met-Enk), like morphine, stimulated GH release in vivo but not in vitro. Since the endogenous opioid peptides (EOP) are present in high concentrations in the hypothalamus and pituitary, they may have a role in maintaining basal hormone secretion. This is particularly indicated by the results with naloxone, which

reduced basal GH concentrations. These substances may be involved in the stress-induced decrease in GH since several workers have reported release of EOP during stress. Although these substances stimulate GH release, stress inhibits GH release in the rat. However, stress may also increase GIF release in the rat which may counteract the action of the EOP. The neural mechanisms whereby the EOP stimulate GH are not clear. However, these substances probably exert their effects through interactions with more than one neuraotransmitter.

Insofar as the role of neurotransmitters on GH release is concerned, the results in this thesis support the concept that GH release is primarily regulated by norepinephrine, dopamine and serotonin. GABA and acetylcholine appear to exert their effects through altering hypothalamic norepinephrine activity. Further work is necessary to determine the mechanisms whereby dopamine exerts its actions on GH release. Other neurally active substances present in the high concentrations in the hypothalamus also require further investigation of their role on GH secretion. The data in this thesis are believed to provide experimental models that should be useful in determining the role of neurotransmitter interactions in control of GH and other anterior pituitary hormones.

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APPENDIX

Appendix A.

List of drugs and actions on GH

Acetylcholine Bromide - Ach - Cholinergic transmitter - increased GH.

Alpha-methyl-paratyrosine-aMPT- Tyrosine - Hyoroxylase inhibitor, decreases dopamine and norepinephrine- decreased GH.

Aminooxyacetic Acid - AOAA - Inhibits GABA transaminase and increases GABA - decreased GH.

Atropine - ATR - Muscarinic receptor blocker - decreased GH.

Bicuculline - BIC - GABA antagonist - increased GH.

Bicuculline methyliodide - BIC. MI. - Same as Bicuculline except does not cross blood brain barrier - increased GH.

Clonidine - CLON - Alpha adrenergic receptor stimulator - increased GH.

Chlorpromazine - CPZ - A catechocamine depleting drug - decreased GH.

Desmethylimipramine - DMI - Catecholamine reuptake inhibitor - did not alter GH.

5,7, Dihydroxytryptamine - 5, 7, DHT - Neurotoxin for serotonergic neurons - decreased GH.

Dopamine - DA - A neurotransmitter - increased GH.

Gamma-amnobutyric acid - GABA - neurally active amino acid - decreased GH.

Haloperidol - HAL - Dopamine receptor blocker.

Methionine -Enkephalin- MET-ENK - Morphinomimetic peptide- increased GH.

Methysergide - MET - Serotonin receptor blocker - decreased GH.

Morphine - MOR - Narcotic opiate - increased GH.

Naloxone - NAL - Opiate antagonist - decreased GH.

Norepinephrine - NE - A neurotransmitter - increased GH.

Parachloroamphetamine - PCA - tryptophan hyrdroxylase inhibitor - decreased 5HT and GH.

Para-chlorophenylalaninc - PCPA - Tyroxine hydroxylase inhibitor - decreased serotonin - decreased GH.

Phenoxybenzamine- alpha adrenergic receptor blocker - decreased GH.

Phentolamine - PHEN - alpha adrenergic receptor blocker - decreased GH.

Physostigmine - PHYSOS - anti-cholinesterase, increased acetylcholine - increased GH.

Picrotoxin - PIC - GABA receptor blocker - decreased GH.

Pilocrarpine - PIL - Cholinergic agonist - increased GH.

Pimozide - PIM - Dopamine receptor blockers - decreased GH.

Propranalol - PROP - Beta adrenergic receptor blocker - Did not alter GH.

Serotonin - 5HT - A neurotransmitter - increased GH.

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61st Annual FASEB Meeting Chicago, Illinois	April, 1977	"Effects of Cholinergic and GABA-ergic Drugs on Serum Growth Hormone in Male Rats"
62nd Annual FASEB Meeting Atalntic City, N.J.	April, 1978	"Naloxne Inhibition of Stressed-Induced Increase in Prolactin Secretion"

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31. Meites, J., D. VanVugt and J.F. Bruni: Possible physiological role of enkephalins and endorphins in regulation of pituitary function. (Will be published as a chapter in the book from the symposium).

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