EFFECTS OF ACETYLCHOLINE, GABA, AND PROLACTIN ON ANTERIOR PITUITARY HORMONE SECRETION

A Dissertation
for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Lindsey James Grandison
1976

This is to certify that the

thesis entitled

Effects of Acetylcholine, GABA, and Prolactin on Anterior Pituitary Hormone Secretion

presented by

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has been accepted towards fulfillment of the requirements for

Ph.D. degree in Physiology

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Date November 12, 1976

O-7639

ABSTRACT

EFFECTS OF ACETYLCHOLINE, GABA, AND PROLACTIN
ON ANTERIOR PITUITARY HORMONE SECRETION

By

Lindsey James Grandison

1. The cholinergic agonist, pilocarpine, significantly reduced the elevated serum prolactin concentration of estrogen-primed male rats and of female rats during the proestrous surge. By contrast, pilocarpine failed to reduce serum prolactin concentration when adrenergic receptors were blocked by chlorpromazine, haloperidol, or pimozide, or when catecholamine stores were depleted by reserpine. The cholinergic (muscarinic) receptor blocker, atropine, did not affect serum prolactin concentration. However atropine, which penetrates the blood brain barrier, prevented pilocarpine from reducing serum prolactin concentration. Methylatropine, which does not readily penetrate the blood brain barrier, did not prevent pilocarpine from reducing serum prolactin concentration. These observations indicate that cholinergic agonists act centrally to reduce serum prolactin concentration, and that they act through dopaminergic neurons. The observed effects of atropine suggest that

cholinergic neurons are not acting tonically and thus are not involved in tonic inhibition of prolactin release.

2. Pilocarpine given before cervical stimulation of female rats on estrous morning, prevented pseudopregnancy induction and lordosis. Pilocarpine prevented pseudopregnancy induction in rats given methyl-atropine but not in rats given atropine. These results suggest that cholinergic neurons in the brain can inhibit pseudopregnancy induction and lordosis.

3. Pilocarpine given to male rats before ether- or restraint-stress prevented the rise in serum prolactin. Pilocarpine also prevented the rise of serum prolactin in stressed rats given methyl-atropine but not rats given atropine. In lactating rats separated from pups for 6 hours, pilocarpine given before pup replacement inhibited suckling. When lactating rats were given methyl-atropine, pilocarpine prevented the rise in serum prolactin during 20 minutes of suckling. These observations show that cholinergic agonists can act centrally to inhibit the rise in serum prolactin concentration following stress or suckling. These and the previous results suggest a role for the cholinergic system in the physiological control of prolactin secretion.

4. Two anterior pituitaries grafted beneath the kidney capsule at the time of ovariectomy reduced the rise in serum LH during the 5th to 11th days postcastration. The pituitary grafts increased serum prolactin concentration up to 5-fold in these castrated rats. In female rats bearing the anterior pituitary tumor $MtTW_{15}$, serum prolactin concentration was greatly elevated (up to 3850 ng/ml) and castration was not followed by an increase in serum LH 20 days after castration. In male rats, 2 anterior pituitaries grafted 4 days prior to orchidectomy reduced the rise in serum LH for two weeks after castration. Median eminence implants of prolactin, or pituitary grafts beneath the kidney capsule in male rats reduced the rise in serum LH to 50% of that in control rats 24 hours after castration. Systemic administration of prolactin, or pituitary grafts did not decrease the amount of LH released by the in situ pituitary following injection of 50 ng LRH/100 gm body weight. In addition pituitary grafts prevented the decrease in hypothalamic LRH content observed in male rats 24 hours after castration. These results indicate that prolactin, acting on the hypothalamus, probably to increase dopamine turnover, can inhibit the release of LH. They also suggest that prolactin may have a physiological role in reducing LH release during such states as lactation.

5. In male rats, the putative neurotransmitter, GABA, significantly reduced serum TSH and increased serum prolactin concentration. Amino-oxyacetic acid, which increases endogenous brain GABA concentration, reduced serum TSH and prevented the rise in TSH following cold exposure. Although amino-oxyacetic acid did not affect basal prolactin concentration, it prevented the fall in prolactin during cold exposure. The GABA antagonist, bicuculline, at non-convulsant doses, reduced serum prolactin concentration but did not affect TSH release. These results suggest that GABA neurons participate in regulation of TSH and prolactin release.

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Lindsey James Grandison

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1976

Dedication

This dissertation is dedicated to
my wife Peggy and my son Timothy.
They have given endlessly to me
during the completion of this work.

ACKNOWLEDGMENTS

I wish to acknowledge thanks to Dr. Joseph Meites for providing me with the support and the opportunity to undertake these studies. Dr. Meites has created a challenging, stimulating atmosphere where extensive experience and scientific judgment are gained. I have developed a deep respect for him because of his genuine excitement with research and vast store of knowledge.

I also wish to thank my fellow graduate students, past and present, for their help and encouragement during my graduate studies.

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INTRODUCTION

The importance of the anterior pituitary gland in physiological function is now well established. After hypophysectomy, mammals are unable to reproduce and incapable of satisfactory adaptation to their changing environment. The anterior pituitary synthesizes polypeptides or proteins and releases them into the circulation. These polypeptide hormones are transported through the vascular system and act on peripheral end organs. The release of anterior pituitary hormones is a dynamic process, responsive to external stimuli and endogenous rhythms. Yet changing rates of hormone release are not characteristic of the isolated anterior pituitary. In the rat translocation of the anterior pituitary from the sella turcica to the renal capsule or to the eye is associated with a steady, reduced rate of release for all anterior pituitary hormones except prolactin (Meites et al., 1963), and with atrophy of the target organs of the anterior pituitary hormones. Similarly, during incubation or organ culture of anterior pituitary tissue in vitro hormone release occurs at a steady, slow rate except for the release of prolactin. Removal of the anterior pituitary from the sella turcica stimulates release of prolactin.

Relocation of a transplanted pituitary to the sella turcica restores normal anterior pituitary function (Nikitovitch-Winer and Everett, 1958). Such observations implicated the hypothalamus, located directly above the pituitary, in the regulation of anterior pituitary hormone release. In agreement with this concept, lesions of the median eminence of the hypothalamus produced effects similar to pituitary translocation, while electrochemical stimulation of the hypothalamus induced hormone release. The hypothalamus is thought to act as a transducer, monitoring environmental stimuli and endogenous function, integrating input, and inducing hormone release to maintain homeostasis and to adapt the organism to its environment.

Early investigation into the anatomical relationship between the hypothalamus and the anterior pituitary demonstrated no neural connections, but revealed a vascular portal connection. In the rat the entire arterial blood supply of the anterior pituitary courses from branch arteries of the circle of Willis through a capillary bed in the median eminence, down the hypophyseal portal vessels and into a second capillary bed in the anterior pituitary. For normal pituitary function the hypophyseal portal system must be intact (Harris, 1955). It is now well recognized that the hypothalamus synthesizes and releases hypothalamic hormones into the hypophyseal portal system, these hormones

induce anterior pituitary hormone release. In turn hypothalamic hormone release appears to be regulated by hypothalamic neurons. Sawyer et al. (1949) provided the first evidence for neural involvement in anterior hormone release when they blocked copulation-induced ovulation in the rabbit with the adrenergic receptor blocker dibenamine. Since then much investigation has been directed toward establishing the identity of neurotransmitters in the hypothalamus which induce release of each anterior hormone, and factors effecting neurotransmitter release. The hypothalamus is rich in several neurotransmitters: dopamine (Carlsson et al., 1962), norepinephrine (Vogt, 1954), serotonin (Amin et al., 1954), histamine (Lipinski et al., 1970) and gamma amino butyric acid (Berl and Wealsh, 1958). The work of Sawyer in the 1940's and the histochemical mapping of catecholaminergic and serotonergic neurons into and within the hypothalamus during the 1960's (Ungerstedt, 1971) provided a great stimulus to investigate the role of catecholamines and serotonin in hypothalamic regulation of hormone release. Such efforts indicated that catecholamines or serotonin are involved in controlling the release of each anterior pituitary hormone. However, only 5% of the nerve terminals in the hypothalamus contain catecholamines (Hokfelt et al., 1970). Little is known about the involvement of the neurotransmitters in hormone regulation in the other 95% of hypothalamic nerve terminals.

The present studies examine the role of the putative transmitters acetylcholine and gamma amino butyric acid (GABA) in regulation of anterior pituitary secretion of prolactin and thyroid stimulating hormone (TSH). In addition, the effects of increased dopamine turnover induced by prolactin on release of luteinizing hormone (LH) were investigated.

A glossary is provided in the Appendix to aid the reader in interpreting abbreviations and effects of drugs.

REVIEW OF THE LITERATURE

I. The Hypothalamus

A. Anatomy

Wherever possible the function of the hypothalamus is related to its anatomy. With a recently developed procedure for isolating individual nuclei (Palkovits, 1973), the role of each hypothalamic nucleus in regulation of hormone release is currently being explored (Kizer et al., 1974, 1976a,b). Consequently the anatomy of the hypothalamus is taking on additional significance for future neuroendocrine research.

The hypothalamus is a bilateral structure forming the floor and lower walls of the third ventricle. It extends caudally from the rostral edge of the optic chiasm to a plane immediately behind the mammillary bodies. The hypothalamic sulcus is the dorsal boundary separating the hypothalamus from the overlying thalamus, while the ventral surface of the hypothalamus forms the base of the brain. Located within the hypothalamus are dense masses of nerve cell bodies referred to as nuclei (Figure 1). The nuclei are paired bilaterally on either side of the third ventricle.

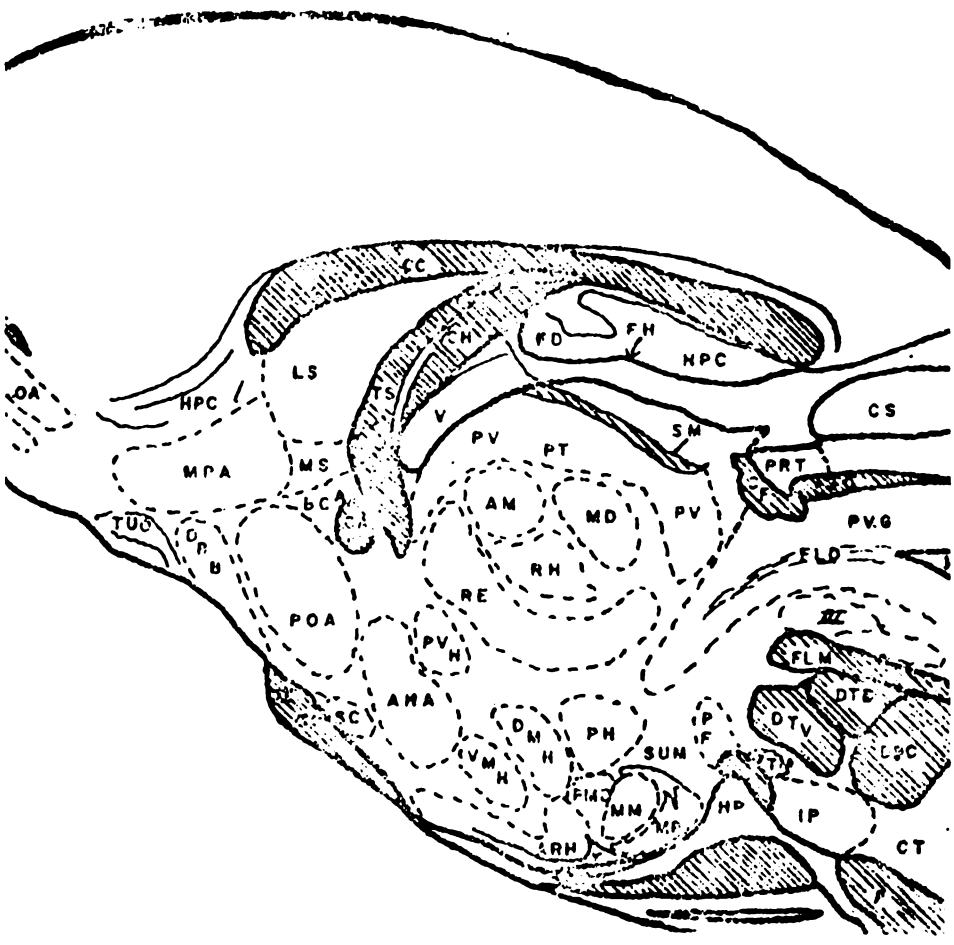


Figure 1. Anatomy of the hypothalamus.

POA	Preoptic Area
AHA	Anterior Hypothalamic Area
SC	Suprachiasmatic Nucleus
PVH	Paraventricular Nucleus
DMH	Dorsal Medial Nucleus
VMH	Ventral Medial Nucleus
PH	Posterior Hypothalamic Area
PMD	Dorsal Premammillary Nucleus
ARH	Archate Nucleus
MM	Medial Mammillary Nucleus
MP	Posterior Mammillary Nucleus

(DeGroot, 1959)

At the anterior hypothalamus of the rat is the unpaired median preoptic nucleus located just dorsal to the third ventricle. The preoptic area composed of a medial and lateral preoptic nucleus is at the rostral end of the third ventricle. The suprachiasmatic nucleus lies directly above the optic chiasm and the supraoptic nucleus is formed from two components, one dorsolateral and the other ventromedial to the optic chiasm. Caudal to the preoptic area and dorsal to the suprachiasmatic nucleus is the anterior hypothalamic nucleus. The paraventricular nucleus is at the dorsal part of the hypothalamus caudal to both preoptic and anterior hypothalamic nuclei. The third ventricle is lined by ependymal cells; lateral to them is the periventricular area. At the rostal part of the hypothalamus the cell bodies in this region are scattered while in the middle portion of the hypothalamus the cell bodies of the periventricular region are grouped together forming the periventricular nucleus. Other nuclei in the middle section of the hypothalamus are the arcuate nucleus located at the ventral wall of the third ventricle. Dorsal lateral to the arcuate is the ventral medial nucleus, and dorsal to it, the dorsal medial nucleus. Lateral to this column of nuclei is the lateral hypothalamic area, a diffuse region of cell bodies. The dorsal hypothalamic area is another region of scattered cell bodies extending along the dorsal part of the hypothalamus from the

anterior hypothalamic nucleus to the caudal limit of the hypothalamus. In the caudal region of the hypothalamus, the mammillary complex is the major anatomical structure consisting of a medial- and lateral-mammillary, and supramammillary nuclei. Surrounding the mammillary complex are the pre-mammillary nucleus anteriorly and the intercalate and mammillary cinereus nuclei basolaterally. The fornix, a nerve tract, terminates in the mammillary complex and is surrounded by the perifornical nucleus. The posterior hypothalamus contains two regions of diffuse cell bodies, the periventricular area and, dorsal to the mamillary complex, the posterior hypothalamic area.

The remaining major hypothalamic structure is the median eminence, the floor of the third ventricle surrounding the tubular infundibular stem. It is organized into three layers: an ependymal layer lining the third ventricle, a fibrous layer, and an outer palisade layer, site of the primary capillary bed. Within the ependymal layer of the median eminence are specialized ependymal cells of unusual shape and variable size, i.e., tanocytes. Processes from tanocytes project down to the palisade layer in the vicinity of the primary capillary plexus. Knigge et al. (1972) proposed that tanocytes participate in regulating anterior pituitary hormone release by transporting releasing factors from the cerebral spinal fluid (CSF) to the primary capillary

bed of the median eminence. Releasing factors have been found in the CSF (Joseph et al., 1974; Knigge and Joseph, 1974; Morris and Knigge, 1975) and in tanocytes (Zimmerman et al., 1974). The tanocytes are able to transport releasing factors, polypeptide hormones and steroids (Ondo et al., 1967; Kumen and Knowles, 1967; Silverman et al., 1973). Intraventricular catecholamine administration induced morphological changes in tanocytes (Schecter and Weiner, 1972) and altered anterior pituitary hormone release. In the monkey changes in tanocyte morphology correlate with stages of the menstrual cycle and in gonadectomized monkeys can be induced with steroid treatment (Knowles and Kumen, 1974). However, the physiological significance of releasing factor transport by tanocytes has not been established.

B. Hypophyseal Portal System

There are no neural connections between the hypothalamus and the anterior pituitary. However, Popa and Fielding (1930) described a portal system between the hypothalamus and the pituitary in which blood flowed from the pituitary to the hypothalamus. Later Wislocki and King (1936) suggested blood flow was in the opposite direction. Harris (1955) conclusively demonstrated that blood flow was from the hypothalamus to the pituitary and proposed factors enter the portal vessels in the hypothalamus, travel to the anterior pituitary and induce hormone release.

In the rat, anterior hypophyseal arteries branch from the internal carotid arteries and form a coiled, looped capillary network in the median eminence and infundibular stem: the primary capillary plexus. The long portal vessels on the surface and inside of the stalk connect the primary capillary bed with a second capillary bed in the anterior pituitary. The inferior hypophyseal arteries form a primary capillary bed in the posterior pituitary. This bed is drained by short portal vessels into a secondary plexus in the anterior pituitary. As a result of these vascular connections, the arterial blood supply of the anterior pituitary first passes through the hypothalamus where it is enriched with releasing factors.

C. Connections to the Hypothalamus and CNS Structures Influencing Hypothalamic Activity

The hypothalamus has the major role in regulating anterior pituitary hormone secretion. The basomedial hypothalamus, separated from the rest of the brain, maintained basal secretion of anterior pituitary hormones and prevented atrophy of the end organs (Halasz, 1969). Removal of end organs still induced hypersecretion of the respective trophic hormones though diurnal ACTH rhythms and ovulation were absent. The preoptic area is responsible for triggering the ovulatory surge of LH. Deafferentation of rostral, dorsal, and lateral connections to the preoptic area did not

inhibit ovulation as long as the connections between the preoptic and basomedial hypothalamus were intact (Halasz and Gorski, 1967).

Other central nervous system structures connected to the hypothalamus do modify its activity. Since drug treatment has been a major approach in examination of hypothalamic regulation of anterior pituitary hormone secretion, these extrahypothalamic structures must also be considered sites of drug action.

The amygdala is connected to the hypothalamus by the stria terminalis and the ventral amygdalofugal pathway. In the rat the stria terminalis connects the corticomedial component of the amygdala with the anterior hypothalamic nucleus, the zone surrounding the ventromedial nucleus, and sparingly with the ventral premammillary nucleus. The ventral amygdalo-hypothalamic pathway extends from the basolateral amygdala to the lateral hypothalamic area. The influence of the amygdala has been most clearly established in hypothalamic regulation of gonadotropin secretion. Stimulation of the amygdala electrochemically (Bunn and Everett, 1957; Van der Schoot, 1974) or by carbachol injection (Velasco and Taleisnak, 1969) caused ovulation in female rats induced into persistent estrus by continuous exposure to light. Similarly electrochemical stimulation of the amygdala induced ovulation in reserpine- or

atropine-treated rats, increased LH in estrogen-primed ovariectomized rats (Velasco and Taleisnak, 1969), and advanced the LH surge on proestrus (Taleisnak, 1976). Cutting the stria terminalis or coagulating the medial amygdala temporarily blocked ovulation for two or three estrous cycles (Velasco and Taleisnak, 1971; Kawakami and Terasawa, 1972; Brown-Grant and Raisman, 1972; Velasco, 1972) while lesion of the cortical amygdala inhibited ovarian compensatory hypertrophy (Smith and Lawson, 1972). In addition to its facilitatory role the amygdala has been found to inhibit gonadotropin release also. Thus, electrochemical stimulation of the basolateral amygdala in proestrous rats inhibited the preovulatory LH surge (Taleisnak, 1976; Kawakami et al., 1976) and in rats on diestrous III blocked ovulation (Van der Schoot, 1974). Lesions of the cortical amygdala in ovariectomized female rats increased serum LH (Lawton and Sawyer, 1970) and electrocoagulation of the basolateral amygdala in male rats increased LH activity in the blood (Eleftheriou et al., 1969). In immature female rats lesions of the amygdala advanced puberty (Elwers and Critchlow, 1960) whereas stimulation delayed it (Bar Sela and Critchlow, 1966). Sawyer (1972), in reviewing the effects of the amygdala on gonadotropin secretion postulated the area of the amygdala stimulatory to gonadotropin release may be estrogen sensitive and dominant to

the amygdala inhibitory area under such conditions. The influence of the amygdala on the secretion of other pituitary hormones is not clearly defined. The amygdala may have a stimulatory role in prolactin secretion since estrogen implants in the amygdala induced lactogenesis (Tindal et al., 1966, 1967) while amygdaloid lesions inhibited mammary tumor growth (Welsch et al., 1968), both prolactin-dependent phenomena. Recently Martin (1974) reported that stimulation of the basolateral amygdala increased growth hormone (GH) release while stimulation of the corticomedial portion decreased GH release.

The hippocampus is another forebrain structure with a major efferent connection to the hypothalamus. The post-commissural fornix column from the hippocampus has its main termination in the lateral subdivision of the medial mammillary nucleus. Offshoots of this column terminate in the rostral hypothalamus. One distinguishable offshoot is the medial corticohypothalamic tract which terminates in the rostral half of the periventricular zone and the arcuate nucleus. Hippocampal influences on hormone release have been reported mainly for gonadotropin secretion. Hippocampal stimulation prolonged vaginal estrous cycles from 4 days to 5 in rats (Kimura and Kawakami, 1972). Electrochemical stimulation of the subiculum in the ventral hippocampus blocked spontaneous ovulation and the ovulation

induced in rats manifesting persistent estrus by stimulation of the amygdala or preoptic area (Velasco and Taleisnak, 1969). The proestrus LH surge and the LH surge after steroid treatment were likewise blocked by electrochemical stimulation of the hippocampus (Taleisnak, 1976; Velasco and Taleisnak, 1969). Release of FSH is also inhibited by the hippocampus (Kawakami, 1972). The effects of hippocampal stimulation on gonadotropin release are reversed in immature rats. Stimulation of the hippocampus advanced vaginal opening and increased FSH release (Kawakami and Terasawa, 1971) and hippocampal lesion delayed puberty (Riss et al., 1963). Sectioning the medial corticohypothalamic tract prevented hippocampal stimulation from effecting gonadotropin release (Velasco and Taleisnak, 1969b; Gallo et al., 1971; Taleisnak, 1976). Prolactin release may be inhibited by the hippocampus (Kawakami et al., 1972) while growth hormone release was stimulated (Martin, 1972, 1974; Martin et al., 1973).

Other forebrain structures project fibers to the hypothalamus in the medial forebrain bundle with terminations in the lateral preopticohypothalamic zone and premammillary nucleus. Stimulation of the bed nucleus of the stria terminalis, the septal nucleus and the nucleus accumbens increased LH release (Kawakami et al., 1970, 1972, 1973; Kawakami and Kumura, 1973). Although the medial forebrain

fibers from the anterior olfactory nucleus, the olfactory tubercle and the periform cortex have not yet been reported to change hormone release, this olfactory input might mediate the effects of pheromones in the Bruce and Whitten phenomena. In both circumstances it has been suggested that prolactin release is inhibited.

Thalamicohypothalamic connections have been reported but not well defined and their significance on pituitary function remains unknown. From the brain stem reticular formation two well described pathways to the hypothalamus exist. The mammillary peduncle in the medial forebrain bundle (MFB) arises in the mediocaudal midbrain and projects to the mammillary body, the lateral hypothalamic area, and the preoptic nucleus. The dorsal longitudinal fasciculus connects the central grey of the mesencephalon mainly with the posterior hypothalamus. In the mesencephalon stimulation of the medial raphe nucleus, the periaqueductal grey of the rostral midbrain or the ventral tegmental area inhibited spontaneous ovulation, progesterone facilitated LH release in proestrous rats and LH release in estrogen primed ovariectomized rats (Carrer and Taleisnak, 1970, 1972). The inhibitory signals from these areas reach the hypothalamus through the dorsal longitudinal fasciculus, the medial forebrain bundle, and the medial corticohypothalamic tract via the MFB and the hippocampus (Carrer and Taleisnak, 1972).

In contrast to the ventral tegmental effects, stimulation of the dorsal tegmentum induced ovulation in persistent-estrous rats, and increased LH release in ovariectomized estrogen-primed rats (Carrer and Taleisnak, 1970).

Other minor tracts from extrahypothalamic structures have been reported. A pallidohypothalamic tract projects from the globus pallidus to the ventral medial nucleus of the hypothalamus where it loses its myelination. However, its actual termination is not established. Direct connections between the cerebral cortex and the hypothalamus via a frontohypothalamic tract were suggested and may convey the effects of cortical spreading depression onto LH and prolactin release (Columbo et al., 1975). Retino-hypothalamic connections were observed by some investigators but remain unconfirmed. Other indirect pathways may convey the effects of light on hypothalamic function. For example, the light-responsive pineal gland, although it lacks a direct neural connection to the hypothalamus, influenced hypothalamic activity. The pineal product melatonin inhibited LH and FSH release and stimulated prolactin release when injected intraventricularly (Kamberi et al., 1970a,b). Since melatonin alters hypothalamic serotonin metabolism, it may effect hormone release by this mechanism as well (Anton Tay et al., 1968). Other pineal compounds also have antigonadotrophic effects (Reiter, 1974). However, pinealectomy in the rat does not produce dramatic effects on

reproductive function (Blake, 1976), so that the physiological significance of the above observations is unresolved. Reiter (1974) suggested that the conventional 12 to 14 hours of light exposure of rats in domestic colonies may produce physiological pinealectomy. Further manipulation of the light-responsive pineal gland would therefore produce negligible effects.

II. Putative Transmitters of the Hypothalamus

Recent advances in biochemical pharmacology have permitted the mapping of biogenic amine-containing neurons, and the measurement of neurotransmitters within individual hypothalamic nuclei. As a result, changes in hormone secretion can now be related to altered neuronal activity in discrete hypothalamic nuclei.

A. Catecholamines

1. Distribution of Catecholamines in the Hypothalamus

a. Norepinephrine

Using the histochemical technique of Hillarp and Falck (1962), Dahlstrom and Fuxe (1964) described groups of noradrenergic cell bodies. Each group is labeled using the letter A followed by a number. The axons from noradrenergic neurons of the medulla oblongata and pons (designated A1, 2, 4-7) enter the medial forebrain bundle as the ventral

noradrenergic system and terminate in the hypothalamus, basal forebrain and limbic structures.

The noradrenergic neurons in the subcaeruleus (ventral part of group A6 and A7) terminate in a periventricular plexus along the third ventricle of the hypothalamus and preoptic area (Olsen and Fuxe, 1972). Lesion of the locus caeruleus reduced norepinephrine concentration in the paraventricular and periventricular nuclei (Kobayashi et al., 1974). The noradrenergic fibers from cell bodies A1, A2 and A5 terminate in the basal and lateral hypothalamus, and the preoptic area (Olson and Fuxe, 1972). Dopamine β-hydroxylase, an enzyme marker for noradrenergic neurons, is lost from the median eminence, and the arcuate, ventromedial and dorsal medial nuclei after hypothalamic deafferentation and in the ventral medial hypothalamus after transection of the ventral noradrenergic bundle (Brownstein et al., 1976; Kizer et al., 1976). Ungerstedt (1971) described in more detail the termination of the noradrenergic neurons of the medulla oblongata and pons. Noradrenergic terminals were found in preoptic, supraoptic, retrochiasmatic, and paraventricular nuclei, in the periventricular, dorsal medial, and arcuate nuclei, the internal layer of the median eminence and the area ventral to the fornix. Measurement of the norepinephrine concentration of hypothalamic nuclei (Palkovits et al., 1974) showed the highest concentration in the

retrochiasmatic nucleus, the periventricular and dorsal medial nuclei and the median eminence agreeing with the histochemical localization of noradrenergic terminals. The distribution of dopamine β-hydroxylase is similar to that of norepinephrine (Saavedra et al., 1974a).

b. Dopamine

Unlike noradrenergic neurons, dopaminergic cell bodies are located in the hypothalamus. The dopamine cell bodies in the arcuate nucleus (cell group A 12) innervate the external layer of the median eminence (Carlsson et al., 1962; Fuxe, 1963). This dopamine system has been the focus of much neuroendocrine research. However, 80 percent of the dopamine in the hypothalamus is found outside the arcuate-median eminence area (Bjorklund et al., 1970; Brown et al., 1972; Kavanagh and Weiss, 1973). Recently Bjorklund et al. (1975) described an incerto-hypothalamic dopamine neurone system. There are two components, not directly connected to each other: a caudal part, and a rostral periventricular-preoptic part. The cell bodies of the caudal component are located in the periaquaductal grey of the rostral mesencephalon, the periventricular grey, and the parafascincular nucleus of the caudal thalamus (the A 11 cell bodies) and in the posterior hypothalamic area and zona incerta (A 13 cell bodies). The dopaminergic terminals from these cells are found in the dorsal part of the dorsomedial

nucleus and the dorsal and anterior hypothalamic areas. The rostral component arises in the anterior periventricular nucleus of the hypothalamus (A 14 cell bodies). It extends laterally to the medial preoptic area, rostrally to the periventricular and suprachiasmatic-preoptic nucleus and into the septal nucleus. These newly described incerto-hypothalamic dopamine neurons are regarded as a system of short intradiencephalic neurons. Substantiating the distribution of dopamine neurons, biochemical measurement of dopamine indicated very high concentrations in the median eminence and arcuate nucleus, with high concentration in the suprachiasmatic, paraventricular, and medial portion of the ventromedial and dorsal medial nuclei (Palkovits et al., 1974). Deafferentation of the medial basal hypothalamus did not significantly reduce its dopamine content (Weiner et al., 1972).

Histochemical fluorescence has been applied at the ultrastructural level in the median eminence to determine the percent of monoamine boutons of total and the number of monoamine boutons per square unit (Ajika and Hökfelt, 1973). In the lateral part of the median eminence about one-third of the nerve endings were monoamine neurons, probably dopamine. In this area only glial cells and nerve endings were found. There was no evidence for axo-axonic synapses in this area but synaptic-like structures were reported

between boutons and tanocytes (Guldner and Wolff, 1973; Kobayashi and Matsui, 1967). Dopamine does effect the tanocytes (Schecter and Weiner, 1972; Hökfelt, 1973, 1974) and may influence releasing factor secretion in this way (Hökfelt, 1973, 1974).

c. Epinephrine

It has been only recently that the distribution of epinephrine containing neurons has been described. Immunohistochemical localization of phenylethanolamine-N-methyltransferase (PNMT), indicated a central tract projecting from the medulla oblongata to the arcuate nucleus, especially the ventral lateral part, the internal and subependymal layers of the median eminence, and the magnocellular portion of paraventricular nucleus (Hökfelt et al., 1973, 1974). Biochemical measurements of PNMT activity and epinephrine substantiate the immunochemical data (Saavedra et al., 1974; Koslow and Schlumpf, 1974; Van der Gugten et al., 1976), but in addition suggest an equally high concentration of epinephrine is located in the periventricular, medial preoptic, supraoptic and dorsal medial nuclei (Van der Gugten et al., 1976).

2. Metabolism of Catecholamines

The catecholaminergic neurons of the hypothalamus regulate anterior pituitary hormone release by depolarizing and releasing catecholamines. There is no method presently

available to monitor the neuronal firing of hypothalamic catecholaminergic neurons selectively. However, catecholamine synthesis is reflective of bioelectrical activity. Changes in catecholamine synthesis and turnover do correlate with changes in rate of anterior pituitary hormone release, and catecholamine metabolism can be altered by hormones. Thus, regulation of catecholamine metabolism is a central concern in neuroendocrinology.

The synthetic pathway of catecholamines is depicted in Figure 2. Tyrosine from the blood enters the brain and is taken up into catecholaminergic neurons by an active transport mechanism (Chirigos et al., 1960). The rate-limiting step in catecholamine synthesis is the hydroxylation of tyrosine to dopa by tyrosine hydroxylase. This enzyme is saturated under physiological conditions and inhibited by its end products. Dopa, dopamine and norepinephrine compete with a pterin cofactor for binding to the enzyme. The remaining steps in catecholamine synthesis occur rapidly. Dopa is decarboxylated to dopamine by ℓ-aromatic acid decarboxylase, a ubiquitous, non-specific enzyme. Noradrenergic neurons contain an additional enzyme, dopamine-β-hydroxylase, which hydroxylates dopamine to norepinephrine. Phenylethanolamine-N-methyltransferase (PNMT) in epinephrine-containing neurons converts norepinephrine to epinephrine by transfer of a methyl group from S-adenylmethionine.

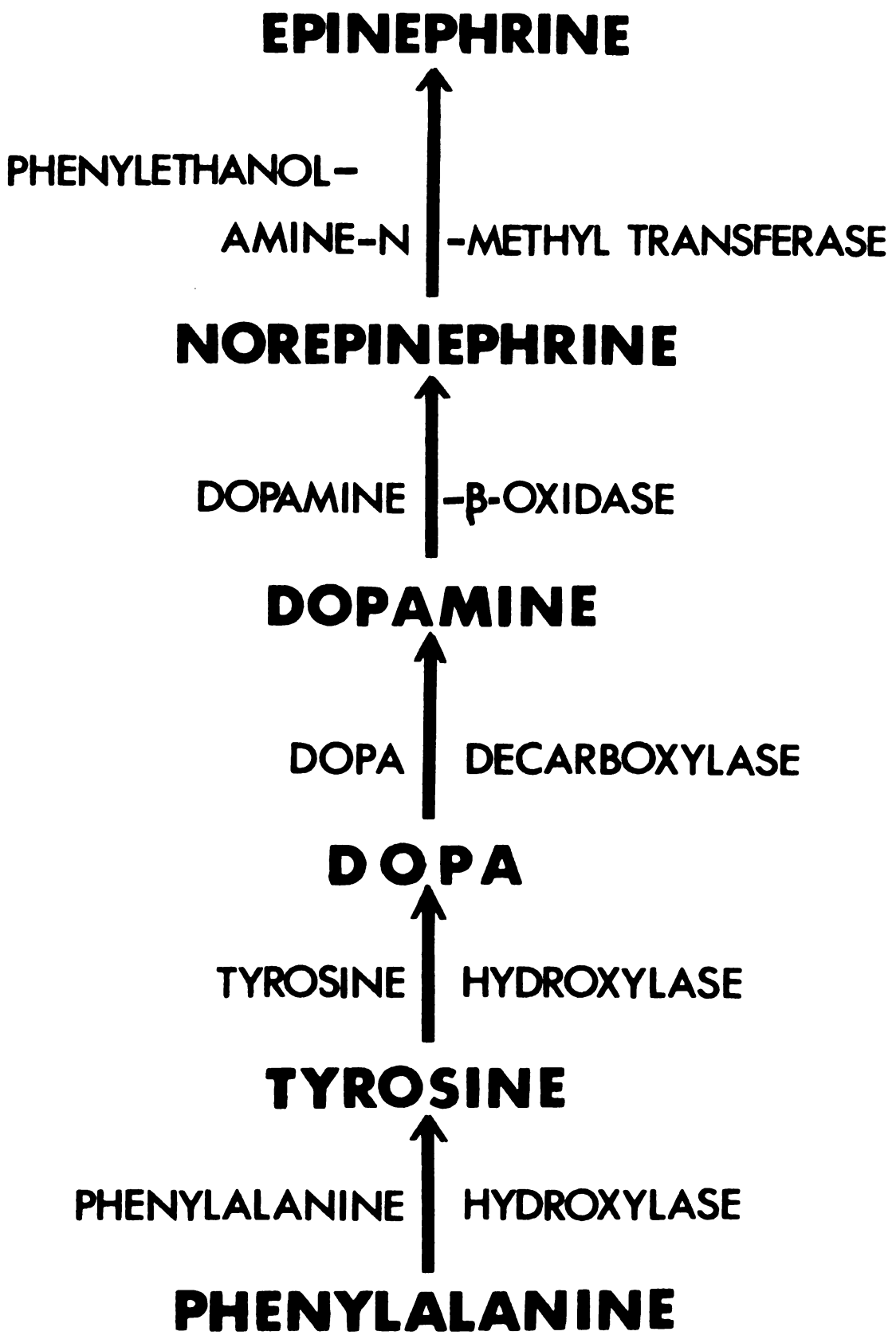


Figure 2. Biosynthesis pathway of catecholamines.

The catecholamines are stored in granules within the nerve terminals and released during cell depolarization. The storage vesicles protect these amines from catabolism by monoamine oxidase (MAO) present in the nerve terminal mitochondria. Catecholamines are converted to their respective aldehydes by MAO and then reduced (Figure 3). MAO also exists extraneuronally along with catechol-o-methyl transferase (COMT). The methyltransfer of catecholamines by COMT is depicted in Figure 3. Catabolism is not the mechanism terminating the action of catecholamines at the postsynaptic membrane. Re-uptake removes the transmitter from the synaptic cleft, thus ending its effect.

B. Serotonin

1. Distribution of Serotonin in the Hypothalamus

Histochemical fluorescent studies located serotonergic cell bodies in the raphe of the mesencephalon that send fibers via the medial forebrain bundle to the hypothalamus (Dahlstrom and Fuxe, 1964; Fuxe, 1965). Serotonergic nerve terminals were observed in the suprachiasmatic nucleus, in the middle of the retrochiasmatic area and in the anterior median eminence (Fuxe, 1965). Biochemical measurement of serotonin has suggested a wider distribution (Saavedra *et al.*, 1974c). A high concentration of serotonin was found in the suprachiasmatic nucleus, the medial forebrain bundle

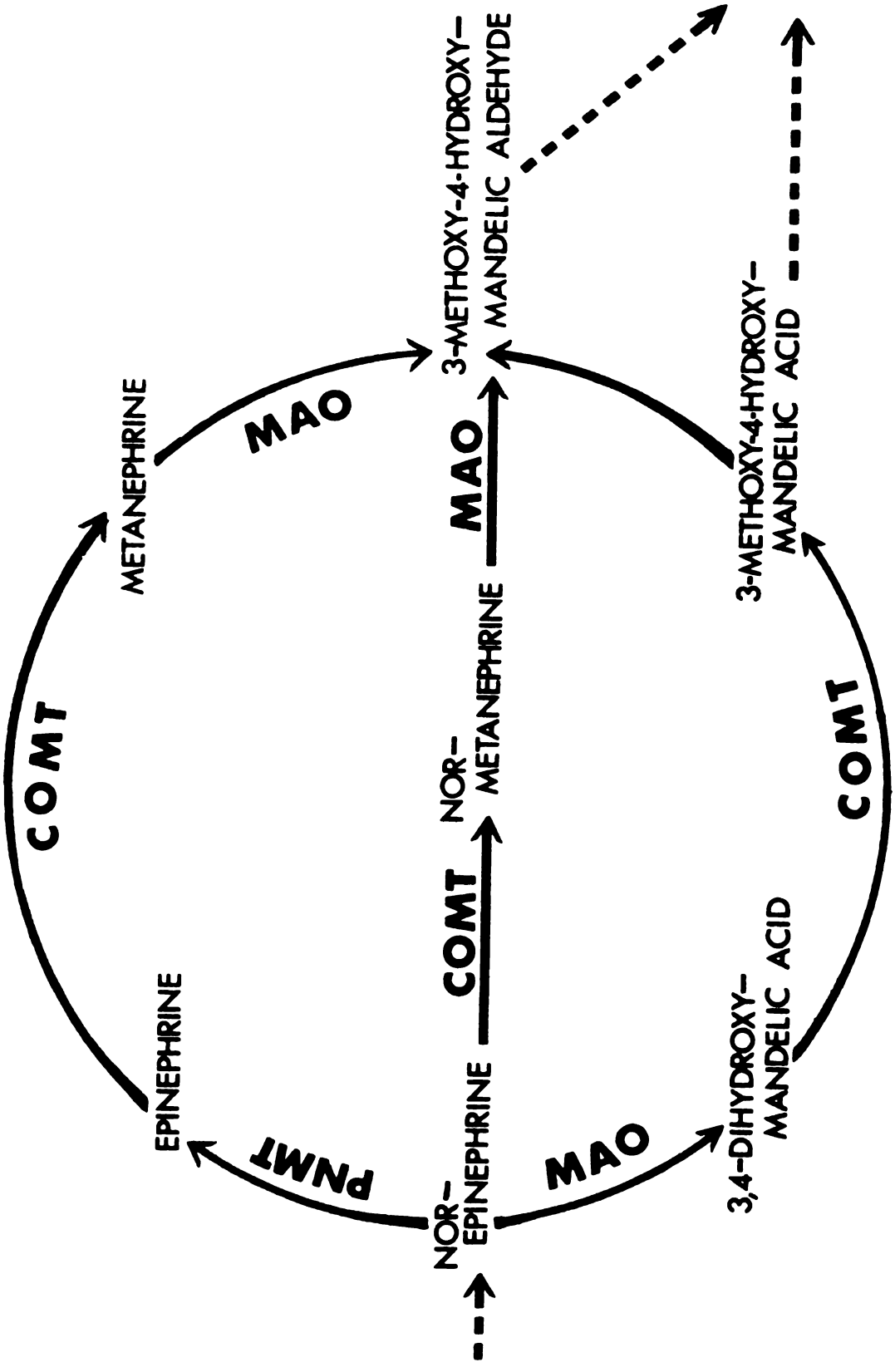


Figure 3. Catabolism pathway of catecholamines.

and the arcuate nucleus. Moderate amounts were detected in the preoptic area, premammillary nucleus, the posterior hypothalamic area and the median eminence. The activity of tryptophan hydroxylase, the enzyme converting tryptophan to 5-hydroxytryptophan, was located in all hypothalamic nuclei so far examined, but high activity correlated with areas having high serotonin concentration (Brownstein et al., 1976).

2. Metabolism of Serotonin

The serotonin precursor, tryptophan, is present in the blood and is actively taken up by brain serotonergic neurons. It is converted to 5-hydroxytryptophan by tryptophan hydroxylase and then rapidly to serotonin by l-amino acid decarboxylase. Tryptophan hydroxylase is not inhibited by 5-hydroxytryptophan or serotonin. Serotonin synthesis is thought to be regulated by precursor availability since under physiological conditions tryptophan hydroxylase is not saturated with substrate. Thus, the daily variation in plasma tryptophan or dietary tryptophan intake can alter serotonin biosynthesis.

Catabolism of serotonin involves deamination by monoamine oxidase to 5-hydroxyindolacetaldehyde and oxidation to 5-hydroxyindolacetic acid (5-HIAA). Termination of postsynaptic nerve stimulation by serotonin results from active reuptake of serotonin by the presynaptic neuron.

C. Acetylcholine

1. Distribution of Acetylcholine in the Hypothalamus

The distribution of cholinergic neurons in the central nervous system has not been well described. Cholinergic tracts have been mapped using histochemical localization of acetylcholinesterase; however, the presence of acetylcholinesterase is not restricted exclusively to cholinergic neurons. Shute and Lewis (1967, 1969) found many acetylcholinesterase containing fibers in the perifornical nucleus and the dorsal posterior region of the hypothalamus. Cholinergic tracts extend from the lateral preoptic area to the supraoptic nucleus and amygdala, from the supramammillary region of the medial and lateral mammillary nuclei and from the reticular system through the hypothalamus. Recently Jacobowitz and Palkovits (1974) described acetylcholinesterase-containing cell bodies in the paraventricular and supraoptic nuclei and in the dorsal medial, ventromedial and arcuate nuclei.

Another enzyme, choline acetyltransferase is closely associated with acetylcholine in the brain. In the hypothalamus the highest choline acetyltransferase activity was observed in the median eminence, with high amounts in the medial forebrain bundle and the premammillary nucleus (Brownstein et al., 1975). Since deafferentation of the basomedial hypothalamus decreased choline transferase

activity in the dorsal medial, ventromedial and arcuate nucleus but not median eminence, Brownstein et al. (1976) suggested cholinergic nerves may project from the area of the dorsomedial, ventromedial and arcuate nuclei to the median eminence.

The distribution of acetylcholine in the hypothalamus is unknown owing to the comparatively low concentration found there and the difficulty of measuring it. However, Cheney et al. (1975) have reported moderate amounts of acetylcholine in the lateral preoptic nucleus and the anterior hypothalamic nucleus. Receptors for acetylcholine (muscarinic) were found in moderate density in the dorsomedial and arcuate nuclei (Snyder et al., 1975). Other hypothalamic regions were not extensively examined.

2. Metabolism of Acetylcholine

In cholinergic neurons the enzyme choline acetyltransferase catalyzes the synthesis of acetylcholine from acetyl coenzyme A and choline. Both choline availability (Cohen and Wurtman, 1976) and uptake (Barker and Mittag, 1975; Mulder et al., 1974; Simon and Kuhar, 1975) have been suggested to regulate acetylcholine synthesis. Other control mechanisms were proposed as well: end-product inhibition (Kaita et al., 1969; Morris et al., 1971), and mass action effects (Glover and Potter, 1971). After release into the synaptic cleft, acetylcholine is hydrolyzed by acetylcholinesterase, thus terminating its postsynaptic effects.

D. Gamma Amino Butyric Acid (GABA)

The hypothalamus contains high concentrations of GABA compared with other brain areas. Kuriyana and Kimura (1976) found that the hypothalamic areas of highest GABA concentration did not coincide with nuclei. GABA content was highest in the lateral hypothalamic area and intermediate in the anterior hypothalamic area and the ventromedial nucleus. The activity of l glutamic acid decarboxylase (GAD) was highest in the anterior hypothalamic, suprachiasmatic, paraventricular and dorsomedial nucleus and the medial forebrain bundle (Brownstein et al., 1976). A synaptic transmitter role for GABA in the hypothalamus is supported by the observation of GABA receptors there (Young et al., 1976).

GABA is formed from glutamate, an intermediate metabolite of the tricarboxylic acid cycle, by the enzyme glutamic acid decarboxylase (GAD). There is some evidence to support end-product inhibition of GAD (Sze and Lovell, 1970). GABA is transaminated with alpha-oxoglutarate by GABA transferase to form succinic semialdehyde (SSA) and glutamate. SSA is then converted to succinic acid by succinic semialdehyde dehydrogenase. This metabolic pathway from glutamate to GABA and to succinic acid forms a shunt (GABA shunt) in the tricarboxylic acid cycle and may account for 10 to 40 percent of total brain metabolism.

III. Hypothalamic Hormones

The effects on anterior pituitary hormone release of pituitary transplantation and of hypothalamic stimulation and lesion demonstrate hypothalamic regulation of anterior pituitary function. Absence of neural connections between the hypothalamus and anterior pituitary along with a hypophyseal portal system suggested that hypothalamic neurohumors affect hormone release. Later, extracts of the hypothalamus were shown to increase release of adrenocorticotropic hormone (ACTH) (Saffran et al., 1955), luteinizing hormone (LH) (McCann et al., 1960), follicle stimulating hormone (FSH) Mittler and Meites, 1964; Igarashi and McCann, 1964; Igarashi et al., 1964), growth hormone (GH) Frantz et al., 1962; Deuben and Meites, 1964), thyroid stimulating hormone (TSH) (Guillerman et al., 1962; Bowers et al., 1964) and inhibit release of prolactin (Pasteels, 1963; Talwalker et al., 1963) and GH (Krulich et al., 1972). Recently three factors from hypothalamic extract effecting pituitary hormone release have been isolated and identified: Thyrotropin releasing factor (TRH) isolated from ovine and porcine hypothalamic extract has the structure: pyro-glutamate-histidine-proline amide (Burgus et al., 1969, 1970; Nair et al., 1970); Luteinizing hormone releasing factor (LRH) is the decapeptide pro-glutamine-histidine-tryptophan-serine-tyrosine-glycine-leucine-arginine-proline-glycine-amide (Matsui et al., 1971; Baba et al., 1971; Burgus et al., 1971); Growth hormone

release inhibiting factor (somatostatin) has been identified as the tetradecapeptide: H-alanine-glycine-cystine-lysine-asparagine-phenylalanine-phenylalanine-tryptophan-lysine-threonine-phenylalanine-threonine-serine-cystine-OH (Brazeau et al., 1973; Burgus et al., 1973; Greibroth et al., 1974).

A. Distribution of Hypothalamic Hormones

The microdissection technique of Palkovits (1973) coupled with radioimmunoassay of hypothalamic hormones, and immunohistochemistry of hypothalamic hormones have provided extensive information on their distribution.

The highest concentration of TRH was found in the median eminence (Brownstein et al., 1975b; Krulich et al., 1974). Significant amounts were observed in the dorsomedial, ventromedial and arcuate nuclei and in the periventricular nucleus (Brownstein et al., 1974b). Unexpectedly, TRH was also observed in extrahypothalamic sites in the CNS. Although concentration of TRH is lower outside the hypothalamus, extrahypothalamic TRH content accounts for 80% of total brain TRH. Outside of the hypothalamus the posterior pituitary had the highest concentration of TRH (Jackson and Reichlin, 1974; Oliver et al., 1974). Significant concentrations were found in the brain stem, mesencephalon, thalamus, preoptic area, septum, basal ganglion and cerebral cortex (Brownstein et al., 1974b; Jackson and Reichlin, 1974; Oliver et al., 1974; Winokur and Utiger, 1974).

The ovine, bovine and porcine (White et al., 1974), but not rat pineal gland (Reichlin et al., 1976), has high TRH concentration. Cerebrospinal fluid also contains TRH (Knigge and Joseph, 1974).

The distribution of luteinizing hormone releasing hormone (LRH) in the hypothalamus has been determined by bioassay of LRH activity in vitro, by radioimmunoassay and by immunocytochemistry of hypothalamic slices. Each procedure indicated LRH concentration was highest in the median eminence (Crighton et al., 1970; Palkovits et al., 1974; Barry et al., 1973). Immunochemical localization of LRH in the median eminence established that the highest concentration was in the zona externa (Barry et al., 1973; Baker et al., 1974; King et al., 1974; Kordon et al., 1974; Hökfelt et al., 1975; Kozlowski et al., 1975), and a high concentration was in the lateral part of the contact zone (Baker et al., 1974), the point of termination of arcuate dopaminergic neurons. LRH was also observed in the zona interna and the subependymal lining of the floor of the third ventricle (Kordon, 1974; Goldsmith and Ganong, 1974). In the median eminence LRH is contained within granules of the nerve terminals opposing hypophyseal portal vessels (Goldsmith and Ganong, 1974, 1975; Pelletier et al., 1974) and tanocytes (Zimmerman et al., 1974; Zimmerman 1976). Low concentration of LRH was observed in the arcuate (Palkovits et al., 1974; Zimmerman

et al., 1974; Wheaton et al., 1975) and ventromedial nuclei (Palkovits et al., 1974).

The catecholaminergic neurons of the basomedial hypothalamus apparently do not contain LRH since 6-hydroxydopamine reduced basomedial hypothalamic catecholamines without altering basomedial LRH content (Kizer et al., 1975). In the rostral hypothalamus LRH was found in the suprachiasmatic nucleus by bioassay (Schneider et al., 1970) and in the suprachiasmatic and supraoptic nuclei by radioimmunoassay (Palkovits et al., 1974). Barry and colleagues were the first to observe LRH-containing cell bodies (Barry and Dubois, 1974; Barry et al., 1974). After inhibiting axonal flow LRH was observed in cell bodies of the arcuate nucleus and preoptic nucleus, and a preoptico-infundibular pathway connecting the preoptic area with the arcuate nucleus was described. Other investigators reported that LRH in the preoptic area was located in the circumventricular organ, the organum vasculosum of the lamina terminalis (OVLT) (Barry et al., 1974; Zimmerman et al., 1974; Kordon, 1975; Wheaton et al., 1975).

The synthesis of LRH in the nerve terminals of the OVLT and the mediobasal hypothalamus are independently regulated. Isolation of the mediobasal hypothalamus reduced LRH content of the mediobasal hypothalamus but did not effect LRH content of the OVLT. Also Araki et al. (1975) reported

differential changes in the LRH content of the median eminence and the preoptic area (presumably the OVLT) during the estrous cycle and after castration.

Reports of extrahypothalamic LRH are few. All the circumventricular organs contain LRH (Kizer et al., 1976); highest concentration was in the median eminence and OVLT but significant amounts were observed in the area postrema, subfornical organ and subcommissural organ. LRH containing neurons project from the hypothalamus into the septum and paraolfactory cortex (Barry and Dubois, 1974; Barry et al., 1974). Hökfelt et al. (1974) reported LRH-containing fibers in the amygdala. LRH is found in ovine, bovine and porcine pineal glands by radioimmunoassay (White et al., 1974) but not in ovine monkey or rat pineal glands by immunocytochemistry (Araki et al., 1975). Controversy likewise exists concerning the presence of LRH in the cerebrospinal fluid; some investigators find it there (Joseph et al., 1975; Morris and Knigge, 1975) while others do not (Cramer and Barraclough, 1975).

Somatostatin (growth hormone release inhibition hormone, SRIF) was the most recently identified hypothalamic hormone, consequently its distribution is still under study. Radioimmunoassay of hypothalamic nuclei indicates SRIF is present to some extent in all nuclei, but the highest concentration is found in the median eminence with moderate

amounts in the ventromedial and arcuate nuclei, the periventricular nucleus and the ventral premammillary nucleus (Brownstein et al., 1976). A similar pattern of distribution was found by bioassay for SRIF activity except for the ventromedial nucleus (Vale et al., 1974). However, SRIF activity in the ventromedial nucleus could be low if growth hormone releasing factor were also present. By immunohistochemistry SRIF was found in the zona externa of the median eminence near portal vessels (Hökfelt et al., 1974; Pelletier et al., 1975). Recently Alpert et al. (1976) reported a few SRIF containing fibers in the preoptic area and the anterior periventricular area, extending from the region between the anterior commissure and optic chiasm to the anterior ventral medial nucleus. Outside the hypothalamus, the amygdala has a high content of SRIF (Hökfelt et al., 1974), while the cerebral cortex (Patel et al., 1975) and posterior pituitary contain lesser amounts. The circumventricular organs, the OVLT and the subcommissural organ, contain SRIF as does the pineal gland (Pelletier et al., 1975). Human cerebrospinal fluid had measureable amounts of SRIF (Patel et al., 1975). In addition SRIF is distributed in the periphery: stomach, pylorus, duodenum, jejunum and pancreatic islets (Luft et al., 1974; Arimura et al., 1975; Patel et al., 1975).

The distribution of other hypothalamic hormones is not known since sensitive procedures for their measurement are not available.

B. Action of Hypothalamic Hormones

Prior to identification and synthesis, hypothalamic hormones were presumed to stimulate or inhibit release of a single anterior pituitary hormone, but this has not proven to be true. TRH does induce synthesis and release of pituitary TSH. In addition, Tashjian et al. (1971) reported that TRH stimulated prolactin release from cultured GH_3 cells, a cell line derived from an anterior pituitary tumor. TRH stimulation of prolactin release from rat (Hill-Samli and MacLeod, 1974) and bovine (Smith and Convey, 1975) pituitaries in vitro and human (Jacobs et al., 1971; Bowers et al., 1973) and rat (Mueller et al., 1973; Takahara et al., 1973) pituitaries in vivo indicated a similar action on normal pituitary tissue. Some investigators have suggested that TRH is the agent inducing prolactin release under physiological conditions. However, prolactin and TSH release are not always parallel. Stress and administration of high amounts of estrogen induce prolactin release (Neill, 1972; Chen and Meites, 1970) while decreasing TSH-thyroid function (Reichlin, 1966; Fisher and D'Angelo, 1972). Cold exposure increases TSH release and decreases prolactin release (Mueller et al., 1974). The release of other hypothalamic

neurohumors along with TRH may explain nonparallelism of TSH and prolactin release. SRIF inhibited TRH induced release of TSH but not prolactin (Siler et al., 1974; Udeschine et al., 1976; Chihara et al., 1976), whereas catecholamines prevented TRH induced release of prolactin but not TSH (Takahara et al., 1974). Thus, the role of TRH as a prolactin releasing factor under physiological conditions remains unresolved. TRH was also reported to induce growth hormone release (Takahara et al., 1974).

The extrahypothalamic distribution of TRH suggests additional effects of this compound. Based on its phylogenic distribution in invertebrates, Reichlin et al. (1976) speculated that TRH regulation of TSH release is an instance in evolution where a pre-existing molecule (TRH) acquired a new function (regulation of TSH release). TRH does affect the central nervous system directly. Iontophoretic application of TRH decreased the spontaneous firing rate of neurons in the hypothalamus (Renaud and Martin, 1974; Dyer and Dyball, 1974). Depression of neuronal firing rate by TRH was also observed in the cerebrum and cerebellum by Renaud and Martin (1974) but not by Dyer and Dyball (1974). TRH was reported to have antidepressant effects in humans (Prange et al., 1972; Kastin et al., 1972; Van der Ves Melren and Weiner, 1972), to have a "relaxing and mild euphoric effect" (Wilson et al., 1973) and to ameliorate symptoms of

schizophrenia (Wilson et al., 1973). Later trials did not confirm the antidepressant effect of TRH (Takahashi et al., 1973; Cooper et al., 1974; Mountjoy et al., 1974; Dimitrihoudi et al., 1974). In rats TRH is a hypothermic agent (Metcalf, 1974). Spontaneous motor activity is increased by TRH (Segal and Mandell, 1974) and when TRH is injected into the medial mesencephalon, shaking, shivering, paw tremor and lacrimation result (Weis et al., 1975). Brown and Vale (1975) suggested that TRH may be a general brain excitant since it raised the LD_{50} of pentobarbital and decreased the LD_{50} of strychine. Earlier reports revealed TRH antagonized the behavioral and temperature-reducing effects of pentobarbital (Prange et al., 1974) and the narcosis and hypothermia induced by ethanol (Breese et al., 1974). However, Nemenoff et al. (1975) found no change in electroshock-induced seizure activity after TRH and an enhancement of the anticonvulsant properties of pentobarbital. TRH also blocked morphine sulfate or pentobarbital-induced hormone release (Brown and Vale, 1975).

The mechanism of TRH action in the central nervous system is unknown, but preliminary reports indicate multifaceted actions. TRH has been reported to increase norepinephrine turnover and release by some researchers (Keller et al., 1974; Horst and Spirit, 1974) but not by others (Reigle et al., 1974). The effects of l-dopa on motor

activity are enhanced by TRH. A direct central action of TRH is indicated since ablation of the pituitary or thyroid did not diminish TRH enhancement of l-dopa action (Plotnikoff, 1972, 1974). Behavioral changes following increased 5-hydroxytryptophan accumulation are also potentiated by TRH (Green and Grahame-Smith, 1974). In evaluating the central actions of TRH, the discrepancy in pituitary and CNS sensitivity is noteworthy. A thousand-fold greater dose is necessary to produce central effects than to induce pituitary hormone release. This discrepancy may reflect poor penetration of TRH into the brain (Stumpf and Sar, 1973). In summary, the effects of TRH on the pituitary are well-established while those on the CNS remain to be confirmed.

The hypothalamic hormone LRH induces synthesis and release of LH and FSH. The magnitude of LH release is much greater than FSH following a single injection of LRH *in vivo*. If a similar dose of LRH is slowly infused the release of FSH is greatly increased (Schally *et al.*, 1973). Since LRH induces release of both, no separate FSH releasing hormone may exist (Schally *et al.*, 1973). Yet the non-parallelism between LH and FSH release after preoptic area stimulation (Kalra *et al.*, 1971) during the proestrous surge (Gay *et al.*, 1970) and during testosterone treatment of castrated male rats, suggests separate regulation of these hormones. Whether the separate control of FSH and LH release consists

of a difference in pituitary response to LRH for FSH or LH release or a separate FSH-RF remains unresolved. In addition to inducing hormone release LRH also primes the anterior pituitary so that subsequent LRH induces greater LH release (Aiyer et al., 1971). A direct effect of LRH on neuronal firing in the preoptic and arcuate-median eminence areas has been reported (Moss et al., 1975; Kawakami and Sakuma, 1974). Most neurons are uneffected while the remaining neurons are either inhibited or stimulated. Such direct neural effects of LRH may explain the ability of LRH to induce lordosis behavior in female rats (Moss and McCann, 1973, 1975; Pfaff, 1973). LRH also potentiates the effect of l-dopa on motor activity and slightly enhances the effects of serotonin (Plotnikoff et al., 1976). As with TRH the amount of LRH required for central effects is much greater than that for hormone release.

The distribution of SRIF is wider than that of LRH or TRH and its range of effects is greater. SRIF reduces basal blood growth hormone concentration and the GH release following stimulation (Reichlin et al., 1976). SRIF inhibits TRH induced TSH release in rats (Borgeat et al., 1974; Vale et al., 1974) and humans (Hall et al., 1973; Siler et al., 1973, 1974; Hall et al., 1974). Release of prolactin is reduced by SRIF in vitro (Vale et al., 1974; Drouin et al., 1976) but not in vivo (Drouin et al., 1976). These effects

of SRIF on pituitary hormone release may result from its ability to reduce anterior cyclic AMP content (Borgeat et al., 1974) although other mechanisms are involved. Within the CNS, SRIF altered neuronal firing rates (Renaud et al., 1975). Several observations suggest SRIF has an inhibitory effect on the CNS. Thus, SRIF reduced spontaneous motor activity (Segal and Mandell, 1974), increased the length of pentobarbital anesthesia (Prange et al., 1974) and reduced the LD_{50} of pentobarbital (Brown and Vale, 1975). Also, duration of strychine induced seizures is reduced and the LD_{50} of strychine is increased (Brown and Vale, 1975) by SRIF. In the periphery blood concentration of gastrin, insulin and glucagon is reduced by SEIF (Alberti et al., 1973; Bloom et al., 1974; Koerber et al., 1974; Gerish et al., 1975).

Other hypothalamic hormones induce anterior pituitary release, but since they have not been identified and synthesized, their full range of activities remains unknown.

IV. Hypothalamic Regulation of Pituitary Prolactin, Luteinizing Hormone (LH), and Thyroid Stimulating Hormone (TSH) Release

A. Prolactin

1. Catecholamines

The release of prolactin, unlike the release of other anterior pituitary hormones, is tonically inhibited by the

hypothalamus. Thus, pituitary transplantation or median eminence lesion increased prolactin release (Everett, 1954; Chen et al., 1970; Sud et al., 1970; Welsch et al., 1971). Extract of hypothalamic tissue reduced prolactin release from pituitary tissue in vitro during short term incubation (Talwalker et al., 1963) and in organ culture (Pasteel, 1961; Gala and Reece, 1963) and also in vivo (Grosvenor et al., 1965). These findings demonstrated the existence of a prolactin release inhibiting factor (PIF) in the hypothalamus. Subsequently catecholamines were found to increase hypothalamic content of PIF and reduce blood concentration of prolactin. Dopamine injected into the third ventricle or its precursor, l-dopa, given systemically, reduced blood concentration of prolactin (Kamberi et al., 1971; Lu and Meites, 1971, 1972). Inhibition of catecholamine metabolism likewise reduced blood prolactin concentration (Lu and Meites, 1971; Donoso et al., 1971; Quadri and Meites, 1973). On the other hand inhibition of catecholamine synthesis (Lu et al., 1970; Lu and Meites, 1971; Carr et al., 1975), depletion of catecholamine stores (Ratner et al., 1965) or blockade of catecholamine receptors by drugs (Lu et al., 1970; Danon and Sulman, 1970; MacLeod et al., 1970; Dickerman et al., 1972; Meites and Clemens, 1972; Quijada et al., 1973), increased blood concentration of prolactin.

Initial observations indicated physiological concentrations of catecholamines did not directly inhibit prolactin release from the pituitary (Talwalker et al., 1963; Koch et al., 1970; Kamberi et al., 1971) although at high pharmacological concentrations they did (Jacobs et al., 1968; MacLeod, 1969; Birge et al., 1970; Koch et al., 1970; MacLeod et al., 1970). It was therefore concluded that catecholamines induced PIF secretion to reduce prolactin release. However, some investigators have proposed that dopamine is a PIF. Recent evidence showed catecholamines act directly on the anterior pituitary to inhibit prolactin release at concentrations lower or equivalent to hypothalamic content. Low concentrations of dopamine and norepinephrine significantly reduced the spontaneous release of prolactin in vitro (Shaar and Clemens, 1974; Dibbet et al., 1974) and in vivo (Takahara et al., 1974; Schally et al., 1974). Higher concentrations of epinephrine were required to produce comparable inhibition. Furthermore, Shaar and Clemens (1974) observed that passage of hypothalamic extract through an aluminum oxide column removed PIF activity along with the catecholamines. Elution of these columns with acid, washed out the catecholamines and PIF activity together. Monoamine oxidase, an enzyme, catabolized catecholamines and destroyed PIF activity of hypothalamic extract as well. Parallel changes in the hypothalamic content of

PIF activity and catecholamines after various drug treatments have been observed (Shaar and Clemens, 1976). The effects of systemically administered catecholamines can result from direct action on the pituitary. In hypophysectomized rats with a pituitary grafted beneath the kidney capsule, an injection of l-dopa reduced serum prolactin (Lu and Meites, 1972; Donoso, 1973). In the periphery l-dopa can be converted to dopamine and directly effect the pituitary. Donoso et al. (1974) observed l-dopa suppression of prolactin release in pituitary grafted rats with a median eminence lesion. Finally dopamine, the catecholamine most potent in inhibiting prolactin release has recently been reported to be present in hypophyseal portal blood using a sensitive enzyme assay (Ben Jonathan et al., 1976). However, before dopamine is assumed to be a physiological PIF, confirmation of its presence in the portal blood is required. Also dopamine release into the portal vessels must be shown to correlate with reduced pituitary prolactin release. In fact the reverse has been observed so far, portal blood concentration of dopamine is highest when prolactin release is greatest (Ben Jonathan et al., 1976).

Other evidence points to a PIF separate from catecholamines. Small polypeptides with PIF activity have been purified from hypothalamic extract (Dular et al., 1974; Greibrok et al., 1974; Takahara et al., 1974; Schally et al., 1975).

Although catecholamines may contaminate some of these extracts, others are free of them (Schally et al., 1976). When dopamine is injected into the third ventricle, it reduced serum prolactin (Kamberi et al., 1971); yet no tritiated dopamine could be extracted from hypophyseal portal blood following administration into the third ventricle (Ben Jonathan et al., 1975). Levo-dopa reduced prolactin release even after its peripheral conversion to dopamine was blocked by MK468 (Jaminez et al., 1976). In this instance inhibition of prolactin release resulted from the effects of l-dopa on the hypothalamus rather than on the pituitary.

Although norepinephrine acts on the pituitary to inhibit prolactin release, norepinephrine injected into the third ventricle induced a small increase in blood prolactin concentration (Kamberi et al., 1971). The norepinephrine agonist clonidine also induced increased prolactin release (Lawson and Gala, 1975). After inhibition of catecholamine synthesis, raising brain norepinephrine concentration with 3,4-dihydroxyphenylserine (DOPS) increased serum prolactin concentration (Donoso et al., 1971). Disulfiram inhibits dopamine-β-hydroxylase and reduces brain norepinephrine; it has also been found to decrease blood concentration of prolactin (Clcmens and Meites, 1972).

In summary, dopamine inhibits prolactin release; yet its physiological site of action is still undetermined. The effect(s) of norepinephrine on prolactin release are not clearly established.

2. Serotonin

In addition to tonic inhibition, the hypothalamus induces or allows surges of prolactin release. While prolactin surges may result from removal of tonic inhibition, secretion of prolactin releasing factor has been suggested as a stimulus for release. When the inhibitory influence of catecholamines on prolactin is eliminated by giving reserpine, stress was still able to induce a further rise in blood prolactin concentration (Valverde et al., 1973; Marchlewski-Kaj and Kurlich, 1975). In this instance prolactin release is not due to lessening of inhibition. During lactation suckling produces a rapid release of prolactin such that the prolactin concentration is twenty times basal levels. Treatment of lactating dams with the catecholamine synthesis inhibitor, alpha-methyl-para-tyrosine, prior to pup suckling did not prevent suckling-induced prolactin release (Voogt and Carr, 1975). In addition suckling-induced prolactin release is not accompanied by a change in hypothalamic catecholamine turnover until maximal blood prolactin concentration is reached. Then dopamine turnover is, in fact, increased (Voogt and Carr, 1974).

These data suggest that stimulation rather than disinhibition is the mechanism producing increased blood prolactin concentration after stress and suckling. These surges of prolactin release may involve stimulation by serotonergic neurons.

Kamberi et al. (1971) found that serotonin injected into the third ventricle stimulated prolactin release. Lu and Meites (1973) showed that systemic injection of the serotonin precursors tryptophan or 5-hydroxytryptophan also increased blood prolactin concentration. Tryptophan in feed increased brain serotonin and serum prolactin of rats on a tryptophan-free diet (Gid Ad et al., 1976). Parachlorophenylalanine (PCPA) and 5,7-dihydroxytryptamine reduced both brain serotonin and serum prolactin of adult rats (Gid Ad et al., 1976). In addition PCPA and methysergide, a serotonin receptor blocker, inhibited surges of prolactin release. Thus, the suckling-induced rise in serum prolactin was inhibited by PCPA (Kordon et al., 1973) while methylsergide blocked suckling-induced prolactin release (Gallo et al., 1975) and prevented the afternoon rise in serum prolactin of estrogen-primed ovariectomized rats (Subramanian and Gala, 1976). The elevated basal concentration of prolactin in estrogen-primed ovariectomized rats was reduced by PCPA, methysergide (Caligaris and Taleisnak, 1974) and by parachloroamphetamine, a specific depletor of serotonin

(Chen and Meites, 1975). From their study Caligaris and Taleisnak (1974) concluded serotonergic neurons participate in estrogen-stimulated prolactin release. Recently Mueller et al. (1976) showed that the rise in prolactin after stress paralleled the increase in serotonin turnover induced by stress. Krulich (1975) found that the serotonin reuptake inhibitor Lilly 11040 potentiated prolactin release after stress. Taken together these data suggest serotonergic neurons stimulate prolactin release.

3. Acetylcholine

Cholinergic neurons also have been implicated in the hypothalamic regulation of prolactin release. Atropine, a muscarinic receptor blocker, prevented depletion of prolactin from the pituitary after suckling (Grosvenor and Turner, 1958). At a high dose atropine inhibited the proestrous surge of prolactin (Libertun and McCann, 1973) and the nocturnal surge during pseudopregnancy (MacLean and Nikitovitch-Winer, 1975). In contrast atropine implanted in the median eminence induced pseudopregnancy (Gala et al., 1970) and when injected into the third ventricle enhanced prolactin release following hypothalamic stimulation (Gala et al., 1972). Yet given over a wide dose range, atropine had no effect on basal prolactin release (Grandison and Meites, 1976). In contrast, the reports on the effects of cholinergic agonists on prolactin release are consistent.

Prolactin release was reduced by intraventricular injection of acetylcholine or carbachol (Grandison et al., 1974; Kuhn and Lens, 1975), and systemic injection of physostigmine and the muscarinic agonist pilocarpine (Grandison et al., 1974; Libertun and McCann, 1974). Hall and Meites (unpublished) recently found that acetylcholine augmented inhibition of prolactin release from pituitaries co-incubated with hypothalamic fragments. In support of a physiological role for cholinergic neurons, pilocarpine blocked the stress-induced release of prolactin (Grandison and Meites, 1976; Krulich et al., 1976; Meltzer et al., 1976). A nicotinic agonist blocked suckling-induced prolactin release and the afternoon surge of prolactin in estrogen-primed ovariectomized rats (Blake and Sawyer, 1972; Subramanian and Gala, 1976). The proestrous afternoon surge of prolactin was delayed by nicotine (Blake et al., 1973). The reported effects of cholinergic drugs on prolactin release are few and at this time no conclusions can be made about the physiological role of cholinergic neurons in regulating prolactin release.

4. GABA, Histamine, and Glycine

Both GABA and histamine are found in high concentration in the hypothalamus. The effect of these putative transmitters on prolactin release is currently being studied. When injected into the ventricles, GABA and histamine each

induce prolactin release (Mioduszewski et al., 1976; Ondo and Pass, 1976; Libertun and McCann, 1974; Donoso et al., 1976). Blockade of histidine decarboxylase, the enzyme catalyzing the synthesis of histamine, reduced blood prolactin concentration. The histamine receptor blocker diphenohydramine blocked stress-induced prolactin release (Libertun and McCann, 1974) but this report has not been confirmed (Meltzer et al., 1976). Another putative transmitter glycine has recently been reported to stimulate prolactin release (Ondo and Pass, 1976).

B. Luteinizing Hormone

1. Catecholamines

Sawyer et al. (1947) were the first to suggest that catecholamines stimulated gonadotropin release. They observed that dibenamine, an adrenergic receptor blocker prevented ovulation in the rabbit. Subsequent studies revealed that the ovulation during the estrous cycle or the induced ovulation in PMS treated immature rats was blocked by another catecholamine receptor blocker, chlorpromazine (Barraclough and Sawyer, 1957; Zarrow and Brown-Grant, 1964), by the depletion of brain catecholamines with reserpine (Barraclough and Sawyer, 1957; Hopkins and Pincus, 1963) and by inhibition of normal catecholamine synthesis with alpha-methyl-para-tryosine (Lippman et al., 1967) or methyl dopa (Coppola, 1969). Interpretation of these early studies was

complicated by the effects of catecholamines at the ovarian level. France (1970) reported that reserpine blocked ovulation in hypophysectomized rats given exogenous gonadotropins (PMS and HCG). Only a few early reports showed that reserpine or alpha methyl-para-tyrosine decreased gonadotropin activity in the blood (Gronoos et al., 1965; Labhsetwar, 1967; Donoso and Santolaya, 1969). With the development of radioimmunoassays for LH, the effects of catecholamines on LH release were then directly observed. It has been found that both dopamine and norepinephrine influence LH release.

Studies reporting the effects of norepinephrine are generally consistent and indicate norepinephrine stimulates LH release. Rubenstein and Sawyer (1970) found norepinephrine given centrally induced ovulation in pentobarbital blocked proestrous rats. Likewise, after intraventricular injection into rabbits norepinephrine induced LH release (Sawyer et al., 1974). The rise in blood LH concentration during proestrus, or following estrogen and/or progesterone treatment is inhibited by alpha-methyl-para-tyrosine, an inhibitor of catecholamine synthesis. However, a rise in LH will take place under the above conditions if brain norepinephrine levels are restored by giving dihydroxyphenylserine (DOPS) (Kalra et al., 1972; Kalra and McCann, 1972, 1974). The preovulatory and estrogen induced LH surge are blocked by diethyldithiocarbamate (DDC) and U-14624.

These compounds inhibit dopamine-β-hydroxylase, the enzyme catalyzing dopamine conversion to norepinephrine. Again restoration of norepinephrine levels with DOPS permits the LH surge to occur. In male rats inhibition of norepinephrine synthesis but not dopamine blocked the postcastration increase in blood LH concentration (Ojeda and McCann, 1973). Similarly blockade of dopamine receptors did not inhibit postcastration LH release but blockade of dopamine and norepinephrine receptors did. Thus, according to the above studies LH release is stimulated by norepinephrine on proestrous afternoon, after estrogen treatment or after castration. In addition, measurement of changing hypothalamic norepinephrine content and synthesis also suggest norepinephrine stimulates LH release. The anterior hypothalamic content of norepinephrine increases during the estrous cycle to a peak at proestrus (Stefano and Donoso, 1976; Donoso et al., 1971). Likewise turnover of norepinephrine and its synthesis from H^3 tyrosine increased at proestrus (Donoso and De Gulierrez-Mozunno, 1970; Zscheck and Wurtman, 1973). After castration LH release increases as does anterior hypothalamic content of norepinephrine (Anton Tay and Wurtman, 1968; Wurtman et al., 1969, Anton Tay et al., 1970). Similar parallel increases in norepinephrine content and turnover and LH release occur at puberty (Coppola, 1968, 1969).

Still unsettled is the role of dopamine in the release of LH. Both stimulatory and inhibitory effects have been reported. Initial investigation indicated dopamine stimulated LRH and LH release. Injection of dopamine into the third ventricle increased LRH activity in the systemic blood of hypophysectomized rats (Schneider and McCann, 1970) and in hypophyseal portal blood of intact rats (Kamberi et al., 1969). Such treatment increased LH and FSH release also (Kamberi et al., 1971; Schneider and McCann, 1970a,b). Norepinephrine and epinephrine were less effective in altering LH release. Dopamine caused LH release in vitro from anterior pituitaries co-incubated with hypothalamic fragments (Schneider and McCann, 1969; Kamberi et al., 1970). These experiments could not be repeated (Miyachi et al., 1973) even in the original laboratories (Quijada et al., 1973; Porter et al., 1972; Cramer and Porter, 1973). McCann and Moss (1975) suggested that in vitro, dopamine may have been converted to norepinephrine by the hypothalamic fragments. Reserpine, which destroys synaptic vesicles where dopamine is converted to norepinephrine, blocked the stimulatory effect of dopamine on LH release in vitro (Schneider and McCann, 1969). In these early studies norepinephrine itself was ineffective, perhaps due to rapid metabolism. However, a stimulatory role for dopamine in regulation of LH release has not been conclusively disproven.

The dopaminergic receptor blocker pimozide inhibited ovulation in pregnant mare serum (PMS) treated immature rats (Corbin and Upton, 1973; Fuxe et al., 1976). Indirect evidence indicates that dopamine synthesis in the median eminence is increased after gonadectomy. The activity of tyrosine hydroxylase in the whole hypothalamus and in the median eminence increased after castration (Beattie et al., 1972; Kizer et al., 1974). Sectioning the ventral noradrenergic bundle destroys noradrenergic neurons in the hypothalamus; yet it did not reduce the activity of tyrosine hydroxylase in the median eminence nor diminish the increase in tyrosine hydroxylase after castration (Kizer et al., 1976a). Also, electrochemical stimulation of the preoptic area induced LH release and at the same time increased fluorescence of dopaminergic neurons in the median eminence (Lichtensteiger and Keller, 1974).

In addition to a stimulatory effect of dopamine on LH release, an inhibitory influence has been reported as well. Administration of testosterone or antifertility steroids to inhibit ovulation increased the fluorescence of dopamine in the median eminence (Klawson et al., 1971; Fuxe et al., 1972; Fuxe et al., 1976). The dopamine agonists, ergotamine and apomorphine, blocked ovulation in proestrous rats and in pregnant mares serum-treated immature rats (Madhwa and Greep, 1973; Fuxe et al., 1976). Injection of dopamine into

the third ventricle of rabbits prevented norepinephrine from stimulating LH release (Sawyer et al., 1974). If dopamine were inhibitory to LH release, dopaminergic neurons might become less active during LH release. A decline in dopamine turnover was observed using histochemical fluorescence during proestrus, the critical period of PMS induced ovulation and after castration (Fuxe and Hökfelt, 1969; Hökfelt and Fuxe, 1972; Fuxe et al., 1973). In summary, the involvement of catecholamines in regulating LH release has been established. Norepinephrine appears to stimulate LH release while the effect(s) of dopamine still require further study.

2. Serotonin

It is generally assumed that serotonin reduces LH release. Stimulation of brain sites containing serotonergic neurons reduced LH release and blocked ovulation (Carrier and Taleisnak, 1970, 1972). In the ewe serotonin content of the hypothalamus fell just before the LH surge (Wheaton et al., 1972). In ovariectomized rats an injection of estrogen reduced LH release and increased tryptophan in the hypothalamus (Bapna et al., 1971) and serotonin in the diencephalon midbrain region (Tonge and Greengross, 1971). When brain serotonin concentration was elevated by giving its precursor 5-hydroxytryptophan or by reducing its catabolism with a monoamine oxidase inhibitor, PMS-induced ovulation was blocked (Kordon et al., 1968). Serotonin given

intraventricularly reduced LH and FSH release in intact and gonadectomized rats (Kamberi et al., 1970, 1971, 1973; Schneider and McCann, 1970) and blocked spontaneous and progesterone-induced ovulation (Kamberi, 1973; Zolovick and Labhsetwar, 1973). Serotonin implanted in the median eminence lowered the pituitary content of LH (Fraschine, 1970). However, there are some reports in which serotonin given intraventricularly failed to block spontaneous ovulation (Rubenstein and Sawyer, 1970; Schneider and McCann, 1970; Wilson and McDonald, 1974). In addition, two reports suggest serotonin may stimulate LH release (Wilson et al., 1974; Cramer and Porter, 1973). Yet the majority of evidence indicates serotonin inhibits LH release.

3. Acetylcholine

The few studies investigating the effects of acetylcholine on LH release generally suggest a stimulatory influence. Acetylcholine injected intraventricularly stimulated progesterone secretion (Endroczi and Hillard, 1965). During co-incubation of pituitaries and hypothalamic fragments acetylcholine facilitated LH and FSH release (Simonovic et al., 1974; Fiorindo and Martini, 1975). There was no direct effect of acetylcholine on the pituitary. In estrogen-primed ovariectomized rats the cholinergic agonist pilocarpine or physostigmine, an inhibitor of acetylcholine catabolism produced a biphasic effect on LH release

(Libertun and McCann, 1974). After injection, LH release was temporarily decreased while at six hours release increased. A similar immediate reduction of LH release followed the elevation of brain acetylcholine concentration with oxotremorine in estrogen primed ovariectomized rats (Marks, 1973). Atropine, a muscarinic receptor blocker, inhibited ovulation (Everett et al., 1949; Benedetti et al., 1971), the proestrous rise in LH and FSH (Libertun and McCann, 1973) and reduced LH in ovariectomized rats (Dickey and Marks, 1971; Libertun and McCann, 1972, 1973). Atropine also prevented ovarian compensatory hypertrophy (Monti et al., 1970) and LH release after electrochemical stimulation of the preoptic area (Lichtensteiger and Keller, 1974). In contrast the nicotinic type of cholinergic receptors appears inhibitory to LH release since nicotine delayed the preovulatory surge of LH (Blake et al., 1972). No definitive conclusion can be made at this point concerning the role of acetylcholine in regulating LH release since the activity of cholinergic neurons has not been correlated with LH release.

4. Other Putative Transmitters

Isolated reports indicate other neuroactive compounds effect LH release. Histamine stimulated progesterone secretion (Endroczi and Hillard, 1965), induced ovulation (Sawyer, 1955) and increased LH release (Libertun and McCann, 1974;

Donoso et al., 1976). LH release was also increased by GABA (Ondo, 1974), glutamate and lysine (Ondo and Pass, 1976).

C. Thyroid Stimulating Hormone

The regulation of TSH release by neurotransmitters in the hypothalamus is poorly understood. Few studies have considered this problem and little concensus exists. Initial investigation indicated that intraventricular injection of dopamine, norepinephrine or serotonin had no effect on TSH release in rats or rabbits (Greer et al., 1960; Harrison, 1961). Recent studies using more sensitive measures of TSH or TRH release demonstrate monoamines can alter TSH release, yet agreement on effect is lacking. Reichlin et al. (1972) described an elegant in vitro procedure for measuring TRH synthesis by murine hypothalamic fragments. Norepinephrine stimulated TRH synthesis (Grimm and Reichlin, 1973). Dopamine also stimulated TRH production, but not when its conversion to norepinephrine was inhibited by disulfiram. In agreement, cold-induced TSH release in rats was inhibited by the alpha-adrenergic blockers phentolamine and phenybenzamine or disulfiram (Kotandi et al., 1973; Tuomisto et al., 1975). Other studies suggest dopamine rather than norepinephrine stimulates TSH release. Dopamine stimulated TRH release from ovine median eminence synaptosomes (Bennett et al., 1975). There appears to be an increase in dopamine

synthesis and TSH release after thyroidectomy, although a causal relationship has not been established. After thyroidectomy tyrosine hydroxylase activity increased in the median eminence, arcuate, ventromedial, and periventricular nuclei (Kizer et al., 1974, 1976). This increase in tyrosine hydroxylase activity still occurs after elimination of noradrenergic input into the hypothalamus, suggesting dopaminergic neurons are responsible. In disagreement with the above, alpha-adrenergic receptor blockers had no effect on basal or cold induced TSH release in humans (Fisher et al., 1971; Woolf et al., 1972) and the inhibitor of catecholamine synthesis alpha methyl-para-tyrosine did not alter TSH release in rats (Chen and Meites, 1975). Levo-dopa did not change TSH release in rats nor in euthyroid human subjects (Eddy et al., 1971; Chen and Meites, 1975). While the reduction of TSH release by reserpine is cited as evidence that catecholamines stimulate TSH, reserpine depletes brain stores of serotonin as well as catecholamines. After reserpine, restoration of catecholamines by administering l-dopa did not increase TSH whereas restoration of serotonin by administering 5-HTP did increase TSH (Chen and Meites, 1975). Still other evidence suggests that dopamine inhibits TSH release. Mueller et al. (1976) found the dopamine agonists apomorphine and peribidil to reduce TSH release.

The reported effects of serotonin on TSH release are equally conflicting. Grimm and Reichlin (1973) reported that serotonin decreased TRH synthesis *in vitro*. The serotonin precursor tryptophan increased brain serotonin and reduced TSH serum concentration *in vivo* (Mueller *et al.*, 1976). On the other hand 5-HTP increased TSH in estrogen-primed ovariectomized rats (Chen and Meites, 1975). Drugs which deplete brain serotonin such as parachloramphetamine and parachlorophenylalanine reduced blood TSH concentration in estrogen-primed ovariectomized female, and male rats (Shenkman *et al.*, 1973; Shapman *et al.*, 1974; Chen and Meites, 1975).

Cholinergic agonists did not alter TRH synthesis or TSH release (Grimm and Reichlin, 1973; Chen and Meites, 1975). However, atropine did inhibit cold-induced TSH release (Kotandi *et al.*, 1973). Based on the above reports one concludes further research on TSH regulation is warranted.

V. Effects of Prolactin on the Hypothalamus

Neurotransmitters regulate secretion of hypothalamic hormones and thus pituitary hormone release. In addition, hypothalamic hormones, anterior pituitary hormones, and hormones from end-organs each can modulate the synthesis and release of neurotransmitters in the hypothalamus.

This circular relationship between the hypothalamus and hormone concentration forms a feedback control loop. The effects on the hypothalamus of end-organ, anterior pituitary, and hypothalamic hormones are independent of one another, although the hypothalamus integrates this type of input along with others such as sensory and extrahypothalamic. Hypothalamic activity is modified by end-organ hormones in 'long-loop feedback', by anterior pituitary hormones in 'short-loop feedback' or autoregulation, and by hypothalamic hormones in 'ultra-short-loop-feedback'. The CNS-anterior pituitary-ovarian axis is an example where each type of feedback loop has been reported. In other cases such as the regulation of prolactin release, long and 'ultra-short-loop feedback' have not been established. No end-organ hormone is specifically associated with prolactin, thus making 'long-loop feedback' difficult to interpret. The existence of a PIF separate from dopamine is controversial, thus 'ultra-short loop feedback' in this instance can not be resolved. These factors make the well documented 'short loop feedback' effect of prolactin even more significant. Furthermore, no other anterior pituitary hormone or other hormone, with the exception of steroids, has such potent or diversified effects on hypothalamic activity.

A. Effects of Prolactin on Prolactin Secretion

The first evidence for autoregulation of prolactin secretion came from anterior pituitary tumor-bearing rats. In rats carrying the pituitary tumor MtTW5 which secretes large amounts of prolactin and growth hormone, or tumor 7315a which secretes prolactin and ACTH, the pituitary weights per 100 grams body weight were significantly reduced (MacLeod et al., 1966, 1968). Also the pituitary content of prolactin was significantly lower than in non-tumor bearing rats. Chen et al. (1968) found that rats bearing the prolactin secreting tumors $MtTW_5$ or $MtTW_{15}$ had pituitaries of lower weight and containing less prolactin. The hypothalamic content of PIF was also reduced. In these cases prolactin acted directly on the CNS since feedback inhibition was observed in ovariectomized, adrenalectomized tumor bearing rats. The pituitary tumors secrete great amounts of prolactin such that the blood concentration of prolactin is well above the physiological range. Thus, the physiological implications of these observations is unknown. Later autoregulation of prolactin was observed in rats given anterior pituitary (AP) grafts or exogenous prolactin. Pituitary grafts secrete mainly prolactin and at a concentration well within physiological limits (Meites and Nicoll, 1965). Prolactin content of in situ pituitaries was reduced by AP grafts in ovariectomized rats but unchanged or increased in

intact female rats (Sinha and Tucker, 1968; Welsch et al., 1968). In the intact female rat prolactin secreted from the AP grafts caused luteinization of the ovaries. The ovarian secretions then prevented depletion of pituitary prolactin content. Reduction in pituitary weight and prolactin content in rats with AP grafts indicated prolactin synthesis was reduced (Sinha and Tucker, 1968). Decreased mammary gland development following median eminence implantation of prolactin demonstrated release was decreased as well (Welsch et al., 1968; Minkhinsky, 1970).

Clemens and Meites (1968) were the first to establish that prolactin acted on the hypothalamus to reduce pituitary prolactin secretion. Implantation of ovine prolactin into the median eminence caused a decrease in pituitary weight, and pituitary prolactin content and concentration. Atrophy of mammary glands and ovaries indicated release was decreased also. Averill (1969) implanted anterior pituitary tissue into the hypothalamus and found corpora lutea were not maintained, another indication of reduced prolactin release.

The physiological consequences of prolactin autoregulation is demonstrated by the effects of median eminence implants of prolactin on several reproductive states. Prolactin implanted into the median eminence one day after cervical stimulation shortened the duration of pseudopregnancy from 14 days to 10 and significantly inhibited the

deciduomata response (Chen et al., 1968). Pregnancy was terminated when median eminence prolactin implants were made during the first eight days (Clemens et al., 1969a). During lactation prolactin implants reduced litter weight gain (Clemens et al., 1969b). The mammary glands of these rats weighed less and vaginal estrous cycles were initiated earlier. These three reproductive states are dependent on prolactin secretion for their continuance. Implants of prolactin in the median eminence also can prevent acute surges in prolactin release. Prolactin implants at 1000 hours on proestrus day prevented the preovulatory prolactin surge. When prolactin was implanted on day four of lactation, it prevented suckling-induced prolactin release on days 6, 8 and 10 (Voogt and Meites, 1973). Recently Advis et al. (1976) found ovine prolactin given intraperitoneally four hours prior reduced the rise in serum prolactin following restraint stress. Estrogen is one of the most potent stimuli for prolactin secretion, yet prolactin implants prevented increases in pituitary weight and pituitary prolactin content and concentration after daily injection on 1 µg estradiol benzoate for five days (Welsch et al., 1968). At higher doses of estrogen, autoregulation was less effective.

Autoregulation is specific for molecules with lactogenic or prolactin-like action. Thus, human growth hormone

and human placental lactogen which have lactogenic properties can reduce prolactin release in rats (Voogt et al., 1971; Clemens and Meites, 1972).

B. Effects of Prolactin on Gonadotropin Secretion

In addition to autoregulation, prolactin also influences release of other anterior pituitary hormones. Clemens et al. (1969c) reported that systemic injections or median eminence implants of prolactin advanced vaginal opening in female rats from an average of 37 days after birth to 30 days. It was suggested prolactin induced precocious puberty by increasing FSH release. Subsequently Voogt et al. (1969) found pituitary FSH concentration was reduced in immature female rats implanted with prolactin in the median eminence suggesting an enhanced release of FSH. In mature female rats, prolactin implants four days after cervical stimulation caused resumption of vaginal estrous cycling in these pseudopregnant rats while LH and FSH concentration in the blood was increased two-fold compared to control pseudopregnant rats. Although these data indicate that prolactin acting centrally can stimulate gonadotropin release, there is an inverse relationship in several reproductive states between blood prolactin and gonadotropin concentration. During pseudopregnancy, pregnancy and lactation blood prolactin concentration is periodically elevated while

gonadotropin concentratin remains at low, diestrous levels. The effects of the nocturnal surges of prolactin on gonadotropin release have not been examined. During lactation ovarian steroids are not responsible for cessation of estrous cycles. Ovariectomy is not followed by an increase in LH or FSH release in dams nursing six or more pups (Ford and Melampy, 1973; Hammons et al., 1973) in contrast to the dramatic postcastration rise in gonadotropin secretion occurring during the estrous cycle (Gay and Midgley, 1969). Inhibition of estrous cycles and gonadotropin release during lactation is associated with pup contact. Physical contact between pups and dam is sufficient to maintain postpartum diestrum (Moltz et al., 1969; Zarrow et al., 1973). The sight and smell of pups causes an increase in prolactin and suckling is one of the most potent stimuli for prolactin release. Prolactin appears to be at least partially responsible for the inhibition of gonadotropin release during lactation. If prolactin release is suppressed by administering ergocornine, serum FSH concentration increases in lactating rats (Lu et al., 1976) and humans (Seke et al., 1974). Although LH release did not increase soon after ergocornine treatment, it must be noted that ergocornine is a dopaminergic agonist and has been found to inhibit LH as well as prolactin release albeit at a higher dose (Fuxe et al., 1976). However, the suckling stimulus alone did prevent

resumption of estrous cycles for several days in ergocornine treated rats (Lu et al., 1976). In contrast, removal of pups at the end of lactation is followed almost immediately by proestrus as indicated by vaginal smears. In conclusion, during lactation prolactin and suckling together inhibit gonadotropin release. Prolactin and suckling during lactation decreased hypothalamic LRH and PIF activity (Minaguchi and Meites, 1967). In addition, prolactin may act at other sites to inhibit the release and action of gonadotropins. The response of the pituitary to LRH is reduced during lactation in rats (Lu et al., 1976; Mougdal et al., 1976) and in humans (Tolis et al., 1973, 1974; Lemaire et al., 1974). Also it has been suggested that the ovaries are less responsive to gonadotropins during lactation (Keeltel and Bradburg, 1961; Zarate et al., 1972; Weiss et al., 1973; Erysthe et al., 1973).

A more direct relationship between high prolactin and inhibition of gonadotropin release is observed in some pathological conditions. Galactorrhea often is associated with ammenorrhea. Forbes-Albright and Chiari-Frommel syndromes are conditions where hyperprolactinemia is associated with infertility. Reduction of prolactin in these circumstances leads to resumption of menstrual cycles and increased gonadotropin release (Turkington, 1972; Varga et al., 1973; Zarate et al., 1973; Seki and Seki, 1974). The mechanism

by which hyperprolactinemia reduced fertility has not been determined but it has been reported that hyperprolactinemia prevented the stimulation of LH release by estrogen (Aono et al., 1976).

C. Effects of Prolactin on ACTH Secretion

The secretory pattern of ACTH is altered during lactation and it appears that prolactin is at least partially responsible for this change. The serum concentration of corticosterone during the morning is higher in lactating as compared to non-lactating rats as reported by some (Voogt et al., 1969) but not by others (Zarrow et al., 1972; Endroczi and Nayakas, 1974). The elevated morning values may result from suckling since suckling has been reported to stimulate ACTH release (Voogt et al., 1969). There is no diurnal rhythm in the release of ACTH (Endroczi and Nyakas, 1974) and the release of corticosterone, ACTH, and CRF in response to stress is significantly less in lactating than in nonlactating rats (Thoman et al., 1968; Kamoun and Haberg, 1969; Endroczi and Nyaka, 1972a, 1974). The buffering of stress-induced corticosterone release during lactation appears to result from elevated blood prolactin since prolactin implanted in the median eminence of nonlactating rats depressed the release of corticosterone following stress (Endroczi and Nyaka, 1972b). Prolactin may affect ACTH

release by lowering the threshold for corticosterone negative feedback. The dose of dexamethasone required to inhibit stress induced corticosterone release is sixteen-fold higher in nontreated than in prolactin treated female rats (Zarrow et al., 1972).

D. Effects of Prolactin on Hypothalamic Activity

Prolactin may reach the hypothalamus through the peripheral circulation or perhaps via the few hypophyseal portal vessels that carry blood from the pituitary to the hypothalamus (Torok, 1954). Clemens and Sawyer (1974) found prolactin in the cerebrospinal fluid of the rabbit and its concentration there paralleled blood concentration during the estrous cycle and after administration of exogenous prolactin.

Following intravenous infusion of prolactin, the activity of hypothalamic neurons is altered: some activated, others inhibited (Clemens et al., 1972). The effects of prolactin are directly on hypothalamic neurons as was shown by iontophoretic application (Yamada, 1975). Fuxe and Hökfelt (1969) found that prolactin increased the turnover of dopamine in the median eminence. The increase in dopamine may explain the higher PIF concentration observed in prolactin treated rats. The reduction in LH and ACTH release may also result from increased dopamine since release of both is reportedly inhibited by catecholamines.

E. Physiological Role for the Central Effects of Prolactin

Although prolactin can modulate other hormone secretion, its physiological significance in this respect is undetermined. The majority of reports dealing with the central effects of prolactin concern experiments where prolactin is given over periods of days. In these cases results are consistent. However, during acute studies lasting hours the reported effects of prolactin are less dramatic or absent. Implantation of prolactin in the median eminence on proestrous morning blocked the afternoon surge (Voogt and Meites, 1972), whereas injection of prolactin four hours prior to stress reduced but did not abolish the stress induced release of prolactin (Advis et al., 1976). In contrast, injection of prolactin prior to suckling did not reduce depletion of pituitary prolactin stores (Grosvenor et al., 1965). Infusion of bovine prolactin in cows for two hours prior to milking did not prevent prolactin release (Tucker et al., 1973). However, infusion of prolactin which raised basal concentration eight-fold diminished the peak concentration of prolactin after milking. More recently it was found that TRH infusion for twelve hours increased endogenous prolactin release, yet it failed to block prostaglandin F_2 alpha stimulated release (Tucker et al., 1975). Perhaps prolonged exposure to high concentrations of prolactin is required for modulation of hypothalamic

functioning. Stimulation of dopamine turnover by prolactin first occurred twelve hours after prolactin administration (Gudelsky et al., 1976). Thus, the physiological effect of prolactin on the central nervous system might be most significant during periods when prolactin is elevated for days, as in lactation.

The physiological function for the central effects of prolactin is as yet unknown. The central action of prolactin during lactation buffers stress induced corticosterone release and may thus protect the developing nervous system of the pups from excessive exposure to corticosterone in milk (Stern et al., 1973). During lactation the autoregulation of prolactin may prevent depletion of pituitary prolactin stores, thus conserving prolactin for future release. However, further research is required to establish the significance of the central action of prolactin.

MATERIALS AND METHODS

I. Animals

Mature male or female Sprague Dawley rats were purchased from Spartan Research Animals (Haslett, MI), and mature female Wistar Furth rats were purchased from Microbiological Associates (Bethesda, MD). All rats were kept in a ventilated temperature controlled room (24°C) illuminated for 14 hours daily (lights on from 0500 to 1900 hours). Lactating rats were housed with 8 pups in large individual plastic cages. Rats implanted with cannulae were placed in individual wire suspension cages. All other rats were housed 3 to 5 per wire suspension cage. Purina Rat Chow (Ralston Purina Company, St. Louis, MO) and tap water were provided ad libitum. To minimize problems with infection, rats received antibiotics after surgical procedures. After castration or implantation of pituitary grafts underneath the kidney capsule, 0.2 ml of Longicil (60,000 units of penicillin G; Fort Dodge Laboratories, Fort Dodge, IA) was given intramuscularly. After implantation of steel cannulae into the median eminence or plastic cannulae into the lateral ventricle, terramycin (oxytetracycline HCL, Pfizer, New York, NY) was supplied in the drinking water.

II. Cannulation of the Lateral Ventricle of the Rat

For injection of GABA or bicuculline methyliodide into the lateral ventricle, cannulae were implanted into the skull according to the description of Verster et al. (1971). The cannulae were constructed from PE#10 polyethylene tubing (Clay Adams, Parsippany, NJ). A piano wire was treaded through the lumen before heating the tubing over a soldering gun. When the plastic was soft, the two ends were pushed toward the middle forming a bulb. After cooling one end was cut diagonally so that there was a 1 mm bevelled point and a total length of 4 mm between tip and bulb. The other end was approximately 5 cm from bulb to end. In preparation for implantation, rats were anesthetized with ether and placed in a stellar stereotaxic instrument (C. H. Stoelting Company, Chicago, ILL). The hair on top of the skull was shaved, and the skin cut and retracted. The underlying fascia was retracted and 3 holes were drilled into the skull. One hole was drilled 2 mm lateral to and 1 mm behind bregma. The dura mater was punctured with a syringe needle and the cannula was then placed so that the 4 mm end projected down through the brain into the right lateral ventricle. The other end was sealed by heating it. Two other holes were drilled 3 mm lateral and 5 mm behind bregma on either side. After the skull was dried, metal screws (browline anchor

screws; Shuron/Continental, Rochester, NY) were placed into the latter 2 holes to serve as support. Dental cement (Nu Weld Caulk; L. D. Caulk Company, Milford, DL) was dusted over the screws and the bulb of the plastic cannula. Caulk liquid (Nu Weld liquid; L. D. Caulk Company, Milford, DL) was applied to harden the powder. After the powder was completely dried, the skin was sewn together. Three days were allowed for recovery and materials were injected from a 10 µl microsyringe (Glenco Scientific Ind., Houston, TX). Following blood collection, an aqueous solution of methylene blue dye was injected into the cannulae and the brain was cut along a coronal plane through the hypothalamus. Data was accepted only from those rats with stain in the median eminence.

III. Radioimmunoassay

After collection, blood was left to clot overnight in a cold room (4°C). Serum was separated by centrifugation (4000 x G, 20 min.) and stored at -20°C until assayed. Prolactin was measured by the radioimmunoassay procedure and reagents of Niswender et al. (1969). LH was measured by the radioimmunoassay of Niswender et al. (1969). In one instance, basal serum LH concentration of intact and intact anterior pituitary-grafted male rats (see EXperiment IV) was determined by a micro-modification of the standard LH

radioimmunoassay (Marshall, Bruni, Campbell, and Meites, in press). Serum TSH measurements were made by the procedures and reagents of the NIAMDD kit. Serum concentration of hormones was expressed as ng/ml of NIAMDD rat prolactin-RP-1, NIAMDD rat LH RP-1 or NIAMDD rat TSH-RP-1. Only when all samples of an experiment were assayed in the same radio-immunoassay, were comparisons of mean hormone concentration made.

EXPERIMENTAL DATA

I. Evidence for Adrenergic Mediation of Cholinergic Inhibition of Prolactin Release

A. Introduction

The hypothalamus contains hypophysiotropic hormones and biogenic amines that can either inhibit or stimulate release of prolactin from the anterior pituitary (Clemens and Meites, 1972; Meites et al., 1972). Under most conditions the mammalian hypothalamus tonically inhibits prolactin release but in some states (e.g., stress, suckling) it can stimulate prolactin release. It is believed that catecholamines, particularly dopamine, induce release of a prolactin inhibiting factor (PIF) from the hypothalamus and/or act directly on the anterior pituitary to inhibit prolactin release (Shaar and Clemens, 1974; MacLeod, 1969; Birge et al., 1970; Dular et al., 1975; Dhariwal et al., 1969). Serotonin, on the other hand, has been shown to stimulate prolactin release, perhaps by promoting secretion of a prolactin releasing factor (PRF).

Recently cholinergic neurons have been implicated in the regulation of prolactin release (Grandison et al., 1974;

Libertun and McCann, 1973, 1974; Kuhn and Lens, 1974) and we have found that acetylcholine, pilocarpine and physostigmine each can significantly reduce serum prolactin in rats. It was of interest to determine whether cholinergic inhibition of prolactin release is mediated via adrenergic neurons.

B. Materials and Methods

Vaginal smears were obtained daily from female rats and only those showing at least two regular, consecutive 4 or 5 day estrous cycles were used. Male rats were handled daily for 3 days prior to experimentation in order to reduce possible stress effects. Blood was collected by cardiac puncture under light ether anesthesia.

Estradiol benzoate (EB) (Nutritional Biochemicals Corporation, Cleveland, OH) was dissolved in absolute ethanol and diluted with corn oil for subcutaneous injection. Pilocarpine nitrate, atropine sulfate and methyl-atropine bromide (Nutritional Biochemicals Corporation, Cleveland, OH), chlorpromazine hydrochloride (Smith, Kline, and French, Philadelphia, PA) and haloperidol (McNeil Laboratories Incorporated, Fort Washington, PA) were dissolved in 0.85% NaCl for intraperitoneal injection. Pimozide (McNeil Laboratories, Fort Washington, PA) was dissolved in 2% tartaric acid. Reserpine (Serpasil, Ciba, Summit, NJ) was given in soluble form (mg/ml).

To determine whether cholinergic inhibition of prolactin release involved activation of adrenergic neurons, the effect of pilocarpine was observed in animals pretreated with drugs known to inhibit catecholamine activity. The doses of drugs and time intervals used previously were shown to elevate prolactin release (Meites et al., 1972; Clemens and Meites, 1972; Lu et al., 1970). Reserpine a depletor of brain catecholamines and serotonin (Sheppard and Zimmerman, 1960), was given at a dose of 10 mg/kg to 16 male rats (350-400 g). Three hours later blood samples were collected: 8 of the rats were then given 5 mg pilocarpine/kg and the remaining 8 were given 0.85% NaCl. At 15 and 45 min after injection of pilocarpine or 0.85% NaCl, blood samples were collected. In a separate experiment chlorpromazine, a catecholamine receptor blocker (McGeer, 1971), was given at a dose of 25 mg/kg to male rats (350-400 g). Blood samples were collected 45 min later, and 8 rats then received 5 mg pilocarpine/kg while the other 8 were given 0.85% NaCl. Blood samples were collected 15, 45 and 90 min after pilocarpine or its vehicle was injected. In a third experiment haloperidol also a catecholamine receptor blocker (Janssen, 1967) was given to 16 male rats (350-425 g) in a dose of 0.5 mg/kg. Forty-five min later blood samples were collected and 8 rats were given 5 mg pilocarpine/kg and 8 other rats were given 0.85% NaCl. Blood samples were collected 15 and

45 min after pilocarpine or 0.85% NaCl injection. Pimozide, a specific dopamine receptor blocker (Janssen et al., 1968) was injected subcutaneously at a dose of 2.5 mg/kg to 16 male rats (330-400 g). Four hours after pimozide injection, blood samples were collected and 8 rats then received 5 mg pilocarpine/kg and 8 other rats were given 0.85% NaCl. At 15, 45, and 90 min after pilocarpine or 0.85% NaCl, blood samples were collected.

Since reserpine, chlorpromazine, haloperidol and pimozide increase the release of prolactin, the effects of pilocarpine were examined in female and male rats with high rates of prolactin release for purposes of comparison. Twenty-four proestrous female rats were bled during the afternoon surge at 1730 hours and immediately 12 were injected intraperitoneally with 10 mg pilocarpine/kg while 12 others were injected with 0.85% NaCl (controls). Blood samples were collected from all rats 20 min after injection of pilocarpine or 0.85% NaCl. In addition 16 estrogen primed (5 µg EB/rat for 10 days) male rats were used since serum prolactin levels are low in normal male rats. On the 11th day, 8 of these rats were given 5 mg pilocarpine/kg while the other 8 received 0.85% NaCl. Blood samples were collected from all male rats 15 and 45 min after pilocarpine or 0.85% NaCl injection.

An attempt also was made to determine whether cholinergic inhibition of prolactin was exerted centrally or

peripherally. Thirty-two male rats were divided into four groups of 8 each. One group received atropine, a central and peripheral cholinergic (muscarinic) receptor blocker, at a dose of 10 mg/kg. The rats were bled 20 min after injection, given 5 mg pilocarpine/kg, and bled 45 min later. A second group received methyl-atropine, a peripheral cholinergic receptor blocker, at a dose of 10 mg/kg. Twenty min after methyl-atropine injection these rats were bled, then given 5 mg pilocarpine/kg and bled again 45 min later. For comparison 16 rats were injected with 0.85% NaCl and bled 20 min later. Eight of these rats were given 5 mg pilocarpine/kg, the other 8 received 0.85% NaCl and the rats were bled 45 min after pilocarpine or 0.85% NaCl injection.

In addition, the effect of atropine sulfate on serum prolactin was examined. A pretreatment blood sample was collected from 48 male rats (350-500 g). Thirty min after the initial blood sample separate groups of 8 each received 0.85% NaCl or 3, 10, 30, 90, or 250 mg atropine sulfate/kg. At 30, 60, 120 and 240 min after injection of atropine sulfate or 0.85% NaCl, a 0.7 ml blood sample was collected from each rat.

Comparisons between treatment and control groups were analyzed by using Student's t test.

C. Results

1. Effects of Pilocarpine on Proestrous Female Rats and Estrogen Primed Male Rats

At 0900 hours on the morning of proestrus the serum prolactin concentration was approximately 40 ng/ml but by 1730 hours a surge had occurred and the level averaged 293±23 ng/ml. A single injection of pilocarpine (10 mg/kg) during the proestrous surge reduced prolactin concentration in 20 min to 109±72 ng/ml ($p < 0.005$) whereas an injection of 0.85% NaCl had no significant effect (230±36 ng/ml) (Figure 4A).

In male rats given 5 µg of EB for 10 days, serum prolactin was increased to 245±15 ng/ml relative to controls (42±5). An injection of 0.85% NaCl into estrogen-primed male rats increased prolactin release when blood was collected 15 min later 352±33 (ng/ml) but by 45 min the serum level (290±26 ng/ml) was not significantly different from the pretreatment value. The increase at 15 min may have been due to stress. An injection of pilocarpine (5 mg/kg) significantly ($p < 0.001$) lowered serum prolactin by 15 min to 202±22 ng/ml and to 165±17 ng/ml by 45 min ($p < 0.005$) (Figure 4B).

2. Effects of Pilocarpine in Drug-treated Rats

Reserpine at a dose of 10 mg/kg increased serum prolactin from 43±6 ng/ml in untreated male rats to 146±11 ng/ml

Figure 4. Effects of pilocarpine on prolactin release in proestrous female and estrogen-primed male rats.

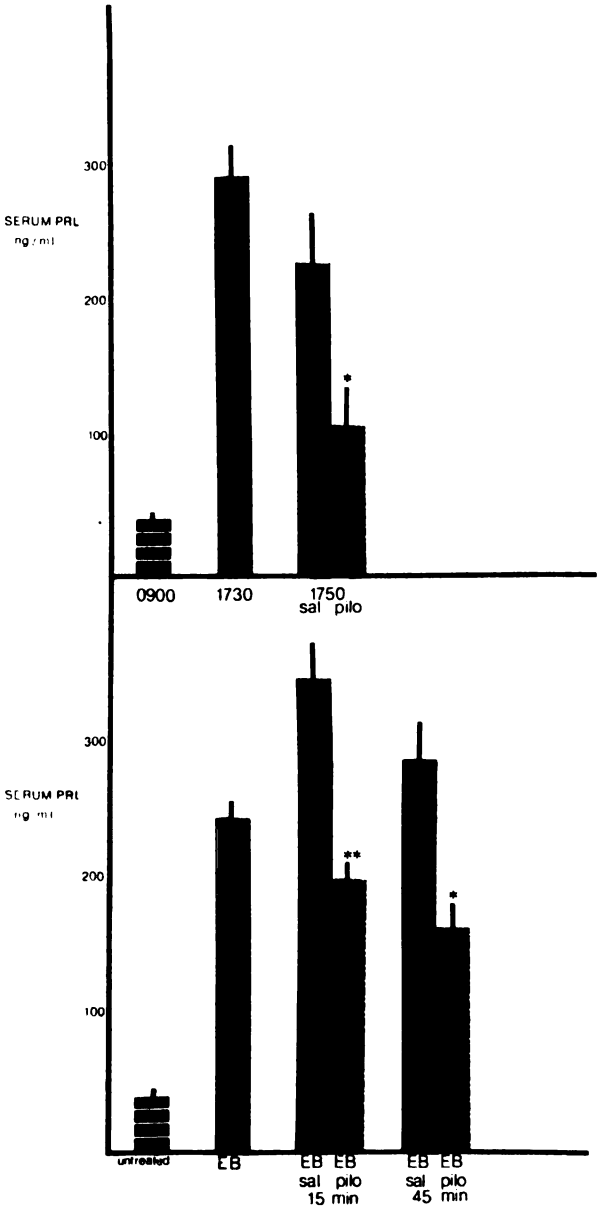
Bars represent serum prolactin concentration ($\bar{X} \pm$ S.E.M.)

A. Female rats on proestrus at 0900 hours (horizontally striped), at 1730 hours (solid) and at 1750 hours 20 min after 0.85% NaCl (solid) or pilocarpine injection (diagonally striped).

B. Untreated male rats (horizontally striped or estrogen primed male rats given 0.85% NaCl (solid) or pilocarpine injection (horizontally striped).

* $p < 0.05$ vs. 0.85% NaCl at same time after injection

** $p < 0.01$


SERUM PRL
300
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*
unfreated
EB
EB EB
sal pilo
15 min
EB EB
sal pilo
45 min

Figure 4

by 3 hours after injection. Fifteen and 45 min after the injection of 0.85% NaCl the reserpine-treated rats the prolactin level was unchanged. Pilocarpine failed to significantly reduce serum prolactin at 15 min (105±17 ng/ml) or 45 min (169±13 ng/ml) after injection (Figure 5A). In male rats an injection of chlorpromazine increased serum prolactin from 38±6 ng/ml in untreated rats to 135±7 ng/ml by 45 min after injection. A subsequent injection of pilocarpine or saline failed to reduce serum prolactin by 15, 45 or 90 min after injection (Figure 5B). A similar inability to reduce prolactin by pilocarpine was observed in rats first treated with haloperidol or pimozide, although each of the latter two drugs by themselves produced significant increases in serum prolactin levels (Figure 6).

3. Effects of Cholinergic Receptor Blockade

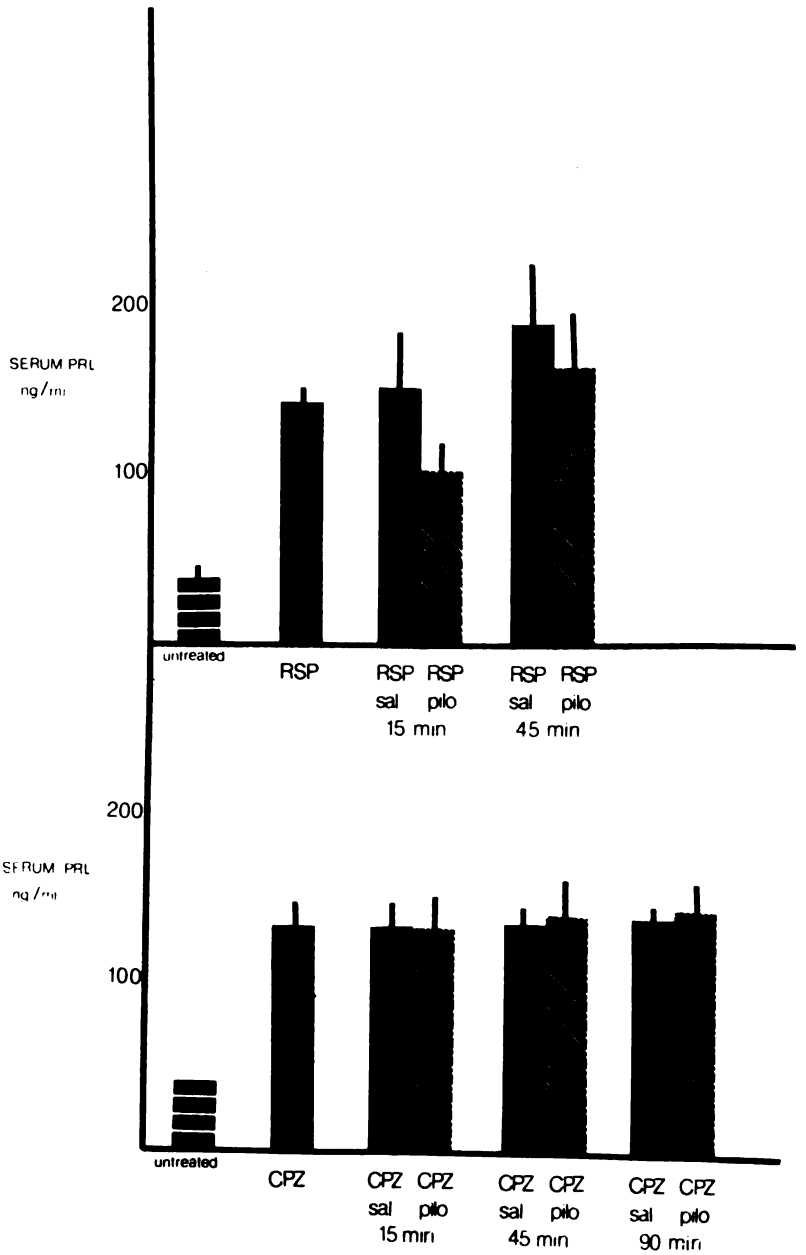
Atropine is known to block the effect of acetylcholine and pilocarpine at muscarinic receptors (Innes and Nickerson, 1970). When atropine sulfate was given alone at doses of 3 to 250 mg/kg intraperitoneally, no significant difference was noted in serum prolactin levels as compared with those in 0.85% NaCl injected rats (Figure 7). However, atropine sulfate prevented pilocarpine from decreasing prolactin release (Figure 8). Methyl-atropine, which does not easily penetrate the blood brain barrier (Innes and Nickerson, 1970), did not prevent pilocarpine from reducing prolactin release.

Figure 5. Effects of pilocarpine on prolactin release in male rats given reserpine or chlorpromazine.

Bars represent serum prolactin concentration ($\bar{X} \pm$ S.E.M.)

A. Untreated male rats (horizontally striped) or reserpinized rats given 0.85% NaCl (solid) or pilocarpine (diagonally striped).

B. Untreated male rats (horizontally striped) or chlorpromazine treated rats given 0.85% NaCl (solid) or pilocarpine (diagonally striped).


SERUM PRL
ng/ml
200
100
untreated
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RSP RSP
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15 min
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100
untreated
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CPZ CPZ
sal pilo
15 min
CPZ CPZ
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45 min
CPZ CPZ
sal pilo
90 min

Figure 5

Figure 6. Effects of pilocarpine on prolactin release in male rats given haloperidol or pimozide.

Bars represent serum prolactin concentration ($\bar{X} \pm$ S.E.M.)

A. Untreated male rats (horizontally striped) or haloperidol treated rats given 0.85% NaCl (solid) or pilocarpine (diagonally striped).

B. Rats given 2% tartaric acid (horizontally striped) and of pimozide treated rats given 0.85% NaCl (solid) or pilocarpine (diagonally striped).

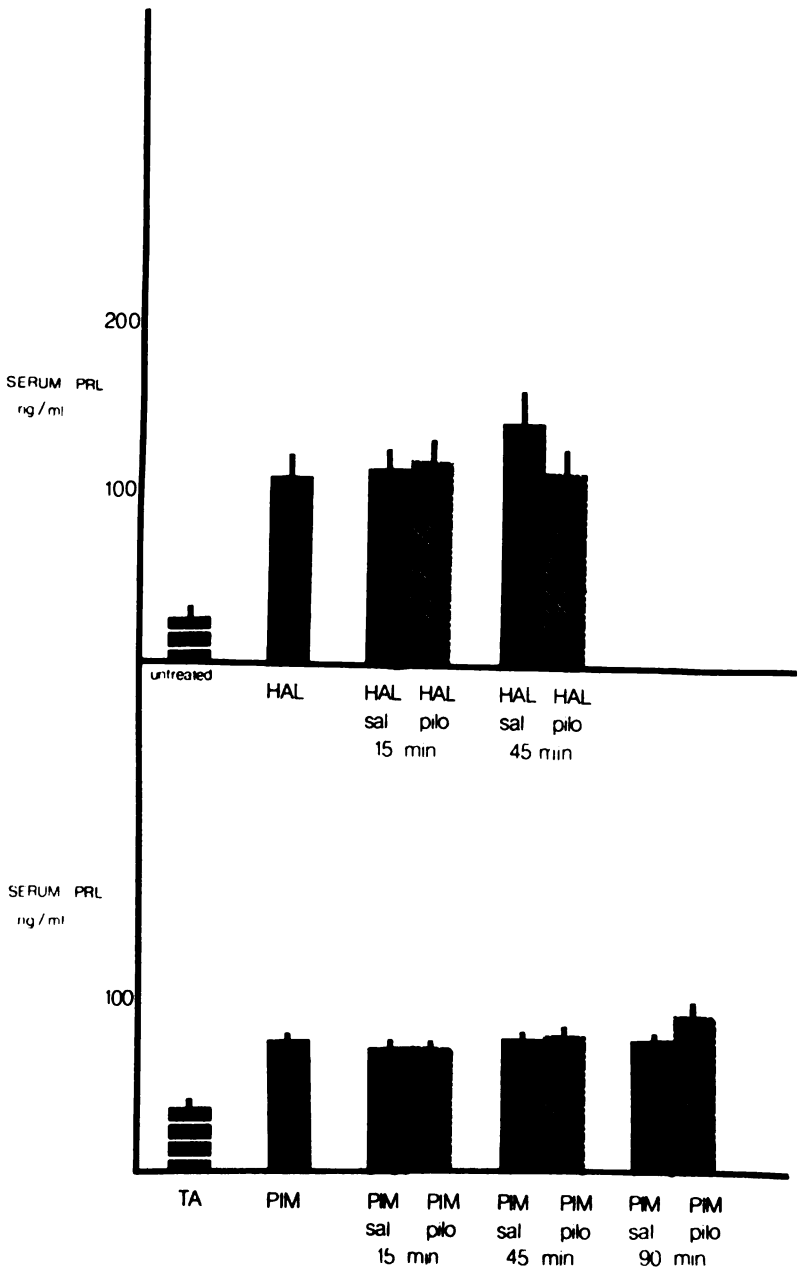
200
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Figure 6

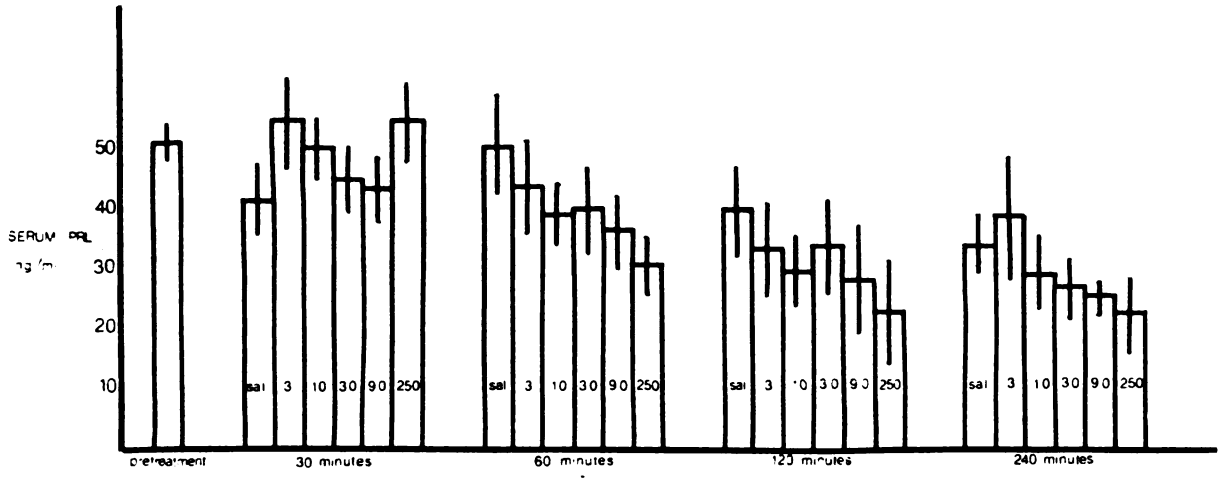

Figure 7. Effects of atropine on prolactin release in male rats.

Bars represent serum prolactin concentration ($\overline{X}$ + S.E.M.) of male rats given 0.85% NaCl (sal) or atropine in doses of 3 to 250 mg/kg.

Indicated on this figure are the minutes after injection of 0.85% NaCl or atropine.

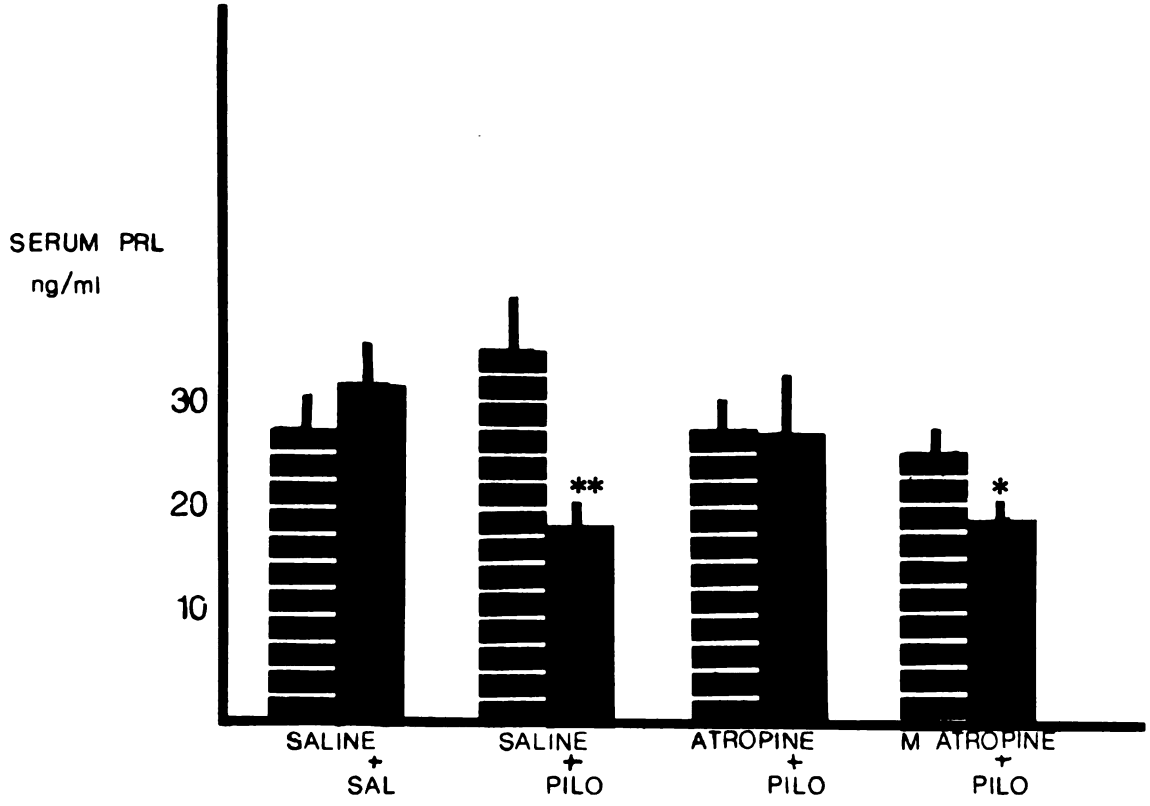

Figure 8. Effects of pilocarpine on prolactin release in male rats given 0.85% NaCl, atropine or methyl-atropine.

Horizontally striped bars represent serum prolactin concentration ($\bar{X} \pm$ S.E.M.) of male rats given 0.85% NaCl (saline) atropine, or methyl-atropine. Solid bars represent serum prolactin concentration of the same groups of rats 45 min after an injection of 0.85% NaCl (sal) or pilocarpine (pilo).

* $p < 0.05$ ** $p < 0.01$

D. Discussion

The cholinergic agonist, pilocarpine, reduced the high serum prolactin concentration in female rats on the late afternoon of proestrous and in estrogen-primed male rats. These results confirm an earlier report on inhibition of prolactin release by pilocarpine in untreated male rats and female rats on the morning of proestrus (Grandison et al., 1974). The 5 and 10 mg/kg doses of pilocarpine used in the present study previously were shown to be equally effective in lowering serum prolactin (Grandison et al., 1974). In this study male and female rats with high initial serum prolactin concentrations were compared to rats given reserpine, chlorpromazine, haloperidol, or pimozide, which also produced high initial serum prolactin values. However, in the rats given the latter drugs, pilocarpine was unable to reduce serum prolactin, suggesting that pilocarpine reduced prolactin release via the catecholamines. The doses of pilocarpine used previously were found to be the most effective for blocking prolactin release at the time intervals used (Grandison et al., 1974). Pilocarpine or acetylcholine at a concentration of 10 µg/ml of medium did not reduce spontaneous prolactin release from incubated anterior pituitary tissue (unpublished), showing that these two drugs do not act directly on the pituitary at these concentrations.

After blockade of catecholamine receptors by chlorpromazine or haloperidol, no reduction in serum prolactin by pilocarpine was seen at any of the time intervals used. Blockade of dopamine receptors by pimozide also was sufficient to prevent pilocarpine from inhibiting prolactin release. In rats given reserpine, pilocarpine produced a small but insignificant reduction in serum prolactin concentration at 15 min and no decrease by 45 min after injection. The slight lowering of prolactin levels at 15 min after pilocarpine injection may have resulted from incomplete depletion of brain catecholamines. These observations suggest that the inhibitory action of pilocarpine on prolactin release is mediated by adrenergic neurons. In agreement with this view, several reports have demonstrated that cholinomimetic drugs can alter brain catecholamine levels (Flynn, 1972; Glisson and Karczman, 1971; Fekete, 1972; Bhatnagar, 1974). Furthermore, Bhatnagar (1974) recently observed that physostigmine, an enzyme inhibitor of acetylcholine metabolism, increased brain dopamine turnover in rats. Physostigmine had earlier been shown to reduce serum prolactin levels (Grandison et al., 1974).

Atropine, a blocker of the receptors for acetylcholine, has been used to determine involvement of cholinergic neurons in physiological functions. Over a wide dose range atropine alone had no effect on prolactin release in male rats.

This observation suggests that cholinergic activity is not involved in tonic inhibition of prolactin release. Large doses of atropine have been reported to inhibit prolactin surges (Libertun and McCann, 1973; MacLean and Nikitovitch-Winer, 1975) in proestrous and pseudopregnant rats, but these effects of the high doses of atropine may be due to central actions not necessarily related to anticholinergic properties of the drug.

The present results suggest that the adrenergic neurons regulating prolactin release may have receptors for acetylcholine, and that cholinergic neurons in the hypothalamus may synapse with adrenergic neurons. Previously Markee et al. (1952) suggested that a cholinergic-adrenergic neuronal pathway existed for activation of LH release and ovulation. Lichtensteiger et al. (1974) provided additional evidence for such a linkage of neurons regulating gonadotropin release. Simonovic et al. (1974) and Fiorindo and Martini (1975) recently reported that cholinergic agents stimulated FSH and LH release in rats. This study provides evidence of a similar cholinergic pathway regulating prolactin release.

II. Effects of Pilocarpine on Pseudopregnancy Induction

A. Introduction

The hypothalamus has a central role in establishing and maintaining various reproductive states. The activity of

the catecholaminergic and serotonergic neurons profusely innervating the hypothalamus, change with the stage of the estrous cycle and during other reproductive states. Also, cholinergic neurons were suggested to participate in reproductive processes such as induced ovulation in rabbits (Sawyer et al., 1949). Recently cholinergic agonists were reported to stimulate LH and FSH release (Simonovic et al., 1974; Fiorendo and Martini, 1975) and inhibit prolactin release (Grandison et al., 1974; Libertun and McCann, 1974). Yet, little is known about the physiological significance that cholinergic activity has on reproduction. In this study the effects of the cholinergic agonist, pilocarpine, on pseudopregnancy induction was examined.

B. Materials and Methods

Vaginal smears were taken daily from mature Sprague Dawley female rats and only those rats showing two regular 4 or 5 day estrous cycles were used. In the first experiment blood samples were collected from 24 rats at 0900 hr on the day of estrus. Twelve rats then received 0.85% NaCl i.p. while the remaining 12 received 5 mg pilocarpine nitrate/kg i.p. The cervix of each rat was stimulated with a glass rod for 60 sec. and 1 ml blood samples were collected by cardiac puncture under light ether anesthesia

1 and 4 hr later. Daily collection of vaginal smears continued for 18 days. Experiment 1 was repeated using 9 rats per group. In another experiment 0.85% NaCl or pilocarpine nitrate at doses of 5, 10, 20, 40, and 50 mg/kg were given to estrous rats 15 min. prior to stimulation of the cervix. Pseudopregnancy induction was determined by the length of the diestrous period. In the third experiment estrous rats were divided into 6 groups; each group received an injection at 30 and again at 10 min prior to stimulation of the cervix. Group I received 0.85% NaCl and 0.85% NaCl; group II received 0.85% NaCl and 5 mg pilocarpine nitrate/kg; group III received 5 mg methylatropine bromide/kg and 5 mg pilocarpine nitrate/kg; group IV received 5 mg atropine sulfate/kg and 5 mg pilocarpine nitrate/kg; group V received 5 mg methylatropine bromide/kg and 0.85% NaCl; and group VI received 5 mg atropine sulfate/kg and 0.85% NaCl. In one trial pseudopregnancy induction was determined by length of diestrum and in a second trial by the occurrence of nocturnal prolactin surges.

C. Results

In estrous rats given 0.85% NaCl, stimulation of the cervix induced a diestrum of 10 to 14 days in length, surges in serum prolactin concentration during the early morning and on estrous afternoon, and behavior characteristic of lordosis during cervical stimulation. Pilocarpine given

prior to stimulation of the cervix blocked the induction of pseudopregnancy. Pilocarpine at 5 mg/kg i.p. blocked induction of pseudopregnancy in 5 of 12 cervically stimulated rats in one trial and 3 of 9 in a second trial (Table 1). In rats given 0.85% NaCl, diestrus lasted 11.5±0.3 days. In rats given pilocarpine diestrus was 12.1±0.4 days in the pseudopregnant rats and 3.2±0.8 days in the remaining rats. A rise in serum prolactin occurred in all rats 4 hrs after cervical stimulation (Figure 9). Of the various doses of pilocarpine given, 10 mg/kg was observed to be most effective in blocking pseudopregnancy induction (Table 2). Doses higher and lower than 10 mg of pilocarpine were less effective in preventing the prolonged diestrum of pseudopregnancy. In the third experiment, pilocarpine prevented pseudopregnancy induction in some but not all rats pretreated with a 0.85% NaCl or methyl atropine bromide, a blocker of peripheral muscarinic receptors (Table 3). However, pilocarpine had no effect on pseudopregnancy induction when estrous rats were given atropine. Methyl-atropine bromide or atropine sulfate alone had no effect at the doses used, on cervical stimulation induced pseudopregnancy.

In the third experiment pseudopregnancy induction was judged by the occurrence of a prolonged diestrum in one trial and by the occurrence of a rise in serum prolactin at 3 A.M. in a second trial (Figure 10). Only in those rats

Table 1. Effects of Pilocarpine Nitrate (5 mg/kg) on Pseudopregnancy Induced by Cervical Stimulation

	Number Pseudopregnant/ Number Cervically Stimulated	
	Controls (0.85% NaCl)	Pilocarpine Nitrate
Trial 1	12/12	7/12
Trial 2	9/9	6/9

Table 2. Dose Response Relationship of Pilocarpine Nitrate on Cervical Stimulation Induced Pseudopregnancy

	Number Pseudopregnant/ Number Cervically Stimulated	
	Trial 1	Trial 2
0.85% NaCl (Controls)	9/10	8/8
Pilocarpine Nitrate		
5 mg/kg	6/11	
10 mg/kg	0/10	1/8
20 mg/kg	4/10	
40 mg/kg	1/3	
50 mg/kg	2/10	

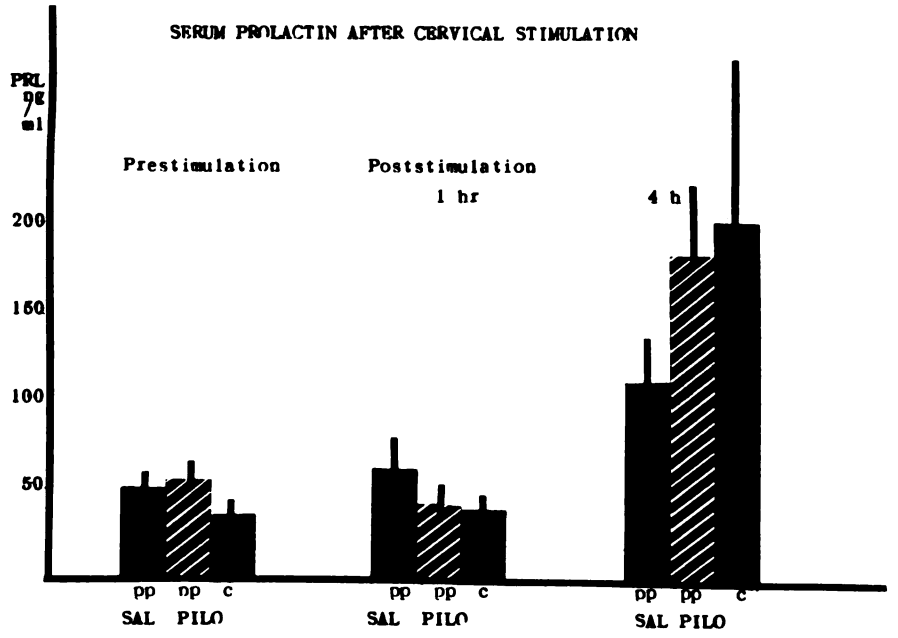

Figure 9. Effects of pilocarpine on the serum concentration of prolactin 1 and 4 hours after cervical stimulation.

Bars represent prolactin levels ($\bar{X} \pm$ S.E.M.) of rats given 0.85% NaCl (SAL) or pilocarpine nitrate (pilo) before cervical stimulation. Pilocarpine treated-rats were separated into pseudopregnant (pp) and nonpseudopregnant (c) groups.

Table 3. Effects of Atropine Sulfate and Methyl-atropine Bromide on Inhibition of Pseudopregnancy by Pilocarpine Nitrate

	Number Pseudopregnant/Number Cervically Stimulated		
	Trial 1	Trial 2	Trial 3
0.85% NaCl-0.85% NaCl (Controls)	9/9	7/7	12/12
0.85% NaCl-Pilocarpine	6/9	4/8	6/14
Methylatropine-Pilocarpine	6/9	2/8	2/11
Atropine-Pilocarpine	9/9	6/6	
Methylatropine-0.85% NaCl	9/9	6/6	6/6
Atropine-0.85% NaCl	9/9	6/6	

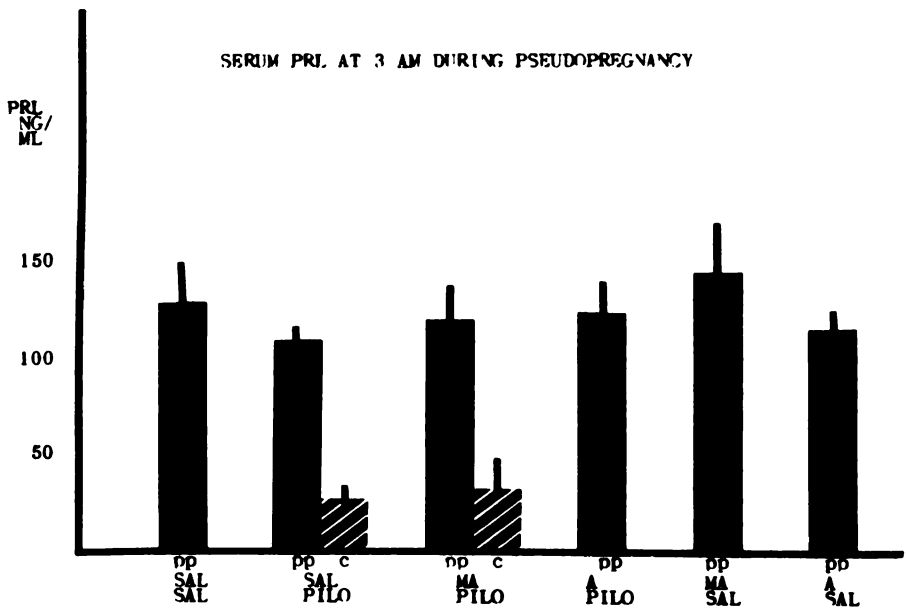

Figure 10. Effects of pilocarpine on the serum concentration of prolactin 2-5 days after cervical stimulation.

Bars represent prolactin levels (X ± S.E.M.) at 0300 hours.

Rats were given 0.85% NaCl (sal), methyl-atropine (MA), or atropine (A) followed by 0.85% NaCl or pilocarpine (pilo) before cervical stimulation. Based on length of diestrum, rats were separated into pseudopregnant (pp) or non-pseudopregnant groups (c).

with a prolonged diestrum was serum prolactin concentration elevated at 3 A.M. Those rats judged not to be pseudopregnant had a serum prolactin concentration similar to that of diestrous rats. In addition to the changes in hormone levels and vaginal histology, behavior of the rats during cervical stimulation was noted. Rats given 0.85% NaCl responded to cervical stimulation by arching of the back, emitting cries and a lack of resistance to stimulation. In contrast, estrous rats given pilocarpine nitrate did not arch their back, were unresponsive to and actively resisted cervical stimulation.

D. Discussion

In estrous rats stimulation of the cervix induced pseudopregnancy. The length of diestrus was prolonged and nocturnal prolactin surges occurred as was previously reported (Freeman and Neill, 1972). A rise in serum prolactin concentration 4 hr after cervical stimulation was also noted in accord with the reports of others (Wuttke and Meites, 1972; Alonso and Deis, 1973/74).

Pilocarpine, a cholinergic agonist, prevented pseudopregnancy induction. When a single injection of pilocarpine was given immediately prior to vaginal stimulation, nocturnal prolactin surges did not occur and diestrus was not prolonged beyond its normal length during the estrous cycle.

Pilocarpine appeared to act within the central nervous system to prevent pseudopregnancy induction since its effect could not be blocked by methyl atropine, a muscarine receptor blocker that does not readily penetrate the blood brain barrier. Atropine, which does enter the CNS, did block the effect of pilocarpine on pseudopregnancy induction. These results suggest that pilocarpine interfered with the programming of the CNS for pseudopregnancy. A likely site of action for pilocarpine is the preoptic area, which is involved in the induction of pseudopregnancy and which contains cholinergic neurons (Cheney et al., 1975). Separation of the preoptic area from the hypothalamus prevented pseudopregnancy following cervical stimulation (Neill et al., 1975; Arai et al., 1974). Electrochemical stimulation of the preoptic area at estrus induced pseudopregnancy (Peters and Gala, 1975). The mechanism by which pilocarpine blocked pseudopregnancy induction is unknown. Catecholaminergic neurons have been suggested to mediate the inhibition of prolactin release produced by pilocarpine (Grandison and Meites, 1976), although it is too early to suggest that catecholaminergic neurons mediate all the effects of cholinergic agonists on hormone release. It is noteworthy that pilocarpine not only inhibited the induction of pseudopregnancy but it also reduced the behavioral response to cervical stimulation. Pilocarpine did not prevent the rise in serum

prolactin concentration on the estrous afternoon following cervical stimulation, suggesting this rise in serum prolactin is separate from and not responsible for the induction of pseudopregnancy. Wuttke and Meites (1972) found that in vaginal stimulated rats suppression of the rise in serum prolactin on estrous afternoon did not block pseudopregnancy, again suggesting this rise in prolactin levels is independent of pseudopregnancy induction. The 10 mg/kg dose of pilocarpine was more effective than a higher dose. A similar dose effect was observed previously in the reduction of prolactin release by pilocarpine (Grandison et al., 1974). At doses above 10 mg pilocarpine/kg stress due to peripheral side effects may counteract the central effects of pilocarpine. Supporting this explanation was the observation of a lower incidence of pseudopregnancy in rats given methyl atropine together with pilocarpine than in rats given pilocarpine alone (Table 3).

In conclusion, these data demonstrate that pilocarpine can prevent pseudopregnancy induction following cervical stimulation. This effect of pilocarpine suggests that reproductive function, and more specifically prolactin release, can be significantly affected by cholinergic agonists and perhaps by cholinergic neurons. These results provide further evidence that cholinergic neurons are involved in regulation of prolactin release.

III. Effects of Pilocarpine on the Rise in Serum Prolactin Concentration After Suckling and Stress

A. Introduction

Putative neurotransmitters of the hypothalamus have been implicated in regulation of prolactin release (Meites et al., 1972; Clemens and Meites, 1972). In addition to catecholamines, serotonin, and GABA, acetylcholine has been recently found to alter prolactin secretion (Grandison et al., 1974). However, there is controversy concerning the effect cholinergic neurons have on prolactin release. At high doses the central cholinergic receptor blocker atropine prevented the nocturnal prolactin surge during pseudopregnancy (MacLean and Nikitovitch-Weiner, 1975) and the preovulatory prolactin surge (Libertun and McCann, 1973), suggesting that cholinergic neurons stimulate prolactin release. Yet over a wide dose range atropine did not alter basal prolactin release (Grandison and Meites, 1976). On the other hand, cholinergic agonists reduced the basal release of prolactin in untreated or estrogen-primed male and ovariectomized female rats (Grandison et al., 1974, 1976; Libertun and McCann, 1974). These observations suggest that cholinergic neurons inhibit prolactin release. The effects of cholinergic agonists on the release of prolactin during physiological stimulation is unknown. It was the objective of this study to determine the effect of the cholinergic

agonist pilocarpine on the release of prolactin after stress and suckling. The findings may help to establish a physiological role for cholinergic neurons in prolactin release.

B. Materials and Methods

Mature Sprague-Dawley rats, male (330-400 gm) and female (220-260 gm) were used throughout the study. In the first series of experiments the effect of cholinergic agonists and/or antagonists on prolactin release after stress was examined. In the first experiment 9 male rats were given 0.85% NaCl i.p. and another 9 received 5 mg of pilocarpine nitrate/kg. Fifteen min later, the rats were put into a sealed ether-saturated chamber until anesthetized, removed and kept anesthetized for 6 min under ether-saturated nose cones. Blood samples were collected by cardiac puncture under light ether anesthesia at the end of ether exposure and 14, 24 and 114 min later. In the second experiment groups of 9 rats each were given 0.85% NaCl or pilocarpine nitrate at a dose of 1, 5, 10 or 20 mg/kg i.p. Fifteen min after injection each rat was securely taped to a test tube rack, inverted for 3 min, removed from the rack and decapitated 7 min later. Blood was collected from the trunk wound. In the third experiment groups of 18 male rats received 0.85% NaCl, 5 mg methyl-atropine bromide/kg or 5 mg atropine sulfate/kg i.p. Fifteen min later each group was divided in half, 9 received 0.85% NaCl and the other 9

received 10 mg pilocarpine nitrate/kg i.p. Fifteen min after injection of pilocarpine or 0.85% NaCl each rat was restrained for 3 min and decapitated 7 min later.

In a second series of experiments the effects of cholinergic agonists and/or antagonists on prolactin release after suckling was investigated. Rats on the 6th through the 14th day of lactation (parturition = day 0) were separated from their litter of 8 pups for 6 hr prior to experimentation. Thirty minutes before replacement of pups with mother rats, the lactating dams were given 0.85% NaCl, methyl-atropine bromide or atropine sulfate; 15 min before replacement dams were then given 0.85% NaCl or pilocarpine nitrate. Blood samples were collected by cardiac puncture under light ether anesthesia 20 min after replacement of pups with dams.

C. Results

In male rats exposure to ether or physical restraint induced a rise in serum prolactin concentration. From a level of 28±3 ng/ml in untreated rats, serum prolactin concentration rose to 50±6 ng/ml after 6 min of ether exposure (Table 4). In rats given 5 mg pilocarpine/kg prior to ether exposure serum prolactin concentration was significantly lower than in rats given 0.85% NaCl at 6, 20, and 30 min after exposure. By 120 min there was no difference in serum prolactin concentration of the rats given pilocarpine

Table 4. Effects of Pilocarpine on the Rise in Serum Prolactin Concentration (ng/ml) after Ether Exposure

	Minutes after Ether Stress				
	-10	6	20	30	120
0.85% NaCl	28±3	50±6	60±11	48±6	40±6
Pilocarpine		33±6*	30±4**	25±3**	29±5

*p < 0.05 vs 0.85% NaCl treated rats at same time.
**p < 0.01 vs 0.85% NaCl treated rats at same time.

Table 5. Effects of Pilocarpine on the Rise in Serum Prolactin Concentration after Restraint

	Serum Prolactin, ng/ml
Nonrestrained	16±2
Restrained	
0.85% NaCl	35±3*
Pilocarpine	
1 mg/kg	22±4**
5 mg/kg	24±3**
10 mg/kg	24±3**
20 mg/kg	22±4**

*p < 0.01 vs nonrestrained rats.
**p < 0.05 vs 0.85% treated restrained rats.
n = 9 per treatment group.

or 0.85% NaCl. Similarly serum prolactin concentration rose from 16±2 ng/ml in untreated rats to 35±3 ng/ml in rats restrained for 3 min (Table 5, on the preceding page). Pilocarpine a cholinergic agonist, prevented the rise in serum prolactin concentration following ether exposure or physical restraint. Prior to physical restraint, injection of pilocarpine at doses of 1 to 20 mg/kg was equally effective in preventing the rise in serum prolactin concentration following this stress. Also, pilocarpine reduced the rise in serum prolactin concentration after restraint in those rats given methyl-atropine but not in rats given atropine (Figure 11). Atropine or methyl-atropine at the doses used had no effect on the rise in serum prolactin concentration after restraint.

In lactating rats, serum prolactin concentration rose from 21 ng/ml to 683±41 ng/ml after 20 min of suckling (Table 6). In rats given pilocarpine dams did not suckle their pups due to the periphenal side effects of this cholinergic agonist. However, when lactating rats were given methyl-atropine and 5 mg pilocarpine/kg, dams did suckle their pups yet serum concentration of prolactin did not increase. Methyl-atropine or atropine alone had no effect on the suckling induced rise in serum prolactin concentration.

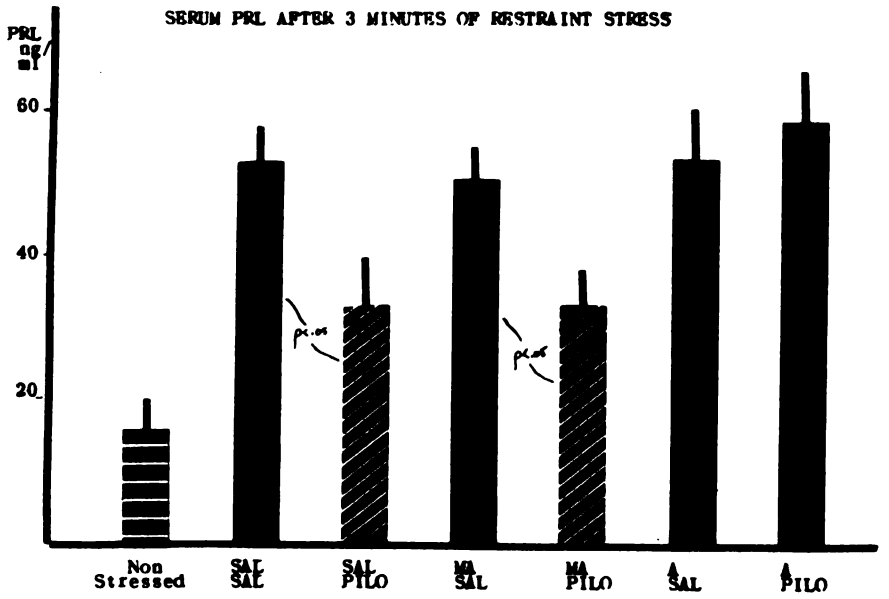

Figure 11. Effects of pilocarpine on serum prolactin concentration of restrained rats given atropine or methyl-atropine.

Bars represent serum prolactin concentration ($\bar{X} \pm$ S.E.M.) of rats given 0.85% NaCl (sal), methyl-atropine (MA), atropine (A) followed by 0.85% NaCl or pilocarpine (pilo). Rats were then restrained for 3 min and decapitated 7 min later.

N = 9 rats per treatment group.

Table 6. Effects of Pilocarpine on the Rise in Serum Prolactin Concentration After Suckling

	Serum Prolactin ng/ml	
	Trial I	Trial II
0.85% NaCl	638±41	
	n = 6	
Pilocarpine (10 mg/kg)	71±9*	
	n = 8	
Methylatropine (5 mg/kg) +0.85% NaCl	609±37	535±27
	n = 14	n = 5
Methylatropine (5 mg/kg) + pilocarpine (10 mg/kg)	59±10*	49±7*
	n = 12	n = 6
0.85% NaCl	560±44	364±77
	n = 12	n = 9
Atropine (10 mg/kg)	447±71	227±50
	n = 12	n = 10

*$p < 0.05$.

D. Discussion

Suckling and the stress of ether exposure or physical restraint induced a rise in serum prolactin concentration as observed previously. A single injection of pilocarpine, blocked the rise in serum prolactin concentration after stress or suckling. In lactating rats it was necessary to pretreat dams with the peripheral cholinergic receptor blocker, methyl-atropine, to overcome the peripheral effects that caused the dams to refuse to suckle their pups. Methyl-atropine at the dose used had no effect on the rise in prolactin concentration after suckling. Pilocarpine acted within the central nervous system to prevent the rises in serum prolactin after stress or suckling, since it was effective in rats given methyl-atropine but not in stressed rats given atropine, a receptor blocker of both peripheral and central cholinergic receptors. The doses of pilocarpine used were previously shown to reduce basal serum prolactin concentration in male and female rats (Grandison et al., 1974).

The mechanisms by which pilocarpine blocked the rise in serum prolactin concentration is presently unknown. The inhibition of basal prolactin release by pilocarpine was believed to involve catecholamines since pilocarpine was ineffective in rats given catecholamine receptor blockers (Grandison and Meites, 1975). Stimulation of catecholaminergic neurons by administration of l-dopa, a catecholemine

precursor, did block the rise in prolactin after suckling (Chen and Meites, 1975). Thus, pilocarpine could prevent the rise in serum prolactin concentration after suckling by stimulating the activity of catecholaminergic neurons.

Blake et al. (1973) reported that nicotine, a cholinergic agonist of nicotinic receptors, blocked the rise in serum prolactin after suckling but not after stress. They concluded that stress and suckling induced prolactin release by separate mechanisms. Since pilocarpine blocked the rise in serum prolactin after both suckling and stress, it probably acts at a final pathway conveying the stimuli of suckling and stress.

These results demonstrate the potent inhibitory influence on prolactin release resulting from stimulation of central cholinergic (muscarinic) receptors. The afternoon surge of prolactin in estrogen-primed ovariectomized female rats also was blocked by cholinergic agonists (Subramanian and Gala, 1976). Thus, under all circumstances examined, cholinergic agonists produced an inhibition of prolactin release suggesting that cholinergic neurons may inhibit prolactin release. The inferences drawn from experiments where atropine blocked prolactin surges must be reconsidered, especially since Donoso and Bacha (1975) found that methylatropine as well as atropine prevented the preovulatory surge of prolactin. Their finding suggests that atropine at high

doses acts on the pituitary to inhibit prolactin release. In conclusion these observations together with the finding of acetylcholine (Cheney et al., 1975), cholinergic neurons (Jacobowitz and Palkovits, 1974) and muscarinic receptors (Snyder et al., 1975) within the hypothalamus strongly suggest that acetylcholine has a physiological role in regulation of prolactin release.

IV. Inhibition by Prolactin of Post-Castration Rise in LH

A. Introduction

Prolactin has been shown to affect directly hypothalamic neural discharge (Clemens et al., 1971; Yamada, 1975). Besides inhibiting its own release, prolactin stimulated gonadotropin release in immature and pseudopregnant female rats (Clemens et al., 1969; Voogt and Meites, 1973). Yet during several reproductive and pathological conditions, there is an inverse relationship between high prolactin and low gonadotropin release, as indicated in the Review of Literature. However, there is no direct evidence that high prolactin levels can reduce LH release. This study sought to examine the relationship between prolactin and LH release by measuring the effects of elevated prolactin on the post-castration LH rise.

B. Materials and Methods

Sixteen mature female Sprague Dawley rats (230-250 g) were ovariectomized and on the same day 2 anterior pituitaries from estrogen-primed female rats were transplanted underneath the left kidney capsule of eight rats. The eight remaining ovariectomized rats underwent sham transplantation: the kidney capsule was incised but no tissue was implanted. One ml blood samples were collected every third day from the 5th to the 44th day postcastration by cardiac puncture under light ether anesthesia. In a second trial of this experiment, nine rats per group were used and blood sampling was done every other day from the 5th to the 17th day after ovariectomy.

In a second experiment, 28 Wistar Furth rats were ovariectomized. Sixteen carried the anterior pituitary tumor $MtTW_{15}$ while 12 others were tumor free and served as controls. One ml blood samples were collected at 5 day intervals from the 5th to the 25th day after castration. In a third experiment LRH at a dose of 50 ng/100 gm BW was given by tail vein injection to 36 Sprague Dawley female rats (230-260 g) 8 days after ovariectomy. Nine of these rats were given 2 anterior pituitaries immediately after ovariectomy while 9 others were sham transplanted. Of the remaining 18 rats, 9 were given 0.85% NaCl and 9 were given ovine prolactin (NIH P-S-11) subcutaneously (1 mg/rat x 7 injections) during the 3 days prior to LRH injection.

Seven-tenth ml blood samples were collected from all rats 2 hr before injection of LRH. Pituitary grafts were removed or sham operations were performed in the grafted rats. At 10, 20, and 40 min after LRH injection, additional blood samples were collected.

In a fourth experiment male rats (350-421 g.) were implanted with 2 anterior pituitaries underneath the kidney capsule or were sham transplanted 4 days before orchidectomy in two separate trials. Blood samples were collected at the time intervals indicated in Results. Finally, 20 male rats (340-410 g) were implanted with a 23 gauge metal cannula in the median eminence. The cannulae in 10 rats contained a mixture of 100 µg ovine prolactin and 100 µg cocoa butter, while the cannulae in 10 other rats contained cocoa butter. Ten different male rats received 2 anterior pituitaries while 10 others were sham transplanted. Four days after implantation or transplantation, these rats together with 20 untreated rats were orchidectomized. Twenty-four hours later, all rats were decapitated. Blood was collected from the trunk wound, and the anterior pituitary from the sella turcica and the hypothalamus were removed from each rat. Student's "t" test was used to determine the significance of difference between groups.

C. Results

1. Effects of Anterior Pituitary (AP) Grafts or Pituitary Tumors on Post-castration LH Release in Female Rats

Serum LH concentration gradually increased from the 5th to the 44th day after ovariectomy (Figure 12A). A similar pattern of LH release was observed in ovariectomized AP grafted rats; however, serum LH concentration was significantly lower in AP grafted rats on days 5, 8 and 11 after castration than in sham grafted rats. From day 14 to 44 there was no difference in serum LH concentration between the two groups. In contrast, serum prolactin concentration was significantly higher in the AP grafted rats during the entire sampling period averaging 222% above that in ovariectomized rats (Figure 12B) (39±1 vs 18±3 ng/ml). In a second trial similar observations were made. Serum LH concentration was significantly lower in AP grafted rats at 5 and 9 days after ovariectomy (Figure 13A), while serum prolactin concentration in AP grafted rats was significantly higher throughout the sampling period (36±2 vs 7±0.7 ng/ml, Figure 13B).

In tumor-free Wistar-Furth rats, serum LH concentration continually increased from day 5 to 25 after ovariectomy (Figure 14). The $MtTW_{15}$ tumor bearing rats were separated into 2 groups: one group of 8 had small tumors (approximate initial size, 1 x 1 cm) growing subcutaneously on their

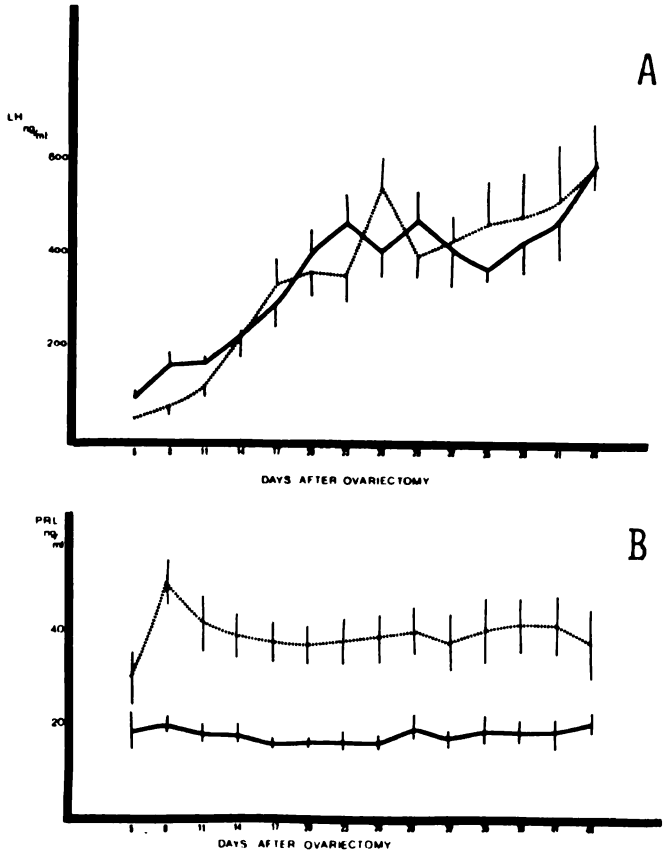

Figure 12. Effects of AP grafts on serum LH and prolactin from 5 to 44 days after ovariectomy.

A. Solid line represents serum LH concentration (X ± 1 S.E.M.) of sham grafted ovariectomized rats and striped line the LH of AP grafted ovariectomized rats.

· $P < 0.05$ vs. sham grafted ovariectomized rats.

B. Solid line represents serum prolactin concentration of sham grafted ovariectomized rats and striped line the prolactin of AP grafted ovariectomized rats.

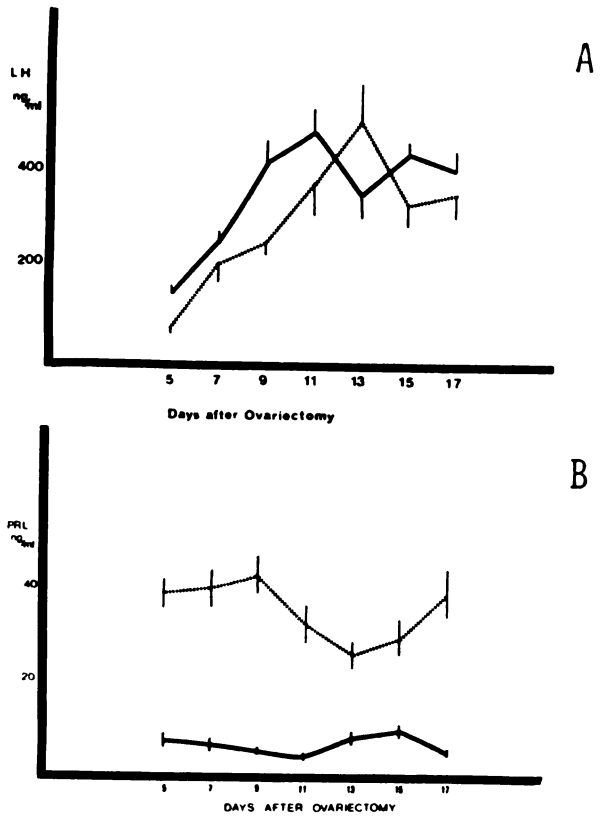

Figure 13. Effects of AP grafts on serum LH and prolactin from 5 to 17 days after ovariectomy.

A. Solid line represents serum LH concentration of sham-grafted ovariectomized rats and striped line, the LH of AP grafted ovariectomized rats.

• $P < 0.05$ vs. sham grafted ovariectomized rats.

B. Solid line represents serum prolactin concentration of sham grafted ovariectomized rats and striped line, the prolactin of AP grafted ovariectomized rats.

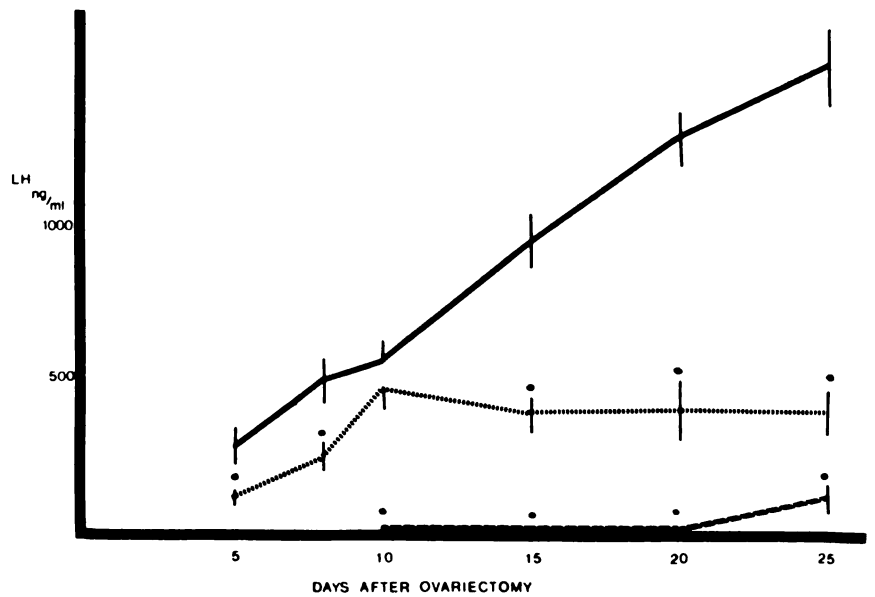

Figure 14. Effects of anterior pituitary tumor on serum LH after ovariectomy.

Lines represent serum LH concentration of rats: solid = nontumor bearing rats, striped = small tumor bearing rats, diagonally striped = large tumor bearing rats.

*$P < 0.05$ vs. non-tumor bearing rats.

backs, and the second group of 8 had large tumors (average initial size 3.1 x 6.2 cm). Serum LH concentration in rats bearing small tumors increased by 5 days after castration. At 10 days a further increase was observed, but thereafter no significant change occurred. In rats with large pituitary tumors serum LH was barely detectable until day 25 after ovariectomy. The serum LH concentration was significantly lower in rats bearing small tumors than in tumor-free rats at all sampling periods after castration except on day 10. Serum LH concentration was significantly lower in rats bearing large tumors than in tumor-free or rats bearing small tumors during the entire experiment. Serum prolactin was very high in all tumor bearing rats. In the small tumor bearing rats, serum prolactin before castration was 964±112 ng/ml and rose to 2601±850 ng/ml by 25 days after castration. In large tumor bearing rats, initial prolactin concentration was 3853±456 ng/ml and rose to 6380±2620 ng/ml by 20 days after castration.

2. Effects of AP Grafts or Prolactin on LRH Induced LH Release

Serum LH concentration was significantly lower in AP grafted rats than in sham grafted rats 8 days after ovariectomy (161±16 vs 280±44 ng/ml) (Figure 15). After removal of the grafted pituitaries or sham operation of sham-grafted rats, LH release in response to LRH was the same in the two groups at 10 and 20 min after injection. By 40 min serum LH

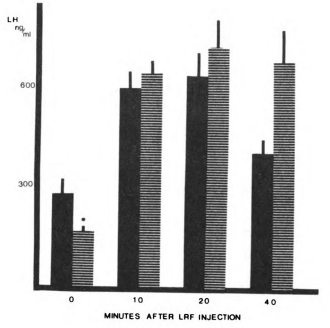

Figure 15. Effects of LRH on serum LH concentration in ovariectomized AP grafted rats.

Solid bars represent serum LH in sham grafted overiectomized rats.

Striped bars represent LH in rats bearing AP grafts for 8 days.

Grafts were removed prior to LRH injection.

*$P < 0.05$.

concentration was higher in those ovariectomized rats which previously had 2 AP grafts. In ovariectomized rats given ovine prolactin serum LH concentration was lower than in rats given 0.85% NaCl; however, the difference was not significant. LH release in response to LRH was the same in both groups at all sampling periods (Figure 16).

3. Effects of AP Grafts or Prolactin on Postcastration LH Release in Male Rats

Serum LH concentration increased rapidly in sham grafted male rats after orchidectomy (Figure 17). However, there was no LH rise in AP grafted rats for 22 hr after orchidectomy. During a second trial serum LH concentration of sham grafted rats was high as compared to intact rats at 18 hr and gradually increased during the next 62 hr (Figure 18). At 10 days and then again 20 days after castration further increases in serum LH concentration occurred. The serum LH concentration in orchidectomized AP grafted rats was significantly lower than in sham-grafted orchidectomized rats at 30, 48, 60 and 80 hrs, and 10 and 14 days after castration.

In rats with median eminence prolactin implants, serum LH concentration was 50% lower than in rats with cocoa butter implants or in non-implanted rats at 24 hrs after castration (Figure 19). Similarly, serum LH concentration was 51% lower in orchidectomized AP grafted rats than in sham grafted orchidectomized rats (Figure 19).

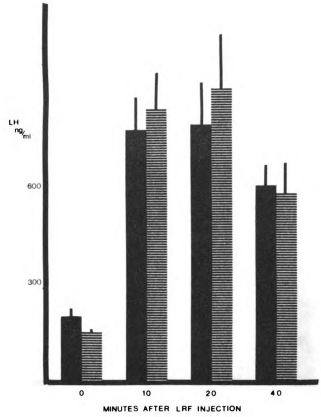

Figure 16. Effects of LRH on serum LH concentration in ovariectomized rats given prolactin.

Solid bars represent serum LH in ovariectomized rats given 0.85% NaCl.

Striped bars represent LH in prolactin treated ovariectomized rats.

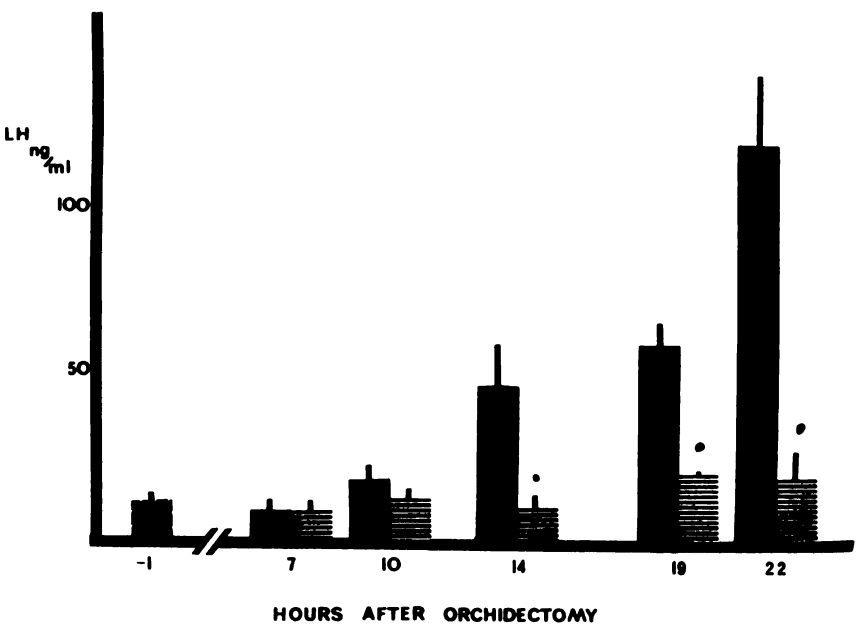

Figure 17. Effects of AP grafts on serum LH during the 22 hours after orchidectomy.

Bars represent serum LH concentration; diagonally striped = intact male rats; solid = orchidectomized rats, striped = AP grafted orchidectomized rats.

*$P < 0.05$.

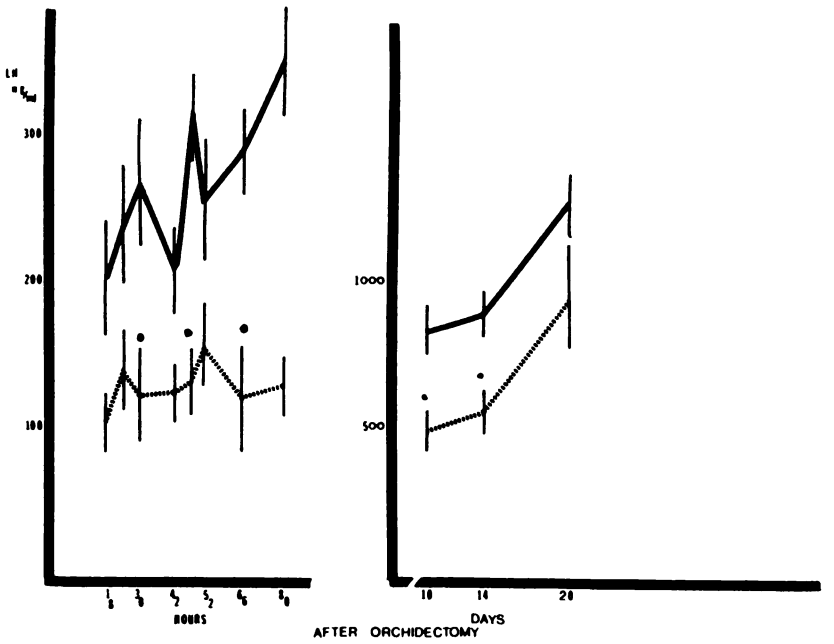

Figure 18. Effects of AP grafts on serum LH concentration of rats 18 hours to 20 days after orchidectomy.

Solid line represents serum LH concentration of orchidectomized rats, striped line of AP grafted orchidectomized rats.

*$P < 0.05$.

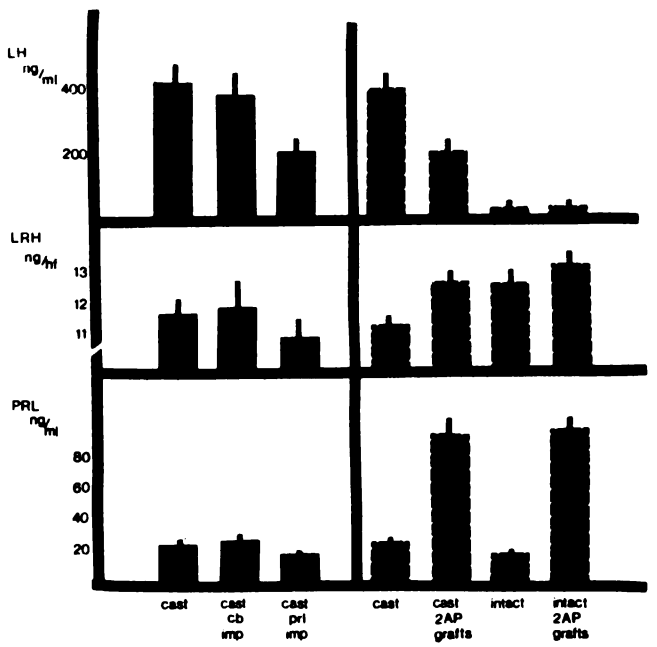

Figure 19. Effects of median eminence prolactin implants or AP grafts on serum LH, hypothalamic LRH and serum prolactin 24 hours after orchidectomy.

Left. Rats were given median eminence implants (imp) of cocoa butter (cb) or prolactin (prl) 4 days prior to orchidectomy (cast) and killed 24 hours later. LRH is expressed as ng per hypothalamic fragment (hf).

Right. Rats were given 2 anterior pituitary (2 AP) transplants beneath the kidney capsule or sham grafted; 4 days later half were orchidectomized (cast) and then all were killed 24 hours later.

Although AP grafts reduced LH release after castration, there was no significant difference in the low basal serum LH concentration between intact and intact AP-grafted male rats (16±3 vs 20±4 ng/ml). AP grafts prevented the reduction in hypothalamic LRH content after orchidectomy. No difference in hypothalamic LRH content was observed among orchidectomized AP grafted, intact, and intact AP grafted rats or between orchidectomized rats with and without median eminence implants. Serum prolactin concentration was significantly higher, three to four-fold, in AP grafted rats than in non-grafted rats. No difference in pituitary LH concentration was observed among treatment groups but pituitary weight was reduced by median eminence implants of prolactin or cocoa butter.

D. Discussion

After castration serum LH concentration rose slowly in female and rapidly in male rats, in agreement with previous observations (Gay and Midgley, 1969). In both sexes, serum LH concentration continued to increase with time after castration. The rise in serum LH was partially inhibited by prolactin. In male rats with AP grafts, the serum LH rise was completely inhibited for 22 hrs. It is well-established that AP grafts mainly secrete prolactin, some GH and little of other anterior pituitary hormones (Meites and Nicoll, 1966). In other trials, serum LH concentration was

significantly lower in AP grafted rats than in sham operated rats at 24 hrs or 30 hrs after orchidectomy, but higher than in intact rats. The complete suppression of the postcastration LH rise at 22 hrs in the first trial may have resulted from a combination of the effects of elevated prolactin with stress due to repeated ether exposure and cardiac puncture. Stress can reduce serum LH concentration (Blake, 1975). Serum LH concentration was still significantly lower in AP grafted rats than in sham operated rats 10 and 14 days after orchidectomy. Similarly serum LH concentration was lower in AP grafted female rats during the 5th-10th day after castration. AP grafts reduced the LH rise after ovariectomy during the first 11 days. With time after castration the inhibitory effect of prolactin on LH release lessened. By day 20 in male or 14 in female castrated rats, serum LH concentration in the AP grafted rats did not differ from sham-operated castrated rats even though serum prolactin concentration continued to be elevated.

Serum prolactin concentration of AP grafted rats is elevated but lower than in postpartum lactating rats (Lu et al., 1976), and thus well within the physiological range. The inhibitory effect of prolactin on LH release in ovariectomized rats could be prolonged by further increasing serum prolactin concentration. When the transplantable AP tumor $MtTW_{15}$ was used to increase prolactin levels, serum prolactin

concentration was greatly elevated and the postcastration LH rise was inhibited for 25 days. The degree of suppression of the LH rise was related to serum prolactin concentration. In rats bearing large AP tumors the LH rise did not occur, and in the small tumor group the LH rise was significantly less than in the tumor free rats during the entire 25 days after castration. Since the $MtTW_{15}$ tumor secretes substantial amounts of GH and perhaps ACTH in addition to prolactin (Meites and Nicoll, 1966), suppression of LH release in the tumor bearing rats may not result solely from prolactin.

AP grafts partially prevented the postorchidectomy rise in serum LH, yet they had no effect on low basal LH concentration in intact rats. The absence of effect in intact rats may be due to readjustment of LH release by gonadal steroids or because AP grafts themselves release LH (Lu et al., in press). The results indicate that prolactin acts on the hypothalamus to inhibit LH release. Median eminence implants of prolactin were as effective as AP grafts in reducing postcastration LH release. AP grafts also prevented the decline in hypothalamic LRH content 24 hrs after orchidectomy. No difference in hypothalamic LRH content was observed between orchidectomized rats implanted with cocoa butter or prolactin perhaps due to inadvertant destruction of neural tissue synthesizing LRH. Pituitary sensitivity to LRH was not reduced by prolactin after ovariectomy

and thus the inhibitory effect of prolactin on LH release is not due to changes at the pituitary level. In contrast, LH release after LRH injection is lower in lactating than in non-lactating rats (Lu et al., 1976) and humans (Tolis et al., 1974). Recently Moudgal et al. (1976) reported that exogenous prolactin given to lactating rats suckling two pups reduced LH release in response to LRH; however, this does not exclude a hypothalamic site of action in this instance. During lactation exogenous prolactin may reduce LRH secretion, thus eliminating the priming effect (Aiyer et al., 1971) of basal LRH levels on later LRH induced LH release.

The inhibitory effect of prolactin on the postcastration LH rise may result from increased dopamine turnover induced by prolactin. Fuxe and Hökfelt (1969) reported that prolactin stimulated the turnover of dopamine in the hypothalamus. In addition, dopamine has been reported to inhibit LH release (Fuxe and Hökfelt, 1969; Sawyer et al., 1974). Recently, administration of prolactin to ovariectomized rats has been shown to inhibit LH release while stimulating dopamine turnover (Gudelsky et al., unpublished).

The lessening of the inhibitory effect of prolactin on post-castration LH rise with time suggests that the stimulus for LH release increases with time or that the dopaminergic neurons or postsynaptic receptors inhibitory to LH release

become insensitive to prolactin. Parallel observations have been observed with alpha-methyl-para-tyrosine, an inhibitor of catecholamine synthesis. Given one day after castration alpha-methyl-para-tyrosine prevented postcastration LH rise (Ojeda and McCann, 1973), but at later times it was ineffective (Donoso et al., 1971). Inhibition of the LH rise produced by alpha-methyl-para-tyrosine results from blockade of the increased noradrenergic activity after castration.

In conclusion, these data indicate that elevated prolactin, acting via the hypothalamus, can reduce the serum LH rise after castration. This inhibition of LH release may have physiological significance during a reproductive state such as lactation where serum prolactin is continuously elevated. During postpartum lactation estrous cycles cease and LH release even after castration is reduced (Ford and Melampy, 1973; Hammons et al., 1973). These findings suggest prolactin has an important role in the postpartum diestrum.

V. Effects of GABA on TSH and Prolactin Release

A. Introduction

Neurotransmitters acting in the hypothalamus have been reported to influence the release of TSH and prolactin (see Review of Literature). Recently GABA, which is highly concentrated in the hypothalamus (Baxter, 1970), was observed

to inhibit ACTH (Makara and Stark, 1974) and stimulate LH and prolactin release (Ondo, 1974; Mioduszewski et al., 1976; Ondo and Pass, 1976). This study was undertaken to examine the effects of GABA agonists and antagonists on TSH and prolactin release.

B. Materials and Methods

Gamma aminobutyric acid (GABA, Nutritional Biochemical Corp., Cleveland, OH) and bicuculline methyliodide (Pierce, Rockford, ILL) were dissolved in 0.85% NaCl and the pH of these solutions was adjusted to 7 by adding NaOH. Picrotoxin (Nutritional Biochemical Corp., Cleveland, OH) and amino-oxyacetic acid (AOAA, Aldrich Chemical Co., Milwaukee, WI) were also dissolved in 0.85% NaCl.

In the first three experiments, rats were given intraventricular injections of GABA or bicuculline methyliodide. Rats were implanted with polyethylene tubing (PE 10) into the lateral ventricle (Verster et al., 1971; see Materials and Methods section). During a 3 day recovery period rats received tetracycline in the drinking water to prevent infection and were handled daily to reduce the stress of handling during experimentation. Three to 5 days after cannulation rats were removed from their cage, and loosely held in a towel for injection. GABA, bicuculline methyliodide, or 0.85% NaCl was slowly infused (60 sec.) in a total volume of 8 µl. The cannulae were rinsed with 2 µl of 0.85% NaCl and

resealed. Rats were replaced in their individual cage and decapitated 20 min later. Blood was collected from the trunk wound. In the first 2 experiments rats were given GABA or bicuculline alone at several doses. In the third experiment estrogen-primed (5 μg EB/rat x 5 days) male rats were given 0.85% NaCl, 10 μM GABA, 0.7 μg bicuculline methyliodide, or 0.7 μg bicuculline methyliodide plus 10 μM GABA.

In separate experiments rats were given intraperitoneal injections of 0.85% NaCl or picrotoxin at doses of 0.5, 1.5 or 4.5 mg/kg and were decapitated 20 min later. Male rats also were given AOAA intraperitoneally at a dose of 25 or 50 mg/kg and were decapitated 1½ or 6 hours later. In the last experiment rats were placed 1 per cage in a ventilated cold room (4°C) for 2 hours. Groups 1-3 received respectively 0.85% NaCl, 25 and 50 mg AOAA/kg i.p. prior to cold exposure. Group 4 received 50 mg AOAA/kg i.p. before cold exposure and 60 min later. Rats of group 5 were taped to test tube racks and placed in the cold room. All rats were decapitated after 2 hours of cold exposure.

Serum samples were assayed for TSH and prolactin by radioimmunoassay. Student's "t" test was used to determine the significance of a difference between control and treatment.

C. Results

GABA at doses of 8 and 15 μM significantly decreased serum TSH and at 15 μM significantly increased serum prolactin concentration (Table 7). Lower doses produced no effects on TSH or prolactin concentration. AOAA, which increases endogenous brain GABA concentration (Baxter and Roberts, 1961), significantly reduced serum TSH concentration in male rats at both the 25 and 50 mg/kg dose at 1½ hours after injection but not at 6 hours (Table 8). AOAA did not affect serum prolactin concentration.

The GABA antagonist bicuculline methyliodide reduced serum prolactin at doses of 0.2 and 0.4 μg without affecting serum TSH concentration (Table 9). At a dose of 0.6 μg bicuculline methyliodide increased serum TSH and prolactin and also induced convulsions. Another GABA antagonist, picrotoxin, did not alter TSH or prolactin release at the doses used nor did it induce convulsions (Table 10).

In estrogen-primed male rats 10 μM of GABA increased serum prolactin concentration from 74±9 ng/ml to 120±18 ng/ml but did not affect TSH concentration (Table 11). Bicuculline methyliodide at a dose of 0.7 μg increased prolactin concentration to 215±15 ng/ml and TSH from 624±57 to 1013±216 ng/ml. When bicuculline methyliodide and GABA were both given, serum prolactin concentration was 125±24 ng/ml, higher than in controls, equal to rats given just GABA but

Table 7. Effects of GABA on Serum TSH and PRL in Male Rats

Treatment	Experiment I		Experiment II	
	TSH	PRL	TSH	PRL
None	575 ± 97	10 ± 1		
0.85% NaCl	504 ± 75	6 ± 1	850 ± 175	32 ± 5
GABA				
0.5 µM	592 ± 99	6 ± 1		
1 µM	431 ± 57	5 ± 1		
5 µM	475 ± 51	7 ± 1		
8 µM			398 ± 45*	25 ± 3
15 µM	227 ± 33*	12 ± 2*		

*Significantly different ($P < 0.05$) vs 0.85% NaCl injected rats.

N = 9 in experiment 1, and 7 in experiment 2.

Table 8. Effects of AOAA on Serum TSH and PRL

Treatment	TSH Hours after Injection 1	TSH Hours after Injection 6	PRL Hours after Injection 1	PRL Hours after Injection 6
None	979 ± 215		11 ± 3	
0.85% NaCl	744 ± 72	620 ± 88	12 ± 3	14 ± 4
25 mg AOAA/kg	176 ± 45*	448 ± 85	10 ± 2	12 ± 3
50 mg AOAA/kg	265 ± 34*	440 ± 150	12 ± 4	9 ± 1.5

N = 8 per treatment.

*P < 0.05 vs control (0.85% NaCl) rats.

Table 9. Effects of Bicuculline Methyliodide on Serum TSH and PRL

Treatment	TSH	PRL
0.85% $NaCl_{N=9}$	391 ± 57	23 ± 4
Bicuculline Methyliodide		
0.2 $\mu g_{N=8}$	299 ± 54	14 ± 1*
0.4 $\mu g_{N=7}$	387 ± 67	14 ± 8
0.6 $\mu g_{N=4}$	828 ± 165*	73 ± 7*

*Significantly different (P < 0.05) vs 0.85% NaCl injected rats.

Table 10. Effects of Picrotoxin on Serum TSH and PRL

Treatment	Experiment I		Experiment II	
	TSH	PRL	TSH	PRL
0.85% NaCl	676 ± 108	15 ± 5	597 ± 69	11 ± 2
Picrotoxin				
0.5 mg/kgm	1052 ± 246	17 ± 7	596 ± 53	16 ± 3
1.5	815 ± 117	19 ± 6		
4.5	704 ± 117	26 ± 12		

N = 8 per treatment group in experiments 1 and 2.

Table 11. Effects of GABA and Bicuculline Methyliodide on Serum TSH and PRL in Estrogen Primed Male Rats

Treatment	TSH	PRL
0.87% NaCl	624 ± 57	74 ± 9
10 μM GABA	545 ± 60	120 ± 18
0.7 μBicMI	1013 ± 216*	215 ± 15*
10 μM GABA + 0.7 μBic MI	654 ± 189	125 ± 24

*Significantly different ($P < 0.05$) vs 0.85% NaCl injected rats.

N = 8 per treatment group.

lower than in rats given bicuculline. Serum TSH concentration in rats given bicuculline and GABA was similar to the TSH concentration of controls or GABA treated rats but lower than that of bicuculline treated rats.

In the last experiment cold exposure increased serum TSH and reduced serum prolactin concentration (Table 12). Fifty mg AOAA/kg given before and during cold exposure not only prevented the rise in serum TSH but decreased TSH concentration. This same dose of AOAA prevented the decline in prolactin concentration associated with cold exposure. Lower doses of AOAA did not significantly affect serum TSH or prolactin concentration during cold exposure. Physical restraint reduced basal TSH concentration, thus preventing the rise in TSH during cold exposure. Prolactin concentration increased approximately four-fold following combined cold exposure and restraint.

D. Discussion

An increase in brain GABA was associated with a reduction in serum TSH concentration. By 20 min after intraventricular injection of GABA, serum TSH concentration was significantly reduced. Also AOAA reduced serum TSH concentration at 1½ hours after injection, a time when accumulation of endogenous GABA occurs most rapidly. By 6 hours serum TSH concentration was similar in 0.85% NaCl and AOAA-treated rats, although brain GABA concentration was reported

Table 12. Effects of AOAA and Cold Exposure on Serum TSH and PRL

Treatment	TSH	PRL
None	1764 ± 283	11 ± 0.7
Cold Exposure		
+0.85% NaCl	3080 ± 533[a]	8.6 ± 0.3[a]
+25 mg AOAA/kg	2271 ± 520	9.0 ± 0.3
+50 mg AOAA/kg	2631 ± 599	9.3 ± 0.3
+2x(50 mg AOAA/kg)	594 ± 198[b]	11.4 ± 0.8[b]
+ restraint	795 ± 187[b]	46 ± 5.4[b]

N = 8

[a] $P < 0.05$ vs nontreated rats.

[b] $P < 0.05$ vs cold exposure + 0.85% NaCl.

to be still elevated in rats given AOAA (Wallach, 1961). By 6 hours after AOAA, regulatory mechanisms may compensate to return serum TSH concentration to pre-injection levels. In addition, AOAA prevented the rise in serum TSH during cold exposure, demonstrating its ability to significantly affect physiological processes. In estrogen-primed rats, GABA at a dose of 10 μM did not reduce serum TSH concentration. Estrogen has been observed to reduce TSH release and may desensitize the release of TSH to certain stimuli. Further, estrogen reduces synthesis of GABA (Baxter, 1970) and may thus affect responses to GABA. However, GABA did prevent the rise in TSH after administration of a GABA antagonist in estrogen primed rats.

Bicuculline increased serum TSH concentration in both estrogen-primed and untreated male rats. Since these doses also produced convulsions, the physiological relevance of these observations remains unknown. Nonconvulsant doses of bicuculline or picrotoxin had no effect on TSH. GABA neurons may not tonically affect TSH release; consequently, GABA antagonists may not alter serum TSH concentration under all conditions.

In contrast to its effects on TSH, GABA increased serum prolactin concentration in estrogen-primed and untreated male rats. Nonconvulsant doses of bicuculline reduced serum prolactin concentration. Although AOAA had no effect on

prolactin release in untreated male rats, it did prevent the fall in prolactin during cold exposure. During cold exposure, stress reduced TSH and increased prolactin release, a pattern unlike that in rats treated with 0.85% NaCl or AOAA. This finding indicates that the effects produced by AOAA during cold exposure does not result from stress.

In conclusion, these data indicate that GABA agonists and antagonists can alter TSH and prolactin release and influence the release of these hormones in response to environmental stimuli. Furthermore, these findings suggest that the putative neurotransmitter, GABA, may participate in regulation of TSH and prolactin release.

GENERAL DISCUSSION

Pioneer research in neuroendocrinology established that the hypothalamus regulated anterior pituitary secretion. In subsequent efforts to explain regulation of pituitary function, hypothalamic substances were administered to determine whether they could evoke release of hormones. This approach indicated catecholamines and serotonin participate in the regulatory process, a finding later substantiated by correlation of amine turnover with hormone release. Since this approach has proven valuable, it was used again in the present study to investigate the effects of acetylcholine, GABA and prolactin on anterior pituitary secretion.

In this study the cholinergic agonist pilocarpine was used to mimic cholinergic discharge. It prevented the release of prolactin after stress and suckling and inhibited pseudopregnancy induction following cervical stimulation. Evidence suggesting that cholinergic agonists reduce prolactin release via adrenergic neurons also was provided.

The results of cholinergic and GABA drugs on hormone release must be interpreted in conjunction with other reports in order to evaluate their physiological relevance. Acetylcholinesterase was found in the arcuate nucleus

(Jacobowitz and Palkovits, 1974) and cholinergic synaptic vessels were observed in the median eminence (Kobayashi and Matsui, 1969) suggesting that cholinergic neurons lie close to the infundibular dopaminergic tract. In addition cholinergic agonists have been reported to stimulate catecholamine turnover in the whole brain (Bhatnagar, 1974; Anden, 1974). These observations provide additional support for the suggestion that cholinergic neurons can stimulate median eminence dopamine neurons. It is well-established that the infundibular dopaminergic neurons produce the tonic inhibition of prolactin release. Blockade of cholinergic receptors with atropine did not stimulate prolactin release nor did it increase release after suckling, stress, heat exposure (not reported here) or after injection of 5 hydroxytryptophan (not reported here). Thus, cholinergic neurons do not chronically stimulate the dopaminergic neurons.

The role of the cholinergic system described above is complicated by the observation that atropine at high doses prevented the rise in prolactin on proestrous afternoon (Libertun and McCann, 1973) and during the mornings of pseudopregnancy (McLean and Nikitovitch-Winer, 1975). These latter reports imply that cholinergic neurons stimulate rather than inhibit prolactin release as reported in the present study. However, atropine has been reported to have non-specific effects, especially at high doses (Curtis and

Phillis, 1960). Furthermore, in the reports by the preceding workers atropine was used in doses equivalent to 90% of the LD_{50} dose. Thus, the relevance of these observations to physiological processes is questionable. An alternate interpretation of the role of cholinergic neurons in prolactin release could encompass the observations reported here and observations of Libertun and McCann (1973) and McLean and Nikitovitch-Winer (1975). There is the possibility that several sites within the neural network regulating prolactin release utilize acetylcholine as a neurotransmitter and that cholinergic neurons may either inhibit or stimulate prolactin release. Systemic or central administration of agonists affect all possible cholinergic receptors. The result could be a summation of inhibitory and stimulatory effects on prolactin release with a predominance of inhibitory action under the above conditions. However, there is little valid evidence for this latter view.

The observations reported here consistently favor the view that cholinergic neurons inhibit prolactin release. It is noteworthy that acetylcholine has been reported to alter not only prolactin but also gonadotropin (Siminovic et al., 1974; Fiorindo and Martini, 1975) and ACTH release (Hedge and Smelik, 1968). Acetylcholine may have a role in depressing prolactin release while at the same time stimulating gonadotropin release such as occurs after castration.

During postpartum lactation prolactin release is high and release of LH and FSH is low (Lu et al., 1976). It is possible that the absence of cholinergic activity is involved in this process. However, further research involving correlation of cholinergic activity with hormone release is required to establish when cholinergic neurons are active in physiological processes.

The putative neurotransmitter, GABA, also was given to determine its effects on hormone release. GABA stimulated prolactin while inhibiting TSH release. Elevation of brain GABA by AOAA prevented the rise in TSH during cold exposure, which is an intense physiological stimulus for TSH release. These findings suggest that GABA reduces TSH release and also confirms earlier reports that GABA stimulates prolactin release (Mioduszewski et al., 1976; Ondo and Pass, 1976). In support of a physiological role for GABA are the observations that GABA is found in the hypothalamus in high concentrations as compared to other brain regions (Baxter, 1970) and that GABA receptors are present in the medial hypothalamus (Makara et al., 1975). Little is known about how GABA affects hormone release. Its action on prolactin release could be mediated by inhibition of dopaminergic discharge. GABA has been reported to inhibit dopamine turnover in the mesolimbic dopaminergic system (Fuxe et al., 1975), although there is no indication that this effect extends to all

dopaminergic neurons. Since GABA inhibits TSH like dopamine, inhibition of the dopaminergic system can not explain the action of GABA on TSH release. Again, as with acetylcholine, the physiological occasions during which GABA release occurs are unknown. Thus, it is not possible to specify a physiological role for GABA on the release of prolactin and TSH at present. However, the potential importance of this neurotransmitter in regulation of hormone release is emphasized by the report of Guidotti and Costa (1975). They recently observed that a depletion of approximately 30% of the hypothalamic GABA content was associated with a nine-fold increase in cyclic AMP concentration of the anterior pituitary. Cyclic AMP is strongly implicated in stimulation of hormone release at the pituitary (Labrie et al., 1976). In addition to prolactin and TSH, GABA was reported to stimulate LH (Ondo, 1974) and inhibit ACTH release (Makara and Stark, 1974).

Prolactin administration reduced the rise in LH after castration. Prolactin has been reported to stimulate the activity of the median eminence dopaminergic neurons (Fuxe and Hökfelt, 1969; Gudelsky, Simpkins, Meites and Moore, unpublished). Since dopamine can reduce LH release (Sawyer et al., 1974), it is probable that prolactin reduced LH release by increasing dopamine turnover. This may constitute the mechanism responsible for the reciprocal relation between

high prolactin and low gonadotropin release. Thus, prolactin may be partially responsible for the diminished release of LH during lactation when prolactin release is high and LH and FSH release are low. The suckling stimulus, by increasing prolactin release, could increase dopamine turnover, and thereby reduce LH and FSH release. A reduction in prolactin secretion during lactation following administration of an ergot drug has been shown to result in enhanced release of LH and FSH (Lu et al., 1976).

To understand the regulation of anterior pituitary function, the organization of the neural network controlling hormone release must be clarified. Also the factors modifying the rate of neural discharge must be determined. This study has suggested the possible participation of two new components in the network of neurons controlling anterior pituitary function: cholinergic and gabergic neurons. It also provides evidence that LH release can be decreased by prolactin, a stimulator of infundibular dopaminergic neurons. Hopefully these observations will provide a stimulus for additional research on the control of anterior pituitary secretion.

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APPENDIX

APPENDIX

GLOSSARY

ACTH--adrenocorticotropin hormone, an anterior pituitary hormone.

Alpha methyl para tyrosine (αmpt)--a drug which inhibits the synthesis of catecholamines.

Aminooxyacetic acid--a drug which increases the endogenous concentration of GABA in the brain.

AOAA--aminooxyacetic acid.

Atropine--a drug which combines with but does not stimulate cholinergic receptors, blocks the effects of acetylcholine and muscarinic cholinergic agonists (pilocarpine) at cholinergic receptors in both the periphery and brain.

Bicuculline--a drug which combines with but does not stimulate GABA receptors, blocks the effects of GABA.

Carbachol--a drug which produces effects like acetylcholine, a cholinergic agonist.

Chlorpromazine--a drug which combines with but does not stimulate catecholamine receptors, blocks the effects of catecholamines.

Clonidine--a drug which produces effects like norepinephrine.

COMT--catecholamine-o-methyl-transferase, an enzyme catabolizing catecholamines.

CRF--corticotropin releasing factor, an hypothalamic hormone stimulating release of ACTH.

DDC--dithiocarbamate.

Dihydroxyphenylserine--a drug which is converted directly to norepinephrine, used to increase the concentration of norepinephrine without increasing the concentration of dopamine.

Disulfiram--a drug which inhibits the enzyme dopamine-β-hydroxylase, used to decrease norepinephrine concentration without decreasing dopamine concentration.

Dithiocarbamate--a drug which inhibits the enzyme dopamine-β-hydroxylase used to decrease norepinephrine concentration without decreasing dopamine concentration.

DOPS--dihydroxyphenylserine.

FSH--follicle stimulating hormone, an anterior pituitary hormone.

GABA--gamma hydroxybutyric acid, a putative neurotransmitter.

GAD--glutamic acid decarboxylase, an enzyme catalyzing the synthesis of glutomic acid to GABA.

GH--growth hormone, an anterior pituitary hormone.

Haloperidol--a drug which combines with but does not stimulate catecholamine receptors, blocks the effects of catecholamines.

HCG--human chorionic gonadotropin, a hormone with LH-like activity.

Histamine--a putative neurotransmitter.

5-HTP--5-hydroxytryptophan.

5-hydroxytryptophan--a precursor of serotonin, used to increase brain serotonin concentration.

l-dopa--a precursor of catecholamines used to increase brain dopamine and norepinephrine concentration.

LH--luteinizing hormone, an anterior pituitary hormone.

Lilly 11040--a drug which inhibits the reuptake of serotonin, used to prolong the effect of serotoninergic discharge.

LRH--luteinizing hormone releasing hormone, a hypothalamic hormone releasing LH and FSH.

MAO--monoamine oxyase, an enzyme which catabolizes catecholamines.

Methylatropine--an atropine derivative which does not penetrate the brain, used to block the peripheral effects of cholinergic agonists.

Methylsergide--a drug which combines with but does not sitmulate serotoniner. Glc receptors, blocks the effects of serotonin.

MK 468--a drug which inhibits the conversion of l-dopa to dopamine in the perphery only.

Nicotine--a drug which acts like acetylcholine at the nicotinic type of cholinergic receptors.

Parachloramphetamine--a drug which depletes the central stores of serotonin.

Parachlorophenylalanine--a drug which depletes the central stores of serotonin.

PCA--parachloramphetamine.

PCPA--parachlorophenylalanine.

Phenlotamine--a drug which combines with but does not stimulate catecholamine receptors, blocks the effects of catecholamines.

Phenylbenzamine--a drug which combines with but does not stimulate catecholamine receptors, blocks the effects of catecholamines.

Physostigmine--a drug which inhibits acetylcholinease, increases endogenous acetylcholine concentration.

Picrotoxin--a drug which combines with but does not stimulate GABA receptors, blocks the effects of GABA.

PIF--prolactin release inhibiting factor, a hypothalamic hormone which inhibits the release of prolactin.

Pilocarpine--a drug which produces effects like acetylcholine at muscarinic cholinergic receptors.

Pimozide--a drug which combines with but does not stimulate dopamine receptors, blocks the effects of dopamine.

PMS--pregnant mares serum, a hormone with FSH activity.

PNMT--phenyl-ethanolamine-n-methyl-transferase; an enzyme catalyzing the synthesis of norepinephrine to epinephrine.

Reserpine--a drug which depletes the concentration of catecholamines.

Somatostatin--a hypothalamic hormone which inhibits the release of GH and TRH-stimulated TSH release.

SRIF--somatostatin.

TRH--thyrotropin releasing hormone, a hypothalamic hormone which stimulates the release of TSH and prolactin.

TSH--thyroid stimulating hormone, an anterior pituitary hormone.

UI 4624--a drug which inhibits dopamine-β-hydroxylase, used to decrease norepinephrine concentration.

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