This is to certify that the

thesis entitled

RELATION OF BRAIN MONOAMINE METABOLISM TO SECRETION OF GONADOTROPINS AND PROLACTIN

presented by

James William Simpkins

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

Joseph Meites
Major professor

Date 9/23/77

O-7639

RELATION OF BRAIN MONOAMINE METABOLISM TO SECRETION OF GONADOTROPINS AND PROLACTIN

By

James William Simpkins

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Physiology

1977

with

a sub

incre

by 6

were

6 and

hypot

after

6 and

dopam

after

concer

thalam

cluded

in AH-

of LH

ABSTRACT

RELATION OF BRAIN MONOAMINE METABOLISM TO SECRETION OF GONADOTROPINS AND PROLACTIN

By

James William Simpkins

1. Treatment of ovariectomized, estrogen-primed rats with 500 μg progesterone (P) per kg body weight resulted in a subsequent LH and prolactin surge. Serum LH concentration increased significantly by 4 h and was elevated dramatically by 6 and 9 after P administration. Serum prolactin levels were elevated by 2 h, peaked at 4 h and remained elevated at 6 and 9 h after P treatment. A 2-fold increase in anterior hypothalamic norepinephrine (NE) turnover was observed by 4 h after P treatment which returned to control (Pre-P) levels by 6 and 9 h after P administration. Anterior hypothalamic (AH) dopamine (DA) turnover decreased significantly by 6 and 9 h after P treatment. No significant difference in catecholamine concentration or turnover was observed in posterior hypothalamic fragments at any of the sampling times. It is concluded that the increase in AH-NE turnover and the decrease in AH-DA turnover may partially mediate the P induced surges of LH and prolactin.

tyros
in ov
the
decay
and
and
trea
at 4
crea
obse
resu
synt

bloc
estr
leve
in A
LH s
nece
to o

into
to o
hypo
Wher

2. The tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (αmpt), completely inhibited the P-induced LH surge in ovariectomized, estrogen-primed rats when administered at the time of P treatment. Alpha-mpt, when injected 1 h before decapitation, was able to block partially LH release at 4,6 and 9 h after P administration, but was ineffective at 2 h and increased LH secretion at 0 h after P treatment. This treatment resulted in elevated prolactin at 0 and 2 h but not at 4 and 6 h after P administration. Since NE turnover increases and DA turnover decreases after P treatment, these observations suggest that the αmpt blockade of NE synthesis results in blockade of the LH surge while its blockade of DA synthesis increases serum prolactin.

3. Sustained administration of the DA agonist, piribedil, blocked the P-induced prolactin increase in ovariectomized, estrogen-primed animals but was ineffective in altering peak levels of serum LH. These findings indicate that the decrease in AH-DA turnover is not essential for mediating the P-induced LH surge, but may indicate that a decreased DA turnover is necessary for mediating prolactin release by P administration to ovariectomized, estrogen-primed rats.

4. Small doses of 6-hydroxydopamine 6-OH-DA implanted into the suprachiasmatic nucleus (SCN) 24 h before P treatment to ovariectomized, estrogen-primed rats, depleted anterior hypothalamus NE by 83% and blocked the subsequent LH surge, whereas median eminence (ME) 6-OH-DA implants decreased ME-NE

by S

Neit

Impla

serum

It i

ses r

mode

this

leve

A 2-

and

alth

mic

at 4

impl

24 h

the

the

invo

the

of n

follo

or la

tonin

by 57%, but were ineffective in altering LH secretion. Neither treatment significantly altered DA concentration. Implants of 6-OH-DA into ME caused a slight decrease in serum LH concentration at the time of P treatment (0900 h). It is concluded that rostral hypothalamic noradrenergic synapses mediate the P-induced LH surge in this experimental model, whereas ME noradrenergic synapses are not involved in this process.

5. Orchidectomy in rats rapidly increased circulating levels of LH (6 h) which remained elevated through 48 h. A 2-fold increase in hypothalamic NE turnover occurred by 6 h and returned to sham castrate levels by 48 h post-castration although serum LH levels continued to be elevated. Hypothalamic DA turnover appeared to increase by 6 h and was elevated at 48 h post-castration. Medial basal hypothalamic (MBH) implantation of the neurotoxin, 6-hydroxydopamine (6-OH-DA), 24 h before castration was effective in partially inhibiting the post-castration LH increase. These studies indicate that the transient increase in NE turnover observed at 6 h may be involved in hastening the post-castration LH increase, although the long term response of LH to castration may be independent of noradrenergic input. The increased DA turnover which follows castration may be in part responsible for the decrease or lack of response of prolactin secretion to castration.

6. The steady state concentration of DA, NE and serotonin (5HT) was determined and turnover estimated in several

brai

rats

were

ing

thal

1 g

cle

cen

hyp

you

NE

in

pro

tio

min

tha

mal

was

les

may

old

SHI

imp

sec

turn

sio

brain regions of young (3-4 mo) and old (21 mo) male Wistar rats. In old male rats, MBH-DA concentration and turnover were significantly lower than in young males. In the remaining hypothalamus, DA concentration was slightly lower in old than young rats although DA turnover was not different in the 2 groups. DA concentration and turnover in olfactory tubercles were the same in both age groups. The steady state concentration of NE in the MBH and remaining hypothalamus and hypothalamic NE turnover were significantly lower in old than young male rats. Since DA inhibits prolactin secretion and NE stimulates LH and FSH secretion, age-related alterations in central CA's may account for the observed increase in serum prolactin and decrease in serum LH and FSH in old male rats.

In both brain and hypothalamus, steady-state concentrations of 5HT were the same in young and old rats, but by 30 min after monoamine oxidase inhibition with pargyline, hypothalamic but not brain 5HT increased more in old than in young male rats. 5-Hydroxyindoleacetic acid (5HIAA) concentration was 25% higher in brains of old than young males but decreased less in response to treatment with pargyline. These results may indicate a greater turnover of 5HT in the hypothalamus in old than young male rats and a deficiency in the clearance of 5HIAA from the brains of old male rats. Since 5HT has been implicated in stimulating prolactin and inhibiting LH and FSH secretion in rats, the apparent increase in hypothalamic 5HT turnover may be involved in elevation of prolactin and depression LH and FSH in old male rats.

Dedication

This thesis is dedicated to my wife, Janet, and my children, Christopher and Gretchen, for joining me in this venture. Their sacrifices en route to this thesis have been so much greater than mine.

ACKNOWLEDGEMENTS

The author wishes to thank the faculty, staff and students of the Department of Physiology, Michigan State University for their contribution to my education. My thanks is also extended to the following individuals who assisted in various aspects of this thesis work: Charles Hodson, Henry Huang, Warren Chen, Juan Advis, William Mallard, Greg Mueller and Claire Twothy. I also wish to express my deepest appreciation to Professor Joseph Meites for his unwavering dedication to my education. His knowledge of endocrinology and his zeal for research has made my tenure in the Neuroendocrine Research Laboratory a very pleasurable and productive experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

INTRODUCTION

The central nervous system (CNS) exerts a profound influence on the endocrine system through its regulation of pituitary gland function. The synthesis and secretion of anterior pituitary (AP) hormones is regulated by neurohormones produced in and released from neurosecretory cells of the hypothalamus. These neurons act as neuroendocrine transducers, receiving input from the autonomic nervous system and providing endocrine output through the release of neurohormones. The input to neurosecretory cells has been extensively studied using pharmacological agents which alter the metabolism and/or synaptic transmission of putative central neurotransmitters. These studies have been valuable in demonstrating a possible role for several putative neurotransmitters in the control of AP function, but have several limitations. First, all drugs have effects other than those for which they are administered. For catecholaminergic and serotonergic drugs, these effects can be exerted on other autonomic inputs affecting AP hormone secretion. Thus the prototype noradrenergic agonist, clonidine may effect both the central serotonergic and the histaminergic systems, and the serotonergic antagonist, methysergide, is a partial dopamine (DA) agonist.

The
(L-
mir
the
cha
str
sec
phy

of
det
neu
sec
neu
vid
inv
hor
ani
tio
Mur
to
dop
AP
AP
stat

The catecholamine precursor, L-dihydroxyphenylalanine (L-dopa), can be taken up and decarboxylated to DA in dopaminergic, noradrenergic, and serotonergic neurons. Second, the ability of a drug which alters CNS function to induce changes in AP hormone secretion does not, in itself, demonstrate a physiological involvement of that system in hormone secretion. Thus, pharmacologic effects do not always have physiological significance.

An alternative approach to the evaluation of the role of putative neurotransmitters in AP hormone secretion is to determine if changes in brain metabolism or transmission of neurotransmitters occur during states of altered AP hormone secretion. Correlations between the metabolism of central neurotransmitters and the rate of AP hormone secretion provide additional support (though not definite proof) for the involvement of a neurotransmitter in the regulation of hormone secretion. Initial attempts at measuring catecholamine (CA) turnover during states of altered hormone secretion have been made primarily in the laboratories of Fuxé, Wurtman, and Donoso. The work in this thesis was conducted to assess further the role of hypothalamic noradrenergic, dopamineragic and serotonergic systems in the regulation of AP function by simultaneously estimating their turnover and AP hormone secretory rates during a variety of experimental states.

Pharmacological studies indicate that drugs which modify central noradrenergic activity affect the rate of secretion of luteinizing hormone (LH), but there is controversy as to the role of central noradrenergic systems in controlling prolactin secretion. Similarly there is general agreement that the tuberoinfundibular dopamine (DA) system inhibits prolactin secretion, but there is no agreement as to the role of DA in the control of LH release. This thesis was in part devoted to an evaluation of alterations in both DA and NE turnover during conditions in which LH and prolactin secretory rates increase simultaneously, diverge in opposite directions, or respond selectively to the stimulus. Comparison of results among these three experimental models has provided further evidence on the role of both CA's in the secretion of LH and prolactin.

Endocrine glands are part of a homeostatic system, responding to a variety of enteroceptive and exteroceptive stimuli. The AP, like other endocrine glands exhibits both acute and chronic responses to changes in the environment. In general, previous studies attempting to relate CA metabolism with endocrine states have employed chronic stimuli. If the CNS is involved in mediating acute changes in AP hormone secretion, one should be able to demonstrate alteration in CA turnover which precedes or accompanies changes in hormone secretion. A part of this thesis is devoted to

determining the time course of the response of the central CA systems to acute endocrine alterations.

Rostral areas of the hypothalamus are believed to be involved in mediating gonadal steroid feedback which results in an increase in secretion of LH. This positive feedback of estrogen and progesterone occurs on the day of proestrus of the estrous cycle in rats, on the day preceding ovulation in human subjects, and prior to the onset of cyclic reproductive function in both animal models and human subjects. In rodents, disruption of rostral (but not caudal) hypothalamic nuclei can block the surge in serum LH on proestrus and LH surges normally induced by administration of gonadal steroids. It would appear then that afferents terminating in the rostral hypothalamus or passing through this area en route to the medial basal hypothalamus (MBH) mediate the steroid induced release of LH. Part of this thesis is devoted to determining whether the rostral hypothalamus functions is a center for integrating afferent information or simply is a hypothalamic area through which nerve tracts pass to reach the MBH.

Finally, senescence in rodents and humans results in a variety of endocrine deficiencies. In rodents, gonadal and thyroid functions decrease as an apparent result of a decreased ability of the AP to secrete hormones in response to appropriate stimuli. Cyclicity in female and fertility in

male rats decreases with age. Old animals are less responsive to several types of stimuli which release LH and follicle stimulating hormone (FSH) and are more responsive to some stimuli which release prolactin. Similarly, old rats are less responsive to stimuli which normally release TSH. Thus exposure to low ambient temperature releases less TSH in old than in young male rats. To assess the possible involvement of CA's in the decreased responsiveness of the AP to physiological stimuli, basal serum concentrations of AP hormones and concentrations and turnover of central biogenic amines are compared in young and old male rats.

A.

cra
bec
cla
ana
be
a p
sto
the
of
lif
and
pit
gro
of
ini
Eva
(Al
193
Smi

LITERATURE REVIEW

I. Hypothalamic Control of Anterior Pituitary Function

A. Classical Observations

The existence of the pituitary located deep within the cranium has been known for some 2,000 years. The pituitary, because of its central location in the brain case, has been claimed to have many supernatural functions by ancient anatomists (Harris, 1972). This structure was considered to be part of the brain until 1838 when Rathke demonstrated that a portion (anterior pituitary) developed from a non-neural stomite. The pioneering work of Cushing and colleagues in the first decade of this century demonstrated the importance of the anterior pituitary gland in the maintenance of normal life and in the genesis of several diseases (Crowe, Cushing and Homans, 1910; Cushing, 1909). The discovery that the pituitary gland contained substances which stimulate body growth (Evans and Long, 1921, 1922), growth and maturation of the ovaries (Smith, 1926a; Zondek and Ashheim, 1926), initiation of milk production (Stricker and Grueter, 1928; Evans and Simpson, 1929), restoration of atrophic thyroids (Allen, 1919; Smith and Smith, 1922; Anderson and Collip, 1932) and restoration of atrophic adrenals (Allen, 1922; Smith, 1926b) left no doubt that the anterior pituitary gland

secretes hormones which exert dramatic effects on physiological processes.

Early indication for a role of the CNS in regulation of AP function came from diencephalon lesions and subsequent atrophy of endocrine glands. Ascher (1912) reported that anterior hypothalamic lesions caused gonadal atrophy in dogs. These observations were later extended to other species (Camus and Roussy, 1920; Dey, 1943). Hypothalamic lesions were subsequently shown to block stress-induced adrenal hypertrophy (Ganong and Hume, 1954) and produce thyroid atrophy (Cahane and Cahane, 1936). Electrical stimulation of the hypothalamus has been shown to induce ovulation in rabbits (Haterius, 1937; Harris, 1948a), and increase thyroid (Harris and Woods, 1958) and adrenal activity (deGroot and Harris, 1950). These effects were not observed with electrical stimulation of the AP (Markee et al., 1946) indicating that the hypothalamus exerts a profound influence on hormone secretion from the AP.

Additional evidence for the influence of the hypothalamus on the normal adenohypophysial function were obtained by interruption of the hypothalamo-hypophysial connection. Dott (1923) and Mahoney and Sheehan (1936) first observed that pituitary stalk transection caused atrophy of both gonads and thyroid glands. Stalk transection was later shown to interfere with normal adrenal responses (Fortier et al., 1957;

Lazolo and DeWied, 1966). Transplantation of the in situ pituitary to the anterior chamber of the eye or under the renal capsule has been shown to cause a variety of metabolic changes including atrophy of the gonads, adrenals and thyroid glands (Harris, 1948, 1955). However, ectopic pituitaries were able to maintain corpora lutea (Everett, 1954, 1956) and mammary gland (Meites, 1967) function due to the increase in PRL release from ectopic pituitaries (Meites et al., 1961; Pasteel, 1961). These early experiments demonstrated both a stimulatory and an inhibitory influence of the hypothalamus on release of AP hormones.

B. Portal Vessels and Neurosecretion

Popa and Fielding (1930) first observed that the blood vessels supplying the anterior pituitary gland were part of a portal system connecting the sinusoids of the AP with a capillary plexus in the median eminence. They proposed blood flow from the pituitary to the hypothalamus. Houssay et al. (1935) followed the direction of blood flow in this portal system of toads and correctly proposed a hypothalamus to pituitary blood flow. One year later, Wislocki and King (1936) observed the movement of dyes from the hypothalamus to the pituitary after a systemic injection, providing strong evidence that the anterior pituitary received blood after its circulation through the medial basal hypothalamus.

The capillary plexus of the superior hypophyseal artery appears to give rise to the long portal vessel which travels along the lateral and anterior aspects of the infundibulum (Netter, 1965). In mammals, 70-90% of the blood supplied to the anterior pituitary is via these long portal vessels (Adams et al., 1963; Porter et al., 1967). In most species, the remaining blood supply to the AP is believed to come from a plexus at the distal end of the infundibulum and travel via short vessels, deep within the infundibulum to the pituitary (Netter, 1965). In mammals, except the rabbit, 100% of the blood supply appears to reach the anterior pituitary via the hypophysial portal system (Harris, 1947; Goldman and Sapirstein, 1962). Thus, anatomical evidence for a link between the nervous and endocrine systems is well documented.

The possibility that nervous tissue was capable of secreting endocrine active substances into the hypophysial portal system (Haterius, 1937; Hensey, 1937) or into the general circulation (Scharrer and Scharrer, 1940) was proposed in the late 1930's and early 1940's. Within the next decade, neurosecretion had been demonstrated and the pathways for oxytocin and vasopressin synthesis, transport and secretion elucidated (Bargman and Scharrer, 1951; Scharrer, 1952; Scharrer and Scharrer, 1954). This pioneering work demonstrated that the paraventricular and supraoptic nuclei of the hypothalamus contained cell bodies of nerves which synthesized

these two hormones. Axons from these nuclei traveled ventrocaudally to the nervous tissue of the pars nervalis (posterior pituitary) where oxytocin and vasopressin are stored prior to release into the circulatory system.

The "chemotransmitter hypothesis" was proposed and popularized by G. H. Harris (1948b) based upon the anatomical relationship between the hypothalamic, hypophysial portal circulation, and the anterior pituitary and the previously proposed concept of neurosecretion. Harris proposed that nervous activity in the hypothalamus induced the release of humors from neurosecretory cells into the intercellular space which bathe the capillary plexus of the median eminence. These humors were proposed to be transported to the anterior pituitary gland via the hypophyseal portal vessels where they were proposed to act on AP cells to alter the release of hormones. This hypothesis has withstood 35 years of experimental testing and today is a basic premise of the science of neuroendocrinology.

C. Hypophysiotropic Hormones

The first demonstration of a hypothalamic factor capable of altering the release of an anterior pituitary hormone was provided by Saffran and Schally (1955) and Guillemin and Rosenberg (1955). Using a hypothalamus-pituitary coincubation system, the former demonstrated that the addition of norepinephrine to the media increased the release of

adrenocorticotropin (ACTH), an effect which was not observed in the absence of hypothalamic tissue in the media. Subsequent to these observations several groups demonstrated the existence of hypothalamic factors capable of altering the release of AP hormones in vitro. In the late 1950's and early 1960's hypothalamic releasing activity was demonstrated for TSH (Shibusawa et al., 1956), LH (McCann et al., 1960), PRL (Meites et al., 1960), FSH (Igarshi and McCann, 1964; Mittler and Meites, 1964) and GH (Deuben and Meites, 1964). Hypothalamic release, inhibiting activity has been demonstrated for PRL (Talwalker et al., 1961, 1963; Pasteels, 1961) and GH (Krulich et al., 1968).

Attempts at purification and identification of hypothalamic releasing and release inhibiting hormones have been only partially successful. Corticotropin-releasing factor, the first hypothalamic releasing factor, is yet to be identified although pepsin sensitive CRF material has been demonstrated and partially purified (Royce and Sayers, 1958) and a proposed 13-amino acid peptide has been published (Schally and Bowers, 1964). Likewise proposed structures for GRF (Schally et al., 1973) and PIF (Schally et al., 1976) have been reported, but as yet unconfirmed.

Thyrotropin-releasing hormone was the first hypothalamic releasing factor purified and synthesized (Burger et al., 1969; Folkers et al., 1969; Boler et al., 1969). This

tripeptide is identical in all species tested, has been shown to release TSH from all species tested, and has been identified in hypophysial portal blood. Thus, TRH appears to be a true hypothalamic hormone. Although releasing factors were originally believed to be specific for one anterior pituitary hormone (and so named), recent studies have shown that TRH releases PRL as well as TSH (Jacobs et al., 1971; Muller et al., 1973; Convey et al., 1973).

Luteinizing hormone releasing hormone (LHRH) was isolated, its amino acid sequence determined, and synthesized in the laboratories of Andrew Schally (Matsuo et al., 1971a; Matsuo, 1971b). This decapeptide has been shown to be effective in releasing LH and FSH (Schally et al., 1971), leading to the common use of the term "gonadotropin releasing hormone" (GnRH) and follicle stimulating hormone releasing hormone (FSH-RH). Since the synthesis of the native LHRH, about 75 analogues have been synthesized (Saffran, 1974), most of which decrease its LH releasing activity.

Growth hormone-release inhibiting hormone (GIF or somatostatin) was first isolated from ovine hypothalami, identified and synthesized by Guillemin's group (Brazeau et al., 1973). Although somatostatin is effective in inhibiting GH release, like the other identified hypothalamic hormones, it is not specific for GH. Somatostatin inhibits TRH induced TSH and prolactin release (Vale et al., 1974; Chen

and Meites, 1975). In addition, somatostatin has been localized in the alimentary tract (Mortimer et al., 1974) and pancreas (Alberti et al., 1973) and may be important in the regulation of both insulin and glucagon release (Gerich et al., 1974). Somatostatin administration has also been shown to exert an antidiabetic effect in both rats and primates (Mortimer et al., 1974).

D. Hypothalamic Anatomy

The hypothalamus is an anatomically diverse structure containing clusters of cell bodies intermixed with a variety of afferent and efferent pathways (Jenkins, 1972). The hypothalamus extends rostrally to the sulcus limitans and caudally to the mammillary bodies at the dorsal border. The hypothalamic sulci separate the hypothalamus from the thalmus. The ventral border is free from nervous connections, except for the extension of the supraopticohypophysial tract to the neurohypophysis. The lateral borders of the hypothalamus are formed in part by the internal capsule. In rats, this area of the diencephalon constitutes about 1/100th of the weight of the whole brain.

In general, cell bodies distribute themselves in three major gray regions in the hypothalamus. The anterior and intermediate gray areas of the hypothalamus appear to be essential for central mediation of anterior pituitary hormone release; whereas, the posterior gray area appears to be

relatively unimportant in this function. Except for the arcuate nucleus and median eminence, hypothalamic nuclei are located bilaterally on either side of the third ventricle. The anterior hypothalamic area includes the preoptic area (POA) located rostral to the optic chiasm; the suprachiasmatic nucleus (SCN) which lays immediately dorasl to the optic chiasm; the anterior hypothalamic nucleus (AHN) which is located dorsolateral to the SCN; the paraventricular nucleus (PVN) which extends rostrally from the POA and dorsocaudally along the wall of the third ventricle; and a rather diffuse supraoptic nucleus (SON). The integrity of the POA-AHN-SCN complex is vital for the cyclic release of luteinizing hormone in rodents (Halasz and Pupp, 1965; Gorski, 1966) but may be less important in premates (Krey et al., 1975).

The intermediate group of nuclei include the lateral hypothalamic nucleus (LHN), the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN), the arcuate nucleus (AN), and the median eminence (ME). In addition to their well documented role in food intake (Mayer, 1953; Mayer and Arees, 1968) the LHN and VMN have been reported to influence the secretion of growth hormone (Martin et al., 1975). The arcuate nucleus is located in the mediobasal region of the tuber cinereum and is continuous with the median eminence. This nucleus has been shown to contain cell bodies of the tuberoinfundibular DA system which extends to the external

layers of the median eminence and nerve terminals containing both norepinephrine and serotonin (Fuxé and Hökfelt, 1966; Ungersted, 1971). In addition to neurons of the arcuate nucleus, specialized ependymal cells at the base of the third ventricle extend through the arcuate nucleus to form foot pads on the vessels of the hypophyseal plexes (Bleier, 1971; Mitchell, 1975). These tanocytes have been proposed to be an alternative route for cerebrospinal fluid born amines and releasing hormones to reach the anterior pituitary gland (Zimmerman et al., 1974).

The very location of the median eminence at the neuro-vascular junction suggests its importance in the control of anterior pituitary physiology. The densely packed nerve terminals of the median eminence surround the capillary plexus of the hypophyseal portal system (Harris, 1948) and contain high concentrations of DA, LHRH, TRH, and somatostatin (Brownstein et al., 1975).

The hypothalamus receives afferents primarily from two brain regions: the brainstem reticular formation and the limbic system (Nauta and Haymaker, 1969). From the brain stem, afferents reach the hypothalamus via the mammillary peduncle to terminate in the mammillary bodies and lateral hypothalamus; the dorsal longitudinal fasiculus which extends from the para-aquaductal gray of the midbrain to the posterior-hypothalamic area; and the medial forebrain bundle which

also originates in the para-aquaductal gray area of the brainstem.

Afferents from the limbic system to the hypothalamus include the fornix, the medial forebrain bundle and the stria terminalis. The fornix follows a course from the hippocampus cranially running ventral to the corpus callosum. At the level of the anterior commissure the fornix divides into two columns and extends caudally to the mammillary bodies of the hypothalamus. The stria terminalis originates in the amygdala and terminates in the anterior gray of the hypothalamus and perhaps other areas (Nauta, 1958). The descending portion of the medial forebrain bundle originates in the olfactory tubercle and septal regions and descends to the preopticohypothalamic region and medial hypothalamus.

Although much evidence has been presented for the above mentioned afferents to the hypothalamus, other pathways to the hypothalamus have been proposed and some evidence presented. The inferior thalamic peduncle appears to extend from the medial thalamic region to the preoptic nucleus (Ingram, 1940; Nauta, 1962). In addition, evidence for afferents reaching from the globus pallidus, the cortex (Ward and McCullock, 1947), and the retina (Riss et al., 1963) has been reported.

A.

inv

cam

reg

his

of

in

196

con

les

in

(Un

adr

(Lo

ent

fro

com

mes

197

inn

Of

obs

II. Monoaminergic Pathways in the Hypothalamus

A. Noradrenergic Pathways

The first evidence that norepinephrine (NE) might be involved as a neurotransmitter in the central nervous system came from the biochemical observation of NE in various brain regions (Amin et al., 1954; Vogt, 1954). The Falck-Hillarp histofluorescence technique provided the first demonstration of the cellular localization of NE (Carlsson et al., 1962) in nerve terminals and cell bodies (Dahlstrom and Fuxé, 1964, 1965; Fuxé, 1965a, 1965b). Because of the relatively low concentration of monoamines in axons, physical or chemical lesions of pathways is required to concentrate monoamines in axons to the extent needed for histofluorescence detection (Ungerstedt, 1971). These techniques demonstrated that noradrenergic cell bodies were located in the locus ceruleus (Loizou, 1969) and midbrain reticular areas, with axons entering the ascending medial forebrain bundle. Projections from the medial forebrain bundle provide the noradrenergic component to the cerebellum, cerebrum, lower brain stem, mesencephalon and diencephalon (Loizou, 1969; Ungerstedt, 1971). The ventral portion of the medial forebrain bundle innervates the entire hypothalamus (Ungerstedt, 1971). Of particular interest to the control of AP function is the observation of noradrenergic projections from the medial

forebrain bundle to the anterior hypothalamic nuclei (POA, SON, and PVN) and the intermedial nuclei (AN and internal layers of the median eminence).

The recent development of a micropunch technique (Palkovits, 1973) and sensitive radioenzymatic assays for monoamines (Coyle and Henry, 1973; Cuello et al., 1973; Moore and Phillipson, 1975; Ben-Jonathan and Porter, 1976) has permitted a systematic evaluation of the monoamine content of discrete hypothalamic areas. Although NE is rather evenly distributed in the hypothalamus, highest concentrations are found in the anterior hypothalamic nuclei and the arcuate nucleus (Palkovits et al., 1974). In general, NE concentration in hypothalamic nuclei correlates well with the activity of dopamine-β-hydroxylase, the enzyme that hydroxylates DA to NE (Saavedra et al., 1974a).

Several lines of evidence indicate that the noradrenergic component of the hypothalamus originates from cell bodies located in extra-hypothalamic brain areas. Histofluorescence studies have been unable to detect any noradrenergic cell bodies in the hypothalamus (Understedt, 1971). Lesions in the midbrain tegmentum (Anden et al., 1966a), locus ceruleus (Loizou, 1969) and medial forebrain bundle (Kabayashi et al., 1974) result in a varying decrease in NE concentration in the hypothalamus. Hypothalamic deafferentation results in a total loss of dopamine-β-hydroxlyase activity from several hypothalamic nuclei (Brownstein

et al., 1976) and a decrease in NE content. These studies suggest that the noradrenergic terminals in the hypothalamus originate from other brain areas. However, all noradrenergic input to the hypothalamus may not come from cell bodies in the locus ceruleus since lesions in this area do not completely eliminate NE in the hypothalamus (Forzon, 1969; Ungerstedt, 1971). Some of the NE remaining in the hypothalamus following midbrain or medial forebrain bundle lesions may be present in neuroglial cells (Iversen, 1974) and thus is unresponsive to removal of noradrenergic input.

B. Dopaminergic Pathways

There is an abundance of evidence for three distance DA systems in the brains of laboratory animals. The nigrostriatal DA system appears to have cell bodies in the A9 cell group of the zona compacta of the midbrain tegmentum and extends rostrally to provide DA terminals to the globus pallidus, the caudate nucleus and the putamen (Anden et al., 1964, 1965, 1966c; Hökfelt and Ungerstedt, 1969). This dopaminergic system appears to be involved in the normal control of extra-pyramidal upper motor neurons, and a deficiency in this system has been demonstrated in Parkinson's disease (Hornykiewicz, 1963). A second dopaminergic system appears to originate just dorsal to the interpeduncular nucleus of the midbrain (A10 cell group) and pass rostrally to innervate the olfactory tubercles and the nucleus accumbens (Anden et al., 1966b; Ungerstedt, 1971). Neither of these two

dopamine systems appear to send branches to the hypothalamus as indicated by the inability of hypothalamic deafferentation to decrease DA content (Weiner et al., 1972a).

The tuberoinfundibular DA system appears to originate from a tightly packed band of cell bodies in the arcuate nucleus and the lateral periventricular nucleus (Fuxé, 1963; Fuxé and Hökfelt, 1966). Axons from these A12 cell bodies pass ventral to terminate in the external layer of the median eminence. This system appears to be the only dopaminergic input to the median eminence.

Dopaminergic cell bodies have been observed in the suprachiasmatic nucleus (Fidbrink et al., 1974). Further, Bjorklund et al. (1975) has presented evidence for a second hypothalamic DA system with cell bodies in the rostral periventricular mucleus (A14 cell group) and axons extending to the medial preoptic area and to the suprachiasmatic nucleus. Palkovits et al. (1976) have presented evidence for decreased DA content in the medial basal hypothalamus and the presence of degenerative hypothalamic axons after lesions placed in the substancia nigra. However, this study has been questioned since total deafferentation of the hypothalamus does not decrease hypothalamic DA content (Weiner et al., 1972a). The bulk of evidence indicates that DA in the hypothalamus originates from intrahypothalamic systems.

C. Serotonergic Pathways

The relatively weak fluorescence of 5HT produced by histofluorometric techniques have made it more difficult to determine the distribution of serotonin than catecholamines in mammalian brains. Lesions and subsequent biochemical analysis have been used to partially map central serotonergic pathways. Yellow fluorescent axons have been shown to emerge from a narrow band of cells in the mid portion of the lower pons and upper medulla, the raphe nuclei (Dahlstrom and Fuxé, 1964; Ungerstedt, 1971). These axons ascend as part of the medial forebrain bundle and appear to provide serotonergic nerve terminals to most if not all of the brain (Kuhar et al., 1972). Lesions of the medial and/or dorsal raphe nuclei and medial forebrain bundle have been shown to decrease 5HT concentration and tryptophan hydroxylase activity in forebrain areas (Kuhar et al., 1972; Ungerstadt, 1971; Herr and Rothe, 1972) including areas of the hypothalamus (Saavedra et al., 1974b). Electrical stimulation of the raphe nuclei increases serotonin turnover in the forebrain (Sheard and Aghajanian, 1968) and the hypothalamus (Advis, Simpkins, Bennett and Meites, unpublished).

In the hypothalamus, serotonin can consistently be visualized in the suprachiasmatic nucleus (SCN) by histofluorescence techniques. This is consistent with the observation that enzymatically determined 5HT and tryptophan

hyd

Ser

con

and

rem

ned

liv

(Mc

sin

(Iv

phe

cho

19

sui

en

app

19

tic

hyd

hyd

et

Spe

hydroxylase are high in this nucleus (Saavedra et al., 1974b). Serotonin and tryptophan hydroxylase appear also to be highly concentrated in the periventricular nucleus, arcuate nucleus and median eminence, and rather evenly distributed in the remaining hypothalamic nuclei.

III. Catecholamine and Serotonin Metabolism

The biosynthesis of catecholamines and serotonin is mediated by enzymes produced in nerve cell bodies and delivered to presynaptic nerve terminals by axonal transport (McClure, 1972; Ochs, 1972). The neutral amino acid L-tyrosine is actively pumped into catecholaminergic neurons (Iversen, 1971) and hydroxylated to the catechol, dihydroxyphenylalanine (DOPA) by the rate limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (Levitt et al., 1965, 1967). Since brain concentrations of tyrosine are sufficiently high under most conditions to saturate this enzyme, changes in circulating levels of tyrosine do not appear to influence catecholamine synthesis (Nagatsu et al., 1964; Copper et al., 1971). Rather intraneuronal concentration of dopamine exerts an inhibitory influence on tyrosine hydroxylase activity by competing with the cofactor, tetrahydropteridine, for binding to this soluble enzyme (Udenfriend et al., 1965; Costa and Neff, 1966; Alousi and Weiner, 1966; Spector et al., 1969). Thus, activation of catecholaminergic

neurons, which decreases intracellular dopamine concentration, activates tyrosine hydroxylase; whereas, reduced activity in catecholaminergic neurons increase dopamine stores and depresses tyrosine hydroxylase activity.

The decarboxylation of DOPA to dopamine is catalyzed by the enzyme L-aromatic amino acid decarboxylase, an enzyme which is common not only to catecholaminergic and serotonergic neurons but to other tissues which metabolize neutral amino acids (Goldstein et al., 1974). In the mammalian brain and in tissue receiving sympathetic innervation, DOPA concentrations are extremely low, a result of the high activity of this soluble enzyme (Km = 4 x 10^{-4}M; Goldstein et al., 1974).

In adrenergic neurons, dopamine-β-hydroxylase (DβH), which hydroxylates dopamine to norepinephrine is present (Kaufman, 1966; Fuxé et al., 1971). This enzyme appears to be localized on membranes of storage granules (Thomas et al., 1973) and has a high copper content (Friedman and Kaufman, 1965; Blumberg et al., 1965), a characteristic which makes it sensitive to chelating agents (Goldstein, 1966; Sulser and Saunders-Bush, 1971). DβH has been used as a specific, sensitive marker for noradrenergic neurons (Geffen et al., 1969; Hartman and Udenfriend, 1970; Hartman, 1973).

Phenylethanolamine-N-methyltransferase (PNMT), a soluble enzyme which catalyzes the N-methylation of

norepinephrine to epinephrine is found in high concentrations in the adrenal medulla (Axelrod, 1962) and in rather low concentrations in the mammalian brain (Axelrod, 1962; McGeer and McGeer, 1964). The presence of this enzyme in low concentrations in the brain suggest a role for epinephrine in central nervous system physiology which, however, has as yet not been elucidated.

Biological inactivation of catecholamines can occur either by enzymation degradation of the neurotransmitter to an inactive form or by the "recapture" of the catecholamine in storage granules of presynaptic nerve terminals. Enzymatic breakdown of catecholamines occurs via the action of monoamine oxidase (MAO) and/or catecholamine-0-methyl-transferase (COMT). MAO deaminates dopamine and norephinephrine to their respective aldehydes, 3,4-dehydroxyphenylactaldehyde and 3,4-dehydroxyphenyl-glycolaldehyde. COMT catalyzes the 0-methylation of dopamine to 3-methoxytyramine and norepinephrine to normetanephrine (Axelrod and Tomchik, 1958; Axelrod et al., 1959). Although high concentrations of both of these enzymes have been observed in mammalian brain, their role in inactivating catecholamines released into the synaptic cleft appears to be very minor, as indicated by the observation that treatment with either MAO or COMT inhibitors does not greatly potentiate the effect of

sympathetic stimulation (Pletscher, 1973).

A major means of inactivation of catecholamines appears to be through an active reuptake into presynaptic vessels for both norepinephrine (Glowinski et al., 1965; Glowinski and Axelrod, 1966) and dopamine (Coyle and Snyder, 1969; Horn et al., 1971). It appears that a large proportion (50-90%) of NE released into the synaptic cleft can be inactivated by this method (Langer, 1970; Haggendal, 1970). The apparent physiological importance of reuptake in terminating catecholamine action is indicated by the observation that inhibition of reuptake by cocaine, phenoxybenzamine, or imipramine caused a marked potentiation of the effects of adrenergic stimulation (Iversen, 1972b).

Considerable evidence indicates that both the secretion and turnover of neurotransmitters are closely coupled to electrical activity in catecholamine neurons. Electrical stimulation of peripheral adrenergic neurons results in increased tyrosine hydroxylase activity (Sedvall et al., 1968; Dairman et al., 1968) and norepinephrine turnover (Alousi and Weiner, 1966; Gordon et al., 1966; Roth et al., 1966; Sedvall and Kopin, 1967; Sedvall et al., 1968; Weiner and Rabadjuja, 1968). Similarly electrical stimulation of the locus ceruleus or its projections results in an increase in norepinephrine turnover in the cerebral cortex and hippocampus (Arbuthnott et al., 1970; Korf et al., 1973a) whereas

lesions of the locus ceruleus decrease norepinephrine turnover in the cerebral cortex (Korf et al., 1973b). Stimulation of the medulla oblongata increases norepinephrine turnover in the spinal cord (Dahlstrom et al., 1965); whereas, spinal transection decreases norepinephrine turnover caudal to the lesion (Anden et al., 1966d, 1969).

A similar relationship between nervous activity and turnover appears to exist in both dopaminergic and serotonergic neurons. Stimulation of the zona compacta of the substancia nigra results in an increase in dopamine turnover in the striatum (VonVoigtlander and Moore, 1971), an effect which appears to be frequency dependent (Roth et al., 1973). Stimulation of the raphe nuclei results in a frequency-dependent increase in serotonin synthesis (Shields and Eccleston, 1973) and accumulation of 5-hydroxyindole acetic acid in the forebrain (Sheard and Aghajanian, 1968). Raphé nucleus lesion decreases forebrain serotonin turnover (Herr and Roth, 1972).

Each of the methods available for estimating monoamine turnover has advantages and disadvantages as indicated by the critical review of Costa (1970). Estimation of catecholamine turnover by measuring the depletion of norepinephrine specific activity after intraventricular infusion of labeled norepinephrine was introduced by Glowinski's group (Mith and Glowinski, 1963; Glowinski et al., 1965). In this method

nore

a re

(Whi

1963

ity

adre

depl

8 h

(Glo

nore

from

synt

Glow

pitf

both

1969

activ

Secon

norep

of ti

requi

metho

over

labele

norepinephrine is transported into noradrenergic neurons by a reuptake mechanism and mixes with endogenous norepinephrine (Whitby et al., 1961; Hertting and Axelrod, 1961; Iversen, 1963). The rate of decrease in specific norepinephrine activity is presumed to reflect its rate of replacement in noradrenergic neurons by newly synthesized norepinephrine. The depletion of specific norepinephrine activity over the first 8 h after injection exhibits an exponential decrease (Glowinski et al., 1965) and yields values for whole brain norepinephrine turnover which are not significantly different from those obtained using the radiolabeled precursors or the synthesis inhibition method to estimate turnover (Iversen and Glowinski, 1966). However, this method has several inherent pitfalls. First, since norepinephrine can be taken up into both dopaminergic and serotonergic neurons (Green et al., 1969) the depletion of labeled norepinephrine may reflect activity in these neurons as well as in noradrenergic neurons. Second, because of the low specific activity of available norepinephrine, turnover can be measured in only large pieces of tissue. And finally, since long periods of time (8 h) are required for the radiolabeled norepinephrine depletion, this method is unacceptable for estimation of norepinephrine turnover in response to acute stimuli.

The measurement of the acute rate of appearance of labeled catecholamines following systemic injection of

catecholamine precursors (Zigmond and Wurtman, 1970; Zschaeck and Wurtman, 1972) has several advantages over the previous technique. The trace doses of labeled precursors used can be easily administered and do not appear to influence endogenous amine concentrations (Zigmond and Wurtman, 1970; Hyyppa et al., 1973). Further, the rate of production of catecholamines can be determined over a very short time interval (less than 10 min.), making this technique acceptable for acute studies. Since this technique measures acutely the accumulation of labeled catecholamines, synthesis rather than turnover is measured. The major disadvantages of this technique are that catecholamine precursors can be taken up by non-catecholaminergic cells. Thus, one can not be certain where the conversion of precursors to dopamine and norepinephrine is occurring. This technique also requires the use of larger pieces of tissue and can not be employed for measuring catecholamine synthesis in very localized areas of the brain.

Two methods have been introduced utilizing synthesis inhibitors to estimate catecholamine turnover. One method used extensively by Carlsson's group (Carlsson et al., 1972a, 1972b) utilizes the inhibition of aromatic-L-amino acid decarboxylase (AAAD) to measure the rate of accumulation of L-dopa. This method has the advantage of allowing acute measurement of catecholamine (30 min.) turnover and the

simultaneous measurement of both catecholamine and serotonin metabolism since AAAD blocks both systems. However, since DOPA is an intermediate in both dopamine and norepinephrine biosynthesis, one cannot distinguish between the two systems. Further, the relative lack of sensitivity of assays for dopa require the use of large pieces of tissue (Kehr et al., 1972).

A final method for measuring catecholamine turnover utilizes the rate of dopamine and norepinephrine depletion following α-methyl-para-tyrosine (αmpt) induced inhibition of tyrosine hydroxylase (Brodie et al., 1966; Costa and Neff, 1966). Catecholamine depletion following αmpt treatment is exponential (Brodie et al., 1966) and yields estimates of turnover rates consistent with those obtained using other techniques (Carlsson et al., 1972a; Costa, 1970). This method has the advantages of convenience and applicability to acute studies. Further, the recent development of sensitive radio-enzymatic assays for both dopamine and norepinephrine (Coyle and Henry, 1973; Cuello et al., 1973; Moore and Phillipson, 1975; Ben Johnathen and Porter, 1976) allows its use in measuring catecholamine turnover in small pieces of brain tissue. However, this method has several drawbacks. First, the intraperitoneal administration of αmpt does not cause an instantaneous inhibition of catecholamine synthesis (Westfall and Osada, 1969) and thus the rate of catecholamine depletion may vary with time after αmpt administration. In addition,

αmpt may have an effect on catecholaminergic neurons other than just tyrosine hydroxylase inhibition which could affect the estimation of catecholamine turnover. Even with these shortcomings, this appears to be the best method available for measuring catecholamine turnover small brain tissue and is the method employed in the research in this thesis.

IV. Hypothalamic Control of Prolactin Secretion

A. Prolactin Levels and Physiological States

A role for prolactin has been demonstrated or implicated in no less than 82 different physiological phenomena in vertebrates (Nicoll and Burn, 1972). In mammals, prolactin has been clearly demonstrated to stimulate mammary gland growth (Talwalker and Meites, 1961: Meites, 1965), initiate and maintain lactation (Stricker and Grueter, 1928; Turner and Gardner, 1931), and mammary tumorogenesis and growth (Liebelt and Liebelt, 1961; Boot *et al.*, 1962). In rats prolactin and LH appear to be luteotrophic (Gospodarowicz and Legault-Demare, 1963). In males, prolactin has been shown to stimulate prostate and seminal vesicle growth (Antliff *et al.*, 1960).

The influence of prolactin on the physiological events enumerated above appears to be mediated in part by increased secretion of prolactin from the anterior pituitary gland. In both male and female rats and in humans, serum levels of

prolactin increase during prepubertal development (Meites and Turner, 1948; Minaguchi et al., 1968; Voogt et al., 1970; Ojeda and McCann, 1975; Dohler and Wuttke, 1975), and seminal vesicle-prostate growth in males. Further, the suckling stimulus, which is necessary to maintain milk production, is a powerful stimulator of prolactin secretion (Sar and Meites, 1969; Amenomori et al., 1970; Reece and Turner, 1937; Grosvenor and Turner, 1958). Increased prolactin release from the anterior pituitary gland occurs in response to increasing serum titers of estrogen during the estrous cycle in rats (Clark and Meites, unpublished; Neill et al., 1971; Chen and Meites, 1970) and appears to be involved in corpora lutea formation and maintenance in rodents (Meites, 1968). Diurnal and nocturnal surges of prolactin release occur during pseudopregnancy and pregnancy in the female rat (Neill, 1975) and may be important to the maintenance of luteal function and progesterone secretion (Everett, 1954).

B. Inhibitory Influence of the Hypothalamus on Prolactin Secretion

The mammalian hypothalamus normally exerts an inhibitory influence on synthesis and release of prolactin from the anterior pituitary gland. Everett (1954) first observed that ectopic relocation of the anterior pituitary resulted in long-term maintenance of functional corpora lutea and

pseudopregnancy. Implantation of 1, 2 or 4 APs underneath the kidney capsule increased circulating prolactin proportional to the number of APs present (Chen et al., 1970). Lesions in the median eminence, which disrupt the hypothalamo-hypophyseal connections initiate lactation in the rabbit (Haun and Sawyer, 1960) and rat (DeVoe et al., 1966), to induce pseudopregnancy in rats (Flerko and Bordos, 1959) and to cause a greater than 10-fold increase in circulating prolactin levels (Welsch et al., 1971). Talwalker et al. (1961) and Pasteels (1961) first observed prolactin-release-inhibiting activity in hypothalamic extracts from rats. Subsequently, these observations were extended to other species (Talwalker et al., 1963; Schally et al., 1965). These acid extracts of the hypothalamus were effective in inhibiting prolactin release both in vivo and in vitro in all species tested. Kamberi et al. (1971a) demonstrated that hypothalamic extracts infused into portal vessels suppressed serum prolactin in a dose-responsive manner. Cerebrocortical extracts had no such effect.

C. Stimulatory Influence of the Hypothalamus on Prolactin Secretion

The mammalian hypothalamus appears to contain a prolactin-releasing factor (PRF) as well as PIF. Injection of acid extracts of rat hypothalamus into estrogen-primed rats initiates milk secretion (Meites et al., 1960; Minhkinsky et al.,

1968). Nicoll *et al*. (1970) later provided evidence for a hypothalamic PRF *in vitro*. Incubation of neutralized hypothalamic extracts with anterior pituitaries appear first to inhibit and later to stimulate prolactin secretion. Methanol extracts of both rat and porcine stalk-median eminence stimulate prolactin release in a dose-response manner (Valverde *et al*., 1972). The chromatographically separated PRF fraction of the methanol extraction was reported to be free of TRH, oxytocin, and vasopressin activity. Recently, Mitnick *et al*. (1973) reported PRF synthesis and release by rat hypothalami incubated *in vitro*.

In avian species, the hypothalamus appears to stimulate rather than to inhibit prolactin secretion. *In vitro* incubation of pigeon pituitaries decreases (Nicoll and Meites, 1962) and addition of hypothalamic extracts increased release of prolactin (Kragt and Meites, 1965). Subsequently, PRF activity was found in several avian species (Meites, 1967; Nicoll, 1965; Gourdji and Tixier-Vidal, 1966, and Chen *et al*., 1968).

The possibility that thyrotropin-releasing hormone (TRH) is the active component in hypothalami responsible for PRF activity is based upon the observation that TRH stimulates release of prolactin in the rat, cow and human (Mueller *et al*., 1973; Vale *et al*., 1973; Convey *et al*., 1973; Snyder *et al*., 1973; Noel *et al*., 1974). This TRH-induced release

of prolactin appears to be through a direct action upon the pituitary rather than via a hypothalamic mechanism since the effect is observed in vitro using normal pituitaries (Mueller et al., 1973) or pituitary tumor cells (Tashjian et al., 1971) and in vivo in animals bearing median eminence lesions (Porteus and Melven, 1974).

The physiological significance of TRH in normal regulation of prolactin secretion has been questioned in that prolactin and TSH secretion rates appear to be independently controlled in many conditions. Low ambient temperature, which has been reported to stimulate hypothalamic TRH synthesis (Reichlin et al., 1972; Hefco et al., 1975) and release (Montoya et al., 1975), increased TSH and decreased prolactin release (Mueller et al., 1974; Chen and Meites, 1975; and Reichlin et al., 1972). Stress caused a rapid increase in prolactin and a decrease in TSH secretion (Brown-Grant et al., 1954; and Nicoll et al., 1960). Suckling, which is a well known stimulator of prolactin secretion, has been reported not to affect TSH release rate (Sar and Meites, 1969; Gautvik et al., 1974). These results indicate that during many conditions, TSH and prolactin secretion are independently controlled and that TRH does not appear to be the physiological PRF. However, Koch et al. (1977) has observed that intraperitoneal injection of TRH antibodies decreases circulating levels of both TSH and PRL in estrogen-primed rats.

This suggests a role for TRH in estrogen stimulation of prolactin secretion. Further, since somatostatin has been reported to block TRH induced TSH release without effecting TSH stimulation of PRL release (Vale et al., 1974), a differential control of TSH and PRL secretion by TRH is possible.

D. Influence of Steroids on Prolactin Secretion

Reece and Turner (1936, 1937) and later Meites and Turner (1942, 1948) were first to present evidence that estrogen increased both synthesis and release of prolactin in male and female rats, male guinea pigs, and rabbits. These initial observations were later confirmed by Chen et al. (1970) using radioimmunoassay to measure both serum and pituitary prolactin, and by MacLeod (1975) using labeled leucine incorporation into de novo synthesized prolactin. In ovariectomized female rats, all doses of estradiol were effective in increasing serum prolactin, although low doses were more effective than higher doses. In the rat and rabbit, estrogen is considerably more effective in females than in males (Meites and Turner, 1948), although no sex difference has been found in human subjects (Frantz et al., 1972).

Increased serum titers of estrogen are associated with an increase in prolactin secretion during several physiological states. The increased prolactin secretion which precedes the onset of puberty coincides with an increase in serum estrogen levels (Brown-Grant et al., 1970; Naftolin

et al., 1972; Dohler and Wuttke, 1974, 1976) and can be blocked by ovariectomy (Simpkins and Meites, unpublished). During proestrus of the estrous cycle increased titers of serum estrogen are followed by a prolactin surge (Meites and Clemens, 1972; Meites et al., 1972). This proestrous prolactin surge can be blocked by ovariectomy on the morning of proestrus or by administration of an antiserum to estrogen (Neill et al., 1971). During diestrus of the estrous cycle and during most of pregnancy, serum levels of both estrogen and prolactin are low (Meites et al., 1972; Yoshinaga et al., 1969).

Estrogen can affect PRL secretion by both a direct action on the anterior pituitary or indirectly through the central nervous system. Administration of estrogen to hypophysectomized rats bearing ectopic pituitary graphs increases serum PRL levels (Chen et al., 1970). A similar effect of estrogen on PRL secretion was observed in animals implanted with pituitary tumors (Mizuno et al., 1964). These studies, which suggested a direct effect of estrogen on the anterior pituitary, did not eliminate the possibility that estrogen increased PRF or decreased PIF release into the circulatory system. Definitive evidence for a direct action of estrogen on pituitary PRL release was provided by the observation of increased PRL release induced by estrogen in 3 h (Lu et al., 1971) and 3 day (Nicoll and Meites, 1962) incubations with

pituitary cell cultures. Further evidence for a direct action of estrogen is the ability of the anterior pituitary to concentrate labeled estrogen (Vertes et al., 1973), perhaps indicating the presence of estrogen receptors.

Evidence for a hypothalamic mediation of the estrogen stimulation of PRL secretion are indirect. Estradiol benzoate (EB) implants in the median eminence (but not other brain areas) tripled serum PRL levels in 25 days (Nagasawa et al., 1969). However, this study did not eliminate the possibility that EB was delivered to the AP through the hypophyseal portal system and exerted its effect directly. Other studies have shown that steroids implanted in the hypothalamus reach the AP (Bogdanove, 1964).

Experimental alteration of plasma estradiol concentrations modify metabolism of hypothalamic neurons. Estrogen administration to female rats has been reported to decrease hypothalamic PIF activity as measured by a bioassay system (Ratner and Meites, 1964). Ovariectomy has been reported to decrease and subsequent estrogen treatment to increase median eminence (Fuxé et al., 1969) and anterior hypothalamic DA turnover (Huang et al., 1977). Since dopaminergic neurons appear to exert an inhibitory influence on PRL secretion, these changes in dopamine metabolism are opposite to the expected effects of ovariectomy and estrogen replacement on serum PRL levels. Further estrogen binding has been reported

in several areas of the hypothalamus and limbic system (Stump et al., 1977). Although evidence exists that estrogen can affect the metabolism of nervous tissue, there is no direct evidence that the estrogen induced increase in PRL secretion is centrally mediated.

Although estrogen is the primary steroid stimulating prolactin release, other circulating steroids may influence the release of PRL from the AP. In vivo, large doses of either progesterone or testosterone have been reported to induce only small increases in serum PRL levels (Meites, 1959). Since both of these steroids are ineffective in altering PRL release rate in vitro (Nicoll and Meites, 1964), the observed effects in vivo may indicate a conversion to estrogen. Progesterone has been reported to inhibit partially the estrogen-induced prolactin release in ovariectomized rats (Chen and Meites, 1970). High concentrations of cortisol have been reported to inhibit PRL secretion both in vivo and in vitro (Nicoll and Meites, 1964). However, the high doses of this steroid needed to inhibit PRL secretion make its physiological significance questionable since such levels of glucocorticoids are never achieved normally.

Evidence has been presented that thyroid hormone can stimulate PRL release both in vivo (Meites et al., 1963) and in vitro (Meites and Nicoll, 1965) at relatively low doses. Since changes in circulating levels of thyroid hormones occur

only very slowly and do not generally coincide with the circulating PRL levels, thyroid hormones are probably not directly involved in the regulation of prolactin secretion. Rather thyroid hormones probably have a permissive function in the synthesis and release of PRL as well as other AP hormones.

E. Dopaminergic Effects on Prolactin Secretion

The inhibitory influence of the mammalian hypothalamus on prolactin secretion appears to be mediated through central catecholaminergic systems. Early evidence indicating an inhibitory influence of catecholaminergic systems on prolactin secretion included the observation that reserpine, which depletes storage pools of monoamines (Holzbauer and Vogt, 1956; Sheppard and Zimmerman, 1960), stimulates lactation in human subjects (Sulman and Winnik, 1956; Rabinowitz and Freedman, 1961) and experimental animals (Meites, 1957). In later experiments, serum prolactin, as measured by radioimmunoassay, has been shown to be elevated following reserpine treatment (Lu et al., 1970). Other pharmacological agents which deplete central catecholamine stores cause a similar increase in serum prolactin. A single injection of alpha-methyl-para-tyrosine, alpha-methyl-meta-tyrosine, M-dopa and 3-odotyrosine increase serum prolactin as measured by radioimmunoassay (Lu et al., 1970; Lu and Meites, 1971; Smythe et al., 1974). L-dopa, which is readily taken up into

both dopaminergic and noradrenergic neurons, and which increases central levels of both DA and NE, has been reported to inhibit postpartum lactation in rats (Mizuno et al., 1964) and to lower serum prolactin levels in rats (Lu and Meites, 1972) and humans (Turkington, 1972).

The antipsychotic drugs which appear to exert their behavioral effect by blocking DA receptors (Clemens, 1975) are among the most powerful stimulators of prolactin secretion. Chloropromazine, perphenazine, and haloperidol, which are believed to block both dopaminergic and noradrenergic receptors all cause a large increase in circulating levels of prolactin (Lu et al., 1970; Ben-David et al., 1971; Dickerman et al., 1972). Pimozide, a specific DA receptor blocker (Janssen et al., 1968) has been reported to induce a rapid increase in prolactin secretion (Clemens et al., 1974). Sulpiride, which is believed to block DA receptor, has also been reported to increase serum prolactin (Clemens et al., 1974; MacLeod et al., 1977; Pass and Meites, 1977).

Apomorphine, a specific DA agonist (Anden et al., 1967; Ferrini and Miragolin, 1972; Lal et al., 1972), has been shown to inhibit prolactin secretion in vivo (Smalstig and Clemens, 1974; MacLeod and Lehmeyer, 1974; Mueller et al., 1976 and in vitro (Smalstig et al., 1974) in rats and in vivo in humans (Martin et al., 1974). Piribedil (ET 495), another

specific DA receptor agonist (Corrodi et al., 1973) has been reported to reduce prolactin secretion in rats (Nicoll et al., 1970; Nagasawa and Meites, 1970; Wuttke et al., 1972; Malven and Hoge, 1971; Shaar and Clemens, 1972) and in humans (Besser et al., 1972; del Pozo et al., 1972, 1974). These DA agonists interfere with the ability of haloperidol and pimozide to increase prolactin secretion (Smalstig and Clemens, 1974; Mueller et al., 1976) indicating that the effects of these drugs on prolactin secretion occurs at DA receptors.

There is little question that a hypothalamic dopaminergic system exerts a tonic inhibitory influence on prolactin secretion, however, there is still controversy as to whether this effect is mediated through a hypothalamic PIF or by DA acting directly on the anterior pituitary. Infusion of DA or its agonist, apomorphine, into the third ventricle or systemic injection of L-dopa have been reported to elevate hypothalamic and portal blood concentration of PIF and decrease serum prolactin (Kamberi et al., 1973; Lu and Meites, 1972; Ojeda et al., 1974). In addition, systemic injection of ergot alkaloids (Wuttke, Cassell and Meites, 1971), monoamine oxidase inhibitors (Lu and Meites, 1971) and prolactin itself (Chen et al., 1967; Clemens and Meites, 1968) have been reported to increase hypothalamic PIF activity. In contrast, the suckling stimulus, systemic reserpine injection, estrogen administration and stress, all of which increase

serum prolactin levels, decrease hypothalamic PIF activity (Mittler and Meites, 1967). The bioassay used in these studies to determine PIF activity could not distinguish between this and other hypothalamic factors which can inhibit prolactin secretion by a direct action on the anterior pituitary. The possibility that DA could act directly on the anterior pituitary in mediating the inhibitory influence of the hypothalamus on prolactin secretion was suggested initially by the observation that doses of DA (but not NE or E) below those found in the medial basal hypothalamus can inhibit prolactin secretion *in vitro* (Clemens, 1975). The observation of tuberoinfundibular dopaminergic nerve endings in the area of the capillary plexus of the hypophyseal portal vessels (Ajeka and Hökfelt, 1975) and the apparent presence of DA in portal blood (Fuxé *et al.*, 1974; Neill *et al.*, 1977) indicates that DA could directly affect the secretion of prolactin. However, no correlation between hypophyseal portal blood DA concentration and circulating levels of prolactin have yet been determined.

Shaar and Clemens (1974) reported that removal of catecholamines from hypothalamic extracts by incubation with monoamine oxidase or by binding catecholamines to aluminum oxide removed PIF activity as measured by the ability of the extract to inhibit prolactin release *in vitro*. Addition of the aluminum oxide bound catecholamines to the extract

restored PIF activity. Further PIF activity was reported to be resistant to pepsin treatment.

The hypothalamus contains factors other than DA which can inhibit prolactin release as demonstrated by the ability of a single rat hypothalamic equivalent (which contains 20-30 ng of DA) to decrease serum prolactin levels shortly after *in vivo* injection (Watson *et al.*, 1971) and its ability to prevent the suckling and stress induced decrease in pituitary prolactin. Lu *et al.* (1970) demonstrated that intravenous administration of 5-10 μg of DA was ineffective in inhibiting prolactin release, and Blake (1976) has reported that infusion of at least 80 μg DA per hour are required to inhibit prolactin secretion in rats. Clearly, the hypothalamus is much more effective in inhibiting prolactin secretion than DA alone. In addition, pretreatment of hypothalamic-anterior pituitary coincubation systems with either pimozide (Vale *et al.*, 1973) or haloperidol (Ojeda *et al.*, 1974) did not prevent hypothalamic inhibition of prolactin secretion. Several laboratories have recently reported evidence for non-catecholamine hypothalamic substances with PIF activity (Takahara *et al.*, 1974; Dular *et al.*, 1974; Greibrokk *et al.*, 1974; Schally *et al.*, 1977), and Kordon (personal communication) has demonstrated PIF activity in a catecholamine-free synaptosome preparation from the rat median eminence. These results indicate that hypothalamic dopamine can inhibit prolactin secretion, but other factors are probably also involved.

F. Noradrenergic Effects on Prolactin Secretion

The influence of the noradrenergic system on prolactin secretion is less clear than hypothalamic dopaminergic effects for two reasons. First, noradrenergic input to the prolactin release mechanism appears to be minor. Thus, removal of noradrenergic afferents to the hypothalamus results in only slight changes in serum prolactin levels (Weiner and Ganong, 1972). Second, few drugs are selective for noradrenergic neurons. The α-adrenergic receptor blocker, phentolamine, is able to partially block the inhibitory effects of dopamine on prolactin secretion in vitro (MacLeod and Lehmeyer, 1974), suggesting that phentolamine is a partial DA antagonist. Clonidine, a reputed α-adrenergic agonist (Anden et al., 1970) may exert its central effect by acting at pre- rather than postsynaptic receptors, thus decreasing central noradrenergic activity (Dollery and Reid, 1973). Clonidine has also been reported to stimulate central histamine receptors. The most common drugs used to block dopamine β-hydroxylase (DBH), the enzyme which hydroxylates DA to NE, acts by chelating the copper ions in DBH (Morgan and Cogan, 1975). Certainly other copper and iron requiring enzymes are also affected. Thus, it is not surprising that conflicting data concerning the role of NE in regulation of prolactin secretion have appeared.

In vitro, high doses of NE incubated with anterior pituitaries has been shown to inhibit prolactin secretion

(Koch et al., 1970; Shaar and Clemens, 1974), however, high doses of NE injected intraventricularly or intravenously are without effect on PRL secretion (Kamberi et al., 1971a; Lu et al., 1970). Administration of L-dihydroxyphenylserine (L-DOPS), a noradrenergic precursor has been reported to increase prolactin secretion (Donoso et al., 1971) and Disulfram, a DBH inhibitor appears to decrease prolactin secretion (Meites and Clemens, 1972). Administration of the α-agonist, clonidine decreases prolactin in male rats (Mueller et al., unpublished) and blocks the proestrous prolactin surge in female rats (Clemens, unpublished). In ovariectomized, estrogen primed rats, clonidine can stimulate prolactin secretion (Lawson and Gala, 1975). Alpha adrenergic receptor blockers have been reported to partially inhibit (Du Ruisseau et al., 1977) and to have no effect (Meltzer et al., 1976) on the stress-induced rise in prolactin. Clearly, the role of the central noradrenergic system in the control of prolactin has not been elucidated.

G. Serotonergic Effects on Prolactin Secretion

The central serotonergic system appears to exert a stimulatory influence on serum prolactin secretion. Systemic injection of serotonin (5 mg daily) has been shown to stimulate mammary growth and lactation in rats and rabbits (Meites et al., 1959; Meites et al., 1960). Lu et al. (1970) was unable to show any effect of systemically administered

serotonin on prolactin levels 60 min. after injection; whereas, Lawson and Gala (1975) reported a transient increase in serum prolactin 30 min. after serotonin injections. The discrepancy in the above two reports appears to be due to the timing of blood sampling. Kamberi et al. (1971a) reported that serotonin injection into the third ventricle of rats causes a prompt rise in serum prolactin. Administration of the tryptophan hydroxylase inhibitor, p-chlorophenylalanine systemically (Caligaris and Taleisnik, 1974; Advis et al., unpublished) or into the third ventricle (Caligaris and Taleisnik, 1974) is able to decrease serum prolactin levels in both male and ovariectomized, estrogen primed female rats. Administration of the serotonin precursor, 5-hydroxytryptophan (5 HTP) partially reversed the PCPA induced prolactin decrease (Caligaris and Taleisnik, 1974), lowers serum prolactin in ovariectomized estrogen primed rats (Lu and Meites, 1973; Chen and Meites, 1975) and blocks suckling-induced prolactin increase (Kordon et al., 1974b). The neutral amino acid precursor, L-tryptophan, has been shown to elevate serum prolactin and to enhance stress-induced prolactin release (Mueller et al., 1976). Enhancement of postsynaptic stimulation of serotonergic receptors by blockade of presynaptic reuptake with Lilly 110140 (fluoxitine) stimulates prolactin secretion when administered with low doses of 5 HTP (Clemens et al., 1977). Blockade of serotonergic receptors

with methylsergide (Gallo et al., 1975) decreases prolactin secretion in lactating and in ovariectomized rats; whereas, quipazine, a serotonin receptor agonist (Hong and Pardo, 1966; Rodriguez et al., 1973) stimulates prolactin secretion (Clemens, 1975; Clemens et al., 1977). Electrolytic or radiofrequency lesions of the raphe nuclei have been shown to impair lactation (Barophy and Harvey, 1975) and decrease serum prolactin in male rats (Advis et al., unpublished); whereas, electrical stimulation of the dorsal raphe increases prolactin secretion (Advis et al., unpublished). These changes in prolactin secretion are thought to be mediated via changes in the turnover rate of serotonin since raphe lesions decrease (Kuhar et al., 1972; Herr and Roth, 1972) and raphé stimulation increases serotonin turnover (Sheard and Aghajanian, 1968).

V. Hypothalamic Control of Luteinizing Hormone Secretion

A. Feedback of Gonadal Steroids on LH Secretion

Luteinizing hormone (LH) secretion appears to be regulated primarily by circulating levels of estrogens and progesterone in the female and testosterone in the male (Martin et al., 1977) through feedback mechanisms acting directly on the anterior pituitary or mediated through the hypothalamus. In female mammals, which show cyclic release of LH, both

stimulatory and inhibitory gonadal steroid feedback mechanisms are well documented (McCann, 1974); whereas, in males inhibitory, but not stimulatory feedback appear to be present (Bloch et al., 1974; Turner, 1973). Teleologically, the existence of a dual effect of gonadal steroids in females allows for the rhythmic surges in LH, FSH, and prolactin which are necessary for the maintenance of ovarian cyclicity. In males, in which the function of LH and FSH is to stimulate continuously testosterone secretion, spermatogenesis and spermiogenesis, a tonic rather than a cyclic control of LH secretion is necessary. This sex difference in adult LH secretory action is determined by the steroid environment of the hypothalamus during a very short postnatal period in the rat (Coy, 1970).

Ovariectomy and orchidectomy causes an increase in serum LH which persists indefinitely (Gay and Midgley, 1969; Yamamoto et al., 1970; Blackwell and Amoss, 1971), an effect which can be inhibited by the administration of estrogen or progesterone (Smith and Davidson, 1974; Chowers and McCann, 1967; Ramirez et al., 1964). Medial basal hypothalamic (MBH) implants of gonadal steroids appear to be more effective in inhibiting LH secretion than intrapituitary implants (Davidson et al., 1969; Davidson, 1969; Sawyer and Hilliard, 1972), suggesting that the MBH is an important hypothalamic site for inhibitory steroid feedback. However, Bogdanove

(1964) has proposed that MBH implants result in a more efficient delivery of steroids to the pituitary than intra-pituitary implants, i.e., the "implantation paradox." In male rats, we have demonstrated that ^{3}H-testosterone implanted into the MBH causes a decrease in LH secretion before substantial label reaches the pituitary gland (Turner and Simpkins, unpublished) suggesting that the MBH can mediate the inhibitory feedback of steroids on LH secretion.

The administration of estrogen and progesterone, alone or in combination can stimulate LH surges in both female rats and humans. Administration of estrogen in both 4- and 5-day cycling rats during diestrus has been shown to advance the time of ovulation (Everett, 1948; Brown-Grant, 1969; Weick et al., 1971). The LH surge which occurs on proestrus of the estrous cycle and that induced by estrogen administration can be blocked by the administration of antibodies against estrogen (Ferin et al., 1969, 1974; Knobil et al., 1974). Estrogen administration to long-term ovariectomized rats causes a daily LH surge (Legan and Karsch, 1975). The ability of progesterone to induce LH surges appears to depend upon the existing estrogen environment. Thus, progesterone can stimulate LH surges if administered on the third day of diestrus in 5-day cycling rats or on proestrus in 4-day cycling rats (Everett, 1948; Zerlmaker, 1966; Brown-Grant and Naftolin, 1972). Administration of progesterone

in early diestrus inhibits LH secretion (Everett, 1948) and interferes with LHRH induced LH release (Martin et al., 1974).

Estrogen and progesterone appear to interact synergistically to stimulate LH surges. Progesterone administration to estrogen primed, long term ovariectomized rats, stimulate an LH surge which is similar to the preovulatory LH surge both in magnitude and timing (Caligaris et al., 1971). The similarity of the response to this sequence of steroid treatments to that which occurs on proestrus is indicated by the observation of both an FSH and a prolactin surge accompanying the LH surge (Caligaris et al., 1974). The possible role of a progesterone-estrogen interaction to induce the preovulatory LH surge has been questioned because of conflicting reports regarding levels of progesterone on the morning of proestrous (Schwartz, 1969; Everett, 1964; Redman, 1968; Hori et al., 1968; Goldman et al., 1969; Piacsek et al., 1971; and Barraclough et al., 1971). However, recent studies utilizing frequent blood sampling times have demonstrated daily morning progesterone peaks during the estrous cycle (Mann and Barraclough, 1973) and a large progesterone surge on the morning of proestrous (Kalra and Kalra, 1974). Thus, a synergistic interaction between progesterone and estrogen may normally occur during the estrous cycle. Lisk and Reuter (1977) have recently reported that progesterone enhanced

binding of ^{3}H-estradiol to both preoptico-anterior hypothalamic and median eminence-arcuate tissue in the rat hypothalamus.

The locus of the stimulatory feedback of gonadal steroids on LH secretion has not yet been conclusively established, although direct anterior pituitary and hypothalamic mediated stimulatory feedback have been described. Removal of afferents to the MBH by deafferentations which exclude the preoptico-anterior hypothalamic area blocks ovulation (Hillarp, 1949; Halasz and Gorski, 1967; Halasz, 1972) and gonadotropin secretion (Palka et al., 1969; Blake et al., 1972; Weiner et al., 1972b). Hypothalamic islands which include the preoptico-anterior hypothalamus in the islands do not interfere with cyclic release of LH (Halasz, 1972). Electrical lesions of the preoptic anterior hypothalamus (Barraclough, 1966) or the suprachiasmatic nucleus (Schneider and McCann, 1969a; Mess et al., 1966) has a similar effect on LH secretion. Deafferentation and anterior hypothalamic lesions also block the steroid induced LH surge (Taleisnik et al., 1970; Bishop et al., 1972). Electrical stimulation of preoptic-anterior hypothalamic areas, as well as the arcuate-median eminence area stimulates ovulation (Sawyer, 1975), LH secretion (Clemens et al., 1971; Cramer and Barraclough, 1973) and increase LHRH levels in portal blood (EsRay et al., 1977). In primates, the role of the

preoptic-anterior hypothalamus in mediating positive steroid feedback has been questioned by Krey et al. (1975) based upon the observation of normal cyclicity and normal response to estrogen after medial basal hypothalamic deafferentation.

In addition to the well known presence of LHRH containing cell bodies and nerve terminals in the median eminence-arcuate nucleus in the mouse (Zimmerman et al., 1974) and rat (Barry and Dubois, 1973; Baker et al., 1974; Kordon et al., 1974), LHRH perikarya have been demonstrated in the preoptic nuclei of the guinea pig (Leonardelli et al., 1973; Barry and Dubois, 1973), the cat and dog (Barry and Dubois, 1975) and the rat (Baker et al., 1975). These rostral LHRH containing cells may send axons to the medial basal hypothalamus (MBH). Deafferentation in male and female rats has been shown to cause a depletion of MBH-LHRH concentration and an increase in preoptic-anterior hypothalamic LHRH (Kalra, 1976; Kalra et al., 1977). In addition to the localization of LHRH containing cell bodies in the rostral aspects of the hypothalamus, there appears to be a large serotonergic and noradrenergic input to rostral hypothalamic nuclei (Ungerstadt, 1971; Brownstein et al., 1976). Pharmacological studies indicate that both of these amines may be important neurotransmitters in mediating stimulatory steroid feedback (Kalra and McCann, 1972; Coen and MacKinnon, 1976).

An alternative site of the positive feedback of gonadal steroid is directly on gonadotropin producing cells of the anterior pituitary gland. Arimura and Schally (1971) first observed that 24 or 48 hours exposure to estrogen increased LHRH induced release of LH in vivo. Later studies have since shown that estrogen can potentiate LHRH induced LH release from the anterior pituitary and from pituitary cell suspensions in vitro (Labrie et al., 1976), and in animals with MBH deafferentation (Greeley et al., 1975). Cyclic variations in pituitary responsiveness of LH secretion to LHRH stimulation have been observed (Gordon and Reichlin, 1974; Aiyer et al., 1974a; Martin et al., 1974; Zeballos and McCann, 1975). The most sensitive stage of the estrous cycle for LHRH-induced LH release is the afternoon of proestrus, when circulating estrogen titers are high. In addition, estrogen appears to increase the self-priming action of LHRH on LHRH induced LH secretion (Aiyer et al., 1974b; Greeley et al., 1975). Consistent with these reports are the observations that specific binding of ^{125}I-LHRH to anterior pituitary membranes is highest on proestrous (Kyringza et al., 1975) indicating that estrogen may increase the synthesis of plasma membrane LHRH receptors. Progesterone appears to have no effect on LHRH induced LH secretion; however, large doses can interfere with estrogen potentiation of LHRH induced LH secretion (Aiyer et al., 1974a; Greley et al., 1975).

B. Noradrenergic Effects on Luteinizing Hormone Secretion

Several pharmacological approaches have been employed to determine the role of catecholamines in the secretion of gonadotropins. These include: a) administration of CA depleting drugs, b) administration of receptor agonists and antagonists, and c) ventricular infusion of monoamines. Alpha-methyl-p-tyrosine (αmpt) which depletes central CA stores by competitively inhibiting the activity of tyrosine hydroxylase (Spector et al., 1965; Corrodi and Hansen, 1965), the rate limiting enzymes in catecholamine synthesis, has been shown to block ovulation (Brown, 1967; Kordon and Glowinski, 1969), the proestrous LH surge (Kalra and McCann, 1973), the release of LH in response to castration (Ojeda and McCann, 1973), and stimulatory gonadal steroid feedback of LH secretion (Kalra et al., 1972). Reserpine, which depletes catecholamine storage vessels (Dahlstrom et al., 1965) is able to block ovulation (Brown, 1967) and prevents LH secretion in response to administration of Pregnant Mare Serum (PMS) (Barraclough and Sawyer, 1957). 6-Hydroxydopamine, which selectively destroys catecholaminergic nerve terminals and axons (Thoenen and Tranzer, 1968; Uretsky and Iverson, 1970) blocks LH release on the afternoon of proestrous and in response to steroid administration. This antigonadotropic effect occurs whether infusion is into the third ventricle (Kalra et al., 1974) or into the ventral noradrenergic tract (Martinovic and McCann, 1977).

High doses of alpha-adrenergic receptor blocking drugs have been reported to block ovulation in the rabbit (Kordon and Glowinski, 1969), to decrease LH levels in castrated females (Schneider and McCann, 1969b), and to block estrogen induced LH release (Schneider and McCann, 1970a). The selectivity of high doses of α-adrenergic receptor blockers has recently been questioned on the basis of the observation that these drugs are able to block the reported DA-induced increase in LHRH secretion into the hypophysial system (Kamberi, 1976) and increased LH secretion (Kamberi, 1970c; Kalra and McCann, 1973).

Intraventricular infusion of norepinephrine induces ovulation (Sawyer et al., 1949; Rubinstein and Sawyer, 1970; Sawyer, 1975; Tima and Flerko, 1975), and increases serum LH in the rat and rabbit (Sawyer, 1975; Krieg and Sawyer, 1976) and increases LHRH release into the hypophyseal portal circulation in female rats (Portal et al., 1976). In vitro incubation of NE in a hypothalamo-pituitary incubation system stimulates LH secretion (McCann and Moss, 1976), an effect which appears to be mediated through an α-adrenergic mechanism (Godde and Schwilling, 1976).

In a series of detailed experiments Kalra and McCann (1972) undertook an analysis of the relative importance of dopaminergic and noradrenergic systems in mediating the stimulatory feedback of estrogen in ovariectomised and

progesterone in ovariectomized estrogen primed rats on LH secretion. Administration of the dopamine-β-hydroxylase inhibitors, diethyldithiocarbarmate (DDC) or 1-phenyl-3-(2-thiazolyl)thiourea (U-14,624), which are believed to depress central NE level while not affecting or increasing central DA levels (Goldstein and Nakajima, 1967; Johnson et al., 1970) blocked the progesterone induced LH surge in ovariectomized, estrogen-primed rats. Administration of L-dopa to DBH blocked rats was ineffective; whereas, dihydroxyphenylserine (DOPS) was effective in reestablishing the LH surge. Since in DBH blocked animals L-dopa should selectively increase DA concentration; whereas, DOPS should selectively increase NE, these studies suggest that progesterone induced LH surge is noradrenergic, but not dopaminergic mediated.

Ojeda and McCann (1973) demonstrated that both αmpt or DDC are able to block the post-castration rise in serum LH in male rats. However, administration of neither L-DOPA or DOPS was successful in restoring the post-castration LH rise. Thus, the involvement of CAs in the post-castration LH rise in male rats is uncertain.

In 4-day cycling rats, systemic administration or third ventricle infusion of either αmpt or FIA-63, a dopamine-β-hydroxylase inhibitor, on the second day of diestrus blocked ovulation and reduced proestrous LH levels (Terasawa et al.,

1975). Administration of L-DOPA or DOPS reversed the αmpt block in about half of the animals tested. These studies indicate that a central noradrenergic mechanism may be involved in stimulating LH secretion in several different conditions.

Increases in NE turnover have been described during states of increased LH secretion. Ovariectomy increases anterior hypothalamic concentration of NE (Donoso et al., 1967), increases rate of depletion of ^{3}H-NE from whole brains at 6 and 21 (but not 2) days post-castration (Anton-Tay and Wurtman, 1968), and triples turnover by 2 weeks post-castration as estimated by measuring the rate of catecholamine depletion following αmpt treatment (Coppola, 1969). The synthesis of NE from ^{3}H-tyrosine also increases after ovariectomy (Anton-Tay et al., 1970; Bapna et al., 1971). Hypothalamic tyrosine hydroxylase activity increases 2-3 fold from 4 to at least 60 days post-ovariectomy in female rats, an effect which can be blocked by progesterone, but not estrogen, replacement (Beattie et al., 1972; Beattie and Soyka, 1973).

Changes in hypothalamic concentration and metabolism of norepinephrine occur during states of stimulatory steroid feedback. On proestrus, anterior hypothalamic (Stefano and Donoso, 1967; Coppola, 1969) and median eminence (Selmanoff et al., 1976) norepinephrine concentration and whole brain

norepinephrine turnover (Zachaeck and Wurtman, 1973) increase. During the first estrous cycle, hypothalamic norepinephrine turnover increases during early proestrus, then decreases in late proestrus and estrus (Advis et al., 1977). A similar transient increase in norepinephrine turnover was observed in ovariectomized, estrogen-primed rats in response to progesterone administration (Simpkins, Huang, Advis and Meites, unpublished). In general, these studies indicate that a central noradrenergic mechanism is involved in stimulating LH secretion during several conditions.

C. Dopaminergic Effects on Luteinizing Hormone Secretion

There is no general agreement concerning the role of central dopaminergic mechanisms in the regulation of LH secretion. Dopamine infusion into the third ventricle has been reported to increase LH and FSH secretion (Kamberi et al., 1970b, 1971c; Schneider and McCann, 1970b,c), portal vessel LRF activity (Kamberi, 1969) and plasma LRF activity in hypophysectomized rats (Schneider and McCann, 1970b). In contrast, Cramer and Porter (1973) observed no effect of third ventricle infusion of DA on plasma LH in intact or estrogen treated rats. Sawyer's group observed that third ventricle DA infusion does not effect LH but is able to block the LH increase stimulated by third ventricle NE infusion (Sawyer et al., 1974; Krieg and Sawyer, 1976).

In an attempt to explain these differences, McCann and Moss (1976) suggested that the steroid environment of the animal is important in modulating the effect of intraventricular DA on LH secretion. During the estrous cycle, intraventricular DA stimulates LH secretion on the second day of diestrus and on proestrus, when estrogen titers are high, but not on estrus or the first day of diestrus (Schneider and McCann, 1970a). Infusion of DA into the third ventricle stimulated LH secretion in ovariectomized, estrogen-progesterone primed animals, but failed to effect LH secretion in ovariectomized animals (Vijayan and McCann, 1977). Furthermore, DA stimulates the in vitro release of LHRH from medial basal hypothalami of ovariectomized, estrogen-primed rats, but not from ovariectomized rats (Rotsztejn et al., 1976). In addition to a possible role of gonadal steroids in modulating the DA effect, it is possible that DA is taken up by noradrenergic neurons and converted to norepinephrine before it affects LH secretion. The α-adrenergic receptor blockers inhibit the reported DA induced LH release both in vitro (Kamberi et al., 1970c; Schneider and McCann, 1969b) and in vivo (Schneider and McCann, 1970a) which indicates that DA may act through a adrenergic system or be converted to NE before interaction with the noradrenergic receptor.

Studies utilizing systemic administration of dopamine receptor stimulators or blockers have produced equally

conflicting data. The neuroleptic haloperidol (Janssen, 1967) has been reported to block ovulation in the rat (Boris et al., 1970) and decrease serum levels of LH and FSH and hypothalamic LHRH activity when administered on early proestrous (Dickerman et al., 1974). In contrast, the dopamine receptor blocker, d-butaclanol (Voith and Herr, 1975; Lippman et al., 1975; Lippman and Pugsley, 1975) appear to have no effect on pulsatile LH release in ovariectomized rats (Drouva and Gallo, 1977) and heloperidol has no effect on basal LH levels in male rats (Mueller et al., 1976). Pimozide administration has been reported to have no significant effect on the post-castration LH rise (Ojeda and McCann, 1973) or on pulsatile LH release in ovariectomized rats (Drouva and Gallo, 1976). Dopamine receptor stimulation with apomorphine and CB-154 blocks pulsatile LH secretion (Drouva and Gallo, 1976), but apomorphine has no effect on basal levels of LH in male rats (Mueller et al., 1976). Piribedil has been reported to decrease basal LH levels in male rats (Mueller et al., 1976), but has no effect on the post-castration LH rise in rats (Grandison, Hodson and Meites, unpublished).

Fuxé and co-workers have provided evidence that the tuberoinfundibular DA system may be involved in inhibiting LH secretion. Castration decreases and administration of estrogen increases tuberoinfundibular DA turnover as

measured by histofluorescence (Fuxé et al., 1969). Tuberoinfundibular DA turnover decreases on proestrous and early estrus of the rat estrous cycle (Ahren et al., 1971). Similarly, dopamine turnover in the whole hypothalamus has been reported to decrease on late proestrous of the first estrous cycle in rats (Advis et al., 1977). During lactation in the rat, serum LH levels are low and tuberoinfundibular DA metabolism is high (Fuxé et al., 1967; Ben-Jonathan and Porter, 1976).

D. Serotonergic Effects on Luteinizing Hormone Secretion

Evidence for both a stimulatory and an inhibitory role of serotonin on LH secretion have been presented. Intraventricular infusion of serotonin has been reported to suppress LH and FSH secretion (Kamberi, 1970b; Schneider and McCann, 1970c) and inhibit the proestrus surge of LH and FSH (Kamberi, 1973). Systemic administration of 5 HTP, a serotonin precursor, blocks ovulation and the proestrous surge of LH and FSH (Kamberi, 1973). Electrical stimulation of the raphe nuclei increases hypothalamic 5 HT turnover and inhibits ovulation (Carrer and Taleisnik, 1970, 1972); whereas, administration of PCPA on the morning of proestrus facilitates ovulation (Kordon and Glowinski, 1972). However, other studies suggest a stimulatory role for 5 HT in LH and FSH release. Coen and MacKinnon (1976) were able to block the

evening surges of LH in ovariectomized, estrogen-primed rats with p-chlorophenylalanine (PCPA), p-chloroamphetamine (PCA) and 5,6-dihydroxytryptamine (5,6 DHT), all of which deplete central serotonin levels. Infusion of 5,6-DHT into medial and dorsal raphe nuclei, which causes complete disappearance of 5-HT terminals in the suprachiasmatic nuclei 10 days after administration (Daly et al., 1973), blocks estrogen induced LH surges. Administration of 5-HTP in PCPA depleted, ovariectomized, estrogen primed rats causes a reappearance of the evening LH surge (Hery et al., 1976). Further, the timing of serotonin depletion by PCPA appears to be important, since administration just before the LH surge was ineffective; whereas, administration earlier causes blockade of the LH surge (Hery et al., 1975).

Kordon's group has suggested two functionally distinct serotonergic systems influence LH secretion. An inhibitory system is indicated by the observation that median eminence-arcuate area is the only hypothalamic area able to mediate the inhibitory effect of implanted serotonin on LH secretion (Kordon, 1969). A stimulatory system is indicated by the observation of a large serotonergic input to the suprachiasmatic nucleus (Fuxé, 1965; Ungerstadt, 1971; Brownstein et al., 1975), an area whose integrity is vital to cyclic release of LH (Clemens et al., 1973), and the observation that serotonergic depleting drugs can block cyclic LH surges

but have little effect on basal LH secretion (Coen and MacKinnon, 1976; Hery et al., 1975, 1976).

E. Inhibitory Effects of Prolactin on LH Secretion

In many physiological, pathological and experimental states an inverse relationship between serum prolactin and gonadotropin exists. During postpartum lactation in rats, serum prolactin is high; whereas, serum LH and FSH are low (Meites, 1966). Ovariectomy in rats results in an increase in gonadotropins and a decrease in prolactin secretion (Meites and Clemens, 1972). In humans, a similar relationship between serum prolactin and LH appears to exist during normal lactation and states of inappropriate lactation (Martin et al., 1977). Forbes-Albright and Chiari-Frommel syndromes are conditions in which hyperprolactinemia is associated with infertility.

In these conditions, the high circulating levels of prolactin are believed to be responsible for the low serum LH. Suppression of serum prolactin in both postpartum rats (Lu et al., 1976) and in humans (Seri et al., 1974) with the DA agonist CB-154 (2-bromo-ergocryptine) results in an increased gonadotropin secretion. In humans, L-DOPA or CB-154 induced decreases in prolactin and results in resumption of menstrual cycles in Forbes-Albright (Turkington, 1974) and Chiari-Frommel (Seri and Seri, 1974) patients. The resumption of

reproductive function is believed to be due to a decrease in circulating prolactin since CB-154 has never been shown to stimulate LH secretion (Fuxé et al., 1976). Induction of hyperprolactinemia by exogenous administration of ovine prolactin (Gudelsky et al., 1977) and by implantation of anterior pituitary glands beneath the kidney capsule or subcutaneous implant of pituitary tumor tissue (Grandison et al., 1977) decreased LH secretion in both castrated males and females.

The prolactin-induced inhibition of gonadotropin secretion appears to be centrally mediated. Systemic injections of prolactin and subcutaneous pituitary tumor implantation increase tuberoinfundibular as well as anterior hypothalamic DA turnover (Hökfelt and Fuxé, 1972; Gudelsky et al., 1977; Hodson et al., unpublished). These treatments do not effect other central dopaminergic systems and have no effect on norepinephrine turnover. Since the inhibitory effect of prolactin on gonadotropin occurs in castrated animals, the prolactin effect does not appear to be secondary to its effect on the gonads. Muralidhar et al. (1977) reported a decreased responsiveness of the pituitary to a single injection of LHRH in rats treated with prolactin. However, using multiple LHRH injections, Hodson et al. (1977) have been unable to demonstrate any effect of prolactin on LHRH induced LH release. The explanation of the difference between these two studies is at present uncertain. However, it is possible

that prolactin could effect pituitary sensitivity by decreasing the release of LHRH. This could decrease the priming of the pituitary which could result in a decreased responsiveness of the pituitary to a single LHRH injection. Multiple LHRH injections would mask this effect.

MATERIALS AND METHODS

I. Animals, Treatments and Blood Collection

Mature male and female rats used in these studies were obtained from four sources (Spartan Research Animals, Haslett, MI; Charles Rivers, Madison, WI; Blue-Spruce Farms, Altamont, N.Y.; and Harlan Industries, Indianapolis, IN). Animals were housed in light (14 h on, 10 h off) and temperature (25°±1°C) controlled rooms and provided with Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum. Animals were orchidectomized or ovariectomized within 3-5 min while under deep ether anesthesia. Cannulae were implanted in ether anesthetized rats with the aid of a Kopf Stereotaxic Instrument using coordinates described in the rat brain atlas of Skinner (1971). Correct placement of annulae was confirmed by macroscopic observation following decapitation of the animals.

Piribedil mesylate (provided by Dr. M. Derome-Tremblay, Les Laboratories Servier, Neuilly sur Seine, France), alpha-methyl-para-tyrosine methyl ester HCl (Regis Chemical Co., Morton Grove, IL) and pargyline hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.89% NaCl just before use. Estradiol benzoate (EB) and progesterone (P) (Nutritional Biochemicals Corp., Cleveland, OH) were dissolved in

corn oil. 6-Hydroxydopamine hydrobromide (6-OH-DA) (Regis Chemical Co., Morton Grove, IL) for intrahypothalamic implantation, was tapped into 26-gauge stainless steel wire cannulae. The combined weights of 50 cannulae determined before and after tapping the drug indicates that 1-2 μg of 6-OH-DA were administered per implant.

Blood samples were taken by decapitation or by orbital sinus cannulation under light ether anesthesia except in Experiment V in which sampling was done via chronically implanted jugular cannulae in unanesthetized, unrestrained animals. Blood samples were stored at 4°±1°C for 24 h to allow clot formation and serum was separated and stored at -20°C until assayed for hormone concentration.

II. Radioimmunoassay of Serum Hormones

Serum concentrations of luteinizing hormones (LH), prolactin, and thyroid stimulating hormone (TSH) were determined using standard double antibody ratioimmunoassay procedures. Serum prolactin was assayed using the method of Niswender *et al*. (1969), while serum LH and TSH were determined by the method described in the NIAMDD-kits. Hormone concentrations are expressed in terms of the standard reference preparations NIAMDD-rat prolactin-RP-1, NIAMDD rat LH-RP-1, and NIAMDD-rat TSH-RP-1. All serum samples were

assayed in duplicate or triplicate. Samples from individual experiments were all tested in the same assay to avoid inter-assay variability. Hormone concentration was determined only from serum volumes which resulted in hormone values on the linear portion of the standard curve.

III. Assay of Dopamine and Norepinephrine in Brain Tissues

A. Isolation and Preparation of Brain Tissue

Immediately after decapitation, brains were removed from the cranium and laid dorsal side down (to avoid deformation of the hypothalamus) in a pool of ice cold 0.89% NaCl. The hypothalamus was excised using the following landmarks: anterior, immediately rostral to the optic chiasm; posterior, middle of the mammilary bodies; lateral, the lateral hypothalamic sulci; dorsal, 2-3 mm dorsal to the ventral hypothalamic surface. In the same experiments (see Experimental) the anterior and posterior portions of the hypothalamus were separated by making a frontal cut just caudal to the infundibular stalk. Such a procedure produced anterior hypothalami (AH) weighting 22.8$\pm$0.4 mg and posterior hypothalami (PH) weighting 11.0$\pm$0.4 mg (N=64). The whole hypothalamus or hypothalamic fragments were immediately frozen on dry ice until assayed for catecholamine concentration.

The median eminence (ME; Experiment IV) was dissected from the remaining hypothalamus using fine iris scissors.

Cuts were made at the posterior border of the infundibular stalk and along the lateral aspects of the tuber cinereum at an angle of about 20° from the ventral hypothalamic surface. The piece of tissue produced by this procedure corresponded roughly to the superficial basal hypothalamic layer described by Kavanagh and Weize (1973) and contained 18±1 µg protein (N=14) as assayed by the micro-method of Lowry et al. (1951). Medial basal hypothalamus (MBH; Experiment VII) was dissected by making cuts similar to those for the ME except at a 45° angle from the ventral hypothalamic surface. This dissection corresponded to the intermediate basal hypothalamic layer described by Kavanagh and Weize (1973) and the tissue contained 34±4 µg proteins (N=32).

The ME was homogenized in 25 µl and the MBH in 40 µl of 0.4 N perchloric acid (PCA) containing 10 mg percent EDTA. Whole hypothalami as well as anterior and posterior hypothalamic portions were hemogenized at a concentration of 1 mg tissue per 10 µl 0.4N PCA (plus 10 mg percent EDTA). Tissues were homogenized using microhomogenizers (Micrometric Instruments Inc., Cleveland, OH) and centrifuged (microcentrifuge, Coleman Inst., Oak Brook, IL) to separate the particulate portion from the supernatant. Dopamine (DA) and norepinephrine (NE) were assayed in 10 µl of supernatant by the method described below.

B. Radioenzymatic Assay of Dopamine (DA) and Norepinephrine (NE)

Tissue DA and NE were assayed by the method of Coyle and Henry (1973) or by a modification of the method of Ben-Jonathan and Porter (1976). Both assays utilized 10 µl of tissue supernatant or standard DA and NE (Sigma Chemical Co., St. Louis, MO) incubated in the presence of buffered catecholamine-o-methyl transferase (COMT) and the methyl donor ^{3}H-S-adenosyl methionine (New England Nuclear, Boston, MA). COMT was partially purified from rat liver by the method of NIkodijevic et al. (1970).

The procedure for extraction and separation of 3-0-methoxynormetanephrine and 3-0-methoxytyramine (the o-methylated metabolites of NE and DA) by the method of Coyle and Henry (1973) is described in Appendix A. Briefly, after side chain cleavage of normethanephrine with sodium metaperiodate (Mallinckrodt Chemical Co., St. Louis, MO), the NE and DA metabolites were separated utilizing a series of acid-case and organic-aqueous phase extractions. As a result of incomplete oxidation of normetanephrine, 4.8% of the counts in the DA fraction were from tissue or standard norepinephrine. Both standard and tissue counts were corrected for this contamination before DA and NE content were calculated.

The Ben-Jonathan and Porter method (1976) utilizes solvent extraction and thin layer chromatography to separate normethanephrine and methoxytyramine. This procedure

simplified the cumbersome Coyle and Henry method and improved the assay sensitivity (see Appendix B). Amine content of samples were determined after separation by counting chromatographic spots containing the ^{3}H-labeled metabolites in class scintillation vials containing 10 ml of Scintiverse (Fisher Scientific Products, Livonia, MI). Samples were counted in a New England Nuclear, Mark II, scintillation counter.

EXPERIMENTAL

I. Effects of Progesterone on Steady State Concentration and Turnover of Dopamine (DA) and Norepinephrine (NE) in Anterior and Posterior Portions of the Hypothalamus, and on Serum Luteinizing Hormone and Prolactin Levels in Ovariectomized, Estrogen-Primed Rats

A. Objectives

Several experimental approaches indicate that central noradrenergic and dopaminergic systems influence the secretion of anterior pituitary hormones (Wurtman, 1971; de Wied and de Jong, 1974; Meites et al., 1976). The anterior hypothalamus is of particular importance in mediating the stimulating influence of gonadal steroids on the secretion of gonadotropins. Destruction of anterior hypothalamic nuclei or surgical separation of the rostral hypothalamus from the MBH can block ovulation (Hillarp, 1949; Halasz and Gorski, 1967; Halsaz, 1972), the proestrus surge of LH (Palka et al., 1969; Blake, 1972), and LH release normally seen in response to progesterone (P) administration to ovariectomized, estrogen-primed rats. The present study was undertaken to determine if alterations in anterior or posterior hypothalamic DA and NE metabolism occur after P treatment of ovariectomized, estrogen-primed rats.

B. Materials and Methods

Female Long-Evans rats, weighing 200-255 g, were bilaterally ovariectomized and 10 days later received a single s.c. injection of estradiol benzoate (EB; 100 μg/kg body weight). Three days after the EB injection, animals received a single s.c. injection of P (500 μg/kg body weight) at 0900 hrs. This treatment has been shown to induce a surge in both LH (Caligaris et al., 1971) and prolactin (Caligaris et al., 1974). Animals were killed by decapitation 0, 2, 4 and 6 h after the P administration. DA and NE turnover were estimated by a modification of the non-steady state method described by Brodie et al. (1966). At the time of sacrifice, animals were randomly placed in two groups. One group of the animals received an i.p. injection of 250 mg alpha-methyl-para-tyrosine per kg body weight 1 h before decapitation. The other group of animals received vehicle (0.89% NaCl). At the time of decapitation, trunk blood was collected for determination of serum LH and prolactin concentration. Anterior and posterior hypothalamic fragments were dissected as described in general "Materials and Methods." Anterior hypothalamus (AH) and posterior hypothalamus (PH) weighed 22.8 $\pm$ 0.4 and 11.0 $\pm$ 0.4 mg, respectively (N=64).

The significance of differences among groups was determined by ANOVA and Student-Newman-Keuls tests (Sokal and Rohlf, 1969). Two-way ANOVA was used to test the significance

of interaction between factors (time after P treatment and drug treatment). The level of significance chosen was $P < 0.05$.

C. Results

The upper panel of Figure 1 shows that the steady state concentration of anterior hypothalamic NE does not change significantly between 0 and 4 h after progesterone administration. However, a significant decrease ($P < 0.05$) in NE concentration occurs between 4 and 6 h post-progesterone. NE turnover, as estimated by the increase in NE depletion after exposure to αmpt, increased 2-fold ($P < 0.05$) at 4 h after P administration. By 6 h post-progesterone turnover was not different from 0900 h controls.

No significant alteration in the steady state concentration of anterior hypothalamic DA occurred at any of the times tested. However, a progressive decrease beginning at 4 and becoming significant ($P < 0.05$) at 6 h, in DA turnover occurred.

Serum LH levels increased slightly by 4 h and were increased dramatically by 6 h after P administration. Serum prolactin levels were elevated by 2 h, peaked at 4 h and remained elevated at 6 h after P administration.

No change in posterior hypothalamic NE or DA concentration or turnover was observed in this study (Figure 2).

Figure 1. Effects of progesterone administration to ovariectomized, estrogen-primed rats on steady state concentration and alpha-methyl-para-tyrosine induced depletion of anterior hypothalamic catecholamines and on serum luteinizing hormone (LH) and prolactin (PRL).

The solid bars indicate steady state concentration and hatched bars indicate amine concentration 1 h after i.p. injection of αmpt (N=6-8). The numbers above the sets of bars indicate percentage depletion of amines induced by αmpt. The star above the solid bar indicates a significant difference in amine concentration ($P < 0.05$) between that group and the 0 h group. Stars above the numbers indicate a significant interaction between αmpt treatment and time after progesterone as analyzed by a Two Way ANOVA ($P < 0.05$). LH and prolactin represented in the lower panel are from sera of non-drug treated, control animals. Stars above the points indicate a significant increase above 0 h values ($P < 0.05$). The verticle line in all 3 panels represents 1 standard error of the means (SEM).

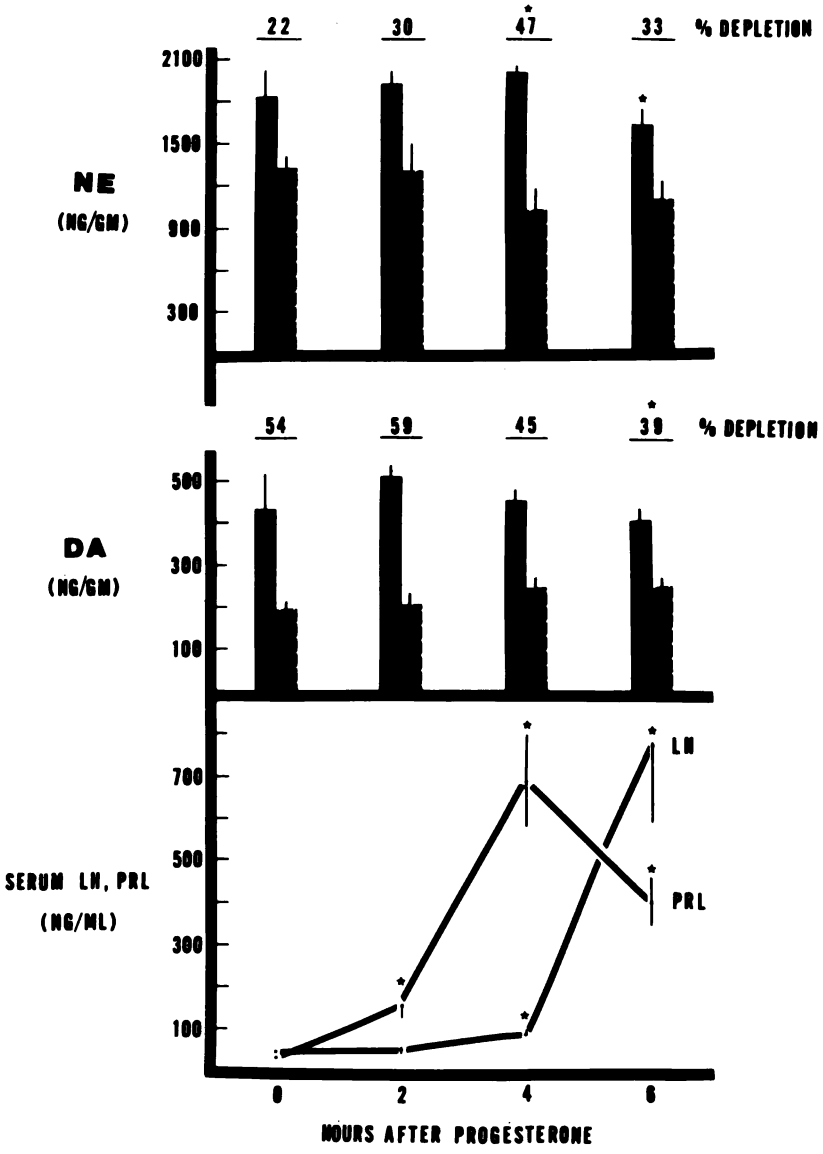

22
30
47
33
% DEPLETION
2100
1500
900
300
NE
(NG/GM)
54
59
45
39
% DEPLETION
500
300
100
DA
(NG/GM)
700
500
300
100
SERUM LH, PRL
(NG/ML)
LH
PRL
0
2
4
6
HOURS AFTER PROGESTERONE

Figure 1

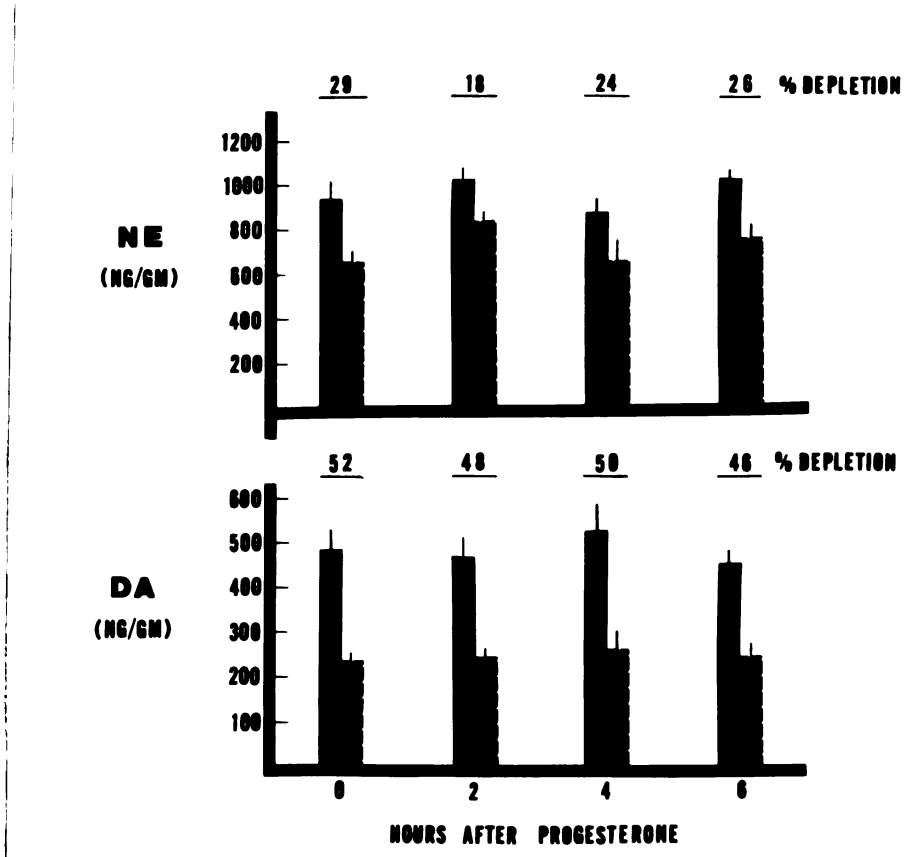


Figure 2. Effect of progesterone administration to ovariectomized, estrogen-primed rats on steady state concentration and alpha-methyl-para-tyrosine induced depletion of posterior hypothalamus.

The solid bars indicate steady state concentration and the hatched bars indicate amine concentration 1 h after i.p. injection of αmpt (N=6-8). Verticle lines indicate 1 SEM. The number above the sets of bars indicates percentage depletion induced by αmpt.

D. Discussion

The observation that anterior, but not posterior hypothalamic catecholamine metabolism is altered in response to a stimulatory regimen of gonadal steroids is consistent with the hypothesis that anterior hypothalamic areas regulate the stimulatory influence of gonadal steroids on LH secretion (Gorski, 1966). Earlier observations indicate that estrogen or a combination of estrogen and progesterone implanted in the anterior hypothalamus stimulate LH secretion (McCann, 1974). Anterior hypothalamic destruction blocks ovulation and the LH surge normally occurring on proestrus and following steroid administration (Hillarp, 1949; Mess et al., 1966; Halasz, 1972; Weiner et al., 1972). However, steroid implants or lesions in the posterior hypothalamus have little or no effect on LH secretion (McCann, 1974).

The doubling of NE turnover by 4 h after the P injection is similar in magnitude to that observed in other conditions of increased LH secretion. Anton-Tay and Wurtman (1968) observed a doubling in the rate of depletion of whole brain ^{3}H-NE at 2 weeks post-castration, whereas Coppola (1969) observed a doubling of total catecholamine turnover in anterior hypothalamus 2 weeks post-castration. Tyrosine hydroxylase activity has been reported to increase 2-3 fold from 4 to 60 days post-ovariectomy in rats (Beattie et al., 1972). The present study indicates that an increase in NE

turnover can occur quickly (4 h) in response to stimulatory steroid feedback, while the increased NE turnover reduced by ovariectomy requires days to develop (Anton-Tay and Wurtman, 1968). The long latency in response to ovariectomy in females may be due to the long half-life of gonadal steroids.

The increased turnover of anterior hypothalamic NE occurs at the onset of the LH surge and at the time of peak serum prolactin. The observation that the dopamine β-hydroxylase inhibitor, diethyldithiocarbarnate (DDC) can block the P induced LH surge in ovariectomized, estrogen-primed rats suggests a causative relationship between the increased NE turnover and the onset of the LH surge. On the afternoon of proestrus in the rat, when a steroid induced LH surge normally occurs (Ferin et al., 1969, 1974; Kalra and Kalra, 1974) hypothalamic (Advis, Simpkins, Chen and Meites, unpublished observation) as well as whole brain (Zachaeck and Wurtman, 1973) NE turnover appears to increase.

A causative relationship between the increase in NE turnover and the prolactin surge seems unlikely since a significant increase in serum prolactin is observed before a significant elevation in anterior hypothalamic NE turnover occurs. Other studies using pharmacological agents which alter central NE metabolism have not demonstrated a consistent affect on prolactin secretion (Meites et al., 1976).

The decreased DA turnover which appears to begin at 4 h and which is significant at 6 h after P administration is associated with both the rise in serum LH and peak in serum prolactin levels. The decreased DA turnover may cause the elevation in serum prolactin since in all mammalian species yet tested, a central dopaminergic mechanism appears to inhibit prolactin secretion (Meites et al., 1972). However, pharmacological observations have produced contradictory data as to the role of DA in controlling LH secretion (McCann and Moss, 1975; Meites et al., 1976). Thus, subsequent studies have been conducted to determine the role of both DA and NE in mediating the P induced LH and prolactin surges in ovariectomized rats.

Further Study on the Effect of Progesterone on Dopamine Turnover in Ovariectomized-Estrogen-Primed Rats

A. Objectives

The effect of P injection on NE turnover appeared to be transient whereas its influence on DA metabolism persisted through 6 h. Since the peak in serum LH levels occurs after 8 to 10 h, in response to P administration into ovariectomized, estrogen primed rats, it was of interest to measure DA and NE turnover in the anterior hypothalamus at these times.

B. Materials and Methods

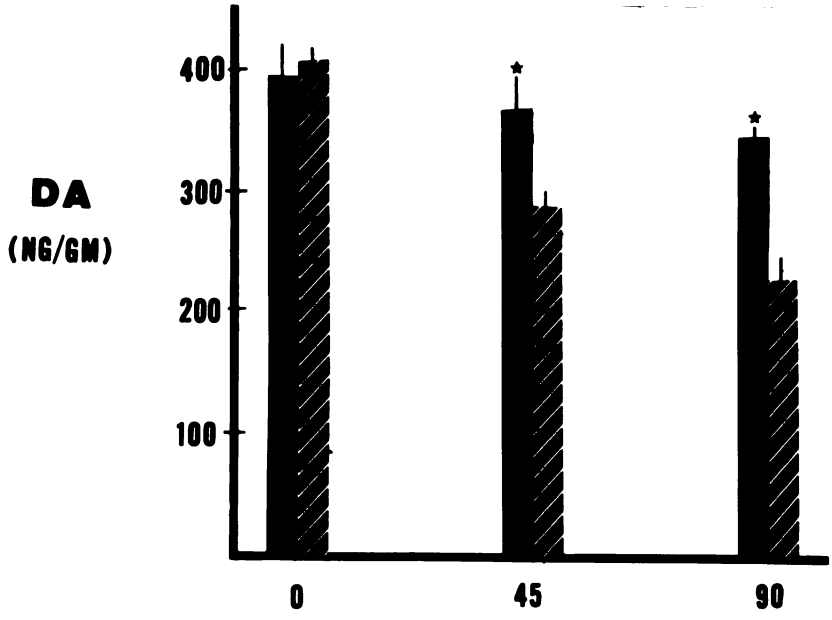
Treatment was the same as in the previous experiment except that animals were killed at the time of and 9 h after P administration. To estimate catecholamine turnover, rats received an injection of αmpt (250 mg/kg body weight) at either 90, 45 or 0 min before decapitation. This treatment allowed the depletion curves of DA and NE to be evaluated. The significance of differences between slopes of the depletion curves was evaluated by ANOVA and Student-Newman Keuls test (Sokal and Rolfh, 1969).

C. Results

Figure 3 shows the effect of αmpt on anterior hypothalamic dopamine (AH-DA) at 0 and 9 h after P administration to ovariectomized, estrogen-primed rats. Nine h of P exposure resulted in a decrease in DA depletion at 45 and 90 min after αmpt treatment. There was no significant difference in the rate of NE depletion between 0 and 9 h after P administration. All vehicle treated control animals showed elevated levels of LH (> 2,000 ng/ml) and high circulating levels of prolactin at 9 h after P treatment.

D. Discussion

The results presented here indicate that the decreased DA turnover which begins between 4 and 6 h after progesterone administration continues through at least 9 h and that the



Hours Post Progesterone		
	0	9
PRL (ng/ml)	105 ±39	383 ±47
LH (ng/ml)	81 ±8	>2,000

Figure 3. Long term effects of progesterone administration to ovariectomized, estrogen-primed rats on alpha-methyl-para-tyrosine induced depletion of anterior hypothalamic dopamine and on serum luteinizing hormone (LH) and prolectin (PRL).

Upper panel shows the effect of progesterone treatment on anterior hypothalamic DA concentration of rats decapitated at 0, 45, or 90 min after i.p. injection of 250 mg αmpt/kg body weight. Solid bars represent animals killed at 9 h after progesterone treatment and hatched bars represent animals killed at 0 h after progesterone treatment. Verticle lines represent SEM. The lower table shows serum LH and prolactin (PRL) concentration in control (non-drug treated) rats at 0 and 9 h after progesterone administration.

increase in NE turnover observed at 4 h post-progesterone was indeed transient, since no significant difference in NE turnover was observed between 0 and 9 h. These data indicate that the P-induced increase in serum prolactin is more closely associated with the decreased DA turnover than the increased NE turnover since NE turnover returned to normal whereas prolactin levels remained elevated. Similarly, the changes in serum LH correlated better with the decreased DA than the increased NE turnover.

Two hypotheses may explain the role of DA and NE in the genesis of the P-induced LH surge in ovariectomized, estrogen-primed rats. First, the increased NE turnover observed at 4 h after P treatment may be sufficient to initiate a series of events (i.e., increased LHRH synthesis and release) leading to the LH surge. Consistent with this hypothesis are the observations that the P-induced LH surge can be eliminated by the interruption of NE metabolism (Kalra and McCann, 1972) or by blockade of α-adrenergic receptors (Kalra et al., 1974). Second, the decreased DA turnover may cause or at least permit the P-induced LH surge. This hypothesis is supported by the observation that third ventricle infusion of DA can block the LH surge induced by subsequent third ventricle infusion of NE (Sawyer et al., 1974; Krieg and Sawyer, 1976). In order to distinguish between these two hypotheses, selective alteration in either DA or NE metabolism is required.

II. Effects of Alpha-Methyl-Para-Tyrosine on Serum LH and Prolactin Surges Induced by Progesterone Treatment of Ovariectomized, Estrogen-Primed Rats

A. Objectives

Alpha-methyl-p-tyrosine (αmpt), which depletes central catecholamine stores by competitively inhibiting tyrosine hydroxylase (Spector et al., 1965; Corrodi and Hansen, 1965), the rate limiting enzyme in catecholamine synthesis, has been shown to block ovulation (Brown, 1967; Kordon and Glowinski, 1967), the proestrus LH surge (Kalra and McCann, 1973), the release of LH in response to castration (Ojeda and McCann, 1973) and stimulatory steroid feedback on LH secretion (Kalra et al., 1972). In view of our observation that NE turnover increases while DA turnover decreases in response to P, the ability of αmpt to block LH secretion may be due to its effect on noradrenergic neurons. If the decrease in DA turnover were critical to the mediation of the P-induced LH surge, a further decrease in DA turnover with αmpt should enhance rather than inhibit LH secretion. The objective of this set of experiments was to characterize further the effects of αmpt on P-induced LH secretion in ovariectomized estrogen-primed rats.

B. Materials and Methods

Female Long Evans rats (Blue Spruce Farms, Altamont, N.Y.) weighing 200-250 g were subjected to surgical and

steroid treatment as described in Experiment I. In one experiment, αmpt (250 mg/kg body weight) was injected i.p. at the time of the 0900 h P administration. Blood samples were obtained via orbital sinus cannulation at 0, 7, and 9 h after P treatment. In a second experiment, 250 mg αmpt/kg body weight was administered i.p. to P treated ovariectomized, estrogen-primed rats at 45, 60 or 90 min before decapitation. Animals were killed at 0, 2, 4, 6, or 9 h after P treatment.

C. Results

Administration of 250 mg in αmpt/kg body weight at the time of progesterone administration completely blocked the P-induced LH surge (Figure 4). Administration of αmpt was ineffective in decreasing serum LH when administered 1 h before the 0 and 2 h blood samples (Table 1). Treatment with αmpt 1 h before the 4 and 6 h blood samples was effective in decreasing serum LH by 46±8 and 84±3%, respectively. At 9 h after P treatment, 45 min of αmpt exposure was ineffective in decreasing serum LH, but 90 min exposure to αmpt decreased serum LH by 69±13%. Treatment with αmpt 45, 60, or 90 min before the 0 h blood sampling caused a slight but significant increase in serum LH.

Exposure to αmpt for 60 min increased serum prolactin in animals sampled at 0 and 2, but not 4 and 6 h after P administration (Table 2).

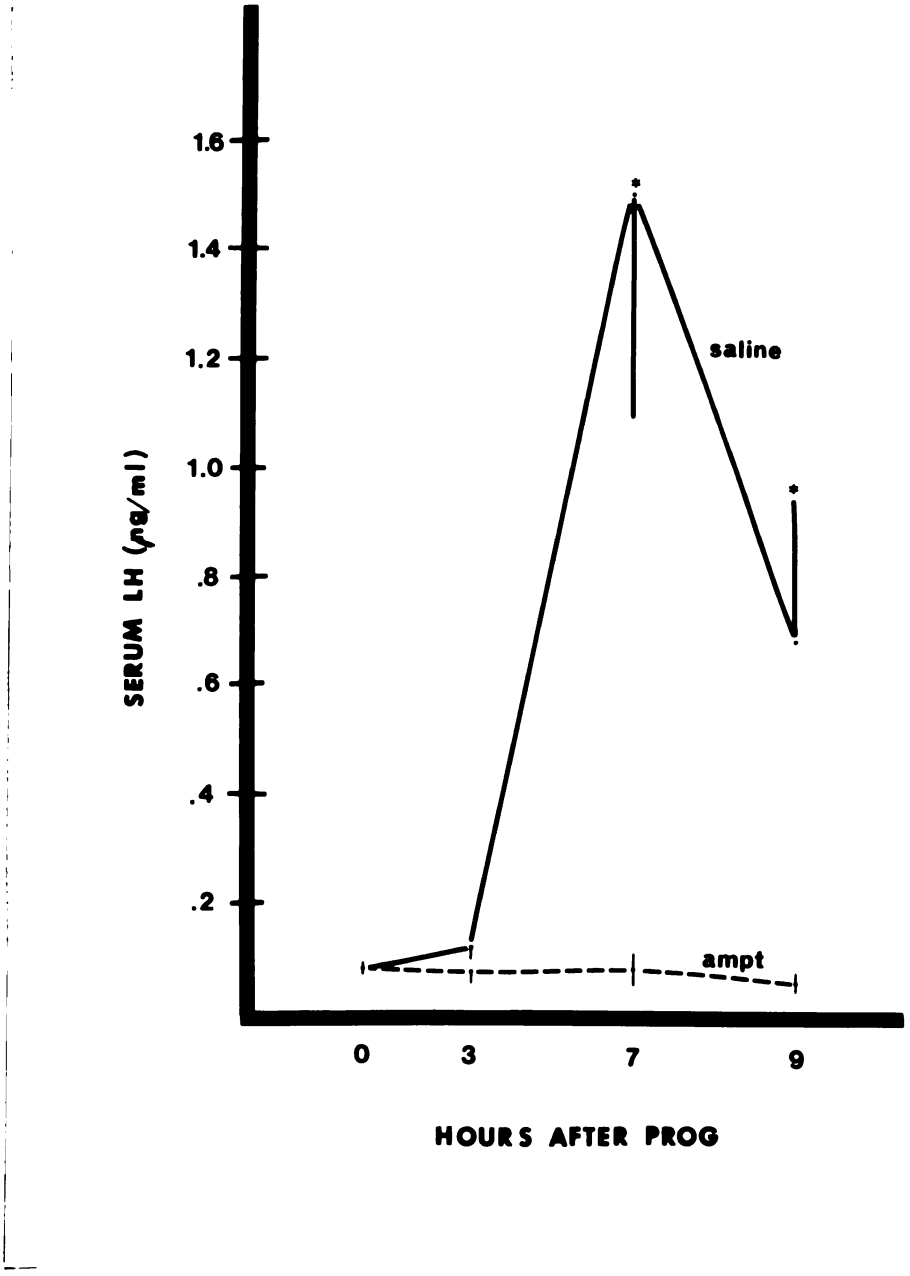


Figure 4. Long term effects of alpha-methyl-para-tyrosine on progesterone induced LH secretion in ovariectomized, estrogen-primed rats.

A single i.p. injection of 250 mg αmpt/kg body weight (solid line) or saline, its vehicle (dashed line) was administered at the time of the progesterone treatment. Each point represents the means of 6 to 8 determinations and the verticle bar represents 1 SEM. Stars above the points indicate that the mean is significantly different from the means of the 0 h sample ($P < 0.05$).

Table 1. Effects of Alpha-Methyl-Para-Tyrosine (αmpt) on Serum Luteinizing Hormone (LH) at Various Times After Progesterone Administration to Ovariectomized, Estrogen-Primed Rats

Min After αmpt	Hours After Progesterone			
	0	2	4	6
	Serum LH			
0†	38 ± 3	46 ± 6	85 ± 9	773 ± 189
60	52 ± 6*	42 ± 6	46 ± 7*	125 ± 23*

Min After αmpt	Hours After Progesterone	
	0	9
	Serum LH	
0††	81 ± 8	2979 ± 1332
45	143 ± 21*	2282 ± 267
90	177 ± 33*	984 ± 401*

† These groups received 0.89% NaCl at 60 min before decapitation.

†† These groups received 0.89% NaCl at 45 min before decapitation.

* Indicates a significant difference when compared with control (0) group ($P < 0.05$).

Table 2. Effects of Alpha-Methyl-Para-Tyrosine (αmpt on Serum Prolactin (PRL) at Various Times After Progesterone Administration to Ovariectomized Estrogen-Primed Rats

Min After αmpt	Hours After Progesterone			
	0	2	4	6
	Serum PRL			
0†	31 ± 2	152 ± 29	685 ± 107	349 ± 55
60	586 ± 48*	665 ± 47*	623 ± 43	378 ± 32

† These groups received 0.89% NaCl at 60 min before decapitation.

* Indicates a significant difference when compared with control (0) group ($P < 0.05$).

D. Discussion

The observation that αmpt injected at the time of P administration can completely inhibit the progesterone induced LH surge is consistent with its ability to block ovulation (Brown, 1967; Kordon and Glowinski, 1973) and the proestrous LH surge (Kalra and McCann, 1973). Kalra et al. (1972) observed that DDC is as effective as αmpt in blocking the progesterone induced LH surge in ovariectomized, estrogen primed rats. This suggests that αmpt inhibits the LH surge by interfering with NE rather than DA synthesis.

The observation that acute exposure to αmpt can decrease LH levels at 4, 6 and 9 h after P administration suggests that NE synthesis is required to maintain sustained LH

secretion. Since αmpt inhibits LH release at the time of increased NE turnover (4 h) and after NE turnover has returned to normal (6 and 9 h), it is possible that some aspect of NE metabolism and transmission other than increased turnover is maintaining the high secretory rate of LH. However, it is also possible that the increased NE turnover seen at 4 h is needed to initiate the LH surge and that a lower turnover rate is required to sustain high LH secretion.

The explanation for the increase in serum LH in response to αmpt at 0 h is not clear. The high DA turnover at 0 h may inhibit LH secretion at this time and the αmpt induced decrease in DA synthesis may release this inhibition. In ovariectomized rats, estrogen administration has been shown to stimulate DA turnover (Fuxé et al., 1969, Gudelsky et al., 1977) and DA has been reported to inhibit LH secretion (Sawyer et al., 1974; Mueller et al., 1976).

The observation that acute exposure to αmpt stimulates prolactin release when DA turnover is high, but has no effect on prolactin release when DA turnover is low, are in accord with the suggested (Experimental I) close relationship between DA turnover and prolactin levels in response to P administration.

III. Effects of Multiple Piribedil Injection on Serum LH and Prolactin Surges Induced by Progesterone in Ovariectomized, Estrogen-Primed Rats

A. Objectives

The observation that DA turnover decreases in response to P treatment of ovariectomized, estrogen-primed rats (Experiment I) suggests that decreased DA activity may be important in mediating the LH and prolactin surges. Since the role of central dopaminergic systems in the control of LH is unclear and reports on this subject are contradictory (see section V.C. of Literature Review), it was of interest to determine the importance of the decreased DA turnover in the steroid-induced LH surge. Therefore, in the present study LH and prolactin levels were determined following P administration to ovariectomized, estrogen primed rats treated with the DA agonist, piribedil (Corrodi et al., 1973; Miller and Iversen, 1973).

B. Materials and Methods

Female Sprague-Dawley rats (Spartan Farms, Haslett, MI), weighing 200-250 g were ovariectomized and subjected to steroid treatment as described in Experimental IB. Animals received a single i.p. injection of piribedil mesylate (1 mg/kg body weight) or 0.89% NaCl (its vehicle) at 0, 3 and 6 h after P injection. This dose of piribedil is the minimally effective dose for decreasing serum LH in intact

male rats (Mueller et al., 1976). Blood samples (1 ml) were obtained via orbital sinus cannulation at 0, 7, and 9 h after P treatment. The significance of differences among means was tested by ANOVA and Student-Neuman Keuls test (Sokal and Rohlf, 1969).

C. Results

Piribedil administration from 0 through 6 h after P treatment had no effect on peak LH concentration (7 h), whereas serum LH concentration was significantly higher in piribedil treated than in control animals at 9 h after P treatment. Serum prolactin levels were significantly lower in piribedil than in saline treated rats at 7 h after P, but did not differ from controls by 9 h after P treatment.

D. Discussion

These data indicated that sustained DA receptor stimulation does not block the P induced LH surge in ovariectomized, estrogen-primed rats, and suggests that the decreased DA turnover observed following P administration is not essential in mediating the steroid induced LH surge. DA receptor blockade with pimozide (Ojeda and McCann, 1973) or DA receptor stimulation with piribedil (Grandison, Hodson and Meites, unpublished observation) has previously been shown to be ineffective in altering the post-castration increase in serum LH in male rats.

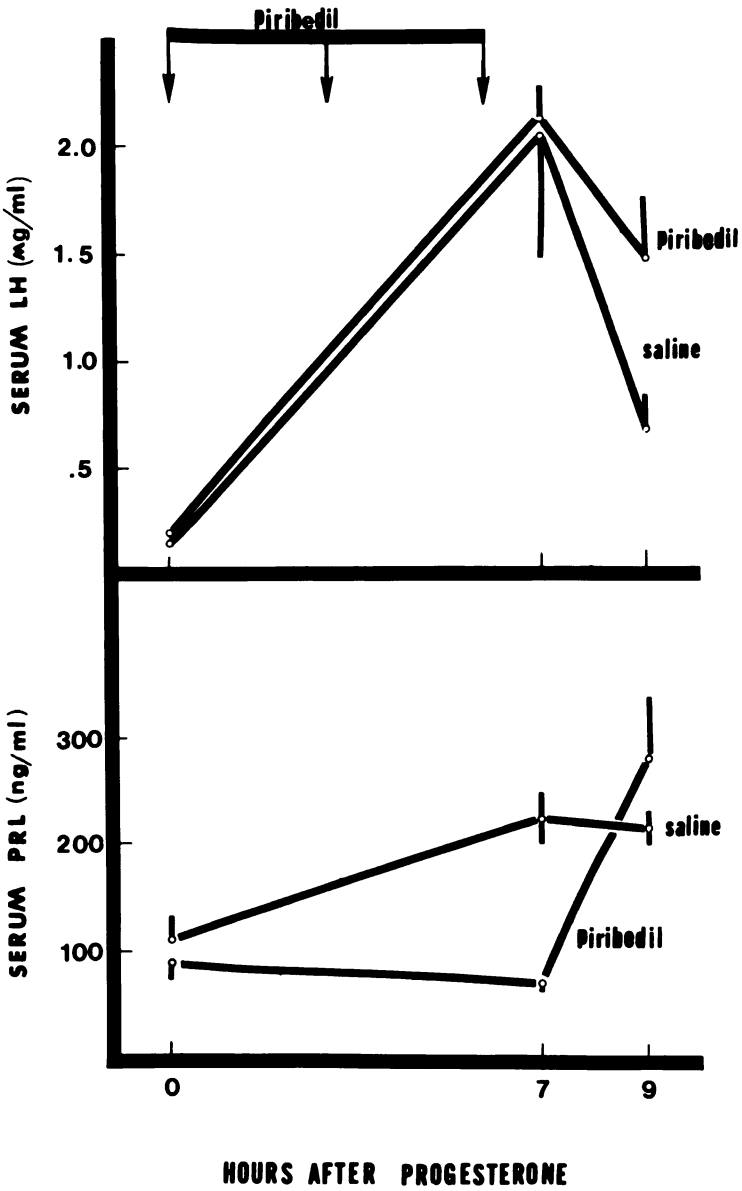


Figure 5. Effect of sustained administration of piribedil mesylate (ET-495) on the progesterone induced luteinizing hormone (LH) and prolactin (PRL) secretion in ovariectomized, estrogen-primed rats.

Rats were injected i.p. with 1 mg peribedil mesylate/kg body weight or 0.89% NaCl, its vehicle, at 0, 3 and 6 h after progesterone administration. Each point represents the means of 8 determinations and the verticle lines indicate 1 SEM. Stars indicate significant difference between means of drug and saline treated animals at a particular sampling time.

The ability of piribedil to block the P-induced increase in serum prolactin at 7 h after P treatment is consistent with the findings of several laboratories (see section IV-E of Literature Review). These investigators demonstrated the inhibitory influence of DA on prolactin secretion. The present study, together with the previous study (Experiment I) suggests that a decrease in DA turnover may be necessary to mediate the P-induced increase in serum prolactin.

The increased rate of prolactin secretion (rebound effect) following termination of piribedil treatment observed in this study, was previously reported in experiments utilizing DA infusion in humans (LeBlanc et al., 1976). A similar rebound effect cannot account for the less rapid rate of decrease in serum LH between 7 and 9 h after P treatment in the piribedil as compared with the saline-treated group since piribedil does not appear to inhibit the LH surge.

IV. Effect of Implants of 6-Hydroxydopamine in the Suprachiasmatic Nucleus (SCN) and Median Eminence (ME) on Anterior Hypothalamic and Median Eminence Dopamine and Norepinephrine Concentration, and on Serum Luteinizing Hormone and Prolactin Surges Induced by Progesterone in Ovariectomized, Estrogen-Primed Rats

A. Objectives

The integrity of rostral areas of the hypothalamus appears to be critical in mediating the stimulatory effect of

steroids on LH secretion in female rats. Rostral deafferentiation (Halasz, 1969) and electrolytic lesions of anterior hypothalamic nuclei (Mess et al., 1966) inhibited whereas electrical stimulation (Clemens et al., 1971) and anterior hypothalamic estrogen implantation (Davidson, 1969) stimulated LH secretion. The observation that NE turnover increases in the anterior (but not the posterior) hypothalamus prior to the LH surge induced by progesterone in ovariectomized, estrogen-primed rats (Experiment I) suggests that noradrenergic nerve terminals in the anterior hypothalamus may mediate positive steroid feedback.

Since the rostral hypothalamus contains both noradrenergic nerve tracts en route to the median eminence (ME) and noradrenergic nerve terminals (Loizou, 1969; Ungerstadt, 1971), physical destruction of the anterior hypothalamus could alter LH secretion by disrupting synapses, nerve tracts or both. The purpose of the present investigation was to selectively disrupt anterior hypothalamic and ME noradrenergic nerve terminals with the neurotoxin, 6-hydroxydopamine (6-OH-DA; Thoenen and Tranzer, 1968), and determine its effect on P-induced LH release in ovariectomized, estrogen-primed rats. To determine the specificity and magnitude of the 6-OH-DA effect, DA and NE concentrations in the anterior hypothalamus and median eminence were determined in both drug and sham-implanted animals.

B. Materials and Methods

Female Sprague-Dawley rats (Spartan Farms, Haslett, MI) weighing 200-250 g, received surgical and steroid treatment as described in Experiment I. Preliminary observations indicated that intrahypothalamic implantation of crystalline 6-OH-DA selectively depleted NE for 24 h after implantation. Thus SCN and ME implants were made 24 h before the P injection. 6-OH-DA was tapped into 26 gauge tubing previously packed with cocoa butter (CB). Cannulae for control animals contained CB alone. Blood samples were taken under light ether anesthesia by orbital sinus cannulation 0, 7, and 9 h after the 0900 h P injection. Immediately after the last bleeding, animals were killed by decapitation and brains were quickly removed from the cranium. The area immediately over the optic chiasm was dissected in the SCN implanted animals by cutting rostral and caudal to the optic chiasm and laterally at the hypothalamic sulci. The cube of tissue produced by cutting at a depth of 2-3 mm was frozen for later catecholamine assay. In ME implanted animals, the external layer of the ME was dissected as described in general Material and Methods section. DA and NE were assayed by the method described in Appendix B and are expressed as ng/g wet weight for the anterior hypothalamus and ng/mg protein for ME.

C. Results

The effect of 6-OH-DA implants into the SCN on anterior hypothalamic DA and NE are shown in the top 2 panels of Figure 6. SCN implants of 6-OH-DA decreased NE concentration by 83% ($P < 0.05$), DA concentration by 24% (not significant) and significantly reduced the P induced LH surges when compared with sham-implanted ovariectomized, estrogen-primed rats (Figure 7). However, a slight, but significant increase in LH was observed at 7 and 9 h in 6-OH-DA implanted animals. Median eminence (ME) 6-OH-DA implants decreased NE by 57% ($P < 0.05$) and DA by 11% in the ME (Figure 6). This treatment had no effect on LH levels at 7 and 9 h after P administration (Figure 7) when compared with sham-implanted animals. ME implants of 6-OH-DA significantly decreased LH levels when measured at 0900 h (before P administration); whereas, SCN implants had no effect (Table 3).

D. Discussion

The present studies indicate that anterior hypothalamic noradrenergic nerve terminals are important in mediating the stimulatory effect of P on the release of LH in ovariectomized, estrogen-primed rats; whereas, ME noradrenergic nerve terminals may be unimportant in this regard. These data are consistent with the observation that 6-OH-DA injected into the third ventricle (Kalra, 1975) or infused into the ventral noradrenergic tract (Martinovic and McCann, 1977), can block

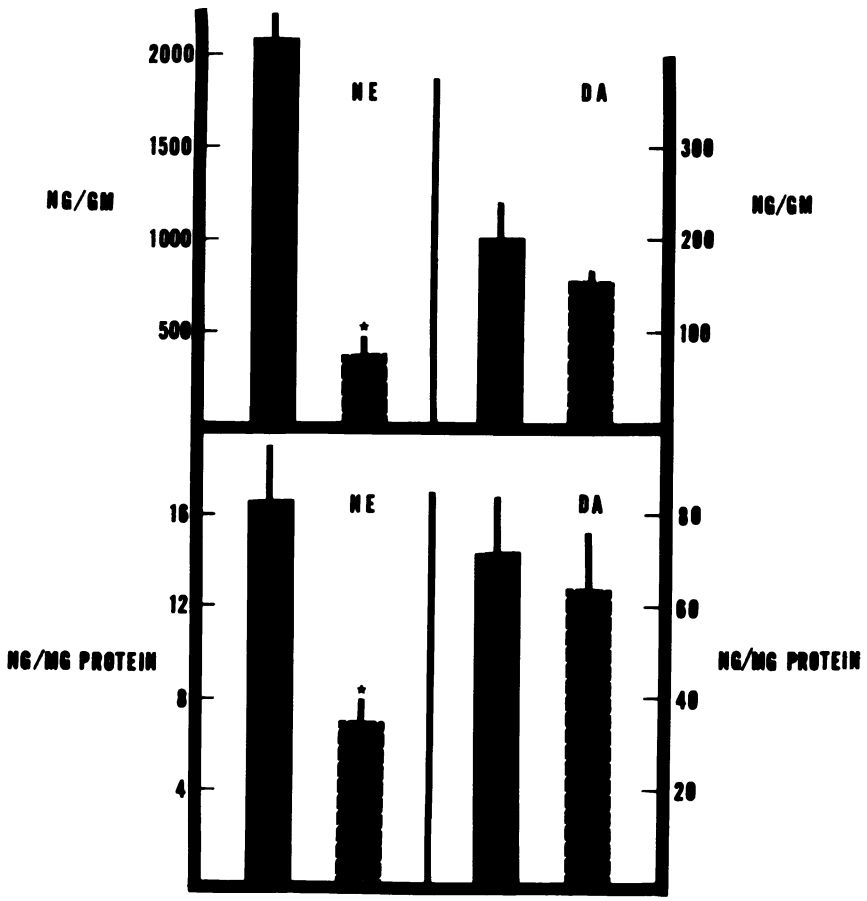


Figure 6. Effects of suprachiasmatic nucleus (SCN) and median eminence (ME) implants of 6-hydroxydopamine (6-OH-DA) on anterior hypothalamic and median eminence concentration of norepinephrine and dopamine.

Upper panels indicate anterior hypothalamus (AH) and lower panels indicate median eminence (ME) norepinephrine and dopamine concentration. Solid bars indicate amine levels in cocoa butter implanted and hatched bars indicate levels 33 h after 6-OH-DA implantation (N=6-8). Verticle lines represent 1 SEM and stars above verticle line indicate a significant ($P < 0.05$) decrease in amine concentration.

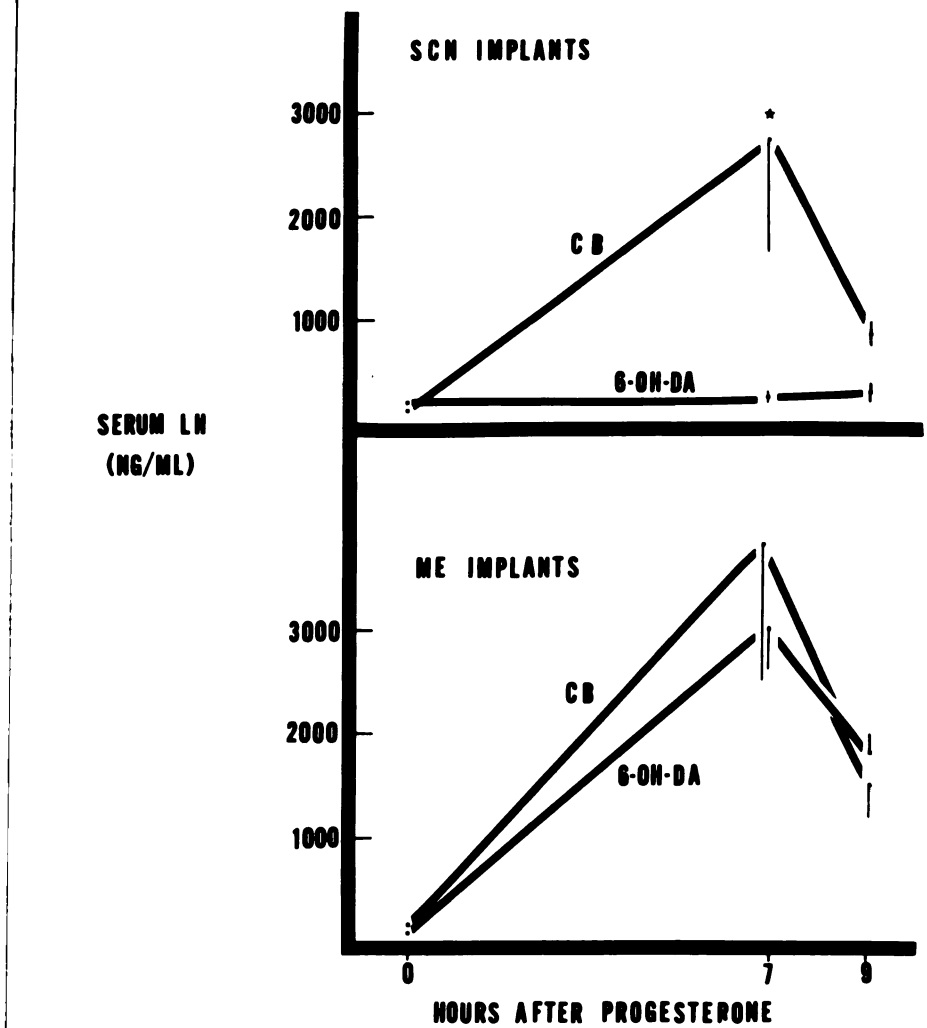


Figure 7. Effect of suprachiasmatic nucleus (SCN) and median eminence (ME) implants of 6-OH-DA or cocoa butter (CB) on progesterone induced luteinizing hormone (LH) secretion in ovariectomized, estrogen-primed rats.

Rats were implanted with 6-OH-DA in the SCN (upper panel) or ME (lower panel) or CB 24 h before progesterone administration. Each point represents the means of 6-8 determinations and the verticle line indicates 1 SEM. Stars above the point indicate a significant difference between means of 6-OH-DA and CB implanted animals at a particular sampling time.

Table 3. Effects of Implants of 6-Hydroxydopamine in Suprachiasmatic Nucleus (SCN) and Median Eminence (ME) on Serum Luteinizing Hormone (LH) in Ovariectomized, Estrogen-Primed Rats

Type of Implant	CB	6-OH-DA
	Serum LH	
ME	109 ± 17	72 ± 10*
SCN	127 ± 25	149 ± 20

† Implants were made 24 h before blood sampling.

†† Values represent serum LH concentration at 900 h (before progesterone administration).

* Indicates a significant difference when compared to cocoa butter (CB) implanted animals (P 0.05).

LH secretion induced by gonadal steroids.

The observation that both noradrenergic nerve terminals (Loizou, 1969; Ungerstedt, 1971) and cell bodies of neurons containing luteinizing hormone-releasing hormone (LHRH) are in rostral hypothalamic nuclei of rats (Baker et al., 1975), provide anatomical support for the present observation indicating the importance of anterior hypothalamic noradrenergic synapses in the mediation of P induced LH secretion. Destruction of noradrenergic nerve terminals in the anterior hypothalamus by local application of 6-OH-DA may interfere with NE stimulation of LHRH containing cells in the anterior hypothalamus. Since ME noradrenergic nerve terminals appear not to be involved in mediation of P induced LH secretion,

the effect of anterior hypothalamic 6-OH-DA implants is not mediated by a destruction of noradrenergic nerve tracts enroute to the ME. These data are supported by the observation of Kalra and McCann (1973) that blockade of NE synthesis decreases LH release in response to anterior hypothalamic, but not ME, electrical stimulation. However, the present study does not eliminate the possibility that noradrenergic nerve terminals in the anterior hypothalamus synapse with non-LHRH containing neurons which transmit impulses to LHRH containing cells in the arcuate-median eminence area.

The observation that 6-OH-DA implants in the ME (but not SCN) decreased LH levels before P administration, may indicate that noradrenergic nerve terminals tonically stimulate LH secretion. This observation is consistent with the hypothesis that the medial basal hypothalamus exerts a tonic stimulatory influence on LH secretion (Gorski, 1966). However, since the present experiment was not designed to test this point, further studies using different experimental models will be needed.

The ability of SCN 6-OH-DA implants to block P induced LH release without significantly affecting anterior hypothalamic DA concentration indicates the relative lack of importance of anterior hypothalamic DA synaptic terminals in mediating LH release. However, this study does not eliminate the involvement of DA in the events leading to the LH surge.

DA and DA agonists have been shown to stimulate, inhibit and to have no effect on LH secretion (see section V-C of Literature Review) which may indicate a dopaminergic modulation of the LH releasing system. Selective depletion of DA nerve terminals in specific areas of the hypothalamus may help clarify the role of this putative neurotransmitter in LH release.

In light of our previous demonstration that anterior hypothalamic NE turnover increased in response to P treatment of ovariectomized, estrogen-primed rats (Experiment I) and the present observation that disruption of anterior hypothalamic noradrenergic synapses blocked the P-induced LH surge, it appears that the noradrenergic component of the anterior hypothalamus is responsible for mediating most, if not all, of the P stimulation of LH secretion in ovariectomized, estrogen-primed rats.

V. Measurement of Dopamine and Norepinephrine Turnover and Serum Luteinizing Hormone After Short Term Castration in Male Rats

A. Objectives

The experimental manipulation used in Experiments I-IV resulted in a surge in both serum LH and prolactin. The LH surges appear to be mediated by an increase in NE turnover; whereas, the prolactin surge is probably mediated by the decreased DA turnover. If alterations in catecholamine

metabolism are a necessary requirement for the central mediation of LH and prolactin secretion, endocrine manipulations which result in selective changes in secretion of either LH or prolactin should result in selective alterations in DA and NE metabolism.

Castration results in a slow rise in serum LH in females (Gay and Midgley, 1969) and a rapid increase in serum LH in males (Gay and Midgley, 1969; Yamamoto et al., 1970; Ojeda and McCann, 1973; Schwartz and Justo, 1977); whereas, serum prolactin levels decrease in both male and female rats following castration (Meites et al., 1972). This rapid selective increase in serum LH makes the acutely castrated male rat a suitable model in which to measure catecholamine metabolism for the purpose of comparison with changes observed during stimulatory steroid feedback in female rats (Experiment I).

B. Materials and Methods

Male Sprague-Dawley rats (Spartan Farms, Haslett, MI), weighing 225-250 g, were maintained in climate-controlled rooms for at least 4 days before the onset of experimentation. Three groups of 16 rats were orchidectomized at 48, 24, or 6 h before decapitation. One group of 16 rats was sham-operated 6 h before decapitation to eliminate the possible influence of surgical stress on catecholamine metabolism and serum LH levels. In sham-operated animals, the testes were externalized, then replaced into the scrotum.

Both operations required the same length of time to complete (3-5 min).

To estimate catecholamine turnover, each group of rats received an i.p. injection of either αmpt or 0.89% NaCl (its vehicle) 1 h before decapitation (see Materials and Methods, Section I). At the time of decapitation (1500 to 1700 h) blood was collected for assay of serum LH and the hypothalamus was dissected. Hypothalamic catecholamines were assayed by the method of Ben-Jonathan and Porter (1976; Appendix B) and are expressed as ng DA or NE per g wet weight. The significance of difference among group means was determined by ANOVA and Student-Neuman Keuls test (Sokal and Rohlf, 1969).

C. Results

Serum LH increased from 7 ± 3 ng/ml serum in sham castrates to 53 ± 14 ng/ml serum by 6 h post-castration (Figure 8). By 24 and 48 h post-castration, serum LH levels were greater than 400 ng/ml. Hypothalamic NE concentration did not change at 6 or 24 h, but was slightly elevated by 48 h post-castration. Hypothalamic NE turnover doubled within 6 h after castration ($P < 0.05$), then returned to sham castration levels by 48 h. A large increase in hypothalamic DA concentration occurred by 6 h post-castration ($P < 0.05$) and returned to sham castrate levels by 24 and 48 h. DA turnover as estimated, by the percent depletion of the

Figure 8. Effect of orchidectomy on concentration and alpha-methyl-para-tyrosine induced depletion of hypothalamic norepinephrine and dopamine and serum LH concentration.

The solid bars indicate steady state concentration and hatched bars indicate amine concentration 1 h after i.p. injection of αmpt (N=8). The numbers above the sets of bars indicate percentage depletion of amines induced by αmpt. The stars above the solid bars indicate a significant difference ($P < 0.05$) in amine concentration between that group and the sham castrated group. Stars above the numbers indicate a significant interaction between time after castration and αmpt treatment as analyzed by Two Way ANOVA ($P < 0.05$). Luteinizing hormone (LH) concentrations (lower panel) are from sera of non-drug treated animals. Stars above the points indicate a significant increase ($P < 0.05$) when compared to sham castrated control rats. The verticle line in all 3 panels represents 1 SEM.

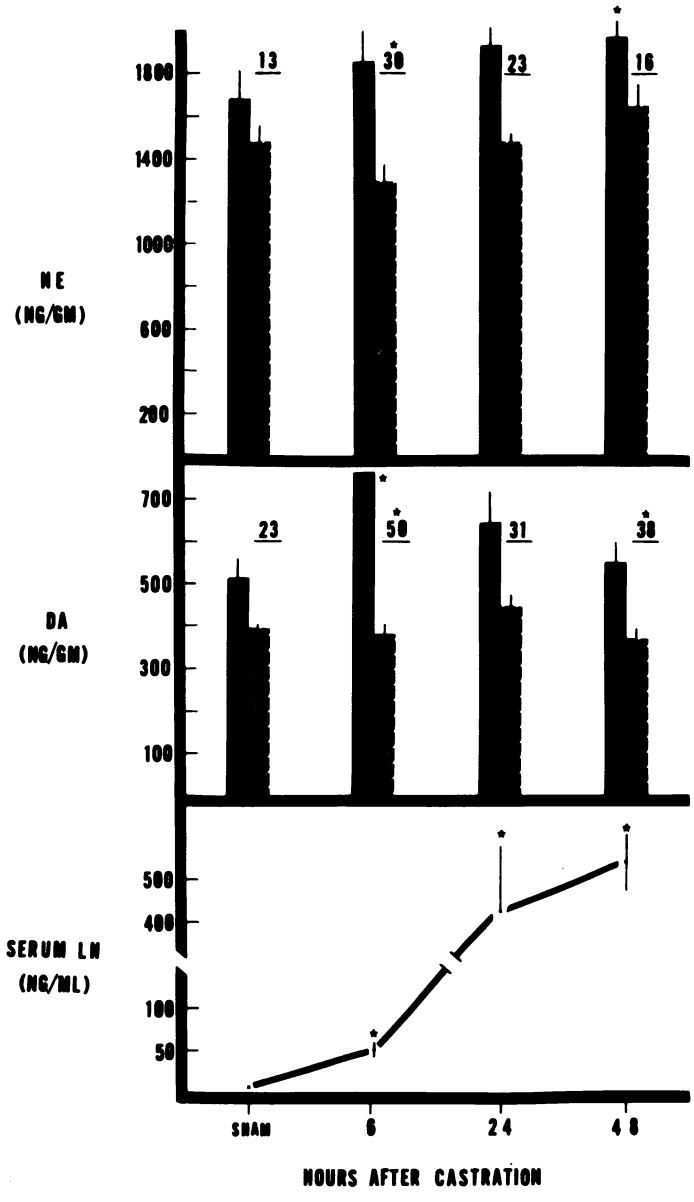

NE
(NG/GM)
1800
1400
1000
600
200
13
30
23
16
DA
(NG/GM)
700
500
300
100
23
50
31
38
SERUM LH
(NG/ML)
500
400
100
50
SHAM
6
24
48
HOURS AFTER CASTRATION

Figure 8

monoamine induced by αmpt, doubled by 6 h ($P < 0.05$) and was elevated at 24 (non-significant) and 48 h ($P < 0.05$) post-castration.

D. Discussion

The rapid doubling in NE turnover, and rise in serum LH concentration, following orchidectomy in male rats is in marked contrast to the very slow changes in both parameters which occurs in female rats following ovariectomy. A 2-3 fold increase in NE turnover occurs by 6 (but not 2) days after ovariectomy in whole brain (Anton-Tay and Wurtman, 1968) and hypothalamus (Coppola, 1969) in rats. This long latent period correlates with the relatively long (3-4 days) post-castration period needed for a significant increase in serum LH in female rats (Gay and Midgley, 1969; Yamamoto et al., 1970). The transient nature of this increase in NE metabolism in the males following orchidectomy also is in contrast to the long term increase in whole brain and hypothalamic NE turnover seen in ovariectomized rats (Anton-Tay and Wurtman, 1968; Coppola, 1969). The LH secretion rate remains high in both male and female rats indefinitely even though NE turnover returns to precastration levels by 48 h in males and remains elevated in females.

The noradrenergic system appears to be important in the acute post-castration rise in serum LH. Alpha-mpt can block the post-castration LH rise if given by 2 h after castration;

and αmpt and the dopamine-β-hydroxylase inhibitor, DDC, are equally effective in decreasing serum LH when administered 18 h after castration (Ojeda and McCann, 1973). Similarly, treatment with the alpha adrenergic receptor blocker, phenoxybenzamine, but not the β-receptor blocker, pronethalol, was as effective as αmpt or DDC in decreasing serum LH when administered 18 h post-castration.

The effects of orchidectomy on DA turnover at 6 h post-castration is difficult to assess in view of the large increase in steady state concentration of DA at that sampling time. The increased hypothalamic DA concentration may indicate an increase in DA synthesis or a decrease in DA release. The large decrease in DA following treatment with αmpt and the increased steady state concentration, suggests that DA synthesis and release is accelerated at 6 h. At 24 h post-castration, DA concentration and depletion are slightly, but not significantly elevated. The increased depletion of DA without a change in steady state concentration indicated an increased DA turnover at 48 h post-castration. This increased DA turnover in the hypothalamus appears to persist at least 14 days after castration in male hypothalamus (Hodson, Simpkins and Meites, unpublished).

Hypothalamic dopaminergic systems do not appear to play a role in the post-castration rise in serum LH. Treatment with the DA receptor blocker, pimozide (Janssen et al., 1968),

or the DA receptor agonist, piribedil (Corrodi et al., 1973; Miller and Iverson, 1973), had no effect on serum LH after castration (Ojeda and McCann, 1973; Grandison and Hodson, unpublished). Further blockade of NE synthesis is as effective as blockade of both NE and DA synthesis in decreasing the post-castration rise in LH (Ojeda and McCann, 1973).

The depressed pituitary and serum prolactin observed after castration in both male and female rats (Meites et al., 1972), may be due to the increased DA turnover which appears to follow castration. Dopaminergic agonists have been shown to inhibit both release and synthesis of prolactin (Meites, 1972; MacLeod, 1976). The differential response of prolactin to gonadal steroids in female rats (Experiment I), and orchidectomy in male rats, may be due to the observed decrease in DA turnover in the former study and the apparent increase in DA turnover in the present study since NE turnover increased transiently in both studies.

VI. Effects of Medial Basal Hypothalamic Implants of 6-Hydroxydopamine on the Post-Castration Increase in Serum Luteinizing Hormone

A. Objectives

The transient increase in NE turnover which follows castration in male rats appears to be primarily responsible for mediation of the acute post-castration LH increase. However, it is not clear if the transient increase in NE

turnover is able to stimulate sustained LH secretion. It is possible that the increased NE turnover is required to initiate LH secretion in response to castration, since a single administration of 250 mg αmpt/kg body weight can block LH secretion through 24 h post-castration (Ojeda and McCann, 1973). However, it is not known whether blockade of NE synthesis can decrease serum LH when administered after long term castration. A second possible mode of noradrenergic involvement is that the increase in LH secretion can occur independently of noradrenergic input, but the increase in NE turnover hastens the post-castration LH surge.

Preliminary observations and Experiment IV indicate that a 24-h exposure to 6-OH-DA implants can depress median eminence NE concentration without significantly affecting DA concentration. The present study was conducted to determine the affect of medial basal hypothalamus NE depletion with 6-OH-DA on both acute and chronic LH secretion.

B. Materials and Methods

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) were implanted bilaterally with permanent cannulae into the ventromedial nuclei. After 1 wk recovery, chronic jugular cannulae were implanted to allow blood sampling from unrestrained animals. Three days later an "inner" cannula containing 6-OH-DA or no drug (sham) was inserted into the permanent cannulae at 0900 h. One day later,

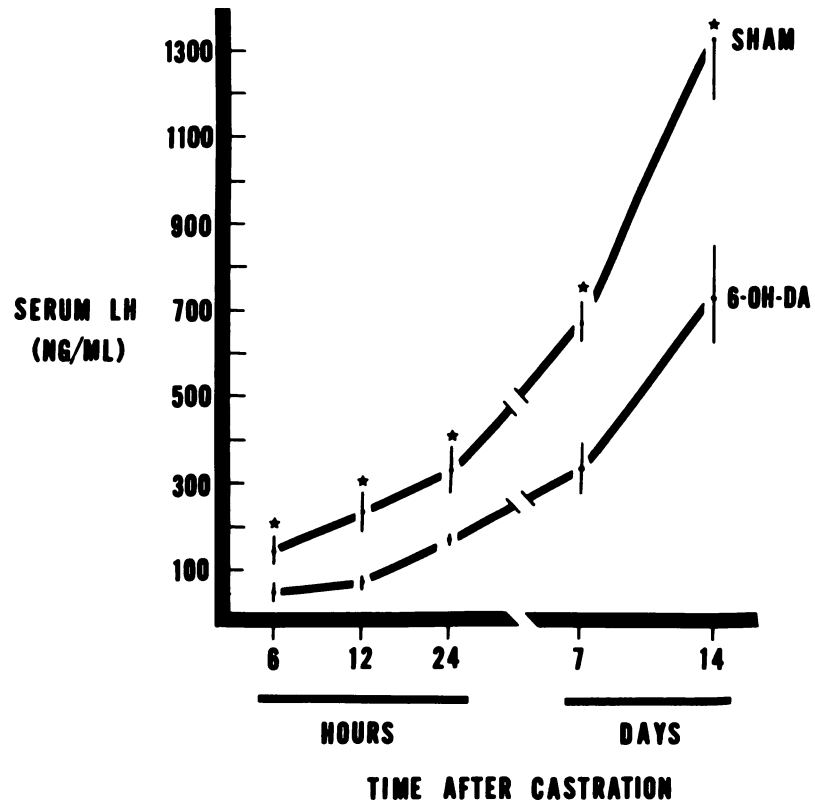


Figure 9. Effect of bilateral ventromedial nuclei (VMN) implants of 6-hydroxydopamine (6-OH-DA) of post-castration rise in serum luteinizing hormone (LH).

6-Hydroxydopamine (6-OH-DA) or empty cannulae (sham) were implanted bilaterally into the ventromedial nuclei (VMN) 24 h before orchidectomy and blood samples were made via chronic jugular cannulae. Each point indicates the means of 7-8 determinations and the verticle bars represent 1 SEM. Stars above points indicate a significant difference ($P < 0.05$) between the drug and sham implanted group at a particular sampling time.

animals were castrated and blood samples were taken 6, 12, 24 h and 7 and 14 days following castration.

C. Results

MBH implants of 6-OH-DA blocked 67±12 and 69±8% of the post-castration rise in serum LH at 6 and 12 h after castration. By 24 h 48±5% inhibition of LH release was observed and persisted through 2 wks post-castration.

D. Discussion

The inability of 6-OH-DA to completely block the post-castration LH increase on the day of and 7 and 14 days after castration may indicate that the LH increase is in part independent of noradrenergic mediation. Halasz et al. (1972) reported that the long term post-castration rise in LH occurs in deafferentated rats and Mitchell and Kalra (1977) recently demonstrated that the depletion of MBH-LHRH which normally follows castration still occurs in deafferentated male rats. Since long term deafferentation destroys noradrenergic nerve terminals in the hypothalamus (Weiner et al., 1972a; Halasz et al., 1972), and since dopaminergic and serotonergic drugs do not appear to alter the post-castration LH increase (Ojeda and McCann, 1973), these data may indicate that LHRH neurons themselves are sensitive to circulating levels of testosterone. Thus ME lesions, which destroy LHRH neurons, are much more effective in blocking the

post-castration LH increase than is deafferentation on 6-OH-DA treatment (Davidson and Sawyer, 1961; Turner and Simpkins, 1977).

An alternative explanation for these data is that 6-OH-DA does not completely destroy the noradrenergic input to the MBH and thus a noradrenergic mediated LH increase can still occur. We have observed that by 2 wks after bilateral VMN implants of 6-OH-DA, MBH-NE is depleted by about 70% (Mallard, Simpkins and Meites, unpublished observations). The remaining NE is probably confined to glial cells and adrenergic nerve terminals of the hypothalamic vascularization (Brownstein et al., 1976) and thus is not involved in the regulation of LH secretion. However, Uretsky et al. (1971) observed that following intraventricular administration of 6-OH-DA, NE turnover increases in surviving noradrenergic neurons. Thus, it is possible that some noradrenergic nerve terminals remain functional following 6-OH-DA implantation and mediate part of the post-castration LH increase.

VII. Measurement of Concentration and Turnover of Brain Dopamine, Norepinephrine and Serotonin and Serum Luteinizing Hormone, Follicle Stimulating Hormone and Prolactin in Young and Old Male Rats

A. Objectives

Aging female rats pass through a series of abnormal reproductive states characterized by irregular estrous cycles,

constant estrus, irregular pseudopregnancies and anestrus (Aschheim, 1961; Clemens et al., 1969; Clemens and Meites, 1971; Huang and Meites, 1975). In general, old female rats show less capacity to secrete LH and FSH and a higher level of prolactin secretion under a variety of experimental conditions. After ovariectomy or estrogen-progesterone treatment of ovariectomized rats, old females release less LH and FSH than young females (Shaar et al., 1975; Watkins et al., 1975; Huang et al., 1976; Meites and Huang, 1976).

Aging male rats also show a gradual decline in fertility (Adams, 1972) and have lower serum LH, FSH and testosterone (Shaar et al., 1975; Bruni et al., 1976) and higher prolactin (Bruni et al., 1976) than young males. In response to castration or ether stress, old male rats release less LH and FSH than young males (Huang et al., 1976; Riegle and Meites, 1976). Thyroid function in old male rats appears to be depressed as indicated by lower circulating T_4, lower thyroid weight and accumulation of colloidal material in the thyroid. Old male rats release less TSH and T_4 in response to low ambient temperature, and exhibit a less pronounced decrease in serum TSH in response to stress (Simpkins et al., 1977; Huang, Chen and Meites, unpublished).

The influence of the autonomic nervous system on the control of anterior pituitary function in young animals is now well established (see section I-V of Literature Review).

The first experimental evidence for a role of the autonomic nervous system in the decreased endocrine function of aging was provided by the demonstration that drugs which stimulate the catecholamine system or inhibit serotonin metabolism could initiate regular estrous cycles in old constant estrous and regular cycling rats (Clemens et al., 1971; Quadri et al., 1973). In aging male mice, NE and DA metabolism appears to be depressed in several brain regions (Finch, 1973). The present study was undertaken to determine if central catechol- and indoleamine metabolism changed in aging male rats and to assess the importance of these changes in terms of their possible influence on anterior pituitary function.

B. Materials and Methods

Study 1

Male Wistar rats 3-4 and 21 mo old (Harlan Industries, Indianapolis, IN) were maintained in climate controlled rooms (see general Materials and Methods) and given Purina Rat Chow and water ad libitum supplemented with whole wheat bread and oranges for at least 2 wks prior to experimentation. To measure basal hormone levels and concentration and turnover of catecholamines, animals received 250 mg αmpt/kg body weight or 0.89% NaCl 1 h before decapitation. At the time of decapitation (0800-0930 h) trunk blood was collected and brains were quickly removed and immersed in ice cold saline.

The MBH was dissected as previously described. The MBH used in this study corresponded roughly to the intermediate basal hypothalamic layer described by Kavanagh and Weisz (1973). The remaining hypothalamus and olfactory tubercles were also dissected and immediately frozen on dry ice.

DA and NE were assayed by the method of Coyle and Henry (1973); see Appendix A) and are expressed as ng/mg protein for MBH ng/g wet weight for remaining hypothalamus and olfactory tubercles. Statistical analysis of data was done by ANOVA and Student-Neuman-Keuls tests. A significant interaction between age and αmpt treatment was taken as evidence for a change in turnover rate. The level of significance chosen was $P < 0.05$.

Study 2

In a second study, 3-4 and 21 mo old male Wistar rats (Harlan Industries, Indianapolis, IN) were injected i.p. with 75 mg pargyline HCl/kg body weight or its vehicle 0.89% NaCl. After 30 min, animals were killed by decapitation and trunk blood was collected. Brains were quickly removed from the cranium immersed in ice cold saline, and the hypothalamus was dissected (as described in general Materials and Methods). The cerebellum was separated from the rest of the brain and discarded. Brain tissues were frozen until assayed for serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA).

Brain and hypothalamic 5HT and brain 5HIAA were assayed according to the method of Curzon and Green (1970) as modified by Hyyppä et al. (1973). The recovery of brain and hypothalamic 5HT was 100% and of brain 5HIAA was 43%. The assay for 5HIAA was not sensitive enough to measure hypothalamic levels. The tissue concentration of 5HT and 5HIAA are expressed as ng/g wet weight. The weights of tissues and the body weights of animals used in both studies are shown in Table 4.

Table 4. Average Body Weights and Weights of Brain Tissue Used for Catecholamine (Study 1) and Serotonin (Study 2) Determinations

	Study 1		Study 2	
	Young	Old	Young	Old
MBH (g protein)	36.1±4.6	32.6±4.1	---*	---
Hypothalamus (mg)	40 ±2	44 ±2	45±1	
Olfactory Tubercles (mg)	14 ±1	15 ±1	---	---
Brain (g)	---	---	1.71±0.02	1.87±0.07
Body weight (g)	391 ±9	906 ±48	405 ±5	834 ±47

*Dashes indicate that the tissue was not assayed.

C. Results

Dopamine concentration in the MBH was significantly lower in old than in young males (53±8 vs 90 ±9 ng/mg protein,

respectively, Figure 10). One h after αmpt treatment, DA decreased by 23±4% in old rats and 37±6% in young rats ($P < 0.05$), suggesting a greater turnover of DA in the young rats. In the remaining hypothalamus, DA content was significantly lower in old than young males. After treatment with αmpt, the percentage of DA depletion was the same in both old and young rats (Figure 11). In the olfactory tubercles, DA concentration before and depletion after αmpt treatment were the same in young and old rats (Figure 12).

Norepinephrine concentration in the MBH was significantly lower in old than in young male rats (Figure 10). Treatment with αmpt had no significant effect on NE content in either age group. In the remaining hypothalamus (Figure 11) NE concentration was 33% lower in old than in young males. Treatment with αmpt resulted in a NE decrease of 24±8% in old rats and 48±3% in young rats ($P < 0.05$), suggesting a greater turnover of NE in the young rats.

Hypothalamic and brain concentrations of 5HT were the same in young and old males. By 30 min after treatment with 75 mg pargyline/kg (Figure 13), hypothalamic 5HT rose 34±5% in old and 20±3% in young males (P 0.05). The increase in brain 5HT after pargyline treatment was the same in both young and old males (Figure 14). Brain 5HIAA levels were 25% higher in old than in young animals ($P < 0.05$). After monoamine oxidase inhibition with pargyline, the decrease in 5HIAA was significantly greater in young than in old rats.

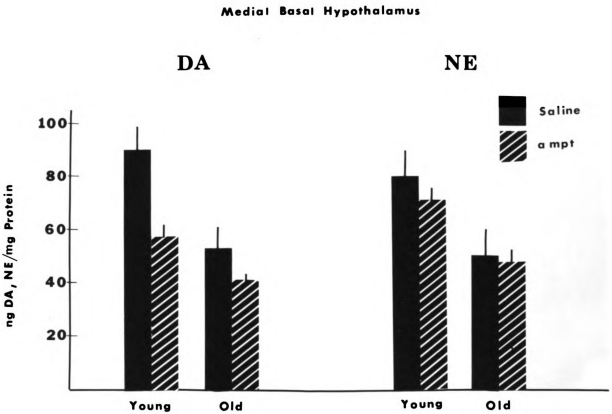


Figure 10. Medial basal hypothalamus (MBH) dopamine and norepinephrine concentration and levels 1 h after alpha-methyl-para-tyrosine treatment in 3-4 and 21 month old male rats.

Rats received a single i.p. injection of 250 mg αmpt/kg body weight, or saline, 1 h before decapitation. Solid bars indicate steady state concentration and hatched bars indicate levels 1 h after αmpt treatment for 6-8 determinations. Verticle lines indicate 1 SEM.

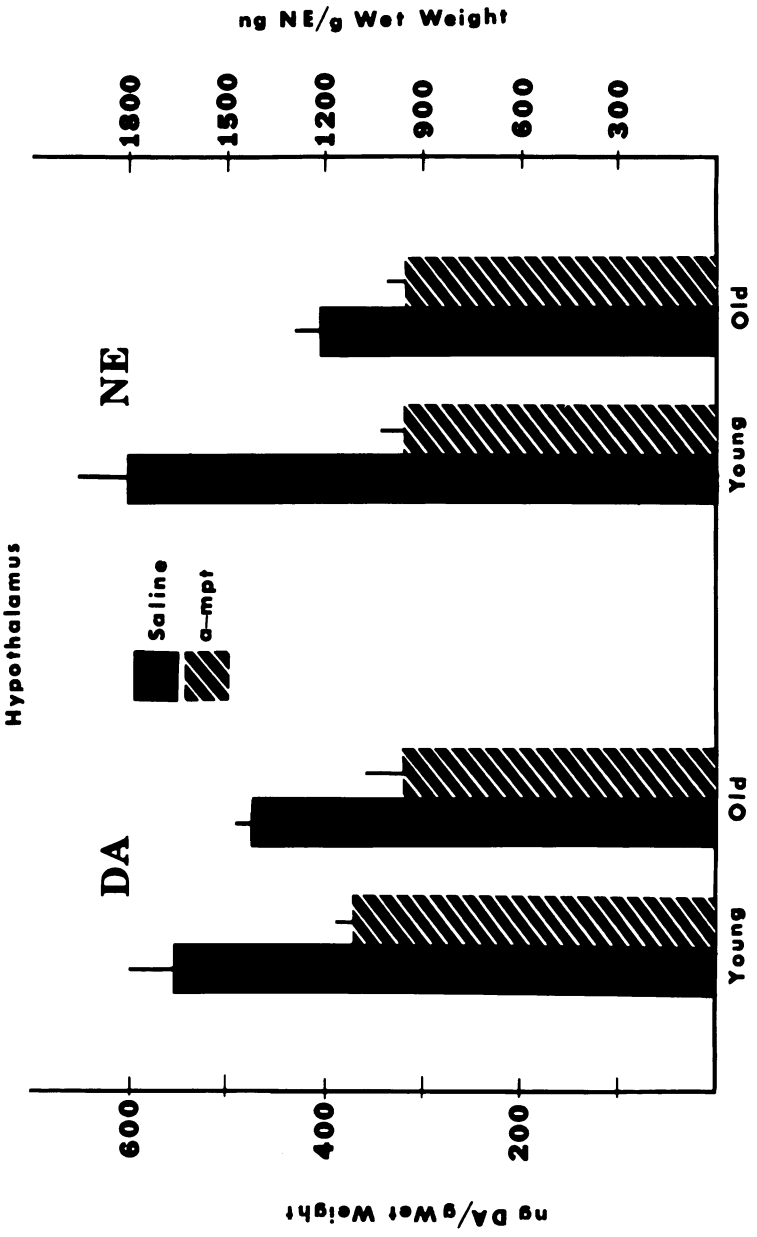


Figure 11. Remaining hypothalamus dopamine and norepinephrine concentration and levels 1 h after alpha-methyl-para-tyrosine treatment in 3-4 and 21 month old male rats. See Figure 10 for explanation.

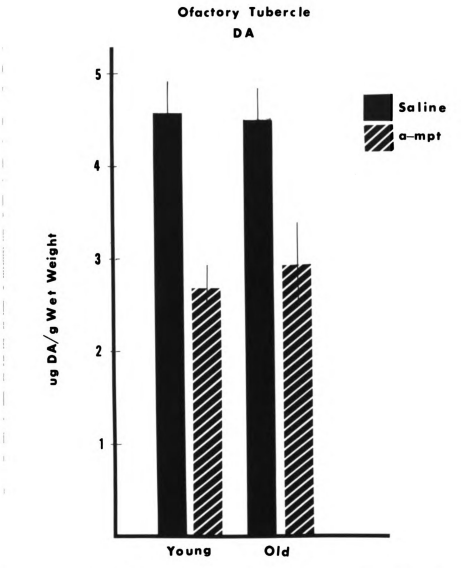


Figure 12. Olfactory tubercle dopamine concentration and levels 1 h after alpha-methyl-para-tyrosine in 3-4 and 21 month old male rats. See Figure 10 for explanation.

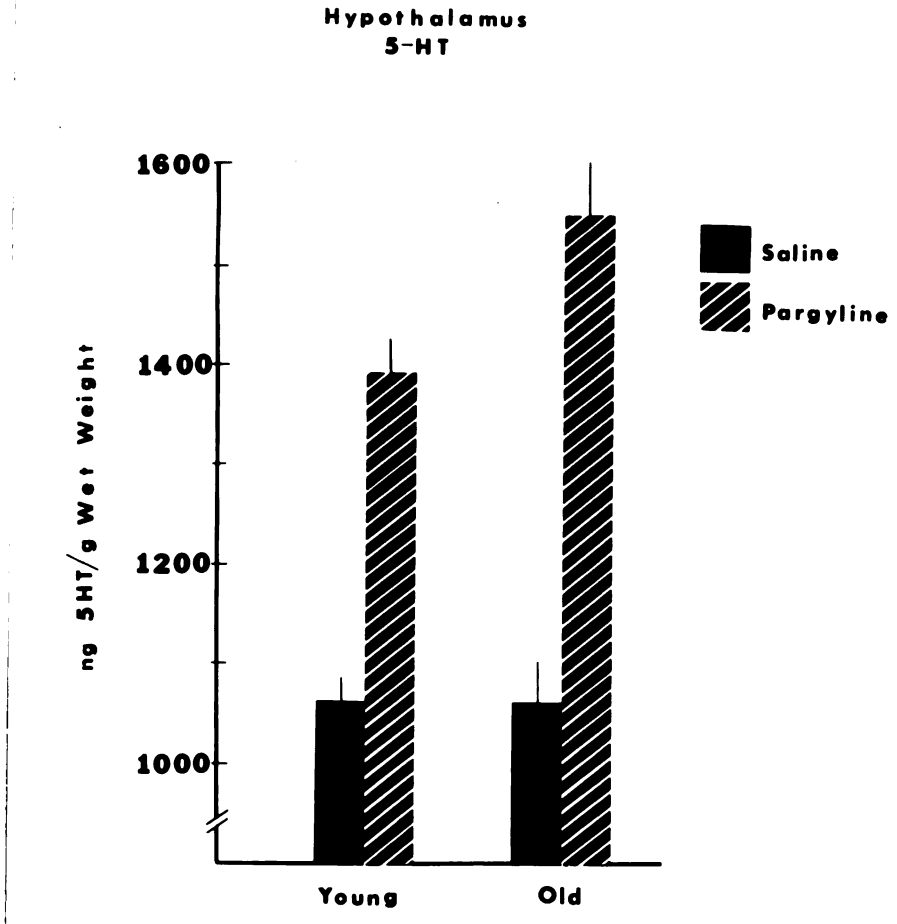


Figure 13. Hypothalamic serotonin (5HT) concentration and levels 30 min after treatment with pargyline in 3-4 and 21 month old male rats.

Rats received a single i.p. injection of 75 mg pargyline HCl/kg body weight or saline, 30 min before decapitation. Solid bars indicate 5HT steady state concentration and hatched bars indicate levels 1 h after pargyline treatment for 7-10 determinations. Verticle lines indicate 1 SEM.

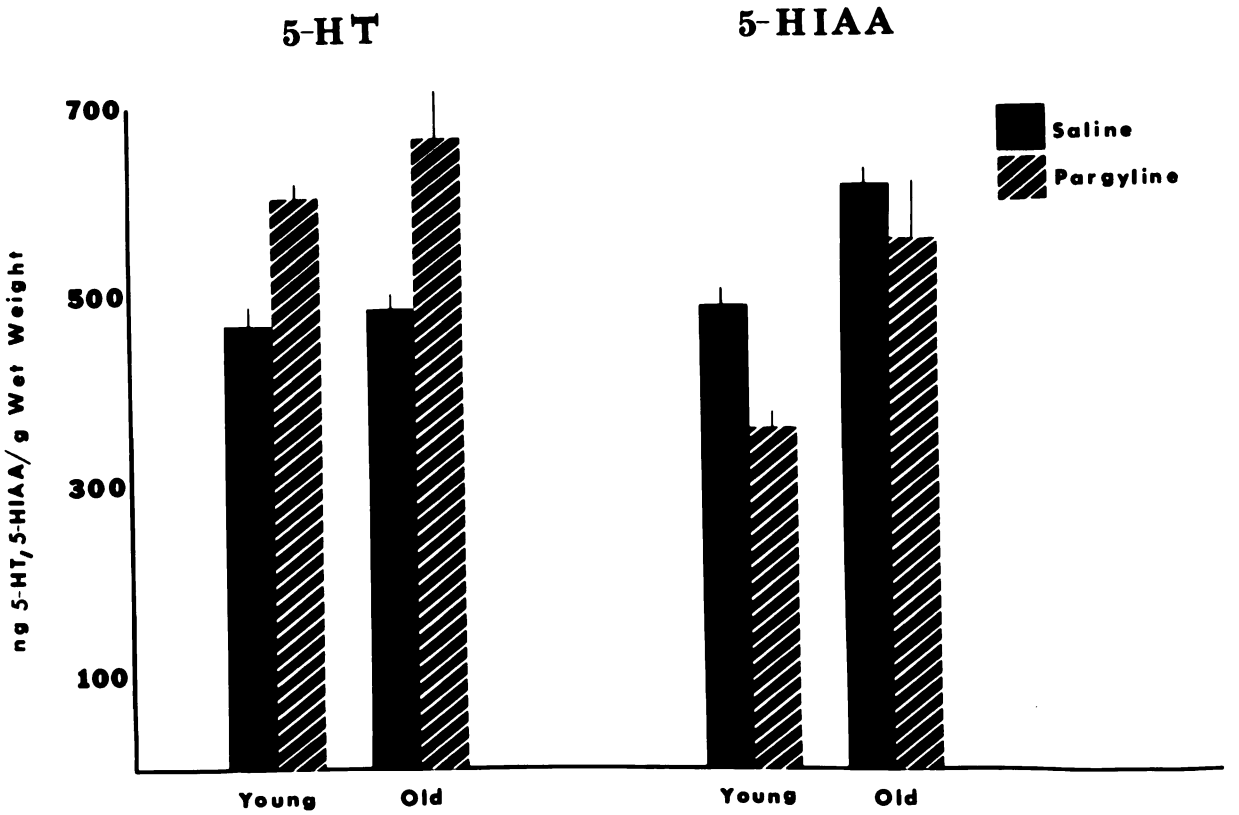


Figure 14. Brain serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) concentration and levels 30 min after treatment with pargyline in 3-4 and 21 month old male rats. See Figure 13 for explanation.

Table 4 shows that the average body weight of the old male rats was much greater than that of the young rats. The old males showed great abdominal fat deposits. The weights of the different brain regions were similar in the old and young rats. Table 5 shows the hormone levels in non-drug treated young and old males used in the catecholamine and serotonin studies. Serum LH and FSH were significantly lower and serum prolactin was significantly higher in old than in young males.

Table 5. Basal SErum Hormone Levels in 3-4 and 21 Month Old Male Rats

	Study 1†		Study 2†	
	3-4 mon	21 mon	3-4 mon	21 mon
LH (ng/ml)	20 ± 3	6 ± 2*	18 ± 4	9 ± 2*
FSH (ng/ml)	220 ± 10	166 ± 14*	330 ± 26	186 ± 48*
PRL (ng/ml)	10 ± 1	29 ± 8*	15 ± 2	37 ± 8*
TSH (ng/ml)	650 ± 112	445 ± 132	576 ± 92	485 ± 134

† Serum hormone levels (mean ± SEM) from control, non-drug treated animals.

* Indicates a significant difference from respective young controls ($P < 0.05$).

D. Discussion

The tuberoinfundibular DA system, the source of DA in the MBH, appears to be less active in old than in young

males, as indicated by a lower steady state concentration and a smaller DA depletion after αmpt treatment. Since first order kinetics have been established for tuberoinfundibular DA depletion after tyrosine hydroxylase inhibition with αmpt (Gudelsky and Moore, 1976), these differences can be interpreted as indicating a decreased turnover rate of MBH-DA in old male rats.

DA in the remaining hypothalamus appears to be localized in part in the incertohypothalamic DA system (Björlund et al., 1975). Steady state concentration of DA in the hypothalamus was lower in old than in young animals. However, depletion of DA 1 h after αmpt treatment was the same in both old and young animals. One interpretation of these data is that a decrease in cell number occurs with age with no change in activity of the DA system. This is consistent with the observation by Brody (1970) that selective loss of neurons in several brain regions occurs in old animals. DA concentration in the olfactory tubercles and αmpt-induced DA depletion were the same in old and in young males. These data may indicate that the mesolimbic and incertohypothalamic DA systems are less affected by age than the tuberoinfundibular DA systems. In contrast, Finch (1973) observed a decreased conversion of precursors to DA in several brain regions, including the hypothalamus, in old male mice. Thus, age-related changes in DA metabolism in the mouse may be more extensive than in the rat.

Steady state concentration of NE in the MBH and remaining hypothalamus was significantly lower in old than in young males. The decreased depletion of NE 1 h after αmpt treatment in old male rats may indicate a lower turnover rate in these animals. A decreased conversion of precursors to NE has been observed in the hypothalamus of old male mice (Finch, 1973).

The possibility exists that the differences observed in depletion of catecholamines after treatment with αmpt represent a difference in the degree of inhibition of tyrosine hydroxylase due to an increase in the per cent of whole body fat which occurs with age in male rats. This possibility seems unlikely since the age-related differences in catecholamine depletion were selective rather than general. DA depletion was reduced in the MBH, but not in the remaining hypothalamus and olfactory tubercles of old males. If tyrosine hydroxylase were inhibited more in young than in old animals, catecholamine depletion would have been greater in all areas in young males. Similar age-related differences have been observed in old female rats, whose body weight (and presumably per cent of whole body fat) are almost identical to those of young females (Huang and Meites, unpublished).

Hypothalamic and brain concentrations of 5HT were the same in young and old rats. However, after monoamine oxidase inhibition with pargyline, 5HT increased to a

significantly higher level in the hypothalamus of old than in young males. In other brain tissue, the rate at which 5HT increased after pargyline treatment was not significantly different in either age group, suggesting that extra-hypothalamic brain 5HT synthesis does not change with age in the rat. The 25% higher concentration in old males of brain 5HIAA, the major serotonin metabolite, probably does not indicate a higher turnover rate of brain 5HT. The high levels and relatively low depletion rate of 5HIAA in brains of old males may indicate a decreased clearance of this metabolite.

It is possible that the alterations in steady state concentration or metabolism of hypothalamic catechol- and indolesamines may be in part responsible for the observed changes in basal levels of serum prolactin, LH and FSH in old male rats observed in this and in previous studies (Reigle and Meites, 1976; Bruni et al., 1976). The increased circulating levels of serum prolactin may be due to the decreased metabolism of the tuberoinfundibular DA, which is believed to be of prime importance in mediating the inhibitory influence of the hypothalamus on prolactin secretion (Meites et al., 1972; MacLeod, 1976). The increased hypothalamic 5HT turnover also could be involved in the enhanced prolactin secretion, since 5HT has been reported to stimulate prolactin release in several physiological states (Kamberi et al.,

1970b; Lu and Meites, 1973; Mueller et al., 1976).

Except for a few contradictory reports (Cramer and Porter, 1973; Craven and McDonald, 1973), most data indicates a stimulatory role of the central noradrenergic system on LH secretion (see Literature Review, Section V-B, Experiments I-VI). If the decreased NE concentration in the MBH and the decreased NE concentration and turnover in the remaining hypothalamus indicate a decreased stimulation of noradrenergic receptors, the age related changes in hypothalamic NE metabolism may in part account for the decreased circulating levels of LH and FSH. Since most data indicate an inhibitory role of 5HT on gonadotropin secretion (see Literature Review, Section V-D), the increased hypothalamic 5HT turnover in old animals may contribute to the lower circulating levels of LH and FSH.

Other factors also may contribute to the lower LH and FSH levels observed in old male rats. Bruni et al. (1977) observed an age related decrease in the ability of LHRH to increase circulating LH in vivo. However, since these data could result from a decreased self-priming action of LHRH (Castro-Vazquez and McCann, 1976) this decreased LH release in response to LHRH may result from an alteration in the hypothalamus rather than the anterior pituitary. To my knowledge, no study has been published demonstrating an age-related change in the responsiveness of the anterior pituitary to LHRH in vitro.

GENERAL DISCUSSION

The data presented in this thesis provide further evidence for the involvement of central catecholaminergic systems in the secretion of anterior pituitary hormones under a variety of conditions. A transient increase in NE turnover has been observed in the AH following P administration to ovariectomized, estrogen-primed rats as well as in the hypothalamus of male rats after orchidectomy. The 2-fold increase in NE turnover observed in the present studies is similar to that observed by other investigators who measured NE metabolism following chronic stimuli which cause LH secretion (Donoso, 1967; Anton-Tay and Wurtman, 1968; Coppola, 1969; Anton-Tay et al., 1970; Bapna et al., 1971; Beattie et al., 1972; Beattie and Soyka, 1973). Since many laboratories have demonstrated that central NE administration can stimulate; whereas, disruption of NE synthesis and transmission can block LH secretion (see IV-B of Literature Review), the present results indicate that a doubling of norepinephrine turnover is sufficient to cause a large surge in the secretion of LH. This may indicate that the increase in NE turnover is amplified through its effect on LHRH secretion or that the increased NE turnover is only one of

many components involved in stimulating LH secretion.

In female rats, the central noradrenergic system appears to be primarily, if not completely, responsible for mediating the steroid-induced LH surge. Thus, blockade of NE synthesis with αmpt (Experiment II; Kalra et al., 1972) or destruction of noradrenergic nerve terminals with 6-OH-DA (Experiment IV) can completely block the P-induced LH surge in ovariectomized, estrogen-primed rats. These observations agree with previous studies suggesting the importance of the noradrenergic system in the proestrous LH surge and subsequent ovulation (Barraclough and Sawyer, 1957; Brown, 1967; Kordon and Glowinski, 1969; Kalra and McCann, 1973; Advis et al., 1977) and the LH increase following ovariectomy (Schneider and McCann, 1970a).

In male rats, the acute LH rise following orchidectomy appears to be noradrenergic dependent while chronic post-castration LH secretion may be in part independent of noradrenergic mediation. An increase in NE turnover is observed during the day of castration (Experiment V) when αmpt and DDC are able to decrease serum LH (Ojeda and McCann, 1973); and MBH-6-OH-DA implants are more effective in attenuating the post-castration LH increase on the day of castration than 7 or 14 days after castration. The observation that in deafferentiated males, long-term castration results in the normally observed depletion of MBH-LHRH (Mitchell and Kalra,

1977) indicates that LHRH neurons themselves may be sensitive to circulating levels of testosterone and thus in part independent of noradrenergic input. Since MBH-6-OH-DA implantation delays the post-castration LH increase, the acute increase in NE turnover following castration may be involved in hastening the post-castration LH increase.

The observation in old male rats that low circulating LH is associated with significantly lower NE concentration in the MBH and lower concentration and turnover in the remaining hypothalamus is consistent with the proposed stimulatory role of NE in LH secretion. The observation that centrally mediated stimuli are less effective in releasing LH in old than in young male and female rats (Shaar et al., 1975; Huang et al., 1976; Meites and Huang, 1976; Reigle and Meites, 1976), and that drugs which stimulate central catecholaminergic systems can reinitiate cycling in old irregular cycling and constant estrous rats (Clemens et al., 1971; Quadri et al., 1973) indicate that the decrease in function of the central noradrenergic system may be involved in the age-related decline in reproductive function in rats.

Results presented in this thesis do not indicate a major role for NE in the regulation of prolactin secretion. In response to P treatment of ovariectomized, estrogen-primed rats both NE turnover and serum prolactin levels increased. However, orchidectomy stimulated NE turnover in male rats

and ocariectomy is known to enhance NE turnover in female rats (Anton-Tay and Wurtman, 1969; Coppola, 1969); whereas, serum prolactin levels decrease in response to both types of surgery (Meites, 1972). Further in old male rats, hypothalamic NE turnover is lower and serum prolactin is higher than in young males. Although NE may be involved in some aspect of prolactin secretion, it does not appear to be a primary stimulator of prolactin secretion in the experimental models used in the present studies.

The observation by many laboratories that hypothalamic dopaminergic systems tonically inhibit the secretion of prolactin from the AP (See section IV-E of Literature Review) are consistent with data presented in this thesis. The increased prolactin secretion which accompanies the decreased DA turnover in response to P treatment of ovariectomized, estrogen-primed rats can be blocked by treatment with the DA agonist, piribedil. Further, DA turnover increased in response to orchidectomy, a treatment which is known to result in a decrease of prolactin secretion (Meites, 1972). In male rats an age-related decrease in MBH-DA concentration and turnover is accompanied by an increase in serum prolactin levels. Thus, in the experimental model used in these studies, high DA turnover is associated with low serum prolactin while low DA turnover is associated with high serum prolactin.

It has been observed that high circulating levels of prolactin achieved by systemic prolactin injections

(Hökfelt and Fuxé, 1972; Gudelsky et al., 1977), or implantation of prolactin secreting pituitary tumors (Hodson, Simpkins and Meites, unpublished observation), stimulate DA turnover in the tuberoinfundibular system and in the AH. These data are not inconsistent with the present observation of a reciprocal relationship between DA turnover and serum prolactin levels, since the prolactin-induced increase in tuberoinfundibular DA turnover requires a relatively long time to develop (24 h; Gudelsky et al., 1977). The alterations in DA turnover observed in the present studies occurred before prolactin stimulation of DA turnover could be expected. The chronically high circulating levels of prolactin and low DA turnover observed in old male rats may indicate that the ability of prolactin to stimulate tuberoinfundibular DA turnover is deficient in old male rats.

The data presented in this thesis together with previous pharmacological observations indicate that DA does not play a major role in the regulation of LH secretion. DA turnover increased following orchidectomy and decreased following P administration to ovariectomized estrogen-primed rats. Both of these treatments resulted in an increased rate of LH secretion. The P-induced LH surge in ovariectomized, estrogen-primed rats could be blocked by NE depletion with 6-OH-DA, but was not greatly affected by sustained treatment with the DA agonist piribedil. Further DDC, which blocks NE synthesis,

has been shown to be as effective as αmpt which inhibits total catecholamine synthesis, in blocking the P-induced LH surge in ovariectomized, estrogen-primed rats (Kalra and McCann, 1972). Treatment with the DA receptor blocker, pimozide (Ojeda and McCann, 1973) or with the DA receptor stimulator, piribedil (Grandison, Hodson and Meites, unpublished observation), did not affect the post-castration LH increase in male rats. Thus, definitive evidence for a role of DA in the regulation of LH secretion has not been presented.

This thesis has attempted to demonstrate a role for central noradrenergic and dopaminergic systems in the regulation of LH and prolactin secretion by simultaneously measuring changes in catecholamine turnover and hormone secretory rates in a variety of experimental models. It can be concluded from these studies that a positive relationship between NE turnover and LH secretion has been demonstrated; whereas, no such relationship between DA turnover and LH secretion exists. An inverse relationship between DA turnover and prolactin secretion has been demonstrated, but no such relationship between DA turnover and LH secretion is apparent. In old male rats, the decreased NE turnover may be responsible for the reduced secretion of LH; whereas, the lower MBH-DA turnover may be involved in the observed increase in serum prolactin. The increase in hypothalamic serotonin turnover also may contribute to these hormonal changes during aging.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams, C. E.: Aging and reproduction. In: Reproduction in Mammals, 4, Reproductive Patterns, 1972, edited by C. R. Austin and R. V. Short, pp. 127-156, Cambridge University Press.

Adams, J. H., P. M. Daniels, and M. M. L. Prichard: Volume of the infarct in the anterior lobe of the monkey's pituitary gland shortly after stalk section. Nature 198:1205-1206, 1963.

Advis, J. A., J. W. Simpkins, J. Bennett, and J. Meites: Serotonergic control of prolactin release in male rats. Neuroendocrinology (in press, 1977).

Advis, J. P., J. W. Simpkins, C. H. Chen, and J. Meites: Relation of biogenic amines to onset of puberty in the female rat. Endocrinology (in press, 1977).

Ahrén, K., K. Fuxé, L. Hamberger, and T. Hökfelt: Turnover changes in the tuberoinfundibular dopamine neurons during the ovarian cycle of the rat. Endocrinology 88: 1415-1424, 1971.

Aiyer, M. S., G. Fink, and F. Greig. Changes in the sensitivity of the pituitary gland to luteinizing hormone releasing factor during the oestrous cycle of the rat. J. Endocrinol. 60:47-64, 1974a.

Aiyer, M. S., S. A. Chiappa, and G. Fink: A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. J. Endocrinol. 62: 573-583, 1974b.

Alberti, K. G. M. M., S. E. Christensen, J. Iversen, K. Seyer-Hansen, N. J. Christensen, A. Prange-Hanse, K. Lundback, and H. Orskov: Inhibition of insulin secretion by somato-statin. Lancet 2:1299-1301, 1973.

Allen, B. M.: The relation of the pituitary and thyroid glands of Bufo and Rana to iodine and metamorphosia. Biol. Bull. 36:405-417, 1919.

Allen, B. M.: Experiments in the transplantation of the hypophysis of adult Rana pipiens to tadpoles. Science 52:274-276, 1920.

Alousi, A., and N. Weiner: The regulation of norepinephrine synthesis in sympathetic nerves: effect of nerve stimulation, cocaine and catecholamine-releasing agents. Proc. Nat. Acad. Sci., U. S. A. 56:1491-1501, 1966.

Amin, A. H., T. B. B. Crawford, and J. H. Gaddum: Distribution of substance P and 5-hydroxytryptamine in the central nervous system of dog. J. Physiol. (London) 126:596-618, 1954.

Andén, N. E., A. Carlsson, A. Dahlström, N. A. Hillarp and K. Larsson: Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sciences 3:523-530, 1964.

Andén, N. E., A. Dahlström, K. Fuxé, and K. Larsson. Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. Amer. J. Anat. 116:329-334, 1965.

Andén, N. E., A. Dahlström, K. Fuxé, K. Larsson, L. Olson, and U. Ungerstedt. Ascending noradrenaline neurons from the pons and the medulla oblongata. Experientia 22: 44-45, 1966a.

Andén, N. E., A. Dahlström, K. Fuxé, K. Larsson, L. Olsen, and U. Ungerstedt. Ascending monoamine neurons to the telencephalon and diencaphalon. Acta. Physiol. Scand. 67:313-326, 1966b.

Andén, N. E., K. Fuxé, B. Hamberger, and T. Hökfelt. A quantitative study of the nigro-neostriatal dopamine neurons system in the rat. Acta. Physiol. Scand. 67: 306-312, 1966c.

Andén, N. E., H. Corrodi, A. Dahlström, K. Fuxé, and T. Hökfelt: Effects of tyrosine hydroxylase inhibition on the amine levels of central monoamine neurons. Life Sciences 5:561-568, 1966d.

Andén, N. E., H. Corrodi, and K. Fuxé: Turnover studies using synthesis inhibition. In: Metabolism of Amines in the Brain, 1969, edited by G. Hooper, pp. 38-47, Macmillan, London.

Andén, N. E., H. Corrodi, K. Fuxé, B. Hökfelt, T. Hökfelt, C. Rydin and T. Svensson: Evidence for a central noradrenaline receptor stimulation by clonidine. Life Sciences 9:513-523, 1970.

Anderson, E. M., and J. B. Collip: Thyrotropic hormone of the anterior pituitary. Proc. Soc. Exptl. Biol. Med. 30:680-683, 1932.

Antiliff, H. R., M. R. N. Prasad, and R. K. Meyer. Action of prolactin on seminal vesicles of guinea pig. Proc. Soc. Exptl. Biol. Med. 103:77-80, 1960.

Anton-Tay, and R. J. Wurtman. Norepinephrine: turnover in rat brain after gonadectomy. Science 159:1245-1246, 1968.

Anton-Tay, F., S. M. Anton, and R. J. Wurtman: Mechanism of changes in brain norepinephrine metabolism after ovariectomy. Neuroendocrinology 6:265-273, 1970.

Arbuthnott, G. W., T. J. Crow, K. Fuxé, L. Olson, and U. Ungerstedt: Depletion of catecholamines in vivo induced by electrical stimulation of central monoamine pathways. Brain Res. 24:471-483, 1970.

Arimura, A., and A. V. Schally: Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by estrogen. Proc. Soc. Exptl. Biol. Med. 136:290-293, 1971.

Ascher, B.: Uber die funktion der hypophyse. Pflügers Arch. Ges. Physiol. 146:1-146, 1912.

Aschheim, P.: La pseudogestation a repetition chez les rattes sencles. C. R. Acad. Sci. 253:1988-1990, 1961.

Axelrod, J.: Purification and properties of phenylethanolamine-N-methyl-transferase. J. Biol. Chem. 237:1657-1660, 1962.

Axelrod, J., and R. Tomchik: Enzymatic o-methylation of epinephrine and other catechols. J. Biol. Chem. 233: 702-705, 1958.

Axelrod, J., W. Albers, and C. D. Clemente: Distribution of catechol-o-methyl transferase in the nervous system and other tissues. J. Neurochem. 5:68-72, 1959.

Baker, B. L., W. C. Dermody, and J. R. Reel. Localization of luteinizing hormone-releasing hormone in the mammalian hypothalamus. Am. J. Anat. 139:129-136, 1974.

Baker, B. L., W. C. Dermody, and J. R. Reel. Distribution of gonadotropin-releasing hormone in the rat brain as observed with immunocytochemistry. Endocrinology 97:125-135, 1975.

Bapna, J., N. H. Neff, and E. Costa: A method for studying norepinephrine and serotonin metabolism in small regions of rat brain: effect of ovariectomy on amine metabolism in anterior and posterior hypothalamus. Endocrinology 89:1345-1349, 1971.

Bargman, W., and E. Scharrer. The site of origin of the hormones of the posterior pituitary. Amer. Scientist 39: 255-259, 1951.

Barophy, A. L., and J. Harney: Impairments in lactation in the rat following electrolytic lesion of midbrain serotonergic neurons. Fed. Proc. 34(3):220, 1975 (Abstract).

Barraclough, C. A.: Modifications in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. Recent Prog. Horm. Res. 22:503-539, 1966.

Barraclough, C. A., and C. H. Sawyer: Blockade of the release of pituitary ovulating hormone in the rat by chlorpromazine and reserpine: possible mechanism of action. Endocrinology 61:341-351, 1957.

Barraclough, C. A., R. Collu, R. Massa, and L. Martini: Temporal interrelationships between plasma LH, ovarian secretion rates and peripheral plasma progestin concentrations in the rat: effects of nembutal and exogenous gonadotropins. Endocrinology 88:1437-1447, 1971.

Barry, J., and M. P. Dubois: Étude on immunofluorescence des structures hypothalamiques á compétence gonadotrope. Annales D'Endocrinologie 34:735-742, 1973.

Barry, J., and M. P. Dubois: Immunofluorescence study of LRF-producing neurons in the cat and the dog. Neuroendocrinology 18:290-299, 1975.

Beattie, C. W., C. H. Rodgers, and L. F. Soyka: Influence of ovariectomy and ovarian steroids on hypothalamic tyrosine hydroxylase activity. Endocrinology 91: 276-279, 1972.

Beattie, C. W., and L. F. Soyka: Influence of progestational steroids on hypothalamic tyrosine hydroxylase activity in vitro. Endocrinology 1453-1455, 1973.

Ben-David, M., A. Davon, R. Benveniste, C. P. Weller, and F. G. Sulman: Results of radioimmunoassays of rat pituitary and serum prolactin after adrenalectomy and perphinazine treatment in rats. J. Endocrinol. 50: 599-606, 1971.

Ben-Jonathan, N., and J. C. Porter: A sensitive radioenzymatic assay for dopamine, norepinephrine and epinephrine in plasma and tissue. Endocrinology 98:1497-1507, 1976.

Besser, G. M., L. Parke, C. R. W. Edwards, I. A. Forsyth, and A. S. McNeilly: Galactorrhea: successful treatment with reduction of plasma prolactin by brain-ergocryptine. Br. Med. J. 3:669-672, 1972.

Bishop, W., P. S. Kalra, C. P. Fawcett, L. Krulich, and S. M. McCann: The effects of hypothalamic lesions on the release of gonadotropins and prolactin in response to estrogen and progesterone treatment in female rats. Endocrinology 91:1404-1410, 1972.

Bjorklund, A., O. Linduall, and A. Noblin: Evidence of an incertohypothalamic dopamine neurons system in the rat. Brain Res. 89:29-42, 1975.

Blackwell, R. W., and M. S. Amoss, Jr.: A sex difference in the rate of rise of plasma LH in rats following gonadectomy. Proc. Soc. Expt. Biol. Med. 136:11-14, 1971.

Blake, C. A.: Effects of intravenous infusion of catecholamines on rat plasma luteinizing hormone and prolactin concentrations. Endocrinology 98:99-104, 1976.

Blake, C. A., R. I. Weiner, R. A. Gorski, and C. H. Sawyer: Secretion of pituitary luteinizing hormone and follicle stimulating hormone in female rats made persistently estrous or diestrous by hypothalamic deafferentation. Endocrinology 90:855-871, 1972.

Block, G. J., J. Masken, C. L. Kragt, and W. F. Ganong: Effect of testosterone on plasma LH in male rats of various ages. Endocrinology 94:947-951, 1974.

Blumberg, W. E., M. Goldstein, E. Lauber, and J. Peisach: Magnetic resonance studies on the mechanism of the enzymic β-hydroxylation of 3,4-hydroxyphenylethylamine. Biochem. Biophys. Acta. 99:188-190, 1965.

Bogdanove, E. M.: Direct gonad-pituitary feedback: an analysis of effects of intracranial estrogenic depots on gonadotrophin secretion. Endocrinology 73:696-712, 1963.

Bogdanove, E. M.: The role of the brain in the regulation of pituitary gonadotropin secretion. Vitamines and Hormones 22:205-260, 1964.

Boler, J., F. Enzmann, K. Folkers, C. Y. Bowers, and A. V. Schally: The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyro-glutamyl-histidyl-proline amide. Biochem. Biophys. Res. Commun. 37:705-710, 1969.

Boot, L. M., O. Muhlbock, and G. Ropcke: Prolactin and the induction of mammary tumors in mice. Gen. Comp. Endocrinol. 2:601-602, 1962.

Boris, A., J. Milmore and T. Trual: Some effects of haloperidol on reproductive organs in the female rat. Endocrinology 86:429-431, 1970.

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

Brodie, B. B., E. Costa, A. Dlabac, N. H. Neff, and H. W. Smookler: Application of steady state kinetics to the synthesis rate and turnover time of tissue catecholamines. J. Pharmacol. 154:493-498, 1966.

Brody, H.: Structural changes in the aging nervous system. Interdisciplinary Topics in Gerontology 7:9-21, 1970.

Brown, P. S.: Antigonadotrophic effects of α-methyltyrosine, methylsergide and reserpine. Nature 214:1268-1269, 1967.

Brown-Grant, K.: The induction of ovulation by ovarian steroids in the adult rat. J. Endocrinol. 43:553-562, 1969.

Brown-Grant, K., D. Exley, and F. Naftolin: Peripheral plasma oestradiol and luteinizing hormone concentrations during the oestrous cycle of the rat. J. Endocrinol. 48:295-296, 1970.

Brown-Grant, K., and F. Naftolin: Facilitation of luteinizing hormone secretion in the female rat by progesterone. J. Endocrinol. 53:37-46, 1972.

Brownstein, M. J., M. Palkovits, J. M. Saavedra, and J. S. Kizer: Distribution of hypothalamic hormones and neurotransmitters within the diencephalon. In: Frontiers in Neuroendocrinology, 1976, edited by L. Martini and W. F. Ganong, pp. 1-23, Vol. 4, Raven Press, New York.

Brownstein, M. J., A. Arimura, A. V. Schally, M. Palkovits, and J. S. Kizer: The effect of surgical isolation of the hypothalamus on its luteinizing hormone-releasing hormone content. Endocrinology 98:662-665, 1976.

Bruni, J. F., S. Marshall, H. H. Huang, H. J. Chen, and J. Meites: Serum prolactin, LH and FSH in old and young male rats. IRCS Medical Science 4:265, 1976.

Bruni, J. F., H. H. Huang, S. Marshall, and J. Meites: Effects of single and multiple injections of synthetic GnRH on serum LH, FSH and testosterone in young and old male rats. Biol. Reproduction (in press, 1977).

Burgus, R., T. F. Dunn, D. N. Ward, W. Vale, M. Amoss, and R. Guillemin: Dérivés polypeptidiques de synthése doues d'activité hypophysiotrope TRF. Comp. Rend. 268:2116-2118, 1969.

Cahane, M., and T. Cahane: Sur certaines medifications des glandes endocrines aprés endocrines aprés une lesion diencephalique. Rev. Franc. Endocrinol. 14:472-487, 1936.

Caligaris, L., J. J. Astrada, and S. Taleisnik. Biphasic effect of progesterone on the release of gonadotropins in the rat. Endocrinology 89:331-337, 1971.

Caligaris, L., and S. Taleisnik: Involvement of neurons containing 5-hydroxytryptamine in the mechanism of prolactin release induced by oestrogen. J. Endorcrinol. 62:25-33, 1974.

Califaris, L., J. J. Astrada, and S. Taleinsnik: Oestrogen and progesterone influence on the release of prolactin in ovariectomized rats. J. Endocrinol. 60:205-215, 1974.

Camus, J., and G. Roussy: Experimental researches on the pituitary body. Endocrinology 4:507-522, 1920.

Carlsson, A., B. Falck, and N. A. Hillarp: Cellular localization of brain monoamines. Acta Physiol. Scand. 56, Suppl. 196:1-28, 1962.

Carlsson, A., W. Kehr, M. Linquist, T. Magnusson, and C. V. Atack: Regulation of monoamine metabolism in the central nervous system. Pharmacol. Rev. 24:371-384, 1972a.

Carlsson, A., J. N. Davis, W. Kehr, M. Lindquist, and C. V. Atack: Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. Naunyn. Schmiedeberg's Arch. Pharmacol. 275:153-168, 1972b.

Carrer, H. F., and S. Taleisnik: Neural pathways associated with the measencephalic inhibitory influence on gonadotropin secretion. Brain Res. 38:299-313, 1972.

Carrer, H. F., and Taleisnik: Effect of mesencephalic stimulation on the release of gonadotropins. J. Endocrinol. 48:527-539, 1970.

Castro-Vazquez, A., and S. M. McCann: Cyclic variations in the increased responsiveness of the pituitary to luteinizing hormone-releasing hormone (LHRH) induced by LHRH. Endocrinology 97:13-19, 1975.

Chen, C. L., and J. Meites: Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Endocrinology 86:503-505, 1970.

Chen, C. L., Y. Amenomori, K. H. Lu, J. L. Voogt, and J. Meites: Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendocrinology 6:220-227, 1970.

Chen, H. J., and J. Meites: Effect of THR and somatostatin (SRIF) on TSH and prolactin release at high and low temperature. Fed. Proc. 34:342, 1975a (Abstract).

Chen, H. J., and J. Meites: Effect of biogenic amines and TRH on release of prolactin and TSH in the rat. Endocrinology 96:10-14, 1975b.

Chowers, I., and S. M. McCann: Comparison of the effect of hypothalamic and pituitary implants of estrogen and testosterone on reproductive system and adrenal of female rats. Proc. Soc. Expt. Biol. Med. 124:260-266, 1967.

Clemens, J. A.: Neuropharmacological aspects of the neural control of prolactin secretion. In: Hypothalamus and Endocrine Functions, 1975, edited by F. Labrie, J. Meites, and G. Pelletier, pp. 283-302, Plenum Press, New York.

Clemens, J. A., and J. Meites: Neuroendocrine status of old constant estrous rats. Neuroendocrinology 7:247-256, 1971.

Clemens, J. A., Y. Amenomori, T. Jenkins, and J. Meites: Effects of hypothalamic stimulation, hormones, and drugs on ovarian function in old female rats. Proc. Soc. Exptl. Bio. Med. 132:561-563, 1969.

Clemens, J. A., C. J. Shaar, J. W. Kleber, and W. A. Tandy: Areas of the brain stimulatory to LH and FSH secretion. Endocrinology 88:180-184, 1971.

Clemens, J. A., E. B. Smalstig, and B. D. Sawyer: Antipsychotic drugs stimulate prolactin release. Psychopharacologia 40:123-127, 1974.

Clemens, J. A., B. D. Sawyer, and B. Cerimele: Further evidence that serotonin is a neurotransmitter involved in the control of prolactin secretion. Endocrinology 100:692-698, 1977.

Coen, C. W., and P. C. B. MacKinnon: Serotonin involvement in oestrogen-induced luteinizing hormone release in ovariectomized rats. Proc. Soc. Expt. Biol. Med. 145: 49P-50P, 1976.

Convey, E. M., H. A. Tucker, V. G. Smith, and J. Zolman: Bovine prolactin, growth hormone, thyroxine, and corticoid response to thyrotropin-releasing hormone. Endocrinology 92:471-476, 1973.

Cooper, J. R., F. E. Bloom, and R. H. Roth: Catecholamines. In: The Biochemical Basis of Neuropharmacology, 1971, pp. 80-141, Oxford University Press, London.

Coppola, J. A.: Turnover of hypothalamic catecholamines during various states of gonadotropin secretion. Neuroendocrinology 5:75-80, 1969.

Carrodi, H., and L. C. F. Hansen: Central Effects of an Inhibitor of Tyrosine Hydroxylation. Psychopharmacologia 10:116-125, 1966.

Carrodi, H., K. Fuxe, T. Hokfelt, P. Lidbrink, and U. Ungersted: Effect of ergot drugs on central catecholamine neurons: evidence for stimulation of central dopamine neurons. J. Pharm. Pharmacol. 25:407-411, 1973.

Costa, E., and N. H. Neff: In: Biochemistry and Pharmacology of the Basal Ganglia, 1966, edited by E. Costa, L. T. Cote, and M. D. Yahr, pp. 141-155, Raven Press, New York.

Costa, E.: Simple neuronal models to estimate turnover rate of noradrenergic transmitters in vivo. Advan. Biochem. Psychopharmacol. 2:169-204, 1970.

Coyle, J. T., and D. Henry: Catecholamines in fetal and newborn rat brain. J. Neurochemistry 21:61-67, 1973.

Coyle, J. T., and S. Snyder: Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different areas. J. Pharmacol. Exp. Ther. 170:221-231, 1969.

Cramer, O. M., and C. A. Barraclough: Effects of preoptic electrical stimulation on pituitary LH release following interruption of components of the preoptico-tuberal pathway in rats. Endocrinology 93:369-376, 1973.

Cramer, O. M., and J. C. Porter: Input to releasing factor cells. Progr. Brain Res. 39:73-85, 1973.

Craven, R. P., and P. G. McDonald: The effect of intrahypothalamic infusions of dopamine and noradrenaline on ovulation in reserpine-treated female rats. J. Endocrinol. 58:319-326, 1973.

Crowe, S. J., H. Cushing, and J. Homens: Experimental hypophysectomy. Bull. Johns Hopkins Hosp. 21:127-169, 1910.

Cuello, A. C., R. Hiley, and L. L. Iverson: Use of catechol-o-methyl transferase for the enzyme radiochemical assay of dopamine. J. Neurochem. 21:1337-1340, 1973.

Dahlström, A., and K. Fuxé: Localization of monoamine in the lower brain stem. Experientia 20:398-399, 1964.

Dahlström, A., and K. Fuxé: Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. 62, Suppl. 232:1-55, 1964.

Dahlström, A., K. Fuxé, and N. A. Hillarp: "Site of action of reserpine." Acta Pharmacol. 22:277-292, 1965.

Dauman, W., R. Gordon, S. Spector, A. Sjoerdsma and S. Udenfriend: Increased synthesis of catecholamines in the intact rat following administration of α-adrenergic blocking drugs. Mol. Pharmacol. 4:457-464, 1968.

Daly, J., K. Fuxé, and G. Jonsson: Effects of intracerebral injections of 5,6-dihydroxytryptamine on central monoamine neurons: Evidence for selective degeneration of central 5-hydroxytryptamine neurons. Brain Research 49:476-482, 1973.

Davidson, E. R., and C. H. Sawyer: Evidence for an hypothalamic focus of inhibition of gonadotropin by androgen in the male. Proc. Soc. Expt. Biol. Med. 107:4-7, 1961.

Davidson, J. M.: Feedback regulation of gonadotropin secretion. In: Frontiers in Neuroendocrinology, 1969, edited by W. F. Ganong, and L. Martini, pp. 343-388, Vol. I, Oxford Univer. Press.

Davidson, J. M., S. Feldman, E. R. Smith, and R. F. Weick: Localization of steroid feedback receptors. Excerpta Med. Int. Congr. Ser. No. 184:542-547, 1969.

del Pozo, E., R. Brun del Re, L. Varga, and H. Friesen: The inhibition of prolactin secretion in man by CB-154 (2-Br-α-ergocryptine). J. Clin. Endocrinol. Metab. 35:768-771, 1972.

del Pozo, E., L. Varga, H. Wyss, G. Tolis, H. Friesen, R. Wenner, L. Vetter, and A. Uittwiler: Clinical and hormonal response to bromocriptin (CB-154) in the galactorrhea syndromes. J. Clin. Endocrinol. Metab. 39:18-26, 1974.

Deuben, R. R., and J. Meites: Stimulation of pituitary growth hormone release by hypothalamic extract in vitro. Endocrinology 74:404-414, 1964.

de Wied, D., and W. de Jong: Drug effects and hypothalamic-anterior pituitary function. Annual Rev. Pharmacol. 14:389-411, 1974.

Dey, F. L.: Evidence of hypothalamic control of hypophyseal gonadotropic function in the female guinea pig. Endocrinology 33:75-82, 1943.

Dickerman, S., J. Clark, E. Dickerman, and J. Meites: Effects of haloperidol on serum and pituitary prolactin and on hypothalamic PIF in rats. Neuroendocrinology 9:632-340, 1972.

Dickerman, S., G. Kledzik, M. Gelato, H. J. Chen, and J. Meites: Effects of haloperidol on serum and pituitary prolactin. LH and FSH, and hypothalamic PIF and LRF. Neuroendocrinology 15:10-20, 1974.

Döhler, K. D., and W. Wuttke: Serum LH, FSH, prolactin and progesterone from birth to puberty in female and male rats. Endocrinology 94:1003-1008, 1974.

Döhler, K. D., and W. Wuttke: Changes with age in levels of serum gonadotropins, prolactin, and gonadal steroids in prepubertal male and female rats. Endocrinology 97:898-907, 1975.

Dollery, C. T., and J. L. Reid: Central noradrenergic neurons and the cardiovascular actions of clonidine in the rabbit. Br. J. Pharmac. 47:206-216, 1973.

Donoso, A. O., and F. J. E. Stefano: Sex hormones and concentration of noradrenalin and dopamine in the anterior hypothalamus of castrated rats. Experienta 23:665-666, 1967.

Donoso, A. O., W. Bishop, C. P. Fawcett, L. Krulich, and S. M. McCann: Effects of drugs that modify brain monoamine concentrations on plasma gonadotropin and prolactin levels in the rat. Endocrinology 89:774-784, 1971.

Drouin, J., L. Lagace and F. Labrie: Estradiol-induced increase of the LH responsiveness to LH releasing hormone (LHRH) in rat anterior pituitary cells in culture. Endocrinology 99:1477-1481, 1976.

Drouva, S. A., and R. V. Gallo: Catecholamine involvement in episodic luteinizing hormone release in adult ovariectomized rats. Endocrinology 99:651-659, 1976.

Drouva, S. A., and R. V. Gallo: Further evidence for inhibition of episodic luteinizing hormone release in ovariectomized rats by stimulation of dopamine receptors. Endocrinology 100:792-798, 1977.

DuRuisseau, P., Y. Taché, and R. Collu: Role of neurotransmitters in acute stress indubed changes of GH and PRL plasma levels. 59th Annual Meeting, Endocrine Society, Abstract #155, 1977.

Eskay, R. L., R. S. Mical, and J. C. Porter: Relationship between luteinizing hormone-releasing hormone concentration in hypophyseal portal blood and luteinizing hormone release in intact, castrated, and electrochemically-stimulated rats. Endocrinology 100:263-270, 1977.

Evans, H. M., and J. A. Long: The effect of the anterior lobe of the hypophysis administered intraperitoneally upon growth and maturity and the oestrous cycle of the rat. Anat. Records 21:62-63, 1921.

Evans, H. M., and J. A. Long: Characteristic effects upon growth, oestrus and ovulation induced by intraperitoneal administration of fresh anterior hypophyseal substance. Anat. Records 23:19, 1922.

Evans, H. M., and M. E. Simpson: Hyperplasia of the mammary apparatus of adult virgin females induced by anterior hypophyseal hormones. Proc. Soc. Exptl. Biol. Med. 26:598, 1929.

Everett, J. W.: Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat. Endocrinology 43:389-405, 1948.

Everett, J. W.: Luteotrophic function of autographs of the rat hypophysis. Endocrinology 54:685-690, 1954.

Everett, J. W.: Functional corpora lutea maintained for months by autographs of rat hypophysis. Endocrinology 58:786-796, 1956.

Everett, J. W.: Central neural control of reproductive functions of the adenohypophysis. Physiol. Rev. 44:373-431, 1964.

Ferin, M., A. Tempone, P. Zimmering, and R. L. Vande Wiele: Effect of antibodies to 17 β-estradiol and progesterone on the estrous cycle of the rat. Endocrinology 85:1070-1078, 1969.

Ferin, M., I. Dyrenfurth, S. Cowchock, M. Warren, and R. L. Vande Wiele: Active immunization of 17 β-estradiol and its effects upon the reproductive cycle of the rhesus monkey. Endocrinology 94:765-776, 1974.

Ferrini, R., and G. Miragolin: Selective antagonism of dopamine by apomorphine. Pharmacol. Res. Commun. 4: 347-352, 1972.

Finch, C. E.: Catecholamine metabolism in the brains of aging male mice. Brain Res. 52:261-276, 1973.

Folkers, K., F. Enzmann, J. Boler, C. Y. Bowers, and A. V. Schally: Discovery of modification of the synthetic tripeptide-sequence of the thyrotropin releasing hormone having activity. Biochem. Biophys. Res. Commun. 37:123-126, 1969.

Fortier, C., G. W. Harris, and I. R. McDonald: The effect of pituitary stalk section on the adrenocortical response to stress in the rabbit. J. Physiol. (London) 136:344-363, 1957.

Frantz, A. G., D. L. Kleinberg, and G. L. Noel: Studies on prolactin in man. Recent Prog. Hormone Res. 28:527-590, 1972.

Friedman, S., and S. Kaufman: 3,4-Dihydroxyphenylethylamine β-hydroxylase: a copper protein. J. Biol. Chem. 240: 552-554, 1965.

Fuxé, K.: Cellular localization of monoamines in the median eminence and in infundibular stem of some mammals. Acta Physiol. Scand. 58:383-384, 1963.

Fuxé, K.: Evidence for the existence of monoamine neurons in the central nervous system. III. The monoamine nerve terminal. Z. Zellforsch 65:573-596, 1965a.

Fuxé, K.: Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. 64, Suppl. 247:39-85, 1965b.

Fuxé, K., and T. Hökfelt: Further evidence for the existence of tuberoinfundibular dopamine neurons. Acta Physiol. Scand. 66:245-246, 1966.

Fuxé, K., T. Hökfelt, and O. Nelson: Changes in amine levels of the tuberoinfundibular dopamine neuron system during implantation in rats. Excerpta Med. Int. Congr. Ser. 133:541-542, 1967.

Fuxé, K., T. Hökfelt, and O. Nelson: Castration, sex hormones, and tuberoinfundibular dopamine neurons. Neuroendocrinology 5:107-120, 1969.

Fuxé, K., L. F. Agnati, B. J. Everitt, T. Hökfelt, G. Jonssen, A. Lofström, and W. Wuttke: Role of monoamines in the control of gonadotropin secretion. In: Neuroendocrine Regulation of Fertility, 1976, edited by T. C. A. Kumar, pp. 101-113, Karger, New York.

Fuxé, K., T. Hökfelt, L. Agnati, A. Lofström, B. J. Everitt, O. Johansson, G. Jonsson, W. Wuttke, and M. Goldstein: Role of monoamines in the control of gonadotropin secretion. In: Neuroendocrine Regulation of Fertility, 1976, edited by T. C. A. Kumar, pp. 124-140, Karger, New York.

Fuxé, K., M. Goldstein, T. Hökfelt, L. Freedman, and B. Anagnosti: The effect of hypophysectomy and ACTH treatment on catecholamine synthesizing enzymes in tissue. Acta Pharmacol. Toxicol. 29, Suppl. 4:15, 1971.

Gallo, R. V., J. Robii, and G. P. Moberg: Effect of methylsergide, a blocker of serotonin receptors, on plasma prolactin levels in lactating and ovariectomized rats. Endocrinology 97:1096-1105, 1975.

Ganong, W. F., and D. M. Hume: Absence of stress-induced and "compensatory" adrenal hypertrophy in dogs with hypothalamic lesions. Endocrinology 55:475-483, 1954.

Gay, V. L., and A. R. Midgley, Jr.: Response of adult rats to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology 84:1359-1364, 1969.

Geffen, L. B., B. G. Levett, and R. A. Rush: Immunohistochemical localization of protein components of catecholamine storage vesicles. J. Physiology 204:593-605, 1969.

Gerich, J. E., M. Lorenzi, V. Schneider, J. H. Karan, J. Rivier, R. Guillemin, and P. H. Forsham: Effect of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. N. Engl. J. Med. 291:544-547, 1974.

Glowinski, J., I. Kopin, and J. Axelrod: Metabolism of ^{3}H-norepinephrine in the rat brain. J. Neurochem. 12:25-30, 1965.

Glowinski, J., and J. Axelrod: Effects of drugs on the uptake, release and metabolism of ^{3}H-norepinephrine in the rat brain. J. Pharmacol. Exp. Ther. 149:43-49, 1966.

Godde, H. H., and C. A. Schweilling: Involvement of catecholaminergic and cholinergic mechanisms in the pulsatile release of LH in the long-term ovariectomized rat. Neuroendocrinology 20:212-223, 1976.

Goldman, B. D., I. A. Kamberi, P. K. Suteri, and J. C. Porter: Temperal relationship of progestin secretion, LH release and ovulation in rats. Endocrinology 85: 1137-1143, 1969.

Goldman, H., and L. A. Saperstein: Nature of the hypophysial blood supply in the rat. Endocrinology 71:857-858, 1962.

Goldstein, M.: Inhibition of norepinephrine biosynthesis at the dopamine-β-hydroxylase state. Pharmac. Rev. 18:77-94, 1966.

Goldstein, M., and K. Nakajima: The effect of disulfiram on catecholamine levels in the brain. J. Pharmacol. Expt. Ther. 157:96-102, 1967.

Goldstein, M., B. Anagonste, E. Lauber, and M. R. McKereghan: Inhibition of dopamine-β-hydroxylase by disulfaram. Life Sciences 3:763-767, 1964.

Goldstein, M., A. F. Battista, A. Anagnoste, P. M. Ceasar, K. Fuxé, and T. Hökfelt: The effect of ventromedial tegmental lesions on the disposition and biosynthesis of dopamine and serotonin. In: Advances in Biochemical Psychopharmacology, 1974, edited by E. Costa, G. L. Gessa, and M. Sandler, pp. 45-53, Vol. 10, Raven Press, New York.

Gordon, J. H., and S. Reichlin: Changes in pituitary responsiveness to luteinizing hormone-releasing factor during the rat estrous cycle. Endocrinology 94:974-978, 1974.

Gordon, R., J. V. O. Reid, A. Sjoerdsma, and S. Udenfriend: Increased synthesis of norepinephrine in the rat heart on electrical stimulation of the stellate ganglion. Mol. Pharmacol. 2:610-613, 1966.

Gorski, R. A.: Localization and sexual differentiation of the nervous structures which regulate ovulation. J. Reprod. Fert., Suppl. 1:67-88, 1966.

Gospodarowicz, D., and Legault-Demare: Étude de l'activité biologique in vitro des hormones gonadotropes. Acta Endocrinol. 42:509-513, 1963.

Goy, R. W.: Early hormonal influences on the development of sexual and sex-related behavior. In: The Neurosciences: Second Study Program, 1970, edited by F. O. Schmitt, pp. 196-206, Rockefeller University Press, New York.

Grandison, L., C. Hodson, H. T. Chen, J. Advis, J. Simpkins, and J. Meites: Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology (in press, 1977).

Greeley, G. H., Jr., M. B. Allen, Jr., and V. B. Mahesh. Potentiation of luteinizing release by estradiol at the level of the pituitary. Neuroendocrinology 18:233-241, 1975.

Green, A. I., S. H. Snyder, L. L. Iversen: Separation of catecholamine storing synaptosomes in different regions of rat brain. J. Pharmacol. Exp. Ther. 168:264-271, 1969.

Green, J. D., and G. W. Harris: The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol. 5:136-146, 1947.

Green, J. D., and G. W. Harris: Observation of the hypophysioportal vessels in the living rat. J. Physiol. 108:359-361, 1949.

Greibrokk, T., B. L. Currie, K. N. Johansson, J. J. Hansen, and K. Folkers: Purification of a prolactin inhibiting hormone and the revealing of hormone DOGHIH which inhibits the release of growth hormone. Biochem. Biophys. Res. Commun. 49:704-709, 1974.

Groot, J. de, and G. W. Harris: Hypothalamic control of the anterior pituitary gland and blood lymphocytes. J. Physiol. 111:335-346, 1950.

Gudelsky, G. A., and K. E. Moore: Differential drug effects on dopamine concentration and rates of turnover in the median eminence, olfactory tubercle and corpus striatum. J. Neural Transm. 38:95-105, 1976.

Guillemin, R., and B. Rosenberg. Humoral hypothalamic control of anterior pituitary: A study with combined tissue cultures. Endocrinology 57:599-607, 1955.

Haggendal, J.: Some further aspects on the release of the adrenergic transmitter. In: Bayer Symposium II: New Aspects of Catecholamines, edited by H. T. Schumann, and G. Kroneberg, 1970, pp. 100-109, Springer-Verlay, Berlin.

Halász, B.: The endocrine effects of isolation of the hypothalamus from the rest of the brain. In: Frontiers in Neuroendocrinology, 1969, edited by W. F. Ganong, and L. Martini, pp. 307-342, Vol. 1, Oxford University Press, New York.

Halász, B.: Hypothalamic mechanisms controlling pituitary function. Prog. Brain Res. 28:97-118, 1972.

Halász, B., and L. Pupp: Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. Endocrinology 77:553-562, 1965.

Halász, B., and G. A. Gorski: Gonadotrophic hormone secretion in female rats after partial or total interruption of neural afferents to the medial basal hypothalamus. Endocrinology 80:608-622, 1967.

Harris, G. W.: Blood vessels of rabbit's pituitary gland, and significance of pars and zona tuberalis. J. Anat. 81:343-351, 1947.

Harris, G. W.: Electrical stimulation of the hypothalamus and the mechanism of neural control of the adrenohypophysis. J. Physiol. (London) 107:418-429, 1948a.

Harris, G. W.: Neural control of the pituitary gland. Physiol. Rev. 28:139-179, 1948b.

Harris, G. W.: Neural Control of the Pituitary Gland, 1955, E. Arnold, London.

Harris, G. W.: Humours and hormones. J. Endocrinol. 54: ii-xxiii, 1972.

Harris, G. W., and J. W. Woods: The effect of electrical stimulation of the hypothalamus or pituitary gland on thyroid activity. J. Physiol. (London) 143:246-274, 1958.

Hartman, B. K.: Immunofluorescence of dopamine-β-hydroxylase application of improved methodology to the localization of the peripheral and central noradrenergic nervous system. J. Histochem. Cytochem. 21:312-332, 1973.

Hartman, B. K., and S. Udenfriend: Immunofluorescent localization of dopamine β-hydroxylase in tissues. Mol. Pharmacol. 6:85-94, 1970.

Haterius, H. O.: Studies on a neurohypophyseal mechanism influencing gonadotrophic activity. Cold Spring Harbor Symp. Quant. Biol. 5:280-288, 1937.

Hensey, J. C.: The relation of the nervous system to ovulation and other phenomena of the female reproductive tract. Cold Spring Harbor Symp. Quant. Biol. 5:269-279, 1937.

Hertting, G., and J. Axelrod: Fate of tritiated noradrenaline at the sympathetic nerve-endings. Nature (London) 192: 172-173, 1961.

Héry, M., E. Laplante, and C. Kordon: Role of pituitary sensitivity and adrenal secretion in the effect of serotonin depletion on luteinizing hormone regulation. J. Endocrinol. 67:463-464, 1975.

Héry, M., E. Laplante, and C. Kordon: Participation of serotonin in the phasic release of LH. I. Evidence from pharmacological experiments. Endocrinology 99:496-503, 1976.

Hillarp, N. A.: Studies on the localization of hypothalamic centres controlling the gonadotrophic function of the hypophysis. Acta Endocrinol. 2:11-23, 1949.

Hökfelt, T., and U. Ungerstedt: Electron and fluorescence microscopical studies on the nucleus caudatus putamen of the rat after unilateral lesions of ascending nigro-neostriatal dopamine neurons. Acta Physiol. Scand. 76: 415-426, 1969.

Hökfelt, T., and K. Fuxé: Effects of prolactin and ergot alkaloids on the tubero-infundibular dopamine neurons. Neuroendocrinology 9:100-122, 1972.

Hong, E., and E. C. Pardo: On the pharmacology of 2-(1-piperazinyl) quinoline. J. Pharmacol. Exptl. Ther. 153:259-265, 1966.

Hori, T., M. Ide, and T. Miyake: Ovarian estrogen secretion during the estrous cycle and under the influence of exogenous gonadotropins in rats. Endocrinol. (Jap.) 15:215-222, 1968.

Horn, A. S., J. T. Coyle, and S. H. Snyder: Catecholamine uptake by synaptosomes from rat brain. Structure-activity relationships of drugs with differential effects on dopamine and norepinephrine neurons. Mol. Pharmacol. 7:66-80, 1971.

Hornzkiewicz, O.: Die topische lokalisation und das verhalten ven noradrenalin and dopamin (3-hydroxytyramin) in der substantia nigra des normalen und Parkinson-Kranken Menschen. Wein Klin. Wschr. 75:309-312, 1963.

Houssay, B. A., A. Biasotti, and R. Sammertino: Modifications fonctionelles de l'hypophyse apres les lesions infundibulotuberiennes chez le crapoud. C. R. Lebd. Seanc. Soc. Biol. (Paris) 120:725-727, 1935.

Huang, H. H., and J. Meites: Reproductive capacity of aging female rats. Neuroendocrinology 17:289-295, 1975.

Huang, H. H., S. Marshall, and J. Meites: Capacity of old versus young fhmale rats to secrete LH, FSH, and prolactin. Biol. of Reproduction 13:538-543, 1976.

Huang, H. H., J. W. Simpkins, and J. Meites: Hypothalamic norepinephrine (NE) and dopamine (DA) turnover and relation to LH, FSH and prolactin release in old female rats. 59th Annual Endo. Soc. Meet., 331, 1977.

Hyyppä, M. T., D. P. Cardinali, H. G. Baumgarten, and R. J. Wurtman: Rapid accumulation of H^3-serotonin in brains of rats receiving intraperitoneal H^3-tryptophan: effects of 5,6-dihydroxytryptamine or female sex hormones, J. Neural Trans. 34:111-124, 1973.

Igarashi, M., and S. M. McCann: A hypothalamic follicle stimulating hormone-releasing factor. Endocrinology 78:533-537, 1964.

Iversen, L. L.: The uptake of noradrenaline by the isolated perfused rat heart. Brit. J. Pharmacol. 21:523-537, 1963.

Iversen, L. L.: Role of transmitter uptake mechanisms in symaptic neurotransmission. Brit. J. Pharmacol. 41: 571-591, 1971.

Iversen, L. L.: Uptake mechanisms for neurotransmitter amines. Biochem. Pharmacol. 23:1927-1938, 1974.

Iversen, L. L., and J. Glowinski: Regional studies of catecholamines in the rat brain. II. Rate of turnover in various brain regions. J. Neurochem. 13:671-682, 1966.

Jacobs, L. S., P. J. Snyder, J. F. Wilber, R. D. Utiger, and W. H. Daughaday: Increase in serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. J. Clin. Endocrinol. Metab. 33:996-998, 1971.

Janssen, P. A. J.: The pharmacology of haloperidol. Int. J. Neuropsychiat. 3:suppl. Vol. 1:10-18, 1967.

Janssen, P., C. Niemegers, K. Schellekens, A. Dress, F. Lengests, A. Pinchard, W. Schoper, J. Van Neuten, and F. Verbruggen: Pimozide, a chemically novel, highly potent, and orally long-acting neuroleptic drug. Arzniemittel-Forsch 18:261-287, 1968.

Jenkins, T. W.: Functional Mammalian Neuroanatomy, Lea and Febiger, Philadelphia, 1972.

Johnson, G. A., S. J. Boukma, and E. G. Kim: In vivo inhibition of dopamine-β-hydroxylase by 1-phenyl-3-(2-thiazolyl)-2-thiourea. J. Pharmacol. Expt. Ther. 171:80-87, 1970.

Kalra, P. S., and S. M. McCann: Involvement of catecholamines in feedback mechanisms. Progr. Brain Res. 39:185-197, 1972.

Kalra, P. S., S. P. Kalra, L. Krulich, C. P. Fawcett, and S. M. McCann: Involvement of norepinephrine in transmission of the stimulatory influence of progesterone on gonadotropin release. Endocrinology 90:1168-1176, 1972.

Kalra, S. P., and S. M. McCann: Effect of drugs modifying catecholamine synthesis on LH release induced by preoptic stimulation in the rat. Endocrinology 93:356-362, 1973.

Kalra, S. P., and P. S. Kalra: Temporal interrelationships among circulating levels of estradiol, progesterone and LH during the rat estrous cycle: effects of exogenous progesterone. Endocrinology 95:1711-1718, 1974.

Kalra, S. P., and S. M. McCann: Effects of drugs modifying catecholamine synthesis on plasma LH and ovulation in the rat. Neuroendocrinology 15:79-91, 1974.

Kalra, S. P.: Modification by 6-hydroxydopamine (6-OH-DA) of ovarian steroid feedback action on LH release mechanisms. 57th Annual Endocrine Soc. Meet., p. 100, 1975 (Abstract).

Kalra, S. P.: Tissue levels of luteinizing hormone-releasing hormone in the preoptic area and hypothalamus, and serum concentrations of gonadotropins following anterior hypothalamic deafferentation and estrogen treatment of the female rat. Endocrinology 99:101-107, 1976.

Kalra, S. P., P. S. Kalra, and E. O. Mitchell: Differential response of luteinizing hormone-releasing hormone in the basal hypothalamus and the preoptic area following anterior hypothalamic deafferentation and/or castration in male rats. Endocrinology 100:201-204, 1977.

Kamberi, I. A.: Role of brain neurotransmitters in the secretion of hypothalamo-pituitary-gonadal principles. In: Neuroendocrine Regulation of Fertility, 1976, edited by T. C. Anand Kumar, pp. 141-154, Karger, Basal.

Kamberi, I. A., R. S. Mical, and J. C. Porter: Luteinizing hormone-releasing activity in hypophysial stalk blood and elevation by dopamine. Science 166:388-390, 1969.

Kamberi, I., R. S. Mical, J. C. Porter: Prolactin-inhibiting activity in hypophyseal portal blood and elevation by dopamine. Experientia 26:1150-1151, 1970a.

Kamberi, I. A., R. C. Mical, and J. Porter: Effect of anterior pituitary and intraventricular injection of catecholamines and indoleamines on LH release. Endocrinology 87:1-12, 1970b.

Kamberi, I. A., H. P. G. Schneider, and S. M. McCann: Action of dopamine to induce release of FSH-releasing factor (FRF) from hypothalamic tissue in vitro. Endocrinology 86:278-284, 1970c.

Kamberi, I. A., R. Mical, and J. C. Porter: Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88: 1012-1020, 1971a.

Kamberi, I. A., R. S. Mical, and J. C. Porter: Effects of melatonin and serotonin on the release of FSH and prolactin serotonin on prolactin release. Endocrinology 88:1288-1293, 1971b.

Kamberi, I. A., R. S. Mical, and J. C. Porter: Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on FSH release. Endocrinology 88:1003-1071, 1971c.

Kamberi, I. A.: The role of brain monoamines and pineal indoles in the secretion of gonadotrophins and gonadotrophin-releasing factors. Progr. Brain Res. 39: 261-280, 1973.

Kaufman, S.: Coenzymes and hydroxylases: ascorbate and dopamine-β-hydroxylase; tetrahydropteridines and phenylalamine and tyrosine hydroxylases. Pharmacol. Rev. 18:61-70, 1966.

Kavanagh, A., and J. Weisz: Localization of dopamine and norepinephrine in the medial basal hypothalamus of the rat. Neuroendocrinology 13:201-212, 1973/74

Kehr, W., A. Carlsson, and M. Lindquist: A method for the determination of 3,4-dehydroxyphenylalamine (DOPA) in brain. Naunym-Schmiedeberg' Arch. Pharmacol. 274: 273-280, 1972.

Kizer, J. S., M. Palkovits, and M. J. Brownstein: The projections of the A8, A9, and A10 dopaminergic cell bodies: evidence for a nigral-hypothalamic-median eminence dopamine pathway. Brain Res. 108:363-370, 1976.

Knigge, K. M., and D. E. Scott: Structure and function of the median eminence. Am. J. Anat. 129:223-244, 1970.

Knobil, E.: On the control of gonadotropin secretion in the rhesus monkey. Recent Progr. Horm. Res. 30:1-46, 1974.

Koch, Y., K. H. Lu, and J. Meites. Biphasic effect of catecholamines on pituitary prolactin release in vitro. Endocrinology 87:673-675, 1970.

Koch, Y., G. Goldhaber, I. Fireman, U. Zor, J. Shani, and E. Tai: Suppression of prolactin and thyrotropin secretion in the rat by antiserum to thyrotropin-releasing hormone. Endocrinology 100:1476-1478, 1977.

Kordon, C.: Effects of selective experimental changes in regional hypothalamic monoamine levels on superovulation in the immature rat. Neuroendocrinology 4:129-138, 1969.

Kordon, C., and J. Glowinski: Selective inhibition of superovulation by blockade of dopamine synthesis during the "critical period" in the immature rat. Endocrinology 85:924-931, 1969.

Kordon, C., and J. Glowinski: Role of hypothalamic monoaminergic neurons in the gonadotropin release-regulating mechanism. Neuropharmacology 11:153-162, 1972.

Kordon, C., B. Kerdelhué, E. Pattau, and M. Juticz: Immunocytochemical localization of LHRH in axons and nerve terminals of the rat median eminence. Proc. Soc. Expt. Biol. Med. 147:122-127, 1974a.

Kordon, C., C. A. Blake, J. Terkel, and C. H. Sawyer: Participation of serotonin-containing neurons in the suckling-induced rise in plasma prolactin levels in lactating rats. Neuroendocrinology 13:213-223, 1974b.

Korf, J., R. H. Roth, and G. K. Aghajanian: Alterations in turnover and endogenous levels of norepinephrine in cerebral cortex following electrical stimulation and acute axotomy of cerebral noradrenergic pathways. Eurpo. J. Pharmacol. 23:276-282, 1973a.

Korf, J., G. K. Aghajanian, and R. H. Roth: Stimulation and destruction of the locus coeruleus: opposite effects on 3-methoxy-4-hydroxyphenylglycol sulfate levels in the rat cerebral cortex. Europ J. Pharmacol. 21:305-310, 1973b.

Krey, L. C., W. R. Butler, and E. Knobil: Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. Endocrinology 96:1073-1087, 1975.

Krieg, R. J., and C. H. Sawyer: Effects of intraventricular catecholamines on luteinizing hormone release in ovariectomized steroid primed rats. Endocrinology 99:411-419, 1976.

Krulich, L., A. P. S. Dhariwal, and S. M. McCann: Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology 83:783-790, 1968.

Kuhar, M. J., G. K. Aghajanian, and R. Roth: Tryptophan hydroxylase activity and synaptosomal uptake of serotonin in discrete brain regions after midbrain raphé lesions: correlation with serotonin levels and histochemical fluorescence. Brain Res. 44:165-176, 1972.

Kyringza, R. P., B. B. Saxina, and H. M. Gandy: Specific binding of LHRH to anterior pituitary gland during the estrus cycle in the rat. Fed. Proc. 33:240, 1975 (Abstract).

Labrie, F., G. Pelletier, P. Borgeat, J. Brouin, L. Ferlund, and A. Belanger: Mode of action of hypothalamic regulatory hormones in the adenohypophysis. In: Frontiers in Neuroendocrinology, Vol. 4, 1976, edited by L. Martini and W. F. Ganong, pp. 63-93, Raven Press, New York.

Lal, S., T. L. Sourkes, K. Misseala, and G. Belendkink: Effects of aporphine and emetine alkaloids on central dopaminergic mechanisms in rats. Europ. J. Pharmacol. 20:71-80, 1972.

Langer, S. Z.: The metabolism of ^{3}H-noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens of the rat. J. Physiol. 208:515-546, 1970.

Lawson, D. M., and R. R. Gala: The influence of adrenergic, dopaminergic, cholinergic and serotonergic drugs on plasma prolactin levels in ovariectomized, estrogen-treated rats. Endocrinology 96:313-318, 1975.

Lázaló, F. A., and D. deWeid: Pituitary-adrenal system in rats bearing lesions in the pituitary stalk. Endocrinology 79:547-553, 1966.

LeBlanc, H., G. C. L. Lachelin, S. Abu-Fadil, and S. S. C. Yen: Effects of dopamine infusion on pituitary hormone secretion in humans. J. Clin. Endocrinol. Metab. 43: 668-674, 1976.

Leonardelli, J., J. Barry, and M. P. Dubois: Mise en évidence par immunofluorescence d'on constituent immunologiquement apparenté au LH-RF dans 1-hypothalamus et l'eminence médiane chez les mammiféres. C. R. Acad. Sci. (D) (Paris) 276:2043-2046, 1973.

Levitt, M., S. Spector, A. Sjoerdsma, and S. Udenfriend: Elucidation of the rate limiting step in norepinephrine biosynthesis using the perfused guinea pig heart. J. Pharmacol. Exp. Ther. 148:1-8, 1965.

Levitt, M., J. W. Gibb, J. W. Daly, M. Lipman, and S. Udenfriend: A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition in vivo. Biochem. Pharmacol. 16:1313-1321, 1967.

Lidbrink, P., G. Jonsson, and K. Fuxé: Selective reserpine-resistant accumulation of catecholamines in central dopaminergic neurons after DOPA administration. Brain Res. 67:438-456, 1974.

Liebelt, A. G., and R. A. Liebelt: Effects of a single pituitary isogra on mammary tumorigenesis in mice. Cancer Res. 21:86-91, 1961.

Lippmann, W., and T. A. Pugsley: The effects of butaclamol hydrochloride, a new neuroleptic drug, on catecholamine turnover. Pharmacol. Res. Commun. 7:371-385, 1975.

Lippmann, W., T. Pugsley, and J. Merker: Effect of butaclamol and its enantiomers upon striatal homovanillic acid and adenyl-cyclase of olfactory tubercle in rats. Life Sci. 16:213-224, 1975.

Lisk, R. D., and L. A. Reuter: In vivo progesterone treatment enhances [^{3}H] estradiol retention by neural tissue of the female rat. Endocrinology 100:1652-1658, 1977.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall: protein measurement with phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Lu, K., Y. Koch, and J. Meites: Direct inhibition by ergocornine of pituitary prolactin release. Endocrinology 89:229-233, 1971.

Lu, K. H., and J. Meites: Effects of L-dopa on serum prolactin and PIF in intact and hypophysectomized, pituitary-grafted rats. Endocrinology 91:868-872, 1972.

Lu, K. H., and J. Meites: Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology 93:152-155, 1973.

Lu, K. H., H. T. Chen, H. H. Huang, L. Grandison, S. Marshall and J. Meites: Relation between prolactin and gonadotropin secretion in postpartum lactating rats. J. Endocrinol. 68:241-250, 1976.

MacLeod, R. M.: Regulation of prolactin secretion. In: Frontiers in Neuroendocrinology, Vol. 4, 1976, edited by L. Martini and W. F. Ganong, pp. 169-194, Raven Press, New York.

MacLeod, R. M., and J. E. Lehmeyer: Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinology 94:1077-1985, 1974.

Malven, P. R., and W. R. Hoge: Effect of ergocornine on prolactin secretion by hypophysial homografts. Endocrinology 88:445-449, 1971.

Markee, J. E., C. H. Sawyer, and W. H. Hollinshed. Activation of the anterior hypophysis by electrical stimulation in the rabbit. Endocrinology 38:345-357, 1946.

Martin, J. B., L. Tyrey, J. W. Everett, and R. E. Fellows: Estrogen and progesterone modulation of the pituitary response to LRF in the cyclic rat. Endocrinology 95: 1664-1673, 1974.

Martin, J. B., S. Lal, G. Tolis, and H. Friesen: Inhibition by apomorphine of prolactin secretion in patients with elevated serum prolactin. J. Clin. Endocrinol. Metab. 39:180-182, 1974.

Martin, J. B., J. Audet, and A. Saunders: Effects of somatostatin and hypothalamic ventromedial lesions on GH release induced by morphine. Endocrinology 96:839-847, 1975.

Martin, J. B., S. Reichlin, and G. M. Brown: Clinical Neuroendoendocrinology, 1977, F. A. Davis, Philadelphia, pp. 93-128.

Martinovic, J. V., and S. M. McCann: Effect of lesions in the ventrol noradrenergic tract produced by microinjection of 6-hydroxydopamine on gonadotropin release in the rat. Endocrinology 100:1206-1213, 1977.

Matsuo, H., Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally: Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43:1334-1339, 1971a.

Matsuo, H., A. Arimura, R. M. G. Nair, and A. V. Schally: Synthesis of porcine LH- and FSH-releasing hormone by the solid phase method. Biochem. Biophys. Res. Commun. 45:822-827, 1971b.

Mayer, J.: Glucostatic mechanisms of regulation of food intake. New England J. Med. 249:13-16, 1953.

Mayer, J., and E. A. Arees: Ventromedial glucoreceptor system. Fed. Proc. 27:1345-1348, 1968.

McCann, S. M., S. Taleisnik, and H. M. Friedman: LH-releasing activity in hypothalamic extracts. Proc. Soc. Exp. Biol. Med. 104:432-434, 1960.

McCann, S. M.: Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. In: Handbook of Physiology, Section 7, Endocrinology, Vol. 4, 1974, edited by R. O. Greep and E. B. Astwood, pp. 489-518, American Physiological Soc., Washington, D. C.

McCann, S. M., and R. L. Moss: Putative neurotransmitters involved in discharging gonadotropin-releasing neurohormones and the action of LH-releasing hormone on the CNS. Life Sci. 16:833-852, 1975.

McClure, W. O.: Effect of drugs on axoplasmic transport. Advances in Pharmacology and Chemotherapy 10:185-220, 1972.

McGeer, P. L., and E. G. McGeer: Formation of adrenaline by brain tissue. Biochem. Biophys. Res. Commun. 17: 502-507, 1964.

Meites, J.: Mammary growth and lactation. In: Reproduction in Domestic Animals I, 1959, edited by H. H. Cole and P. T. Cupps, pp. 539-593, Academic Press, New York.

Meites, J.: Maintenance of the mammary lobuloalveolar system in rats after adreno-orchidectomy by prolactin and growth hormone. Endocrinology 76:1220-1223, 1965.

Meites, J.: Control of mammary growth and lactation. In: Neuroendocrinology, Vol. I, 1966, edited by L. Martini and W. F. Ganong, pp. 569-708, Academic Press, New York.

Meites, J.: Assay of prolactin and luleotropic effects. Archives d'Anatomie Microscopique 56, Suppl. 3-4: 401-412, 1967.

Meites, J.: Control of prolactin secretion. Archives d' Microscopique 56, Suppl. 3-4:516-529, 1967.

Meites, J., and C. W. Turner: Effects of estrone on lactogen content in pituitary and blood of male rabbits. Proc. Soc. Expt. Biol. Med. 49:190-192, 1942.

Meites, J., and C. W. Turner: Studies concerning the induction and maintenance of lactation. I. The mechanism controlling the initiation of lactation at parturition. Mo. Agr. Expt. Sta. Res. Bull. 415, 1948.

Meites, J., and C. S. Nicoll: In vivo and in vitro effects of steroids on pituitary prolactin secretion. In: Proc. First Intnl. Cong. Hormonal Steroids, 2, 1965, Academic Press, New York, pp. 307-316.

Meites, J., and J. A. Clemens: Hypothalamic control of prolactin secretion. Vitamins and Hormones 30:165-221, 1972.

Meites, J., and H. H. Huang: Relation of the neuroendocrine system to loss of reproductive function in aging rats. In: Neuroendocrine Regulation of Fertility, Int. Symp. Simila, 1974, Karger, Basel, pp. 246-258, 1976.

Meites, J., C. S. Nicoll, and P. K. Talwalker: Effects of reserpine and serotonin on milk secretion and mammary growth in the rat. Proc. Soc. Expt. Biol. Med. 101: 563-565, 1960.

Meites, J., P. K. Talwalker, and C. S. Nicoll: Initiation of lactation in rats with hypothalamic or cerebral tissue. Proc. Soc. Expt. Biol. Med. 103:298-300, 1960.

Meites, J., P. K. Talwalker, and C. S. Nicoll: Induction of mammary growth and lactation in rabbits with epinephrine, acetylcholine and seroton. Proc. Soc. Expt. Biol. Med. 104:192-194, 1960.

Meites, J., C. S. Nicoll, and P. K. Talwalker. The central nervous system and the secretion and release of prolactin. In: Advances in Neuroendocrinology, 1963, edited by A. V. Nalvandov, pp. 238-277, University of Illinois Press, Urbana, IL.

Meites, J., K. H. Lu, W. Wuttke, C. W. Welsch, H. Nagasawa, and S. K. Quadri: Recent studies on functions and control of prolactin secretion in rats. Recent Prog. Hormone Res. 28:471-526, 1972.

Meites, J., J. Simpkins, J. Bruni, and J. Advis: Role of biogenic amines in control of anterior pituitary hormones. IRCS J. Med. Sci. 5:1-7, 1977.

Meltzer, H., V. S. Feng, and S. Daniels: Biogenic amines and serum prolactin during stress in male rats. Fed. Proc. 35:306, 1976 (Abstract).

Mess, B., F. Fraschini, M. Motta, and L. Martini: The topography of the neurons synthesizing the hypothalamic releasing factors. In: Proc. 2nd Intnl. Cong. Hormonal Steroids, 1966, Exerpta Medica Int. Congr. Ser. No. 132, p. 1004.

Milhaud, G., and J. Glowinski: Métabolisme de la noradrénaline-C^{14} dans le cerveau du rat. C. R. Acad. Sci. (Paris) 256:1033-1035, 1963.

Miller, R. J., and L. L. Iversen: Stimulation of a dopamine-sensitive adenylate cyclose in homogenates of rat striatum by a metabolite of peribedil. Naunym-Schmiedeberg's Arch. Pharmacol. 282:213-216, 1973.

Minaguchi, H., J. A. Clemens, and J. Meites. Changes in pituitary prolactin levels in rats from weaning to adulthood. Endocrinology 82:555-558, 1968.

Mittler, J. C., and J. Meites. In vitro stimulation of pituitary follicle-stimulating hormone release by hypothalamic extract. Proc. Soc. Expt. Biol. Med. 117:309-313, 1964.

Mizuno, H., P. K. Talwalker, and J. Meites: Influence of hormones on tumor growth and plasma prolactin levels in rats bearing a pituitary "Mammotropic" tumor. Cancer Res. 24:1433-1436, 1964.

Moore, K. E., and O. T. Phillipson: Effects of dexamethasone on phenylethanolamine N-methyltransferase and adrenaline in the brains and superior cervical ganglin of adult and neonatal rats. J. Neurochem. 25:289-294, 1975.

Morgan, R., and E. J. Cagan: Acute alcohol intoxication, the disulfiram reaction, and methyl alcohol intoxication. In: The Biology of Alcoholism, Vol. 3, Clinical Pathology, 1974, edited by B. Kissin and H. Begleiter, pp. 163-189, Plenum Press, New York.

Mortimer, C. H., D. Carr, T. Lind, S. R. Bloom, C. N. Mallison, A. V. Schally, W. M. G. Tunbridge, L. Yeomens, D. H. Coy, A. J. Kastin, G. M. Basser, and R. Hall: Effect of growth-hormone release inhibiting hormone on circulating glucagon, insulin, and growth hormone in normal, diabetic acromegali, and hypopituitary patients. Lancet 2:267-701, 1974.

Mueller, G. P., H. J. Chen, and J. Meites. In vivo stimulation of prolactin release in the rat by synthetic TRH. Proc. Soc. Exptl. Biol. Med. 144:613-617, 1973.

Mueller, G. P., C. P. Twohy, H. T. Chen, J. P. Advis, and J. Meites: Effects of L-tryptophan and restraint stress on hypothalamic and brain serotonin turnover and pituitary TSH and prolactin release in rats. Life Sci. 18:715-724, 1976.

Muralidher, K., R. Maneckjee, and N. R. Moudgal: Inhibition of in vivo pituitary release of luteinizing hormone in lactating rats by exogenous prolactin. Endocrinology 100:1137-1142, 1977.

Naftolin, F., K. Brown-Grant, and S. C. Corker: Plasma and pituitary luteinizing hormone and peripheral plasma oestradiol concentrations in normal oestrus cycle of the rat and after experimental manipulation of the cycle. J. Endocrinol. 53:17-30, 1972.

Nagasawa, H., C. L. Chen, J. Meites: Effects of estrogen implants in median eminence on serum and pituitary prolactin levels in the rat. Proc. Soc. Expt. Biol. Med. 132:859-861, 1969.

Nagatsu, T., N. Levitt, and S. Udenfriend: Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910-2917, 1964.

Nata, W. J. H.: Neural associations of the amygdaloid complex in the monkey. Brain 85:505-520, 1962.

Neill, J. D., M. E. Freeman, and S. A. Tillson: Control of the proestrus "surge" of prolactin and LH secretion by estrogens in the rat. Proc. 55th Ann. Meet., Fed. Amer. Soc. Expt. Biol., Chicago, p. 474, 1971. (Abstract).

Neill, J. D., P. M. Plotsky, D. M. Gibbs, and R. C. Tsou: Electrochemical measurement of dopamine in rat pituitary stalk blood: correlation with prolactin levels. 59th Annual Endocrine Soc. Meet., 1977 (Abstract).

Netter, F. H.: Endocrine System, Vol. 4, 1965, Ciba Pharmaceutical Products, Summit, N. J.

Nicoll, C. S., and J. Meites: Estrogen stimulation of prolactin production by rat adenohypophysis in vitro. Endocrinology 70:272-277, 1962.

Nicoll, C. S., and J. Meites: Prolactin secretion in vitro: effects of gonadal and adrenal cortical steroids. Proc. Soc. Exptl. Biol. Med. 117:579-583, 1964.

Nicoll, C. S., and H. A. Bern: On the action of prolactin among vertebrates: is there a common denominator. In: Lactogenic Hormones, 1972, edited by G. E. W. Walstenholme and J. Knight, pp. 299-324, Churchill Livingston, London.

Nikodijevic, B., S. Senoh, J. W. Daly, and C. R. Creveling: Catechol-o-methyltransferase. II. A new class of inhibitors of catechol-o-methyltransferase; 3,5-dihydroxy-4-methoxybenzoic acid and related compounds. J. Pharmacol. Expt. Ther. 174:83-90, 1970.

Niswender, G. D., C. L. Chen, A. R. Midgley, Jr., J. Meites, and S. Ellis: Radioimmunoassay for rat prolactin. Proc. Soc. Expt. Biol. Med. 130:793-797, 1969.

Ochs, S.: Fast transport of materials in mammalian nerve fibers. Science 176:252-260, 1972.

Ojeda, S. R., and S. M. McCann: Evidence for participation of a catecholaminergic mechanism in the post-castration rise in plasma gonadotropins. Neuroendocrinology 12:294-315, 1973.

Ojeda, S. R., P. G. Harms, and S. M. McCann: Effects of blockade of dopaminergic receptors on prolactin and LH release: median eminence and pituitary sites of action. Endocrinology 94:1650-1657, 1974.

Ojeda, S. R., P. C. Harms, and S. M. McCann: Effect of blockade of dopaminergic receptors on prolactin and LH release: median eminence and pituitary sites of action. Endocrinology 94:1650-1657, 1974.

Palka, Y., D. Coger, and V. Critchlow: Effects of medial basal hypothalamus on pituitary-adrenal and pituitary-ovarian functions. Neuroendocrinology 5:333-349, 1969.

Palkovits, M.: Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. 59:449-450, 1973.

Pass, K. A., and J. Meites: Enhanced growth of carcinogen-induced mammary tumors in rats by sulpiride. IRCS J. Med. Sci. 5:241, 1977.

Pasteel, J. L.: Secretion de prolactine par l'hypophyse in culture de tissue. Comp. Rend. Soc. Biol. 253:2140-2142, 1961.

Piacsek, B. E., T. C. Schneider, and V. L. Gay: Sequential study of luteinizing hormone (LH) and "progestin" secretion on the afternoon of proestrus in the rat. Endocrinology 89:39-45, 1971.

Pletscher, A. The impact of monoamine research on drug development. In: Frontiers in Catecholamine Research, 1973, edited by E. Usden and S. H. Snyder, pp. 27-37, Pergamon Press, New York.

Popa, G. T., and U. Fielding: A portal circulation from the pituitary to the hypothalamic region. J. Anat. (London) 54:88-91, 1930.

Porter, J. C., M. F. M. Hines, K. R. Smith, R. L. Repass, and A. J. K. Smith: Quantitative evaluation of local blood flow of the adenohypophysis. Endocrinology 80: 583-598, 1967.

Quadri, S. K., G. S. Kledzik, and J. Meites: Reinitiation of estrous cycles in old constant-estrous rats by central-acting drugs. Neuroendocrinology 11:248-255, 1973.

Ramirez, V. D., R. M. Abrams, and S. M. McCann: Effect of estradiol implants in the hypothalamo-hypophyseal region of the rat on the secretion of luteinizing hormone. Endocrinology 75:243-248, 1964.

Ratner, A., and J. Meites: Depletion of prolactin-inhibiting activity of rat hypothalamus by estradiol or suckling stimulus. Endocrinology 75:377-382, 1964.

Redmond, W. C.: Ovulatory response to brain stimulation or exogenous luteinizing hormone in progesterone-treated rats. Endocrinology 83:1013-1022, 1968.

Reece, R. P., and C. W. Turner: Influence of estrone upon galactin content of male rat pituitary. Proc. Soc. Expt. Biol. Med. 34:402-404, 1936.

Reece, R. P., and C. W. Turner. The lactogenic and thyrotropic hormone content of the ant-rior lobe of the pituitary gland. Mo. Univ. Agr. Expt. Sta. Res. Bull. 266, 1937.

Riegle, G. D., and J. Meites: Effects of aging on LH and prolactin after LHRH, L-DOPA, Methyl-DOPA, and stress in male rat. Proc. Soc. Expt. Bio. Med. 151:507-511, 1976.

Riss, W., H. D. Knapp, and F. Scala: Optic pathways in crytobranchus allegheniensis as revealed by nauta technique. J. Comp. Neurol. 121:31-40, 1963.

Rodriquez, R., J. Rojos-Ramirez, and R. Drucker-Colin. Serotonin-like action of quipazine on the central nervous system. Eur. J. Pharmacol. 24:164-171, 1973.

Roth, R. H., L. Stjarme, and U. S. vonEuker: Acceleration of noradrenaline biosynthesis by nerve stimulation. Life Sci. 5:1071-10 , 1966.

Roth, R. H., J. R. Walters, and G. K. Aghajanian: Effect of impulse flow on the release and synthesis of dopamine in the rat striatum. In: Frontiers in Catecholamine Research, 1973, edited by E. Usdin and S. H. Snyder, Pergamon Press, pp. 567-574, 1973.

Rotsztejn, W. H., J. L. Charli, E. Pattou, J. E. Pelbaum, and C. Kordon: In vitro release of luteinizing hormone-releasing hormone (LHRH) from rat mediobasal hypothalamus: effects of potassium, calcium and dopamine. Endocrinology 99:1663-1666, 1976.

Royce, P. C., and G. Sayer: Corticotropin-releasing activity of a pepsin-labile factor in the hypothalamus. Proc. Soc. Expt. Biol. Med. 98:677-680, 1958.

Rubinstein, L., and C. H. Sawyer: Role of catecholamines in stimulating release of pituitary ovulating hormone(s) in rats. Endocrinology 86:988-995, 1970.

Saavedra, J. M., M. Brownstein, M. Palkovitz, J. S. Kizer, and J. Axelrod: Tyrosine hydroxylase and dopamine-β-hydroxylase: Distribution in individual rat hypothalamic nuclei. J. Neurochem. 23:869-871, 1974a.

Saavedra, J. M., M. Palkovitz, M. Brownstein, and J. Axelrod: Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Res. 77:157-165, 1974b.

Saffran, M., and A. V. Schally. Release of corticotrophin by anterior pituitary tissue in vitro. Canad. J. Biochem. 33:408-415, 1955.

Saffran, M.: Chemistry of hypothalamic hypophysiotropic factors. In: Handbook of Physiology, Vol. 4, 1974, edited by R. A. Greep and E. B. Astwood, pp. 563-586, American Physiology Soc., Washington, D. C.

Sawyer, C. H., J. E. Markee, and B. F. Townsend: Cholinergic and adrenergic components in the neurohumoral control of the release of LH in the rabbit. Endocrinology 44:18-37, 1949.

Sawyer, C. H., and J. Hilliard: Sites of feedback action of estrogen and progesterone. Excerpta Medica Int. Congr. Ser. No. 219:716-721, 1972.

Sawyer, C. H., J. Hilliard, S. Kanematsu, R. Scaramuzzi, and C. A. Blake: Effects of intraventricular infusions of norepinephrine and dopamine on LH release and ovulation in the rabbit. Neuroendocrinology 15:328-337, 1974.

Sawyer, C. H.: First Geoffrey Harris Memorial Lecture. Some recent developments in brain-pituitary-ovarian physiology. Neuroendocrinology 17:97-124, 1975.

Schally, A. V., and C. Y. Bowers: Corticotropin-releasing factor and other hypothalamic peptides. Metab. Clin. Expt. 13:1190-1205, 1964.

Schally, A. V., A. Arimura, A. J. Kastin, H. Marsuo, Y. Baba, T. W. Redding, R. M. G. Nair, L. Debeljuk, and W. F. White: Gonadotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle stimulating hormone. Science 173:1036-1038, 1971.

Schally, A. V., A. Arimura, and A. J. Kastin: Hypothalamic regulatory hormones. Science 179:341-350, 1973.

Schally, A. V., T. W. Redding, A. Arimura, A. Dupont, and G. L. Linthicum: Isolation of gamma-amino butyric acid from pig hypothalami and demonstration of its prolactin release-inhibitory (PIF) activity in vivo and in vitro. Endocrinology 100:618-691, 1977.

Scharrer, E.: The general significance of the neurosecretory cell. Scientia 87:176-182, 1952.

Scharrer, E., and B. Scharrer: Secretory cells with the hypothalamus. Res. Publ. Assoc. Res. Nervous Mental Diseases 20:170-194, 1940.

Scharrer, E., and B. Scharrer: Hormones produced by neurosecretory cells. Recent Progr. Horm. Res. 10:183-232, 1954.

Schneider, H. P. G., and S. M. McCann: Suprachiasmatic LH-releasing factor. Neuroendocrinology 5:271-280, 1969a.

Schneider, H. P. G., and S. M. McCann: Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. Endocrinology 85:121-132, 1969b.

Schneider, H. P. G., and S. M. McCann: Estradiol and the neuroendocrine control of LH release in vitro. Endocrinology 87:330-338, 1970a.

Schneider, H. P. G., and S. M. McCann: Release of LH-releasing factor into the peripheral circulation of hypophysectomized rats by dopamine and its blockade by estradiol. Endocrinology 87:249-253, 1970b.

Schneider, H. P. G., and S. M. McCann: Mono- and indolamines and control of LH secretion. Endocrinology 86:1127-1133, 1970c.

Schwartz, N. B.: A model for the regulation of ovulation in the rat. Recent Progr. Horm. Res. 25:1-55, 1969.

Schwartz, N. B., and S. N. Justo: Acute changes in serum gonadotropins and steroids following orchidectomy in the rat: role of the adrenal gland. Endocrinology 100: 1550-1556, 1977.

Sedvall, G., and I. J. Kopin: Acceleration of norepinephrine synthesis in the rat submaxillary gland in vivo during sympathetic nerve stimulation. Life Sci. 6:45- , 1967.

Sedvall, G. C., V. K. Weise, and I. J. Kopin: The rate of norepinephrine synthesis measured in vivo during short intervals; influence of adrenergic nerve impulse activity. J. Pharmacol. Expt. Ther. 159:274-2 , 1968.

Seki, K., and J. Seki: Successful ovulation and pregnancy achieved by CB154 (2-Br-α-ergocryptine) in a woman with Chiari-Frommel syndrome. J. Clin. Endocrinol. Metab. 38:508-509, 1974.

Seki, K., M. Seki, and T. Okumura: Serum FSH rise induced by CB-154 (2-Br-α-ergocryptine) in postpartum woman. J. Clin. Endocrinol. Metab. 39:184-186, 1974.

Shaar, C. J., and J. A. Clemens: The role of catecholamines in the release of anterior pituitary prolactin in vitro. Endocrinology 95:1202-1212, 1974.

Shaar, C. J., J. S. Euker, G. D. Reigle, and J. Meites: Effect of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. J. Endocrinol. 66:45-51, 1975.

Sheard, M. H., and G. K. Aghajanian: Stimulation of the midbrain raphé: effect on serotonin metabolism. J. Pharmacol. 163:425-430, 1968.

Shibusawa, K., S. Saito, K. Nishi, T. Yamamoto, C. Abe, and T. Kawai: Effects of thyrotropin releasing principle (TRF) after section of the pituitary stalk. Endocrinology (Jap.) 3:151-157, 1956.

Shields, P. J., and D. Eccleston: Effects of electrical stimulation of rat midbrain on 5-hydroxytriptamine synthesis as determined by a sensitive radioisotope method. J. Neurochem. 19:265-272, 1972.

Simpkins, J. W., C. A. Hodson, and J. Meites: Differential effects of stress on TSH release in young and old male rats. Proc. Soc. Expt. Biol. Med. (in press, 1977).

Simpkins, J. W., H. H. Huang, and J. Meites: Effects of positive steroid feedback on hypothalamic norepinephrine and dopamine metabolism in female rats. Fed. Proc. 36:366, 1977 (Abstract).

Skinner, J. E.: Neuroscience: a laboratory manual. In: Saunders Books in Psychology, 1971, edited by R. D. Singer, W. B. Saunders.

Smalstig, E. B., and J. A. Clemens: Pharmacological characterization of rat pituitary receptors mediating the inhibition of prolactin release. Endocrinology Suppl. A-185:259, 1974.

Smalstig, E. B., B. D. Sawyer, and J. A. Clemens: Inhibition of rat prolactin release by apomorphine in vivo and in vitro. Endocrinology 95:123-129, 1974.

Smith, P. E.: Hastening development of female genital system by daily hymoplastic pituitary transplants. Proc. Soc. Expt. Med. 24:131-132, 1926a.

Smith, P. E.: Ablation and transplantation of the hypophyses in the rat. Anat. Records 32:221, 1926b.

Smith, P. E., and I. P. Smith: The effect of intraperitoneal injection of fresh anterior lobe substance in hypophysectomized tadpoles. Anat. Records 23:38-39, 1922.

Smith, E. R., and J. M. Davidson: Location of feedback receptors: effects of intra-cranially implanted steroids on plasma LH and LRF response. Endocrinology 95:1566-1573, 1974.

Sokal, R. R., and F. J. Rohlf: Biometry, 1969, W. H. Freeman, p. 239, San Francisco.

Spector, S., A. Sjoerdsma, and S. Udenfriend: Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. Expt. Ther. 147:86-95, 1965.

Spector, S., R. Gordon, A. Sjoerdsma, and S. Udenfriend: End-product inhibiyion of tyrosine hydroxylase as a possible mechanism for regulation of norepinephrine synthesis. Mol. Pharmacol. 3:549-555, 1967.

Stefano, F. J. E., and A. O. Donoso: Norepinephrine levels in the rat hypothalamus during the estrous cycle. Endocrinology 81:1405-1406, 1967.

Stricker, P., and F. Grueter: Action du lobe anterieur de l'hypophyse sur la montie laiteuse. Compt. Rend. Soc. Biol. 99:1978-1980, 1928.

Takahara, J., A. Arimura, and A. V. Schally: Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophysial portal vessel. Endocrinology 95:462-465, 1974.

Taleisnik, S., M. E. Velasco, and J. J. Astrada: Effect of hypothalamic deafferentation on the control of luteinizing hormone secretion. J. Endocrinol. 46:1-8, 1970.

Talwalker, P. K., and J. Meites: Mammary lobulo-alveolar growth induced by anterior pituitary hormones in adreno-ovariectomized and adreno-ovariectomized-hypophysectomized rats. Proc. Soc. Expt. Biol. Med. 197:880-883, 1961.

Talwalker, P. K., A. Ratner, and J. Meites: In vitro inhibition of prolactin synthesis and release by hypothalamic extract. Symposium on Neuroendocrinology, Miami, Florida, 1961.

Talwalker, P. K., A. Ratner, and J. Meites. In vitro inhibition of pituitary prolactin synthesis and release by hypothalamic extracts. Am. J. Physiol. 205:213-218, 1963.

Terasawa, E., and W. E. Bridson, J. W. Davenport, and R. W. Goy: Role of brain monoamines in release of gonadotropin before proestrus in the cyclic rat. Neuroendocrinology 18:345-358, 1975.

Thoenen, H., and J. P. Tranzer. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. Naunyn-Schmiedebergs Arch. Parakol. 261:271-288, 1968.

Thomas, J. A., L. S. VanOrden III, J. A. Redick, and I. J. Kopin: Immunological localization of dopamine-β-hydroxylase on the chromaffin granule membrain. In: Frontiers in Catecholamine Research, 1973, edited by E. Usdin and S. H. Snyder, pp. 79-81, Pergamon Press, New York.

Tima, L., and B. Flerkó: Ovulation induced by the intraventricular infusion of norepinephrine in rats made anovulatory by neonatal administration of various doses of testosterone. Endokrinologie 66:218-220, 1975.

Turkington, R. W.: Inhibition of prolactin secretion and successful therapy of the Forbes-Albright syndrome with L-DOPA. J. Clin. Endocrinol. Metab. 34:306-311, 1974.

Turner, C. W., and W. U. Gardner: The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agr. Expt. Sta. Res. Bull. 158, 1931.

Turner, J. W., Jr.: Effect of hypothalamic lesions and androgen on plasma LH levels in castrated male gerbils. Endocrinology 92:227-234, 1973.

Turner, J. W., and J. W. Simpkins: Effects of hypothalamic lesions on androgen regulation of plasma LH in the castrate male rat. Neuroendocrinology (in press, 1977).

Udenfriend, S., P. Zaltzman-Nirenberg, and T. Nagatsu: Inhibitors of purified beef adrenal tyrosine hydroxylase. Biochem. Pharmacol. 14:837-845, 1965.

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. Scand. Suppl. 367: 1-48, 1971.

Uretsky, N. J., and L. L. Iverson: Effects of 6-hydroxydopamine on catecholamine containing neurons in the rat brain. J. Neurochem. 17:269-278, 1970.

Uretsky, N. J., M. A. Simmonds and L. L. Iversen: Changes in the retention and metabolism of ^{3}H-l-norepinephrine in rat brain in vivo after 6-hydroxydopamine pretreatment. J. Pharmacol. Expt. Ther. 176:489-496, 1971.

Vale, W., R. Blackwell, G. Grant, and R. Guillemin; TRF and thyroid hormones on prolactin secretion by rat anterior pituitary cells in vitro. Endocrinology 93:26-33, 1973.

Vale, W., C. Rivier, P. Brazeau, and R. Guillemin: Effect of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology 95:668-677, 1974.

Vijayan, E., and S. M. McCann: Possible sites of action of dopamine in control of LH release. Fed. Proc. 36:301, 1977 (Abstract).

Vogt, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123:451-481, 1954.

Voith, K., and F. Herr: The behavorial pharmacology of butaclamol hydrochloride (AY-23,028), a new potent neuroleptic drug. Psychopharmacologia 42:11-20, 1975.

VonVoigtlander, P. F., and K. E. Moore: Nigro-striatal pathway: Stimulation-evoked release of [^{3}H] dopamine from caudate nucleus. Brain Res. 35:580-583, 1971.

Voogt, J. L., C. L. Chen, and J. Meites: Serum and pituitary prolactin levels before, during and after puberty in female rats. Am. J. Physiology 218:396-399, 1970.

Ward, A. A., and W. S. McCullock: The projection of the frontal lobe on the hypothalamus. J. Neurophysiol. 10:309-314, 1947.

Watkins, B. E., J. Meites, and G. D. Riegle: Age-related changes in pituitary responsiveness to LHRH in the female rat. Endocrinology 97:543-548, 1975.

Weick, R. F., E. R. Smith, R. Domingues, A. P. S. Dharival, and J. M. Davidson: Mechanism of stimulatory feedback effect of estradiol benzoate on the pituitary. Endocrinology 88:293-301, 1971.

Weiner, N., and M. Rabadjija: The regulation of norepinephrine synthesis. Effect of puromycin on the accelerated synthesis of norepinephrine associated with nerve stimulation. J. Pharmacol. Expt. Ther. 164:103-110, 1968.

Weiner, R. I., J. E. Shryne, R. A. Gorski, and C. H. Sawyer: Changes in the catecholamine content of the rat hypothalamus following deafferentation. Endocrinology 90:867-873, 1972a.

Weiner, R. I., C. A. Blake, and C. H. Sawyer: Integrated levels of plasma LH and prolactin following hypothalamic deafferentation in the rat. Neuroendocrinology 10: 349-357, 1972b.

Westfall, T. C., and H. Osada: Influence of adrenalectomy on the synthesis of norepinephrine in the rat heart. J. Pharmacol. Expt. Ther. 167:300-308, 1969.

Whitby, L. G., J. Axelrod, and H. Weil-Malherbe: The fate of H^3-norepinephrine in animals. J. Pharmacol. Expt. Ther. 132:193-201, 1961.

Wislocki, G. B., and L. S. King: The permeability of the hypophysis and hypothalamus to vital dyes, with a study of the hypophyseal vascular supply. Am. J. Anat. 58: 421-472, 1926.

Yamamoto, M., N. D. Diebel, and E. M. Bogdanove: Analysis of initial and delayed effects of orchidectomy and ovariectomy on pituitary and serum LH levels in adult and immature rats. Endocrinology 86:1102-1111, 1970.

Yoshinaga, K., R. A. Hawkins, and J. F. Stocker: Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy. Endocrinology 85:103-112, 1969.

Zeballos, G., and S. M. McCann: Alterations during the estrous cycle in the responsiveness of the pituitary to subcutaneous administration of synthetic LH-releasing hormone (LHRH). Endocrinology 96:1377-1385, 1975.

Zeilmaker, G. H.: The biphasic effect of progesterone on ovulation in the rat. Acta Endocrinol. (Kbh) 51:461-468, 1966.

Zigmond, M. J,, and R. J. Wurtman: Daily rhythm in the accumulation of brain catecholamines synthesized from circulation ^{3}H-tyrosine. J. Pharmacol. Expt. Ther. 172:416-422, 1970.

Zimmerman, E. A., K. C. Hsu, F. Ferin, and C. P. Kozlowski: Localization of gonadotropin-releasing hormone (Gn-RH) in the hypothalamus of the mouse by immunoperoxidase technique. Endocrinology 95:1-8, 1974.

Zondek, B., and S. Aschheim: Uber die funktion des ovariums. A. Geburtshilfe Gynaekal 90:372-376, 1926.

Zschaeck, L., and R. J. Wurtman: Brain ^{3}H-catechol synthesis and the vaginal estrous cycle. Neuroendocrinology 11:144-149, 1973.

APPENDICES

APPENDIX A

COYLE AND HENRY CATECHOLAMINE ASSAY PROCEDURE

1. Homogenize pieces of brain tissue in desired volume of 0.4 N perchloric acid (plus 10 mg percent EDTA) using matched glass microhomogenizers (Micrometric Instruments, Cleveland, Ohio).

2. Transfer homogenate to microcentrifuge tubes (Kew Scientific, Inc., Columbus, Ohio) and centrifuge for 45 sec in a microcentrifuge (Coleman Instruments, Oak Brook, Illinois).

3. Transfer 10 µl of supernatant (or of working NE and DA standard solution) to glass culture tubes and add 25 µl of the following mixture:

Reagent	Proportion
20 mM EGTA-Na salt (0.760 gm/100 ml H_2O and pH to 7.2)	1
Pargyline Solution (to 4 mg pargyline add 25 µl β-mercaptoethenol and 225 µl H_2O)	1
1 M Tris base (with 3 mM $MgCl_2$) (to 6.05 gm Tris add 50 ml H_2O) plus 30.5 mg $MgCl_2$)	6.5
S-adenosyl-1-methionine-(Methyl-^{3}H) [11.6 Ci/m mole in Sulfuric acid: ethanol solution (90:10, v:v), pH 1-3]	3.0
Catecholamine-o-methyl transferase (COMT, partially purified by the method of Nikodijevic *et al.*, 1970).	2.5
1 mM sodium phosphate buffer	2.5

4. Incubate for 40 min at 37°C.

5. Add 30 µl of mixture of 5 volumes 0.45 M borate buffer (pH 10.0) and 1.0 volumes of carrier methoxyamine mix prepared as follows:

   add 5.0 ml H_2O to the following salts:
   50 mg 3-methoxytyramine, 50 mg DL-metanephrine,
   50 mg DL-normetanephrine and 5 mg Na-bisulfite

6. Add 500 µl to toluane: isoamyl alcohol solution (3:2, v:v), vortex for 30 sec and centrifuge for 5 min at 3,000 RPM (RC2-B, Sorvall, Dupont Instruments, Newtown, Connecticut).

7. Transfer 400 µl of organic phase to conical centrifuge tubes containing 400 µl borate buffer (pH 10.0), vortex for 30 sec and centrifuge at 5/7 speed in IEC clinical centrifuge (International Equipment Co., Needham Hts., Mass.).

8. Transfer 300 µl of organic phase to conical centrifuge tubes containing 500 µl of 0.1 N HCl, vortex for 30 sec and centrifuge as in step 7.

9. Aspirate organic phase.

10. To remaining aqueous phase add 7 ml toluene: isoamyl alcohol (3:2, v:v), vortex, centrifuge as in step 7 and discard organic phase.

11. To remaining aqueous phase neutralize with 500 µl of 0.5 M sodium phosphate buffer (pH 7.5), add 50 µl of 3% sodium metaperiodate wait 2 min and add 50 µl of 10% glycerol.

12. Add 10 ml toluene, vortex for 30 sec, centrifuge as in step 7.

13. Transfer 9 ml of organic phase to conical centrifuge tubes containing 1 ml of 1 N NaOH for final extraction of NE metabolites. Vortex for 30 sec, centrifuge and discard organic phase. Add 100 µl glacial acetic acid, 10 ml Scintiverse (Fisher Scientific, Livonia, Michigan), and transfer to 20 ml glass scintillation vials for counting.

14. From the remaining aqueous phase of step 12, the residue toluene is aspirated, 500 µl of 1 M borate buffer is added and tubes are vortexed. Add 8 ml of toluene isoamyl alcohol (3:2, v:v), vortex and centrifuge. 0.6 ml of the organic phase is added to 10 ml of Scintiverse in 20 ml glass scintillation vials and counted for dopamine.

APPENDIX B

BEN-JONATHEN AND PORTER CATECHOLAMINE ASSAY PROCEDURES

1. Same as step 1 in Appendix A.

2. Same as step 2 in Appendix A.

3. Transfer 10 µl of supernatant (or of working NE and DA standard solutions) to conical centrifuge tubes and add 25 µl of the following mixture:

Reagent	Proportion
20 mM EGTA-Na salt (0.760 gm/100 ml H_2O and pH to 7.2)	1
Pargyline solution (to 4 mg pargyline add 25 µl β-mercaptoethanol and 225 µl H_2O)	1
1 M Tris base (with 3 mM $MgCl_2$) (to 6.05 gm Tris add 50 ml H_2O plus 30.5 mg $MgCl_2$)	6.6
S-adenosyl methionine (Methyl-^{3}H) (11.6 Ci/m mole diluted 1:3.5 with H_2O)	3.0
Catecholamine-o-methyl transferase (COMT; partially purified by the method of Nikodijevic et al., 1970)	5.1

4. Incubate for 60 min at 37°C.

5. Add 30 µl of 0.45 M borate buffer (pH 10.0) and 5 µl of carrier methoxyamine mix (50 mg 3-methoxytyramine, 50 mg DL-metanephrine and 50 mg DL-normetanephrine; 10 mg of each amine/ml of 0.1 N HCl). Add 500 µl of toluene: isoamyl alcohol (3:2, v:v), vortex for 30 sec, and centrifuge at 5/7 speed on IEC clinical centrifuge (International Equipment Co., Neddham Hts., Mass.).

6. Transfer 400 1 of organic phase to conical centrifuge tubes containing 40 1 of 0.1 N HCl. Vortex for 30 sec and centrifuge as in step 5. Carefully aspirate organic phase.

7. Apply 25 1 of acid phase to LQ-60 silica gel plates previously spotted with 5 1 of carrier methoxyamine mix. Allow plates to dry.

8. Place plates in thin-layer chromatography tanks containing chloroform, ethanol and methylamine (40:18:5 by volume). Allow plates to run 1 1/2 to 2 h and remove from tank to allow for drying.

9. Visualize and outline spots under ultraviolet light.

10. Scrape plates and place scrapings into scintillation vials containing 1.0 ml of ethlacetate, acetic acid and H_2O (3:3:1 by volume) and shake for 30 min. Add 10 ml of Scintiverse and count.

APPENDIX C

RESEARCH PUBLICATIONS

1. Simpkins, J. W., J. F. Bruni, R. J. Mioduszewski and J. Meites. Serum and pituitary TSH and response to TRH in developing male and female rats. Endocrinology 98: 1365-1369, 1976.

2. Mueller, G. P., J. W. Simpkins, J. Meites and K. E. Moore. Differential effects of dopamine agonists and haloperidol on release of prolactin, thyroid stimulating hormone, growth hormone, and luteinizing hormone in rats. Neuroendocrinology 20:121-135, 1976.

3. Grandison, L., J. Advis, C. Hodson, J. Simpkins and J. Meites. Effects of prolactin on postcastration LH release. IRCS Medical Science 4:427, 1976.

4. Simpkins, J. W., G. P. Mueller, H. H. Huang and J. Meites. Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats: possib le relation to gonadotrophin secretion. Endocrinology 100:1672-1678, 1977.

5. Meites, J., J. Simpkins, J. Bruni and J. Advis. Role of biogenic amines in control of anterior pituitary hormones. IRCS Medical Science 5:1-7, 1977.

6. Chen, H. T., J. W. Simpkins, G. P. Mueller and J. Meites. Effects of pargyline on hypothalamic biogenic amines and serum prolactin, LH, and TSH in male rats. Life Sciences 21:533-542, 1977.

7. Gudelsky, G. A., J. W. Simpkins, G. P. Mueller, J. Meites and K. E. Moore. Selective effect of prolactin on dopamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology (in press, 1977).

8. Turner, J. W. and J. W. Simpkins. Effect of hypothalamic lesions on androgen regulation of plasma LH in castrate male rat. Neuroendocrinology (in press, 1977).

9. Advis, J. P., J. W. Simpkins, H. T. Chen and J. Meites. Relation of biogenic amines to onset of puberty in female rats. Endocrinology (in press, 1977).

10. Grandison, L., C. A. Hodson, H. T. Chen, J. P. Advis, J. W. Simpkins and J. Meites. Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology (in press, 1977).

11. Advis, J. P., J. W. Simpkins, J. Bennett and J. Meites. Serotonergic control of prolactin secretion. Submitted to Neuroendocrinology, 1977.

12. Meites, J., H. H. Huang, J. W. Simpkins. Recent studies on neuroendocrine control of reproductive senescence in rats. Gerontology Research (in press, 1977).

13. Hodson, C. A., J. W. Simpkins and J. Meites. Inhibition of LH release and LHRH action by the ovaries of post-partum lactating rats. Submitted to Endocrinology, 1977.

14. Meites, J., K. H. Lu, L. Grandison, C. Hodson and J. Simpkins. Relation of prolactin to gonadotropin secretion during postpartum lactation and after castration, in: International Symposium on Prolactin, 20-23 October, 1977, C. Robyn and M. Harter (eds.), Elsevier/North-Holland (in press).

15. Simpkins, J. W., G. P. Mueller, H. H. Huang and J. Meites. Changes in metabolism of dopamine, norepinephrine and serotonin and relation to gonadotropin secretion in aging male rats. Physiologist 19:368, 1976 (Fall APS meetings).

16. Gudelsky, G. A., J. W. Simpkins, J. Meites and K. E. Moore. Effect of prolactin and haloperidol on dopamine turnover in tuburoinfundibular and nitrostriatal pathways of rat brain (Neuroscience meetings, 1976).

17. Simpkins, J. W., H. H. Huang and J. Meites. Effects of positive steroid feedback on hypothalamic norepinephrine and dopamine metabolism in female rats. Fed. Proc. 36:366, 1977.

18. Advis, J. P., J. W. Simpkins and H. T. Chen. Hypothalamic biogenic amines at onset of puberty in female rats. Fed. Proc. 36:311, 1977.

19. Chen, H. T., J. W. Simpkins, G. P. Mueller and J. Meites. Effects of pargyline on hypothalamic biogenic amines and serum prolactin (PRL), LH and TSH in male rats. Fed. Proc. 36:366, 1977.

20. Hodson, C. A., J. W. Simpkins and J. Meites. Ovarian inhibition of LH release in post-partum lactating rats. Fed. Proc. 36:301, 1977.

21. Bruni, J. F., R. J. Mioduszewski, L. J. Grandison, J. W. Simpkins and J. Meites. Effects of cholinergic and GABA-ergic drugs on serum growth hormone (GH) in male rats. Fed. Proc. 36:323, 1977.

22. Huang, H. H., J. W. Simpkins and J. Meites. Hypothalamic norepinephrine and dopamine turnover and relation to LH, FSH, and prolactin release in old female rats. 59th Endocrine Society meetings, 254, 1977.

23. Advis, J. P., J. W. Simpkins, J. Bennett and J. Meites. Serotonergic control of prolactin release in male rats. 59th Endocrine Society meetings, 331, 1977.

CURRICULUM VITAE

NAME: James William Simpkins

DATE OF BIRTH: September 22, 1948

PLACE OF BIRTH: Port Clinton, Ohio

NATIONALITY: American

SEX: Male

MARITAL STATUS: Married Two children (Chris and Gretchen)

PRESENT ADDRESS: Department of Physiology, Michigan State University, East Lansing, MI 48824

FUTURE ADDRESS: Department of Physiology, University of Florida, Gainesville, FL 32610

EDUCATION:

University of Toledo	1971	BS	Biology
University of Toledo	1974	MS	Biology (C.J.V. Smith)
Michigan State Univ.	1977	Ph.D.	Physiology (J. Meites)

POSITIONS HELD:

Teaching Assistant, Department of Biology, University of Toledo, Toledo, Ohio, September 1971 to June 1974.

Technician, Department of Physiology, Medical College of Ohio at Toledo, Toledo, Ohio, June 1972 to September 1974.

Instructor, Science Department, Lansing Community College, Lansing, Michigan, January 1975 to September, 1977.

Research Assistant, Department of Physiology, Michigan State University, East Lansing, Michigan, September 1974 to October 1977.

MAJOR RESEARCH INTERESTS:

Mechanism of hypothalamic control of anterior pituitary function with particular interest in the role of catechol- and indoleamines in the secretion of pituitary hormones during development, aging, and the feedback of steroids and peptide hormones.

AWARDS:

Outstanding Graduate Student Award from the Graduate Affairs Committee, Department of Physiology, Michigan State University, East Lansing, MI, 1977.