EFFECTS OF BIOGENIC MONOAMINES, ERGOT DRUGS, ESTROGEN AND SYNTHETIC THYROTROPIN-RELEASING, HORMONE ON PITUITARY PROLACTIN RELEASE

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
KUEW-HSIUNG LU
1972

This is to certify that the

thesis entitled

Effects of Biogenic Monoamines, Ergot Drugs, Estrogen and Synthetic Thyrotropin-Releasing Hormone on Pituitary Prolactin Release

presented by

Kuew-Hsiung Lu

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Physiology

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Date October 20, 1972

O-7639

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ABSTRACT

EFFECTS OF BIOGENIC MONOAMINES, ERGOT DRUGS, ESTROGEN AND SYNTHETIC THYROTROPIN-RELEASING HORMONE ON PITUITARY PROLACTIN RELEASE

By

Kuew-Hsiung Lu

1. A single intracarotid or intraperitoneal injection of dopamine, norepinephrine, or epinephrine had no effect on serum prolactin levels in proestrous female rats. The failure of catecholamines given systemically to alter serum prolactin is due to the "blood-brain barrier" which prevents them from reaching the hypothalamus.

2. A single intraperitoneal injection of L-DOPA, the immediate precursor of dopamine, produced a rapid decrease in serum prolactin and an increase in pituitary prolactin concentrations. Monoamine oxidase inhibitors, pargyline, iproniazid and Lilly compound-15641, also significantly reduced serum prolactin. These reductions in serum prolactin by the 4 drugs were associated with increased PIF activity in the hypothalamus. L-DOPA also produced rapid decrease in serum prolactin in hypophysectomized, pituitary-grafted rats.

This was associated with an increase in hypothalamic PIF activity and appearance of PIF in the serum. Methyl-DOPA, d-amphetamine, reserpine, chlorpromazine, and alpha-methyl-para-tyrosine, drugs known to depress hypothalamic catecholamines, all elevated serum prolactin and reduced pituitary prolactin concentrations by increasing pituitary release of prolactin. These results indicate that hypothalamic catecholamines inhibit pituitary prolactin release by increasing PIF release from the hypothalamus. Drugs that decrease catecholamines stimulate prolactin release by decreasing PIF activity.

3. A single intravenous injection of 5-hydroxytryptophan, the immediate precursor of serotonin, or melatonin, a product of serotonin, produced a rapid elevation in serum prolactin in proestrous female rats. 5-Hydroxytryptophan also increased serum prolactin in hypophysectomized, pituitary-grafted rats. Tryptophan produced only a small rise in serum prolactin. Serotonin itself did not significantly alter serum prolactin, presumably because it failed to cross the "blood-brain barrier." These results suggest that serotonin and melatonin in the brain stimulate pituitary release of prolactin. The mechanism of 5-hydroxytryptophan stimulation of prolactin release is unknown. It had no effect on hypothalamic PIF activity.

4. Ergocornine methanesulfonate (ERG) significantly inhibited the stimulatory effects of estradiol benzoate (EB)

on pituitary and serum prolactin concentrations, mammary growth, and increase in anterior pituitary (AP) weight in ovariectomized rats. In hypophysectomized, pituitary-grafted rats EB increased both AP and serum prolactin and stimulated mammary growth, whereas ERG reduced prolactin release from the AP graft and inhibited mammary growth. When given together, ERG partially counteracted EB stimulation of pituitary prolactin secretion and mammary growth. When normal rat AP halves were incubated in vitro, ERG completely inhibited estrogen stimulation of prolactin release and increased pituitary accumulation of prolactin. These results indicate that ERG can inhibit prolactin release by a direct action on the AP, and can counteract the stimulatory effect of estrogen on prolactin secretion.

5. In female rats bearing pituitary "mammotropic" (MtT. W 15) tumor transplants, the size and number of pituitary tumors developed in each rat were closely related to the serum prolactin concentration. A single injection of Lilly compound-55327, an ergot derivative, into these rats produced a highly significant reduction in serum prolactin. Daily injections of ergocornine, and to a lesser extent, ergonovine and Lilly compound-55327, also significantly inhibited pituitary tumor growth. Ergocornine produced a loss of nuclei and disappearance of cells in the tumor tissue. These results suggest that the major action of the ergot drugs was exerted directly on the tumor to inhibit its growth and suppress prolactin secretion.

6. Synthetic thyrotropin-releasing hormone (TRH) in doses of 3 and 30 ng produced no effect on prolactin release by the end of 4, 8 or 12 hours of incubation when incorporated into a medium containing an AP half from normal male rat or rat pituitary tumor tissue. TRH increased prolactin release by about 30% by AP halves from thyro-parathyroidectomized rats after 4 hours of incubation, but had no effect after 8 and 12 hours of incubation. An intravenous injection of 5 or 7.5 ug of TRH into normal male rats had no effect on serum prolactin at 15, 30 and 60 minutes after the injection. Daily injections of 50 ug TRH for 6 days significantly increased AP prolactin concentration and produced a small elevation in serum prolactin. This stimulation of pituitary prolactin secretion presumably was due to activation of the TSH-thyroid system, since no such increases were observed when TRH was given to thyro-parathyroidectomized rats. It is concluded from these experiments that synthetic TRH is not a specific releaser of prolactin in the rat.

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By

Kuew-Hsiung Lu

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Physiology

1972

Dedicated to my parents,
En-Gie and Jan-Mei Lu,
and to my wife, Mann

ACKNOWLEDGMENTS

Only those who have sailed on the boundless ocean of learning appreciate the importance of a great teacher, and only those who have been away from home alone in a foreign country treasure the helping hands of a professor, who in addition to guidance, loves and cares. During his course of graduate work at this university, the author was able to reach where he is only under the capable guidance of Dr. Joseph Meites. As a foreigner from the other side of the world, fumbling in the darkness in search for advanced education in a country very much strange to him when he first came, the author is forever grateful to Dr. Meites who has rendered not only academic advice but also loving care and compassion so much needed by a foreign student.

The author has been exposed to numerous science literature so appropriately assigned by Dr. Meites whose enlightening direction and profound insight have given the author unique training which is second to none in this field of science. Whatever honors and credits the author has received during his graduate work at this university are due solely to Dr. Meites, to whom the author feels it extremely difficult to find proper words to express his heartfelt gratitude and appreciation.

The author wishes to express his sincere gratitude to the members of the guidance committee: Drs. W. D. Collings, E. P. Reineke, J. B. Scott, T. W. Jenkins, and A. J. Morris, who willingly counseled the author in preparing this thesis and other academic matters toward the completion of this graduate education. Sincere gratitude is expressed to Drs. E. M. Convey and G. D. Riegle who extemporarily requested by the author upon short notice willingly served at the guidance committee. The opportunity for consultation with Dr. C. W. Welsch is also gratefully acknowledged.

Appreciation is also expressed to Drs. Y. Koch, Y. Amenomori, C. L. Chen, and H. Nagasawa, Miss M. C. Gelato and Mr. C. J. Shaar, from whom the author received encouragement and assistance during collaboration in some of his studies. The author is indebted to Mrs. Claire Twohy, Mr. Gary Kledzik, and Mr. Eldon Cassell for their invaluable technical assistance, especially in carrying out many radioimmunoassays and preparing histology sections. Special thanks are given to Mrs. Amylou Davis and Mrs. Pam Rashid for their willingness and tolerance in typing many forms, letters, and manuscripts.

The author also wishes to thank Dr. Meites and the members of the Graduate Affairs Committee of Physiology Department, Michigan State University, for the assistantship granted him from September, 1968 to the completion of this graduate work.

Last, but not the least, the author wishes to express his many thanks to his wife, Mann, who helped and collaborated a great deal to make these graduate years at Michigan State meaningful in his career.

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INTRODUCTION

It long has been recognized that the functions of the anterior pituitary are influenced by the central nervous system, particularly by the hypothalamus. Early in the 1950's when neuroendocrinology evolved as a new field of science, it was proposed that secretion of anterior pituitary hormones was controlled by specific neurohormones produced by the hypothalamus. Among the six anterior pituitary hormones, it is now known that synthesis and release of prolactin is uniquely regulated by a prolactin release-inhibiting factor (PIF) produced by the hypothalamus. The secretion of the other five anterior pituitary hormones requires stimulation by releasing factors produced in the hypothalamus.

During recent years several significant "breakthroughs" have occurred in neuroendocrinology. The most exciting discovery has been the determination of the chemical structure and synthesis of two releasing factors, thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing factor (LRF). The identification of these two hypophysiotropic hormones of the hypothalamus has essentially proved the "chemotransmitter hypothesis" of hypothalamic control of anterior pituitary function as first stated by G. W. Harris (1955). These two

releasing hormones are now being synthesized, are available commercially and are now employed in both basic and clinical investigations. Currently attempts also are being made to chemically characterize the remaining hypothalamic hypophysiotropic hormones, including the prolactin-inhibiting factor (PIF).

Another significant advancement in neuroendocrinology has been the recent clarification of the role of biogenic amines in secretion of anterior pituitary hormones, particularly in relation to prolactin and gonadotropins. Norepinephrine and serotonin are highly concentrated in the nerve terminals located in the basal hypothalamus. The median eminence is rich in nerve endings containing dopamine and serotonin. Catecholamines have been shown to release PIF, LRF, and FRF into the pituitary stalk portal blood, with resultant inhibition of prolactin release and stimulation of LH and FSH release from the pituitary (Kamberi et al., 1969, 1970b). Catecholamines have no effects on pituitary release of these hormones when they are infused directly into stalk portal vessels. Thus the concept has evolved that biogenic amines, particularly catecholamines, act as neurotransmitters to control the release of hypophysiotropic hormones from the hypothalamus.

One aspect of this thesis is an investigation of the role of biogenic monoamines on release of PIF (and possibly prolactin-releasing factor, PRF) by the hypothalamus and on

release of prolactin. One particularly significant compound employed in these studies was L-dihydroxyphenylalanine (L-DOPA), the immediate precursor of dopamine. L-DOPA has been known for its ability to ameliorate Parkinson's disease. More recently this catechol amino acid was first shown by us to inhibit prolactin release and to suppress mammary tumor growth in rats. It is now being used successfully to treat human breast cancer. In patients with Forbes-Albright syndrome, L-DOPA administration also has been reported to induce cessation of persistent lactation and cause resumption of menstrual cycles. This thesis has attempted to elucidate the possible mechanism by which L-DOPA inhibits pituitary prolactin release and induces mammary tumor regression.

The recent development of specific radioimmunoassays for anterior pituitary hormones permits investigators to measure hormone levels in the blood and other tissues with high accuracy. By use of the standard radioimmunoassay for rat prolactin, it is possible to detect changes in hormone levels simultaneously in three different organ-systems: PIF in the hypothalamus and its release into the systemic circulation; the amount of prolactin present in the pituitary; and prolactin levels in the circulation and peripheral tissues. Most of the studies in this thesis include results on changes in hormone activity in these three systems, in an effort to provide a more complete profile of changes in the hypothalamic-pituitary system under different experimental conditions.

There is little doubt that pituitary prolactin secretion is mainly under the control of hypothalamic PIF. But several drugs and hormones also can influence prolactin release by a direct action on the pituitary. Part of this thesis deals with studies on the direct action of ergot drugs and estrogen on pituitary release of prolactin, and on prolactin release by pituitary "mammotropic" tumor. An attempt was made to delineate the mechanisms by which ergot drugs induce regression of pituitary and mammary tumors.

There has been much speculation recently that synthetic TRH, pyro-glutamyl-histidyl-proline amide, may be identical or similar to the postulated PRF in the hypothalamus. This is based on observations that synthetic TRH increases blood levels of prolactin when given to humans, monkeys and cattle. In an attempt to clarify the possible action of TRH on pituitary release of prolactin, the author has spent much of his last period of graduate work on TRH. This thesis describes the findings with TRH in the rat under several different endocrine states, and compares these with results reported in other mammalian species.

REVIEW OF LITERATURE

I. Functional Neuroanatomy of the Hypothalamic-Pituitary Axis

A. General Anatomy of the Hypothalamus

Several books and reviews have been written on the neuroanatomy of the hypothalamus (Jenkins, 1972; Truex and Carpenter, 1969; Netter, 1967; Daniel, 1966; deGroot, 1959; Gurdjian, 1927). The recent volume by Jenkins (1972) has an excellent description of the functional neuroanatomy of the mammalian hypothalamus. The colored illustrations by Netter (1967) provide a brief but clear view of the role of the hypothalamus in pituitary function.

The hypothalamus is the most ventral portion of the diencephalon and is visible from the ventral surface of the brain. It comprises the lateral walls of the third ventricle below the hypothalamic sulcus and the structures of the ventricular floor, including the optic chiasm, the tuber cinereum and infundibulum, the neurohypophysis, and the mammillary bodies. The region immediately in front of the optic chiasm, extending to the lamina terminalis and the anterior commissure, is known as the preoptic area. Dorsal to the

hypothalamus is the thalamus, and lateral is the subthalamic areas. Caudally, the mammillary bodies are considered as the limiting border of the hypothalamus, but there is a melting into the posterior perforated substance and tegmentum of the midbrain. Medially, the vertically oriented third ventricle divides the hypothalamus into right and left halves.

The pituitary is attached to the brain by the infundibulum (stalk of the pituitary). The latter has a hallow center, which is the ventral extension of the third ventricle. The neurohypophysis or posterior lobe of the pituitary is composed of nervous tissue continuous with the basal portion of the hypothalamus.

In a rostro-caudal sequence, three distinct regions can be identified in the hypothalamus: supraoptic, tuberal, and mammillary areas with their specific groups of nuclei. The supraoptic area contains two functionally well-known nuclei, the supraoptic and paraventricular. In the tuberal region, the infundibulum attaches to the median eminence of the tuber cinereum. The ventral portion of the periventricular nucleus and the arcuate nucleus collectively constitute the gray matter enveloping the base of the third ventricle. Also located in the tuberal region are the dorsomedial, ventromedial, and lateral hypothalamic nuclei. The mammillary, or caudal hypothalamic, area is composed of the prominent mammillary bodies.

The hypothalamus receives projections from many biber tracts coming in from broad areas of the brain. These different pathways include the fornix, medial forebrain bundle, thalamo-hypothalamic fibers, mammillary peduncle and stria terminalis. In addition, the hypothalamo-hypophyseal tract, periventricular fibers and mammillary body efferents are the three major efferent fiber tracts originated from specific nuclei of the hypothalamus. It long has been known that oxytocin and anti-diuretic hormone (ADH) are synthesized in the supraoptic and paraventricular nuclei (Bargmann and Scharrer, 1951), and subsequently traverse the hypothalamo-hypophyseal tract to the posterior pituitary.

B. Hypothalamo-Pituitary Portal System

The secretion of hormones by the anterior pituitary is under the direct control of the hypothalamus. This hypothalamic regulation is by way of the pituitary portal vessels rather than through direct innervation. No direct neural connection between the hypothalamus and the anterior pituitary is evident.

Popa and Fielding (1930) first described the pituitary portal circulation and indicated that the portal blood flowed from the pituitary to the hypothalamus. However, subsequent studies suggested that the flow was from the hypothalamus to the anterior pituitary (Wislocki and King, 1936; Houssay et al., 1935; Green, 1947). Green and Harris (1949)

directly observed the pituitary portal circulation in living rats under the microscope. They indicated that the portal vessels originated in the median eminence of the tuberal cinereum and in the infundibular stem, and observed that the blood flowed caudally toward the anterior pituitary. Similar observations also were reported in the dog by Torok (1954) and in the mouse by Worthington (1955). Since then, the "chemotransmitter hypothesis" of Harris (1955) has received strong support from morphological evidence (see Section II, A).

The median eminence receives blood from branches of the anterior pituitary artery. These arterioles break up into tortuous capillary loops in the median eminence. The venous blood goes from the median eminence to the stalk portal vessels which run down to the anterior pituitary tissue. The free part of the pituitary stalk (infundibular stem) is supplied by the peduncular artery which springs from the posterior communicating and internal carotid arteries. Stalk portal vessels also originate from the capillary loops in this region. These stalk portal vessels constitute the only venous drainage from the capillary beds in either the median eminence or the infundibular stem.

The anterior pituitary derives its entire blood supply from the portal circulation. There is no arterial blood supply directly perfusing the anterior pituitary tissue, except in humans and in rabbits. The dorso-caudal portion of the

anterior pituitary also receives blood from the posterior pituitary through a group of small, short portal vessels. A more thorough discussion of the anatomy of the pituitary portal system is given by Daniel (1966), Green (1966), Adams et al. (1965), Landsmeer (1963), and Daniel and Prichard (1956).

C. Sites of Origin of Hypothalamic Hypophysiotropic Hormones

The anterior pituitary loses most of its histological characteristics and secretory capacity when its vascular connection with the median eminence of the hypothalamus is interrupted (Harris and Jacobsohn, 1952; Nikitovitch-Winer and Everett, 1959). Halasz et al. (1962) introduced the term "hypophysiotropic" to refer to the need for connections with the hypothalamus for the maintenance of normal anterior pituitary histology and function. The "chemotransmitter hypothesis" of Harris (1955) proposed that neurohormones released by the hypothalamus are conveyed to the anterior pituitary via the stalk portal vessels. These neurohormones of the hypothalamus are responsible for regulating anterior pituitary function and hence have the name "hypothalamic hypophysiotropic hormones."

By transplanting fragments of anterior pituitary tissue into different parts of the hypothalamus, it was observed that pituitary grafts showed some histological maintenance and functional activity, particularly when they were placed

in certain areas of the ventral hypothalamus (Halasz et al., 1962; Knigge, 1962; Desclin and Flament-Durand, 1963; Flament-Durand, 1964). But greater functional activity was found when the pituitary grafts were in direct contact with the median eminence tissue (Halasz et al., 1965; Flament-Durand, 1965). Halasz et al. (1962) have proposed the name "hypophysiotropic area" for the hypothalamic zone in which pituitary grafts maintained a nearly normal histological picture. This area includes the arcuate nucleus, the ventral part of the anterior periventricular nucleus, and the medial parvicellular region of the "retrochiasmatic area." Here are located the cell bodies of the tubero-infundibulular fibers to the median eminence (Halasz and Szentagothai, 1962; Szentagothai, 1962, 1964). It is hypothesized that the hypophysiotropic hormones are synthesized or present in the cell bodies within the hypophysiotropic area and are normally released at the nerve endings in the median eminence (Halasz et al., 1962; Szentagothai and Halasz, 1964). It will be seen subsequently that biogenic amines serve as neurotransmitters and participate in the release of hypophysiotropic hormones by the hypothalamus (see Section II, D).

In recent years the evidence has essentially proved the existence of the presumed hypophysiotropic hormones in the hypothalamus. Two such substances, thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing factor (LRF), have been purified and synthesized by several laboratories.

The chemical structure of TRH was determined to be a tripeptide, pyro-glutamyl-histidyl-proline amide (Burgus et al., 1969; Bowers et al., 1970). A decapeptide, pyro-glutamyl-histidyl-tryptophan-serine-tyrosine-glycine-leucine-arginine-proline-glycine amide, was shown to be the structure of LRF (Baba et al., 1971; Burgus et al., 1971). These 2 hormones are now available commercially and are now employed in both basic and clinical investigations. Efforts also are being made to purify and to synthesize the remaining hypophysiotropic hormones, including the prolactin-inhibiting factor (PIF).

II. Control of Pituitary Prolactin Secretion by the Hypothalamus and by Other Systems

A. Hypothalamic Control of Anterior Pituitary Function

The hypothalamus, by regulating synthesis and release of specific hypophysiotropic hormones, controls the secretion of anterior pituitary hormones. Early work by Taubenhaus and Soskin (1941) suggested that the rat hypothalamus secretes an acetylcholine-like substance into the portal vessels to elicit pituitary LH release. Later, Markee et al. (1948) proposed that an adrenergic mechanism also might be involved in the control of LH secretion in rats. Harris and Jacobsohn (1952) were the first to demonstrate the direct influence of anterior pituitary function by the hypothalamus. They observed

return of normal reproductive function and milk secretion in hypophysectomized rats when an anterior pituitary was grafted under the median eminence. By contrast, normal estrous cycles were not resumed in hypophysectomized rats when the pituitary was transplanted under the temporal lobe of the brain. In 1955 Harris proposed the "chemotransmitter hypothesis," according to which neurohormones released from the hypothalamus were responsible for regulating pituitary function. This hypothesis has been substantiated in recent years by the demonstration that specific hypophysiotropic hormones are present in the hypothalamus (see review by Guillemin and Schally, 1963; Harris, 1970); several of these have been synthesized by several laboratories (Burgus et al., 1969; Bowers et al., 1970; Burgus et al., 1971).

Hypothalamic control of prolactin secretion is unique, since it is the only anterior pituitary hormone that appears to be chronically inhibited by the mammalian hypothalamus under most conditions. Thus, it has been demonstrated that transplantation of pituitary away from the median eminence results in sustained secretion of prolactin at a high level, whereas secretion of all other anterior pituitary hormones is sharply reduced (Everett, 1954; 1956; Nikitovitch-Winer and Everett, 1958; Chen et al., 1970). Similar results also were observed in rats after sectioning the pituitary stalk (Dempsey and Uotila, 1940), after lesioning the median eminence or "hypophysiotropic area" of the hypothalamus (Chen

et al., 1970; Welsch et al., 1971), by culturing or incubating anterior pituitary tissue in vitro (Meites et al., 1961) and by administering appropriate drugs (Meites, 1962; Meites et al., 1963). These observations indicate that predominant influence of the mammalian hypothalamus on pituitary prolactin secretion is inhibitory in nature.

B. Hypothalamic Prolactin Release-Inhibiting Factor (PIF)

1. In Vivo and in vitro demonstrations of a hypothalamic PIF

Inhibition of pituitary prolactin release by the mammalian hypothalamus is exerted via the action of a prolactin release-inhibiting factor. Addition of crude extract of rat hypothalamus to incubations or cultures of pituitary decrease prolactin release (Meites et al., 1961; Pasteels, 1961; Talwalker et al., 1963). Extracts from the hypothalami of sheep, swine, cattle, humans, but not birds also have been demonstrated to inhibit prolactin release in vitro (Schally et al., 1965; Kragt and Meites, 1965). Talwalker et al. (1963) were the first to propose the name "prolactin-inhibiting factor" (PIF) for the presumed hypothalamic hypophysiotropic hormone which inhibits pituitary secretion of prolactin. They observed a decrease of both synthesis and release of prolactin by the rat pituitary by adding a crude extract of rat hypothalamus to an incubation medium. Subsequently, Kragt and Meites (1967) demonstrated a negative dose-response

relationship between the quantity of hypothalamic extract added and the amount of prolactin released in vitro. This has been confirmed by Chen (1969) by use of a specific radioimmunoassay for rat prolactin (Niswender et al., 1969). PIF activity also has been demonstrated in vivo by showing that hypothalamic extracts prevented pituitary prolactin depletion by suckling during lactation (Grosvenor et al., 1964) or in response to cervical stimulation during estrus in rats (Kuroshima et al., 1966; Arimura et al., 1967). Amenomori and Meites (1970) reported that an injection of crude extract of 8 rat hypothalamic equivalent markedly reduced serum prolactin in cycling and lactating rats. Watson et al. (1971) also observed that a single injection of crude hypothalamic extract decreased serum prolactin in normal and orchidectomized male rats. The chemistry of hypothalamic PIF has not been characterized. It has been shown that PIF activity is stable in acid medium and not destroyed by boiling, suggesting it may be a small peptide molecule like the other hypophysiotropic hormones (Burgus et al., 1969; Bowers et al., 1970; Baba et al., 1971; Burgus et al., 1971). Little is known about the mechanism of action by which PIF inhibits the pituitary release of prolactin. It is believed that the membrane of the prolactin-secreting cell spontaneously depolarizes when the pituitary is freed from hypothalamic influence. Thus, Nicoll (1971) indicated that PIF may act by depressing spontaneous depolarization of prolactin cells,

thereby reducing the entry of Ca ions into the cells and inhibiting the release of secretory granules.

2. Control of hypothalamic PIF secretion

PIF activity in the hypothalamus is readily altered by a variety of drugs, hormones and stimuli. Perphenazine (Danon et al., 1963), reserpine (Ratner et al., 1965), haloperidol (Dickerman et al., 1972), epinephrine and acetylcholine (Mittler and Meites, 1967), estrogen (Ratner and Meites, 1964), progesterone, testosterone and cortisol (Sar and Meites, 1968), a norethynodrel-menstranol combination (enovid) (Minaguchi and Meites, 1967a) and the suckling stimulus (Ratner and Meites, 1964; Minaguchi and Meites, 1967b) were found to decrease hypothalamic PIF activity in rats, whereas ergocornine (Wuttke et al., 1971), L-DOPA and monoamine oxidase inhibitors (pargyline, iproniazid, and Lilly compound-15641) (Lu and Meites, 1971) and prolactin itself (Chen et al., 1967; Clemens and Meites, 1968; Voogt and Meites, 1971) were shown to increase PIF activity in the hypothalamus. Work by Kamberi et al. (1970) demonstrated that a single injection of dopamine into the third ventricle of rats elevated PIF activity in the pituitary stalk blood. We have extended this observation in a recent study and demonstrated that L-DOPA, the immediate precursor of dopamine, increases PIF in the hypothalamus and elicits the presence of PIF activity in

the systemic blood of intact and hypophysectomized rats (Lu and Meites, 1972). These results indicate that drugs that increase hypothalamic catecholamines also stimulate synthesis and release of PIF, whereas drugs that reduce catecholamines in the brain also depress hypothalamic PIF activity (Meites et al., 1972). Evidence has been presented that prolactin increases dopamine activity in the hypothalamus (Fuxe and Hokfelt, 1970).

C. Hypothalamic Prolactin-Releasing Factors (PRF) ?

1. Observations suggesting the possible presence of a PRF in the mammalian hypothalamus

The inhibitory influence on prolactin release by the mammalian hypothalamus suggests a unique control mechanism. Demonstrations of the presence of a prolactin-releasing factor (PRF) in the avian hypothalamus (Kragt and Meites, 1965; Nicoll, 1965; Gourdji and Tixier-Vidal, 1966; Meites, 1967; Chen et al., 1968) has raised the question that the mammalian hypothalamus also may contain a hypophysiotropic hormone(s) that stimulates pituitary release of prolactin.

Meites et al. (1960) observed that injections of a crude extract of rat hypothalamus initiated lactation in estrogen-primed rats, indicating release of prolactin and probably ACTH by the pituitary. However, these responses may not be specific since lactation also was induced in similar

rats by injecting cerebral cortical extract or other pharmacological agents. Later, Mishkinsky et al. (1968) confirmed these observations and concluded that the rat hypothalamus contains a PRF.

Oxytocin was considered to be responsible for pituitary release of prolactin (Benson and Folley, 1956) after it was found to be secreted by hypothalamic nuclei (Bargmenn and Scharrer, 1951). Peterson (1942) also postulated that the posterior pituitary may be responsible for the rapid decrease in pituitary content observed after suckling. However, no direct evidence was demonstrated that oxytocin can stimulate prolactin release. Oxytocin is ineffective in altering pituitary prolactin content (see review by Meites et al., 1963) and has little effect on serum prolactin levels in sheep (Greenwood, 1972). Inhibition of mammary gland involution by oxytocin appears to be via mechanisms other than release of prolactin (see review by Meites et al., 1963). More recently, Valverde and Chieffo (1971) reported that a single injection of a procine hypothalamic extract increased serum prolactin in estrogen, progesterone-treated male rats. This apparently conflicts with the report by Schally et al. (1965) that extracts from porcine hypothalami inhibited prolactin release under in vivo and in vitro conditions.

Several in vitro studies suggested the possible presence of a PRF in the rat hypothalamus. Nicoll et al. (1970) indicated that both prolactin-inhibiting and prolactin-

stimulating activities are present in the rat hypothalamus. They demonstrated that hypothalamic extract inhibited pituitary prolactin release during the first 4 hours of incubation, but stimulated prolactin release during the subsequent 4 hours. However, using a somewhat different method of incubation, a consistent inhibition of pituitary prolactin release throughout 8 hours was observed when extract from rat hypothalamus was added to an incubation medium (Chen, 1969; Meites, 1970). Krulich et al. (1971) reported that, in rat hypothalamus, the median eminence and a narrow basal portion of the preoptic area mainly contains PRF activity, while the dorsolateral region of the preoptic area is rich in PIF. These findings based on in vitro studies apparently conflict with results from numerous in vivo studies in which it was demonstrated that pituitary release of prolactin is increased and sustained by lesioning the median eminence (Chen et al., 1970; Welsch et al., 1971), indicating that this area of the hypothalamus is concerned with the release of LH rather than prolactin (Everett and Quinn, 1966). In vitro studies have been extended by Chen et al. (1972) who assayed PIF and PRF activities in fresh slices of rat hypothalamus, but their results did not sustain the observation of Krulich et al. (1971). These studies suggest that the mammalian hypothalamus may contain a hypophysiotropic hormone that releases prolactin although the results do not permit any definite conclusion to be made at this time (Meites et al., 1972).

2. Is synthetic pyro-glutamyl-histidyl-proline amide (TRH) a PRF in mammals?

More recently several laboratories have reported that synthetic pyro-glutamyl-histidyl-proline amide (TRH) can induce prolactin release in humans, monkeys, bovine, and rats. Tashjian et al. (1971) observed that TRH increased prolactin and decreased GH release when added to cultures or short-term incubations of clonal cells from rat pituitary tumors. However, these workers also reported that extracts from rat hypothalamus, cerebral cortex, kidney or liver significantly increased prolactin and decreased GH release when added to cultures of clonal strains of rat pituitary tumor cells (Tashjian et al., 1970). This raises the question about the specificity of the reported effects of TRH on prolactin and GH release by the pituitary tumor cells in vitro. Recent work from our laboratory (Lu et al., 1972) indicated that TRH had no effect on prolactin release when added to an incubation medium containing normal male rat pituitary halves or "mammotropic" pituitary tumor (MtT. W15) tissue. Single injections of TRH also failed to alter serum prolactin levels, but when TRH injected daily for 6 days there was a significant increase in pituitary prolactin concentration and a small elevation in serum prolactin. These increases of prolactin were not observed when TRH was injected into thyroparathyroidectomized rats, suggesting that its effects were mediated through the TSH-thyroid system. Previous studies

indicated that thyroid hormones stimulated prolactin secretion in the rat (Chen and Meites, 1969) and could act directly on the rat pituitary to increase prolactin release *in vitro* (Nicoll and Meites, 1963). Bowers (1971) and McCann (1971) also failed to observe any effect on prolactin release by normal rat pituitary tissue when it was added to an incubation system. These observations do not support the report of Tashjian *et al.* (1971) that TRH increases prolactin release in the rat.

The relation of these observations in the rat to reports that TRH can produce a rapid rise in blood prolactin in humans, monkeys, and bovine is not clear. A single intravenous injection of TRH was reported to evoke a prompt increase in blood prolactin levels in humans (Hwang *et al.*, 1971; Bowers *et al.*, 1971; Jacobs *et al.*, 1971; Bowers *et al.*, 1972) and monkeys (Knobil, personal communication). In view of the rapidity of TRH stimulation on prolactin release in the primates, and the observation that TRH is even more effective in rising blood prolactin in hypothyroid than in euthyroid humans (Bowers *et al.*, 1971; Jacobs *et al.*, 1971), it is unlikely that the stimulation of prolactin release by TRH is effected via the TSH-thyroid system in humans. A single intravenous injection of TRH also was observed to elicite a rapid increase in serum prolactin in bovine (Convey et al., 1972). However, no increase in prolactin release was observed when TRH was added to an incubation medium containing

bovine pituitary slices (LaBella and Vivian, 1971; Convey et al., 1972). There is as yet no evidence that TRH acts directly on the human pituitary to induce prolactin release. Thus, the possibility that the synthetic pyro-glutamyl-histidyl-proline amide is identical or similar to the postulated PRF in the mammalian hypothalamus (Jacobs et al., 1971; Tashjian et al., 1971) remains to be determined.

In contrast to the well established alterations of hypothalamic PIF activity by a variety of agents and stimuli (see Section II, B), changes in the activity of the presumed PRF have not been reported. A prompt and big increase in pituitary prolactin release is seen in rats after suckling (Sar and Meites, 1969; Amenomori et al., 1970) and after injecting 5-hydroxytrytophan, the immediate precursor of serotonin (Lu and Meites, unpublished). However, the serum or hypothalamus from rats injected with 5-hydroxytryptophan or the serum of rats after suckling has no effect on pituitary prolactin release when added to an incubation medium (Lu and Meites, unpublished). Evidence has been presented that suckling reduces hypothalamic PIF activity (Rather and Meites, 1964; Minaguchi and Meites, 1967). Recent work from our laboratory (Dibbet et al., unpublished) has indicated that TRH does not inhibit the ability of PIF in a rat hypothalamic extract to reduce pituitary release of prolactin in vitro. We also have observed that TRH has no ability to alter hypothalamic PIF activity when it is injected into normal male rats

(Lu and Meites, unpublished). These results from recent and earlier studies indicate that synthetic TRH is not a specific releaser of pituitary prolactin in rats. It remains to be determined how synthetic TRH evokes a rapid rise in blood prolactin in primates and the bovine. There is as yet no definite evidence that synthetic pyro-glutamyl-histidyl-proline amide is the presumed hypophysiotropic hormone that stimulates pituitary release of prolactin in mammals (Lu et al., 1972).

D. Role of Biogenic Amines

1. Biogenic amines in the brain

The neural signal emitted by each mammalian neuron is presumably a chemical substance, a neurotransmitter. This substance is released at the synapse and diffuses across the synaptic cleft to reach the receptor surface of the post-synaptic neuron. Upon interaction with the neurotransmitter, the post-synaptic neuron generates an action potential and transmits a nerve impulse. The substances most generally accepted as serving as neurotransmitters in the brain include acetylcholine and three monoamines, i.e., dopamine, norepinephrine, and serotonin. Serotonin is an indoleamine; dopamine and norepinephrine are catecholamines. With the development of histochemical fluorescence techniques for identifying biogenic amines in the brain (see review by Hillarp

et al., 1966), it has been demonstrated that norepinephrine and serotonin are highly concentrated in the hypothalamus and midbrain (Vogt, 1954; Brodie et al., 1959). It also has been shown that the nigrostriatal system and the median eminence are rich in dopaminergic nerve terminals (Dahlstrom and Fuxe, 1965; Anden et al., 1964). Some noradrenergic terminals are also found in the median eminence and infundibulum (Bjorklund et al., 1970). Chemical assays also have shown that relatively large amounts of norepinephrine and dopamine are present in the median eminence (Laverty and Sharman, 1965; Rinne and Sonninen, 1968). More recently, Pizzi et al. (1970) have observed that the bovine median eminence also contains high concentrations of serotonin. In addition to serving as neurotransmitters as mentioned above, dopamine and norepinephrine may also function as neurohormones. These catecholamines may be released into the hypothalamo-pituitary portal system from neurons whose cell bodies lie in the medial hypothalamus and whose nerve endings are terminated in the median eminence and the infundibulum near the primary capillary loops of the portal system (Fuxe and Nilsson, 1967; Anton-Tay et al., 1969; Knigge et al., 1968). This has led some workers to speculate that the biogenic amines may be the "hypophysiotropic hormones" which act directly on the anterior pituitary for hormone secretion. Based on the observations that catecholamines, including dopamine, norepinephrine and epinephrine inhibited pituitary

release of prolactin *in vitro*, MacLeod (1969) and Birge *et al*. (1970) concluded that the catecholamines in the hypothalamus were the not as yet defined prolactin release-inhibiting factor. There is no clear evidence that dopamine or norepinephrine is present in the portal blood which perfuses the anterior pituitary (Wurtman, 1970). However, there is evidence that little if any of the catecholamine released from nerve endings in the brain can gain entry into the blood stream without first undergoing oxidative deamination (Glowinski *et al*., 1965).

2. Biosynthesis of brain monoamines

The first biochemical transformation in the synthesis of brain catecholamines involves the hydroxylation of the amino acid, tyrosine, Tryosine is taken up from the circulation by neurons. The hydroxylation of tyrosine is catalyzed by the enzyme, L-tryosine hydroxylase, and results in the formation of a catechol amino acid, L-dihydroxyphenylalanine (L-DOPA). L-DOPA is rapidly converted to dopamine by the enzyme aromatic L-amino acid decarboxylase (DOPA decarboxylase), after it is formed (Anton and Sayre, 1964). The transformation of dopamine to norepinephrine is through the action of the enzyme dopamine-B-oxidase. Both tyrosine hydroxylase and dopamine-B-oxidase are only found in catecholamine-producing cells. Dopamine-B-oxidase is localized within norepinephrine storage vesicles at the nerve terminals (Wurtman, 1966). It

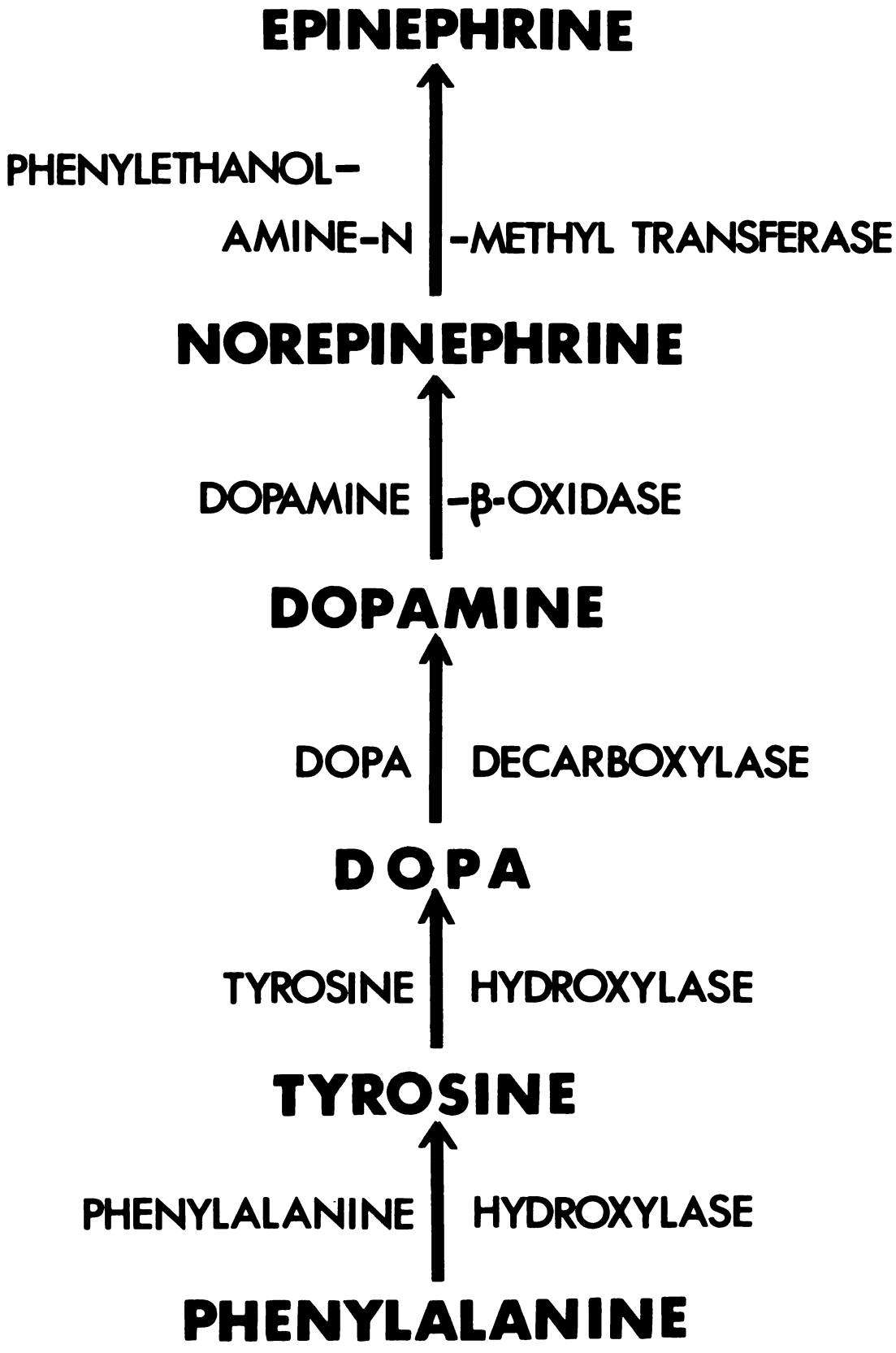


Figure 1. Biosynthesis Pathway of Catecholamines.

is believed that the action of the enzyme tyrosine hydroxylase is the rate-limiting step for the overall biosynthesis of catecholamines (Costa and Neff, 1970). There is as yet no clear evidence that epinephrine is synthesized by any part of the mammalian brain and serves as a neurotransmitter. However, phenylthanolamine-N-methyl transferase, an enzyme which catalyzes the conversion of norepinephrine to epinephrine, has been identified within the olfactory bulb and the hypothalamus (Pohorecky et al., 1969).

The biosynthesis of brain serotonin (5-hydroxytryptamine) involves the transformation of tryptophan to 5-hydroxytryptophan. This process is catalyzed by the enzyme, tryptophan hydroxylase. 5-hydroxytryptophan, like L-DOPA, is catalyzed by the enzyme, aromatic L-amino acid decarboxylase, to form serotonin almost immediately after it is formed (Wurtman, 1970).

3. Physiological disposition of brain monoamines

Using histochemical fluorescence techniques, it has been demonstrated that catecholamines are more concentrated within synaptic vesicles at the nerve terminals than they are in other portions of the neuron (Dahlstrom and Fuxe, 1965; Potter and Axelrod, 1963; Wurtman, 1966). By contrast, serotonin is homogeneously distributed within serotonin-containing neurons (Anden et al., 1965). Nerve stimulation causes the release of catecholamine molecules from the

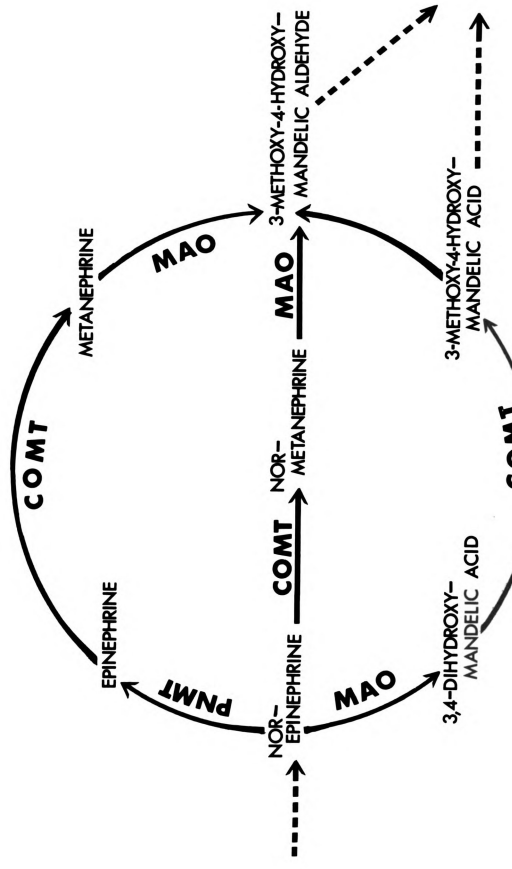


Figure 2. Catabolism Pathway of Catecholamines.

synaptic vesicles. The catecholamines transverse the synaptic cleft and interact with the post-synaptic membrane receptors. The physiological actions of norepinephrine within the synaptic cleft are mainly terminated by the process of reuptake into the nerve terminals. Most of the catecholamine present in a neuron is degraded within that neuron by the action of the enzyme, monoamine oxidase (MAO), and released as oxidative deaminated metabolites. Norepinephrine also can be inactivated by the enzyme, catechol-O-methyl transferase (COMT) within the synaptic cleft (Anton-Tay and Wurtman, 1971).

The main pathway for serotonin metabolism appears to be oxidative deamination by monoamine oxidase, then followed by oxidation or reduction. In the mammalian pineal gland, the transformation of serotonin to melatonin is of a greater significance as a pathway for its metabolism (Wurtman, 1970). Serotonin is first N-acetylated to form the compound, N-acetylserotonin. By the enzyme action of hydroxyindole-O-methyl transferase, a methyl group from S-adenosylmethionine is transferred to N-acetylserotonin resulting in the synthesis of 5-methoxy-N-acetyltryptamine (melatonin).

4. Alterations of brain monoamine activity by pharmacological agents

It is generally accepted that the enzymatic action of tyrosine hydroxylase is the rate-limiting step for the overall biosynthesis of catecholamines. This enzyme is

readily attacked by methylated tyrosine analogs such as alpha-methyl-para-tyrosine and alpha-methyl-meta-tyrosine and results in the inhibition of catecholamine synthesis in the brain. Methyl-DOPA competes for DOPA-decarboxylase to synthesize methyl-dopamine and methyl-norepinephrine (false neurotransmitters) instead of dopamine and norepinephrine (normal neurotransmitters). The methylated catecholamines have much weaker function as neurotransmitters than the normal catecholamines (Coppola, 1968; Innes and Nickerson, 1970). The brain catecholamine activity also can be decreased by several tranquilizers: reserpine interfers with storage and/or induces depletion of catecholamines; chlorpromazine inhibits catecholamine action by blocking the receptor sites on the post-synaptic membrane (Coppola, 1968). On the other hand, increased brain catecholamine activity can be achieved by administering precursor catecholamine substrates, such as L-DOPA. This catechol amino acid is taken up by catecholamine-containing neurons and is rapidly transformed to dopamine and norepinephrine in the brain (Wurtman, 1971), even though the amount of L-DOPA gaining entry to the brain represents only a small fraction of the total (Wurtman et al., 1970). Since oxidative deamination is the major pathway for monoamine metabolism, brain monoamine activity is readily enhanced by administering monoamine oxidase inhibitors such as pargyline, iproniazid and amphetamine (Koelle, 1971).

p-Chloroamphetamine and p-chlorophenylalanine induce depletion of brain serotonin by blocking the enzymatic action of tryptophan hydroxylase (Fuller et al., 1965; Koe and Weissman, 1966; Sanders-Bush and Sulser, 1970). p-Chlorophenylalanine also reduces norepinephrine levels in the brain (Koe and Weissman, 1966).

5. Changes in brain monoamine activity during different reproductive states and diurnal rhythm

Sandler (1968) observed no changes in hypothalamic catecholamines during the estrous cycle of rats, whereas Donoso and Stefano (1967) reported that norepinephrine levels are high in proestrus, low after ovulation and lowest at estrus. By contrast, Lichtensteiger (1969) and Coppola (1971) observed increased hypothalamic catecholamine content at estrus and reduced levels at diestrus. These inconsistencies are probably due to the different method used for measuring brain catecholamine (Coppola, 1971). Nevertheless, more consistent results on the effects of gonadal hormones on brain norepinephrine metabolism have been published. Castration of male or female rats enhanced the synthesis of norepinephrine in the hypothalamus (Wurtman et al., 1969; Anton-Tay and Wurtman, 1968; Anton-Tay et al., 1970); this led to a slight increase in the steady-state concentration of norepinephrine (Coppola, 1968) and a marked acceleration in the turn-over rate of norepinephrine (Anton-Tay and Wurtman,

1968). Estrogen prevented these effects of castration (Donoso and Stefano, 1967; Coppola, 1968, 1969; Anton-Tay and Wurtman, 1968). The mechanism by which castration accelerates norepinephrine synthesis appears to be due to a direct action of FSH, since injections of FSH to intact or hypophysectomized, ovariectomized rats mimiced the effect of castration on norepinephrine turn-over (Anton-Tay et al., 1969). On the day of estrus, a diurnal rhythm of brain norepinephrine levels was reported in rats and cats (Donoso and Stefano, 1967; Reis and Wurtman, 1968; Manshardt and Wurtman, 1968), with higher levels in the morning than in the afternoon. In cycling female rats, the prolactin and gonadotropin surges all appear approximately at the same time on the afternoon of proestrus (Wuttke and Meites, 1970; Gay et al., 1970). It remains to be determined, however, whether hypothalamic catecholamines participate in the control of onset of these hormonal peaks, since the former is stimulatory to pituitary release of gonadotropins but inhibitory to prolactin release. Preliminary results from a recent study in our laboratory have indicated that L-DOPA is ineffective in preventing the proestrous surge of prolactin (Lu and Meites, unpublished). Paralleling those changes in hypothalamic catecholamines, alterations in the hypothalamic enzyme activity of monoamine oxidase also were reported under different reproductive states in rats (Kobayashi et al., 1964; 1966). Monoamine oxidase in the rat hypothalamus was increased

during proestrus and estrus, but decreased at diestrus. Increased activity of hypothalamic monoamine oxidase was observed in rats with the onset of puberty. A similar increase in the enzyme activity also was seen in ovariectomized rats, but estrogen blocked this rise in monoamine oxidase.

Plasma and brain levels of tryptophan, the precursor serotonin substrate, exhibit significant daily fluctuations (Wurtman et al., 1968; Fernstrom and Wurtman, 1971). The serotonin content in rat brain also exhibits a diurnal rhythm, with a maximum at about the 8th hour after the onset of light and a minimum at the 4th hour after the onset of darkness (Synder et al., 1965; 1967); this is in opposition to the circadian rhythm of norepinephrine which shows a peak in the dark phase (Asano, 1971). A diurnal variation in serum prolactin in rats was reported by Koch et al. (1971), with higher serum prolactin values on the late afternoon of each day than in the morning. Similar variation was also demonstrated in pituitary prolactin content (Clark and Baker, 1964). Dunn et al. (1972) found a circadian periodicity in serum prolactin concentration with a peak at 11 PM. These observations suggest that there is a dual control in the hypothalamus over prolactin secretion, with a predominant adrenergic tonus to inhibit prolactin release under most conditions, and a serotonergic system that may be responsible for the diurnal rise on the late afternoon and perhaps under other conditions in which serotonin is increased (Meites et al., 1972).

6. In vivo and in vitro fates of exogenous monoamines

Any monoamines administered systematically can reach the hypothalamo-pituitary portal system only to a minor degree (Fuxe and Hokfelt, 1969; Steinman et al., 1969). Monoamines are rapidly inactivated by enzymes in the liver and kidneys. Norepinephrine is cleared from the circulation by uptake into adrenergic nerve endings (Wurtman, 1970). Any monoamine that gained entry into brain arteries still would face great difficulty in reaching the neurons which release into the pituitary protal system. The operation of a "blood-brain barrier" hinders the entry of circulating monoamines into brain tissue. The "barrier" is relatively weak at the level of the hypothalamus and the area of postrema than in most other regions of the brain, but still is highly resistant to monoamines (Wurtman, 1966; 1970).

Catecholamines are degraded rapidly in vitro to form adrenochromes which exert powerful pharmacological effects on cellular metabolism (Wurtman, 1970).

7. Role of biogenic amines in prolactin and gonadotropin secretion

Reports over many years have indicated that biogenic amines in the brain, particularly those present in the hypothalamus, participate in the control of secretion of several anterior pituitary hormones, including prolactin and gonoadotropins. Taubenhaus and Soskin (1941) believed that an

acetylcholine-like substance from the hypothalamus acted via the pituitary protal vessels to elicit LH release in rats. Markee et al. (1948) and Sawyer et al. (1949) demonstrated that adrenergic drugs produced ovulation and therefore presumably elicited LH release in rabbits. These investigators proposed that an adrenergic mechanism was involved in the control of LH secretion. Anti-adrenergic drugs blocked ovulation in rabbits and rats (Sawyer et al., 1949; Everett et al., 1949, Markee et al., 1952). However, the adrenergic blockers were only effective when administered before coitus in rabbits or prior to the "critical period" on the day of proestrus in rats. Reserpine and cholropromazine were reported to inhibit ovulation induced by pregnant mare serum in immature rats when administered prior to the expected time of LH release (Hopkins and Pincus, 1963; Zarrow and Brown-Grant, 1964; Coppola et al., 1966; Coppola, 1968). It also was reported that reserpine blocked ovulation in mature rats when given prior to the "critical period" on the day of proestrus (Barraclough and Sawyer, 1957; Meyerson and Sawyer, 1967). Alpha-methyl-para-tyrosine, a potent inhibitor of catecholamine synthesis, also was shown to inhibit pregnant mare serum-induced ovulation in immature rats (Lippmann et al., 1967; Coppola, 1968; Kordon and Glowinski, 1969). Subsequently, it was observed that inhibition of LH secretion by sympatholytic agents such as reserpine, alpha-methyl-DOPA, tetrabenazine and syrosingopine was associated

with a marked reduction in hypothalamic catecholamines; whereas drugs that specifically deplete catecholamines in peripheral tissues but not in the brain (bretylin, tyramin and guanethidine) were ineffective (Coppola et al., 1966; Lippmann et al., 1967; Coppola, 1968). It also was demonstrated that inhibition by reserpine of LH secretion could be prevented by concurrent treatment with a monoamine oxidase inhibitor (iproniazid) or a precursor catecholamine substrate (L-DOPA). These observations provide convincing evidence that sympatholytic agents act on a central mechanism possibly through the hypothalamic catecholamine, which control the pituitary secretion of LH. In more recent studies, Kamberi et al. (1970) and Schneider and McCann (1970) demonstrated that catecholamines, particularly dopamine, induced release of LH and FSH by increasing hypothalamic LRF and FRF activities.

Reserpine and chlorpromazine were reported to produce lactation in humans (Sulman and Winnik, 1956; Rabinowitz and Friedman, 1961) and rabbits (Meites, 1957; Kanematsu et al., 1963), and to induce pseudopregnancy in rats (Barraclough and Sawyer, 1959). Early work from our laboratory indicated that injections of epinephrine and norepinephrine induced lactation in estrogen-primed rats and rabbits (Meites, 1959; 1962; Meites et al., 1963). These latter responses may be nonspecific, since anti-adrenergic and nonspecific agents were also effective in initiating lactation in these animals. The

effective drugs included acetylcholine, atropine, pilocarpine, serotonin, morphine, amphetamine, reserpine, chlorpromazine, and meprobamate. Since lactation is not a specific indicator of prolactin release, these early experiments did not prove that biogenic amines could induce prolactin release (Meites et al., 1972). Nevertheless, the observation that reserpine and chlorpromazine stimulated mammary growth and lactation in rats (Meites, 1957, 1962; Meites et al., 1963) suggested these two drugs stimulated prolactin release. Ratner et al. (1965) further demonstrated that reserpine decreased hypothalamic production of PIF, providing an explanation of how reserpine evoked increase in prolactin secretion. Similar result was also reported on perphenazine, a compound related to chlorpromazine (Danon et al., 1963). The work by Mizuno et al. (1964) demonstrated that injections of iproniazid, a monoamine oxidase inhibitor and therefore a depressor of catecholamine metabolism, inhibited post-partum lactation in rats, suggesting decreased secretion of prolactin. This provided the first evidence that brain catecholamines are inhibitory to anterior pituitary secretion of prolactin. We have extended this in a more recent study and observed that a single injection of iproniazid decreases pituitary release of prolactin by increasing hypothalamic PIF activity in cycling female rats (Lu and Meites, 1971). Alpha-methyl-para-tyrosine, a catecholamine synthesis inhibitor, and sympatholytic agents including reserpine, alpha-methyl-DOPA,

tetrabenazine and syrosingopine were reported to stimulate prolactin secretion in rats (Coppola *et al.*, 1965; Lippmann *et al.*, 1967; Coppola, 1968). Drugs which reduce hypothalamic catecholamines stimulated prolactin secretion, whereas agents which deplete catecholamines in tissues other than the brain were ineffective (Coppola, 1968). The stimulation of prolactin secretion by sympatholytic agents, or by catecholamine synthesis inhibitors could be blocked by concurrent treatment with monoamine oxidase inhibitors, or precursor catecholamine substrates respectively (Coppola *et al.*, 1965; Lippmann *et al.*, 1967; Coppola, 1968; van Maanen and Smelik, 1968). These observations provide evidence that catecholamines in the hypothalamus participate in the inhibitory control of pituitary prolactin secretion. Drugs which stimulate pituitary release of prolactin exert their effects by reducing hypothalamic catecholamine levels.

In vitro studies by Talwalker *et al.* (1963) indicated that norepinephrine and epinephrine had no direct effect on pituitary release of prolactin, whereas Gala and Reece (1965) observed that some doses of epinephrine increased prolactin release by rat pituitary. Jacobs *et al.* (1968), MacLeod (1969), and Birge *et al.* (1970) reported that catecholamines including dopamine, norepinephrine and epinephrine profoundly inhibited prolactin release by rat pituitary tissue *in vitro*, and concluded that the catecholamines may represent the not as yet defined hypothalamic PIF. In our laboratory we

confirmed the observation (Koch et al., 1970) that relatively large doses of catecholamines inhibited prolactin release in vitro, but in addition we found that the actions of catecholamines on pituitary release of prolactin in vitro is dose-dependent, with high doses producing inhibition, intermediate doses no effect, and low doses stimulation of prolactin release. The low stimulating doses of catecholamines are approximately equal to the amounts reported to be present in the rat hypothalamus (Lippmann, 1968; Donoso et al., 1967). Catecholamines are rapidly degraded in vitro to form adrenochromes which exert powerful pharmacological effects on cellular metabolism (Wurtman, 1970). In our laboratory we also observed that drugs unrelated chemically to catecholamines could decrease prolactin release by rat pituitary in vitro (Lu and Meites, unpublished). Thus, reserpine and chlorpromazine are well known to stimulate prolactin release in vivo (Lu et al., 1970), but can inhibit prolactin release by rat pituitary tissue in vitro. This provides evidence that the effects of drugs in vitro do not necessarily reflect their actions in vivo. Since there is as yet no evidence that dopamine and norepinephrine released in the median eminence of hypothalamus can enter the pituitary portal vessels (Wurtman, 1970), it is doubtful that hypothalamic catecholamines exert by direct effect on pituitary release of prolactin.

By use of a specific radioimmunoassay for rat prolactin (Niswender et al., 1969), we observed that systemic injections of a single dose of various catecholamines produced no change in serum prolactin levels in the rat, although dopamine and epinephrine evoked a small but statistically significant decrease in pituitary prolactin (Lu et al., 1970). Inasmuch as the "blood-brain barrier" in the hypothalamus effectively hinders the entry of circulating catecholamines into the brain tissue, no definite conclusions could be made at that time as to the role of catecholamines on prolactin release. In a more recent study, drugs that readily pass through the "blood-brain barrier" and can either increase or decrease hypothalamic catecholamines were used to assess their effects on prolactin release (Lu and Meites, 1971; Lu et al., 1970). Reserpine, chlorpromazine, alpha-methyl-para-tyrosine, alpha-methyl-meta-tyrosine, methyl-DOPA and d-amphetamine, each is known to reduce brain catecholamines, greatly stimulated pituitary release of prolactin in cycling female rats, thus elevating serum prolactin levels and reducing pituitary prolactin concentrations. A single injection of L-DOPA (the immediate precursor of dopamine) or of monoamine oxidase inhibitors (pargyline, iproniazid, or Lilly compound-15641), each known to enhance hypothalamic catecholamine activity, significantly decreased serum prolactin values. It was further demonstrated that the latter group of drugs (L-DOPA and the three monoamine

oxidase inhibitors) increased PIF activity in the hypothalamus (Lu and Meites, 1971). We have extended these observations in a more recent study and demonstrated that L-DOPA reduced serum prolactin values in intact and pituitary-grafted, hypophysectomized rats by increasing PIF activity in the hypothalamus and eliciting prolactin release-inhibiting activity in the systemic circulation (Lu and Meites, 1972). In a related study, Kamberi et al. (1970) reported that a single injection of dopamine into the third ventricle of rats decreased serum prolactin by increasing PIF activity in the pituitary portal blood. These results suggest that L-DOPA and dopamine increase both synthesis and release of PIF by the rat hypothalamus. On the other hand, reserpine and chlorpromazine reduce hypothalamic PIF and stimulate prolactin release. Haloperidol, another neuroleptic drug that reduces brain catecholamines also markedly elevated serum prolactin by decreasing PIF in the hypothalamus (Dickerman et al., 1972). From these and other related observations the concept evolved that hypothalamic catecholamines, including dopamine and norepinephrine, act as neurotransmitters to increase the release of hypophysiotropic PIF, which in turn enters the pituitary portal vessels to inhibit pituitary prolactin release (Meites et al., 1972; Wurtman, 1970; Fuxe and Hokfelt, 1969; Coppola, 1968).

Reports on the role of other biogenic amines on prolactin secretion also have appeared recently. Work by Gala

et al. (1970) indicated that hypothalamic cholinergic fibers had an inhibitory influence on pituitary prolactin secretion; they observed deciduomata formation by implanting atropine, an anti-cholinergic drug, into the rat hypothalamus. Kamberi et al. (1971) reported that injection of serotonin or melatonin into the third ventricle of rats stimulated prolactin release, suggesting involvement of indoleaminergic nerves in the hypothalamic control of prolactin secretion. In our laboratory we reported that a single intraperitoneal injection of serotonin had no definite effect on serum prolactin values (Lu et al., 1970). We have extended this in a more recent study and demonstrated that a single intravenous injection of 5-hydroxytryptophan, the immediate precursor of serotonin, melatonin, or to a lesser extent, tryptophan, significantly elevated serum prolactin levels in cycling female rats (Lu and Meites, unpublished). Indoleamines and catecholamines in the rat hypothalamus appear to work in opposition on the control of pituitary prolactin secretion (Meites et al., 1972; Clemens and Meites, 1972). It is noteworthy that indoleamines exert an inhibitory influence on pituitary secretion of gonadotropins (Kamberi et al., 1970; 1971), a mechanism which also is in opposition to the effects of the catecholamines (Kamberi et al., 1970; Schneider and McCann, 1970). The mechanisms of action by which hypothalamic indoleamines stimulate prolactin release remain to be determined, since no significant change in PIF activity is observed

in the hypothalamus or serum from rats given a single injection of tryptophan, 5-hydroxytryptophan, or serotonin (Lu and Meites, unpublished).

E. Short-Loop Feedback Control of Pituitary Prolactin Secretion

Most anterior pituitary hormones have well defined target glands or tissues that produce hormones that inhibit secretion of their tropic hormones by the pituitary. However, this classic endocrine feedback system does not appear to operate in the cases of GH and prolactin. Many years ago Sgouris and Meites (1953) postulated that prolactin may act to control its own secretion by the pituitary. This hypothesis has been sustained by many recent studies, although its physiological significance is not yet clear. Motta et al. (1969) proposed the term "short feedback loop" referring to the mechanism by which the anterior pituitary hormones themselves act to control their own secretion.

Transplantation of pituitary "mammosomatotropic" tumors into intact rats was reported to decrease pituitary prolactin content (MacLeod, 1966; 1968). Chen et al. (1967) made similar observations in rats with a "mammotropic" tumor and further demonstrated that these pituitary tumor transplants increased hypothalamic PIF content. Injections of prolactin were shown to reduce pituitary weight and prolactin concentration in intact female rats (Sinha and Tucker, 1968). Multiple pituitary grafts underneath the kidney

capsule were reported to lower pituitary weight and prolactin concentration of the in situ pituitary in intact and ovariectomized rats (Welsch et al., 1968). Enormous amount of prolactin are released by the transplanted pituitary, but secretion of other anterior pituitary hormones are greatly reduced (Meites, 1966). Implants of prolactin into the median eminence of rats also reduced serum prolactin values (Voogt and Meites, 1971). These results indicate that prolactin can act to depress its own secretion by the pituitary.

Clemens and Meites (1968) showed that implantation of a minute amount of prolactin into the median eminence of intact or ovariectomized rats decreased pituitary prolactin content, inhibited mammary growth, reduced the number of corpora lutea, and increased PIF content in the hypothalamus. Inhibition of lactation was observed when prolactin was implanted into the medium eminence of lactating rats (Clemens et al., 1969). These results suggested that prolactin implants into the median eminence acted by increasing PIF to inhibit both synthesis and release of prolactin.

Chen et al. (1968) observed that an implant of prolactin in the median eminence shortened the length of pseudopregnancy and prevented the formation of deciduomata. An implant of LH or FSH had no effects. Pregnancy was terminated when an implant of prolactin was placed into the median eminence of early pregnant rats (Clemens et al., 1969). However, the pregnancy could be maintained by daily injections

of progesterone. These observations indicated that prolactin implants into the median eminence inhibited pituitary prolactin secretion, thereby resulting in luteal regression and reduced progesterone secretion necessary for maintenance of pregnancy and pseudopregnancy.

A prolactin implant into the median eminence not only can shut off its own secretion, but stimulates release of gonadotropins by the pituitary, thus resulting in follicular growth, ovulation, and resumption of cycling in pseudopregnant, early pregnant or post-partum lactating rats (Clemens and Meites, 1968; Clemens et al., 1969; Voogt and Meites, 1971). Systemic injection or implantation of prolactin into the median eminence of immature female rats hastened the onset of puberty (Clemens et al., 1969) and resulted in elevated pituitary FSH levels and a probable increase in LH release (Voogt et al., 1969). The action of proactin implant in the median eminence is unique, since implants of other hormones such as LH, FSH, ACTH or GH each appear to inhibit selectively only their own secretion by the anterior pituitary, and have no effect on the secretion of other pituitary hormones (Motta et al., 1969).

Averill (1969) grafted a pituitary into the hypothalamus of rats and observed no luteotropic effects by the pituitary grafts. Although many of the grafts were distant from the median eminence and portal vessels, prolactin secretion by the grafts was inhibited. This strongly indicated

that there was a local feedback between the graft and hypothalamic tissue that secretes PIF. Thus, it becomes obvious that the site of the inhibitory action of prolactin in its own secretion is in the hypothalamus. Work by Nicoll (1971) has indicated that prolactin does not act directly on the pituitary to inhibit its own secretion *in vitro*. It is significant that prolactin injections to rats markedly activated the tubero-infundibular dopaminergic neurons (Fuxe and Hokfelt, 1970). This activation of dopaminergic fibers in the hypothalamus provides an explanation of how prolactin implants in the median eminence increase the release of PIF and inhibit prolactin secretion. Evidence has been presented that L-DOPA, the immediate precursor for dopamine synthesis, increased PIF activity in the hypothalamus (Lu and Meites, 1971; 1972). This increase in dopamine activity in the hypothalamus also explains why prolactin implants increase pituitary release of FSH and LH, since dopamine has been shown to increase the release of FSH-RF and LH-RF into the portal vessels (Kamberi *et al.*, 1970).

F. Direct Effects of Drugs and Hormones on Pituitary Prolactin Release

The foregoing considered the control of pituitary prolactin secretion through the hypothalamus. The biogenic amines, short-loop feedback, prolactin-inhibiting activity and prolactin-stimulating activity of the hypothalamus constitute the principal regulatory mechanisms of prolactin

secretion. However, several hormones and drugs can influence prolactin secretion by a direct action on the pituitary. In addition, some agents can act both on the pituitary and hypothalamus to regulate prolactin secretion.

Injections of estrogen were reported to stimulate prolactin secretion in hypophysectomized rats with a pituitary graft underneath the kidney capsule (Desclin, 1956; Chen et al., 1970; Lu et al., 1971). Although these observations suggested a direct action of estrogen on the grafted pituitary, the possible intervention of the hypothalamus could not be completely excluded. Nicoll and Meites (1962) reported that estradiol could directly stimulate rate pituitary to release prolactin in a 3-day organ culture. Subsequent studies confirmed this finding (Nicoll and Meites, 1963; 1964). More recently, by use of a specific radioimmunoassay for rat prolactin, a direct stimulation by estradiol of rat pituitary prolactin release in a 12-hour incubation was demonstrated by Lu et al. (1971). The direct action of estrogen on the pituitary can be completely blocked when an ergot derivative, ergocornine, is administered simultaneously (Lu et al., 1971). Since estrogen injections in vivo can depress hypothalamic PIF activity in the rat (Ratner and Meites, 1964), this indicates that estrogen acts to stimulate prolactin release both via the hypothalamus and by a direct action on the anterior pituitary. The direct action of estrogen on the pituitary may have a role in the control of

prolactin surge on the afternoon of proestrus. Ovariectomy (Clark and Meites, unpublished) or administration of an estradiol-antiserum on the day before proestrus prevented any rise in serum prolactin on the following day (Neill et al., 1971).

It has been well demonstrated that thyroid function can influence pituitary secretion of prolactin (see Meites, 1960, 1966). Reports from our laboratory elucidated the mechanism of action by which thyroid hormones influence pituitary prolactin secretion. Incorporation of small amounts of thyroxine and triiodothyronine into a culture system was found to directly increase prolactin release by the rat pituitary (Nicoll and Meites, 1963). Subsequently, Chen and Meites (1969) reported that in vivo injections of thyroxine had no effect on hypothalamic PIF activity in rats. This suggests that thyroxine stimulates prolactin release only by a direct action on the anterior pituitary.

Mention has already been made of the ability of synthetic TRH to increase blood prolactin levels in primates and bovine. However, there is as yet no clear evidence that TRH acts directly on the primate or bovine pituitary to release prolactin. Tashjian et al. (1971) reported an increase in prolactin and decrease in GH release by TRH when the latter was added to cultures or incubations of clonal cells from rat pituitary tumors. These workers also reported that clonal strains of rat pituitary tumor cells showed significant

increases in prolactin and decreases in GH release when extracts of rat hypothalamus, cerebral cortex, kidney or liver were added to the culture medium (Tashjian et al., 1970). It was reported that TRH had no effect on prolactin release when incubated with bovine pituitary tissue in vitro (LaBella and Vivian, 1971; Convey et al., 1972), despite the increase by TRH of blood prolactin levels in bovine (Convey et al., 1972). Bowers (1971) and McCann (1971) also failed to observe any effect of TRH on prolactin release by normal rat pituitary in vitro. A recent study from our laboratory (Lu et al., 1972) indicated that TRH has no effect on prolactin release when added to incubation of normal rat pituitary halves or "mammotropic" pituitary tumor tissue. A single injection of large dose of TRH to rats has no effect on serum prolactin either. However, incorporation of TRH into an incubation medium was found to evoke a limited increase (about 30%) in prolactin release by pituitary halves from rats 5 weeks after thyro-parathysoidectomy. Thus the pituitary of hypothyroid rats may respond somewhat differently to TRH than the pituitary of normal rats. These observations suggest that synthetic TRH is not a specific releaser of prolactin in the rat. The possibility that synthetic TRH is identical with or similar to the presumed PRF of mammals remains to be determined (see Section II, C).

Catecholamines have been shown to alter pituitary prolactin release in vitro, although its physiological

significance is not clear. Jacobs et al. (1968), MacLeod (1969) and Birge et al. (1970) reported that dopamine, norepinephrine and epinephrine inhibited prolactin release by the incubated rat pituitary, and suggested that catecholamines themselves might be identical with hypothalamic PIF. Subsequently, Koch et al. (1970) demonstrated that the effects of catecholamines on pituitary prolactin release in vitro is dose-dependent, with high doses producing inhibition and low doses stimulation of prolactin release. The low stimulating doses are approximately equal to the amounts of catecholamines reported to be present in the rat hypothalamus (Lippmann, 1968; Donoso et al., 1967). Catecholamines were not detected in the pituitary portal blood (Wurtman, 1970; Porter et al., 1970), and had no effect on prolactin release when infused into a single pituitary portal vessel (Kamberi et al., 1970). There is as yet no definite evidence that epinephrine is synthesized in the mammalian brain (Anton-Tay and Wurtman, 1971). In view of these observations and the reported pharmacological effects by catecholamine metabolites on cellular metabolism in vitro (Wurtman, 1970), it is probable that the direct effects of catecholamines on pituitary prolactin release observed in vitro are non-specific in nature (Koch et al., 1970).

Early studies in the rat indicated that injections of ergot drugs induced termination of pseudopregnancy and early pregnancy (Shelesnyak, 1955; Carlsen et al., 1961),

suppression of implantation (Shelesnyak, 1964), and inhibition of lactation (Zeilmaker and Carlsen, 1962), suggesting inhibition of pituitary prolactin secretion. Later, it was found that ergot drugs inhibited mammary tumor growth in rats (Nagasawa and Meites, 1970; Yanai and Nagasawa, 1970). Definite evidence that ergocornine, an ergot derivative, reduces pituitary and serum prolactin levels was reported by Nagasawa and Meites (1970) and by Wuttke et al. (1971). Subsequently, it was found that ergocornine acted directly on rat pituitary to diminish prolactin release in vitro, resulting in an increase in pituitary prolactin stores (Lu et al., 1971). Ergocornine also can prevent estradiol from increasing prolactin release in vitro. When ergocornine was injected into hypophysectomized rats with a pituitary graft underneath the kidney capsule, it significantly inhibited prolactin secretion by the graft. When ergocornine was injected together with estrogen into rats, it prevented estrogen from producing enlargement of the pituitary, counteracted estrogen stimulation of pituitary prolactin secretion. Results from other studies also suggest that ergot drugs act directly on the pituitary. Our laboratory recently reported that ergot drugs induced significant regression or inhibition of pituitary tumor growth in rats (Quadri et al., 1972). Ergocornine caused a decrease in cell number and a disappearance or pycnosis of nuclei in the pituitary tumor tissue. It will be seen in a subsequent experiment that ergot drugs

inhibit prolactin release in rats bearing pituitary tumor transplants (see Experiment VII). It was reported that ergocornine increased hypothalamic PIF content (Wuttke et al., 1971), suggesting that it acted at least in part via the hypothalamus. Inasmuch as ergocornine inhibits prolactin release from the incubated pituitary and by the pituitary transplant in vivo, it can be assumed that inhibition of prolactin release is due mainly to its direct action on the pituitary (Lu et al., 1971).

Implants of sodium pentobarital into the median eminence of rats reduced hypothalamic PIF activity and elevated serum prolactin values (Wuttke et al., 1971). On the other hand, sodium pentobarbital directly inhibited prolactin release by the rat pituitary in vitro, apparently accounting for its ability to depress serum prolactin when given in vivo (Wuttke and Meites, 1970). It appears that sodium pentobarbital exerts a biphasic effect on pituitary prolactin release, initially stimulating and subsequently inhibiting prolactin release (Wuttke et al., 1971).

In addition to the above drugs, several other drugs can exert direct pharmacological effects on pituitary prolactin release in vitro. Thus, reserpine, chlorpromazine, alpha-methyl-para-tyrosine, and alpha-methyl-meta-tyrosine each can inhibit prolactin release when incubated with rat pituitary in vitro (Lu et al., 1970b), despite their marked

ability to increase prolactin release *in vivo* (Lu *et al.*, 1970a). It is apparent, therefore, the effects of drugs *in vitro* do not necessarily reflect their actions *in vivo*.

G. Multiple Control of Pituitary Prolactin Secretion

It is evident from the preceding discussions that pituitary secretion of prolactin is controlled mainly by the hypothalamus but also by other systems. Prolactin secretion normally is inhibited by hypothalamic PIF under most conditions. But it may also be influenced by the presumed PRF under some conditions. An increase in prolactin release could result from a decrease in PIF or by stimulation from PRF in the hypothalamus. Biogenic amines apparently participate in the control of pituitary prolactin secretion by regulating the release of PIF (and probably PRF) by the hypothalamus. There appears to be a dual control in the hypothalamus over pituitary secretion of prolactin, with a predominant adrenergic tonus acting to depress release of prolactin under most conditions, and a serotonergic system that may be responsible for the diurnal rise in pituitary and serum prolactin (Clark and Baker, 1964; Koch *et al.*, 1971) on the late afternoon of each day and perhaps under other conditions (Meites *et al.*, 1972). No feedback mechanism is evident for the control of prolactin secretion by its "target" gland hormones. The "auto regulation" of prolactin secretion by a "short-loop" feedback may represent a

physiological means for its own control. Extrahypothalamic structures also may influence pituitary prolactin secretion (Mena and Beyer, 1968; Rubenstein and Sawyer, 1969; Tindal and Knaggs, 1969, 1970), providing an explanation of how emotional and stressful stimuli influence prolactin release (Clemens and Meites, 1972). In addition to the hypothalamic (central nervous) mechanism mentioned above, several hormones and drugs also can alter prolactin secretion by a direct action on the pituitary. Some agents can influence pituitary prolactin secretion by acting both through the hypothalamus and directly on the pituitary.

MATERIALS AND METHODS

I. Animals

All intact mature male and female rats of Sprague-Dawley strain used for experiments, and as pituitary and hypothalamus donors, were purchased from Spratan Research Animals, Inc. (Haslett, Michigan). Mature female, hypophysectomized rats of Sprague-Dawley strain were obtained from Hormone Assay Labs., Inc. (Chicago, Illinois). Inbred female rats of Wistar-Furth strain were purchased from Microbiological Associates, Inc., Walkersville, Maryland. All rats were housed in metal wire cages in temperature-controlled (75 ± 1°F) and artificially illuminated (lights on from 5:00 AM until 7:00 PM daily) rooms, and were maintained on a standard diet of Wayne Lab Blox pellets (Allied Mills, Chicago, Illinois) and tap water ad libitum. Thyro-parathyroidectomized rats were given 1% calcium lactate solution for one week post-operation, and hypophysectomized rats were fed a supplement of fresh orange slices and sugar cubes throughout the experimental period. All surgical procedures except blood vessel cannulation were performed with ether anesthesia, and surgically treated rats were given 0.2 ml of Bicillin

(Wyeth Labs, Inc., Philadelphia, Pa.), a wide spectrum antibiotic, post-operatively by intramuscular injection to prevent infection.

II. Pituitary Transplantation Technique

Mature male rats (250-300 g bw) and female rats (200-220 g bw) of the Sprague-Dawley strain were used as pituitary donors. The donor rats were decapitated with a guillotine. The pituitary gland was immediately removed and placed on a filter paper moistened with physiological saline in a petri dish. The anterior pituitary was carefully separated from the posterior lobe using a fine forceps. Seven days after hypophysectomy, the rats were anesthetized and an abdominal skin incision (2-3 cm long) was cut just beneath the ribs to expose the left kidney. A small slit was made through the transparent renal capsule membrane, and a single anterior pituitary was placed underneath the kidney capsule close to the renal hilus.

III. Blood Vessel Cannulation Technique

Intact and thyro-parathyroidectomized mature male rats were anesthetized by a single intraperitoneal injection of sodium-pentobarbital (4.5 mg/100 g bw). The rat was placed on an operation board in a supine position. A lateral ventral neck skin incision (2-3 cm long) was cut with a razor

blade. The skin and fascia were retracted to expose the left carotid artery or jugular vein. With the aid of a pair of closed forceps placed underneath, the blood vessel was pulled out and freed from surrounding tissues and nerves. Polyethylene tubing (# PE-20) filled with heparin solution was inserted into and tied on the carotid artery or jugular vein. The arterial cannula was inserted anteriorly with its opening facing to the head, whereas the tip of the jugular vein cannula was inserted down to the junction of the vena cavae. The external portion of the tubing was passed subcutaneously around the neck region and emerged through postcervical skin on the neck. The cannulated rats were maintained in separate cages to avoid intermingling and chewing of cannulas. After cannulation, at least 3 days of recovery were permitted before the rats were used for experiments, unless otherwise stated. During the experiment, each rat was kept in a cage (Figure 3). A segment of 40 cm-long polyethylene tubing of the same size was connected to the cannula. Collection of blood and infusion of agents through the cannula were performed outside the cage usually without appreciable notice or disturbance to the rat.

IV. Pituitary Tumor Transplantation Technique

Furth pituitary mammotropic tumors (MtT.W 15) were removed by sterile technique from inbred Wistar-Furth strain female rats. The pituitary tumors were cut into fine pieces

Figure 3. View of a Rat with a Jugular Cannula Attached by an Extension Segment of Polyethylene Tubing.

in sterile saline solution using an iris scissors. A volume of 0.2 ml tumor mince was injected subcutaneously to 50-day-old female rats of the same strain in the postcervical region on the neck in order to develop transplantable tumors. This transplantable pituitary tumor, originally obtained through the courtesy of Dr. Jacob Furth, Department of Pathology, Columbia University, is known to secrete large amounts of prolactin and GH (Furth, 1961).

V. Preparation of Hypothalamic Extract, Pituitary Homogenate, and Serum

Hypothalamus-donor rats and rats at the end of the experiments were decapiated by guillotine. The hypothalamus including the stalk-median eminence was quickly removed with a small, curved forceps coated with epoxylite. The hypothalamic fragment was immediately placed in a plastic centrifuge tube containing chilled 0.1 N HCl (0.15 ml/hypothalamic fragment) kept in an ice water bath. All hypothalami from each experiment were pooled in one tube and kept frozen at -20°C until assayed by *in vitro* incubation. Just prior to incubation, the pooled hypothalami were quickly thawed in lukewarm water and homogenized with a Sonifier cell disruptor (Heat Systems-Ultrasonics, Inc., Plainview, New York). The acid homogenates were centrifuged at 12,000 x G for 40 minutes at 4°C in a Sorvall RC2B automatic refrigerated centrifuge (Ivan Sorval, Inc., Norwalk, Conn.). The supernatant was then incorporated into pH 7.4 medium 199 (Difco Labs.,

Detroit, Michigan). The acid mixture was again neutralized to pH 7.4 by adding 1.0 N NaOH a drop at a time. The volume and concentration were so adjusted that each hypothalamic equivalent was contained in 2.0 ± 0.1 m. of incubation medium.

Rats were killed by decapitation at the end of the experiments. The pituitary gland was quickly removed, placed in a petri dish over a filter paper moistened with physiological saline. The anterior pituitary was separated from the posterior lobe, blotted on filter paper, weighed on a Mettler electrical balance (Mettler Instrument Corp., Highstown, New Jersey) and homogenized with neutral phosphate buffer saline (PBS) in a 5-ml disposable culture tube using a Sonifier cell disruptor. The volume and concentration were so prepared that each ml of homogenate contained 0.1 mg of female, or 0.2 mg of male rat anterior pituitary tissue. The homogenates of anterior pituitary tissue after in vitro incubations were similarily prepared with a concentration of 0.25 mg per ml. The pituitary homogenates were kept frozen at -20°C until assayed.

Collection of blood samples was performed by cardiac puncture under light ether anesthesia using a 1-ml Tuberculin syringe with a # 26 1/2 hypodermic needle. The blood was collected into a 5-ml disposable culture tube (Kimble Products, Owens, Illinois and Scientific Products, Mcgaw, Illinois). The blood sample tube was first placed obliquely (Figure 4) in a refrigerator (4°C) for 4 hours before centrifuging at 5,000 rpm for 10 minutes in a Sorvall refrigerated centrifuge.

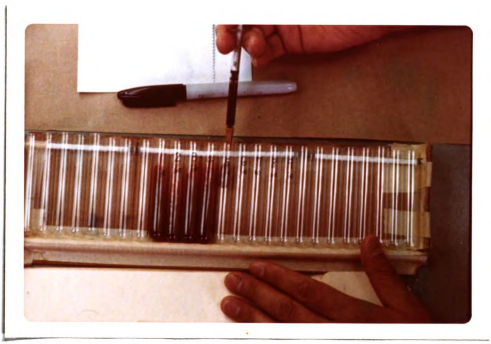


Figure 4. View of a Rack Holding Tubes with Blood Samples in an Oblique Position to Increase the Contact Surface of Blood to Glass.

Serum was pipetted into another 5-ml culture tube using a 1-ml Tuberculin syringe with a # 26 1/2 hypodermic needle. The serum tubes were capped with parafilm and kept frozen at -20°C until assayed.

VI. In Vitro Incubation Technique

Mature male rats (250-300 g bw) used as pituitary-donor were decapitated with a guillotine. The pituitary gland was immediately removed, placed in a petri dish over a filter paper moistened with neutralized medium 199. The anterior pituitary was hemisected using a razor blade after separation from the posterior lobe. For comparison, one half of the anterior pituitary tissue was placed in a control incubation tube and the other half in an experimental tube. Each anterior pituitary half was blotted on filter paper, weighed on a Mettler electrical balance, and placed in a 5-ml culture tube containing 2 ml of medium 199 at a pH of 7.4. Incubations were carried out in a Dubnoff metabolic shaking Incubator (Labline, Inc., Chicago, Illinois), 60 cycles per minute, under constant gassing with 95% O_2 - 5% CO_2 at 37 ± 0.5°C. After 30 minutes pre-incubation, the medium was removed and replaced with 2 ml of fresh medium 199 containing hypothalamic extract, serum, drug or hormone for incubation. During the incubation, 100 µl of medium were collected and replaced with 100 µl of fresh medium at different time intervals. The anterior pituitary tissue was removed from the

medium at the end of incubation, blotted on filter paper, weighed and homogenized according to the methods described under Section V. Both the media and pituitary homogenates were kept frozen at -20°C until assayed.

VII. Radioimmunoassay of Rat Prolactin

In all experiments, prolactin in individual serum samples, pituitary homogenates and incubation media was measured by a double-antibody radioimmunoassay. This radioimmunoassay for rat prolactin was developed by a collaborative effort between this laboratory and Dr. Midgley's laboratory in the Department of Physiology, the University of Michigan at Ann Arbor, Michigan (Niswender et al., 1969). The Ph.D. dissertation by Dr. C. L. Chen (Michigan State University, 1969), who devoted much of the initial efforts to the development of this method, describes the procedures and validation for this radioimmunoassay.

Purified rat prolactin (HIV-8-C and H-10-10-B), obtained from Dr. S. Ellis (NASA, Ames Research Center, Moffett Field, California), was used for radioiodination with I^{125} of I^{131} (Cambridge Nuclear Radiopharmaceutical Corp., Billerica, Massachusetts). The radioiodinated rat prolactin was collected by elution through a 1 x 15 cm Bio-Gel P-60 column, and was diluted with 0.1% egg white-neutral phosphate buffer saline (EW-PBS) to a working concentration of 30,000 cpm per 100 µl as counted under optimal conditions inside an

automatic gamma well counter (Nuclear-Chicago Corp., Des Plaines, Illinois). This amount of I^{125}- or I^{131}-rat prolactin was added to each incubation tube for competitive binding with un-labelled rat prolactin to the anti-rat prolactin antibody.

The antibody against rat prolactin was developed in a rabbit, and was diluted to a working concentration of 1:4,000 or 1:5,000 which consistently bound 35-45% of the radioiodinated rat prolactin added to the incubation tube.

The antiserum against rabbit gamma globulin (Anti-RGG) was developed in sheep. Optimal dilutions (1:3 to 1:9) of the antisera from different bleedings were titrated to precipitate the rabbit gamma globulin.

The incubation of rat prolactin with the antibodies was carried out in 12 x 75 mm disposable culture tubes (Kimble Products, Owens, Illinois and Scientific Products, McGaw Park, Illinois) placed in a refrigerator at 4°C. On day one, 200 µl of the first antibody was added to each incubation tube containing known (standard) or unknown (assay sample) amounts of rat prolactin plus the diluent 1% EW-PBS in a total volume of 500 µl. Twenty four hours later, 100 µl of radioiodinated rat prolactin was added to each tube, and the incubation was carried out for another 24 hours to achieve maximal binding of the first antibody to both labelled and un-labelled rat prolactin. On day three, 200 µl of

anti-RGG was pipetted into each tube, and the incubation was continued for the next 3 days to precipitate maximal amounts of both labelled and un-labelled antigen-antibody complex.

At the end of the 5 day-incubation, 3 ml of PBS were added to each tube before centrifuging in a Model-K International Centrifuge (International Equipment Co., Needham Heights, Massachusetts) at 2,000 rpm for 35 minutes. The supernatant was decanted. The tube with precipitate in the bottom was air-dried before being placed into a plastic capsule and counting in an automatic gamma well counter. The counting time for all tubes was set for 10,000 specific counts from the "zero hormone"-first antibody binding tube, after subtraction of non-specific counts from the normal rabbit serum (NRS) -antigen binding tube. Non-specific counts by NRS in each tube were both corrected on the standard curve and automatically subtracted by background setting on the gamma counter.

Purified rat prolactin preparations (HIV-8-C and H-10-10-B obtained from Dr. S. Ellis and NIAMD-rat prolactin RP-1 received from NIH Rat Pituitary Hormone Program, Bethesda, Maryland) were used as reference standards in different experiments of this study. A standard curve was drawn on semi-logarithmic paper on the results of duplicate sets of 16 different doses of standard rat prolactin ranging from 0.4 to 40 ng. The NIAMD-rat prolactin-RP-1 in the radioimmunoassay,

using I^{125}-labelled rat prolactin as tracer, consistently gave a value of 4.0 ± 0.3 ng at the 50% binding point on the standard curve.

For the assay, 50-200 µl of serum sample or 25-100 µl of pituitary homogenate were pipetted into each incubation tube, depending on the experiment. For in vitro experiments, 50-150 µl of diluted incubation medium were used for prolactin assay. The dilution of the incubation medium depends on the nature and the time of incubation. In all experiments, samples from comparable experiment groups were usually assayed on the same date, and each sample was assayed at 2 or 3 different dose levels to insure accurate measurement of prolactin values. Prolactin values were expressed in terms of purified rat prolactin reference standard as indicated above.

VIII. Mammary Gland Growth Rating System

Inguinal mammary pads were removed from rats after decapitation, spread flat on cork, and fixed in Bouin's fluid for whole mount evaluation. Each mammary pad was stained with hematoxylin by a standard procedure (Nandi, 1959) as follows, for gross examination:

1. The mammary pad on cork is fixed in Bouin's fluid overnight
2. Wash in running water for at least 24 hours until no picric acid is retained

3. Remove extraneous tissues from the pad
4. Stain the pad directly in Mayer's hematoxylin
5. Wash in running water overnight
6. Destain in acid alcohol until the right degree of stains has been obtained
7. Pass the mammary tissue through a series of alcohol for dehydration (30% - 50% - 70% - 85% - 95% - 100% - 100% - 100%)
8. Immerse (store) the whole mammary tissue in methyl salicylate for clearance.

Each mammary pad is rated under a dissecting microscope for development, according to the following scale (Talwalker and Meites, 1961):

1 = Few ducts; few or no end buds;
2 = Moderate duct growth; moderate number of end buds;
3 = Numerous ducts and branches; many end buds;
4 = Numerous ducts and branches; moderate lobulo-alveolar growth;
5 = Numerous ducts and branches with dense lobulo-alveolar growth, as in mid or late pregnancy.

IX. Methods of Statistical Analysis

The prolactin values obtained from radioimmunoassay of 2 or 3 dose levels of the same sample were averaged, and the mean value was used as the prolactin value for that

particular sample. Mean and standard error of the mean were calculated from the averaged prolactin value of each sample within an experimental group. Mammary gland growth ratings, organ weights, and body weights were similarly analyzed. Student's "t" test was used to determine the significance of difference between the control and the experimental groups. In experiments with 2 or more "experimental" groups, a test of significant differences was carried out by an "F" test for approximation. If the result of the "F" test showed significant differences, Duncan's new multiple range test (Duncan, 1955) was used to evaluate the significance of differences between groups.

EXPERIMENTAL

I. In Vivo and In Vitro Effects of Catecholamines on Pituitary Prolactin Release

A. Objectives

Both *in vivo* and *in vitro* studies have indicated that catecholamines may influence prolactin release by the rat pituitary. It was considered of interest to determine the effects of catecholamines on pituitary prolactin release. Dopamine, norepinephrine and epinephrine were tested for their effects on serum and pituitary prolactin levels of female rats, as measured by radioimmunoassay. The direct effects of catecholamines on release of prolactin by the incubated pituitary also were studied.

B. Materials and Methods

1. Animals

Mature, 3- to 4-month-old, virgin female Sprague-Dawley rats, weighing 200-250 g each, were used in all *in vivo* experiments. Two estrous cycles (4-5 days/cycle) were followed by taking daily vaginal smears on all female rats before they were given a drug on the day of proestrus, when

serum prolactin levels are significantly higher than during diestrus (Amenomori et al., 1970; Kwa and Verhofstad, 1967). Since the purpose of these experiments was to determine whether catecholamines could depress serum prolactin levels, rats with higher serum prolactin than the low basal levels present during diestrus were used.

Mature male rats of Sprague-Dawley strain, weighing 250-300 g each, were used as pituitary-donors. The method for removal of anterior pituitary tissue from the rat is described under Materials and Methods.

2. Catecholamines

The catecholamines used were dopamine hydrochloride (Mann Research Labs., Inc., New York, N.Y.), L-norepinephrine bitartrate hydrate (K & K Labs., Plainview, N.Y.), and epinephrine chloride (Parke, Davis and Co., Detroit, Michigan). Each catecholamine was directly dissolved in 0.85% NaCl saline for injection, or first dissolved in saline, and then incorporated into medium 199 in volumes of 10-20 µl for incubation.

3. In vivo experiments

The catecholamine solutions in a volume of 0.6 ml were injected intraperitoneally into cycling rats at 10:00 AM on the day of proestrus. The catecholamines also were administered intravenously via the left carotid artery, and the

central end of the artery was immediately ligated after the injection to prevent bleeding. The doses of catecholamines and the routes of injection used are shown in Tables 1 and 2. Individual blood samples (0.4-0.5 ml) were taken from the rats at 0, 0.5, 1 and 2 hours following catecholamine injection. A pre-treatment blood sample was taken from each rat prior to catecholamine injection for comparison with subsequent blood samples, and the rats were killed after the last blood sample was collected. The methods for preparation of pituitary homogenate and serum sample for prolactin radioimmunoassay are described under Materials and Methods.

4. In vitro experiments

Each anterior pituitary half was pre-incubated with 1 ml of medium 199 at a pH of 7.4 for 30 minutes. The method for in vitro incubation is described under Materials and Methods. After pre-incubation, the medium was removed and replaced with 1 ml of fresh medium 199 containing a given amount of catecholamine as shown in Tables 3 and 4. Six tubes were used for each dose of catecholamine. Catecholamines were not incorporated into the control tubes. The incubations were carried out for 4 hours. At the end of incubation, the anterior pituitary halves were removed, and the media were kept frozen at -20°C until assayed.

5. Prolactin assay

Prolactin in individual serum samples, pituitary homogenates and incubation media were measured by radioimmunoassay. Each sample was assayed at 3 different dose levels, and the prolactin values were averaged and expressed in terms of the purified rat prolactin reference standard, HIV-8-C.

Student's "t" test, or analysis of variance followed by Duncan's new multiple range test was used to determine the significance of differences between groups.

6. Test for possible influence of drugs on radioimmunoassay

For the purpose of testing possible interactions between drugs and prolactin during radioimmunoassay, 8 series of assay tubes were prepared with the same concentrations of purified rat prolactin (HIV-8-C, 0.25-64 ng) as used for plotting the standard binding curve. To each assay tube one of the following amounts of drugs was added 12 hours before initiation of the antigen-antibody reaction: 10, 20, 30 or 40 µg of norepinephrine; 10 or 20 µg of epinephrine; 235 or 470 µg of alpha-methyl-para-tyrosine. Two additional series of assay tubes with prolactin but no drugs served as controls. At the end of the 5 day-incubation, the per cent binding of labelled rat prolactin was plotted on semi-logarithmic paper, and the curves obtained from the experimental tubes were compared with the standard curve from the control tubes.

C. Results

1. Effects of a single intraperitoneal injection of catecholamines on pituitary prolactin release

The data in Table 1 show that none of the 3 catecholamines produced any significant change in serum prolactin values as compared to pre-treatment values or saline controls by 2 hours following treatments.

2. Effects of a single intracarotid injection of catecholamines on pituitary prolactin release

A single intravenous injection of catecholamines produced no significant changes in serum prolactin levels after 30 minutes, 1 hour or 2 hours, when compared with pre-treatment or saline-control values (Table 2). The variations in the serum prolactin levels can be attributed at least in part to the surgical stress and ligation of the left carotid artery. Small but statistically significant decreases were observed in pituitary prolactin concentration after treatment with the larger doses of dopamine or epinephrine.

3. Effects of different doses of catecholamines on pituitary prolactin release in vitro

The data in Table 3 show that dopamine had no effect on pituitary prolactin release at doses of 2-40 ng, but produced marked inhibition at doses of 80-640 ng. In two

Table 1. Effects of intraperitoneal injections of catecholamines on serum prolactin levels.

Treatment	Dose/200 g bw	Serum prolactin levels, ng/ml		
		Pre-treatment	1 hr	2 hr
Saline (controls)	0.6 ml	57.0± 7.5[a](11)	48.7± 7.4	40.6±5.7
Dopamine	0.5 mg	56.0±11.9 (5)	61.1±19.9	47.2±8.5
Norepinephrine	0.25 mg	58.2±10.7 (5)	57.2± 3.7	51.6±3.6
Epinephrine	0.3 mg	56.8±10.6 (5)	53.2±10.4	45.8±4.9

[a]Mean and standard error of the mean.

( ) Number of rats in parentheses.

Table 2. Effects of intracarotid injections of catecholamines on serum and pituitary prolactin concentrations.

Treatment	Dose/ 200 g bw	Serum prolactin levels, ng/ml				Pit. prolactin concentration, µg/mg
		Pre-treatment	0.5 hr	1 hr	2 hr	
Saline (controls)	0.6 ml	44.4± 8.5[a] (9)	42.2± 7.9 (9)	36.4± 6.7 (9)	77.2±16.8 (9)	3.76±0.08 (8)
Dopamine	5 µg	55.4± 4.4 (4)	30.3± 5.9 (4)	37.3±11.9 (4)	75.8±24.7 (4)	3.55±0.15 (8)
Dopamine	10 µg	41.0± 8.8 (10)	30.4± 4.1 (10)	43.3± 9.3 (10)	34.0± 8.0 (10)	3.05±0.16 (8)[b]
Norepinephrine	5 µg	46.4± 6.6 (5)	34.6± 3.0 (5)	38.0±11.6 (5)	54.6±18.6 (5)	3.67±0.07 (8)
Norepinephrine	10 µg	47.1± 6.7 (11)	37.5± 4.5 (11)	43.2±12.1 (11)	61.8±19.3 (11)	3.52±0.19 (8)
Epinephrine	5 µg	51.0±11.5 (5)	57.0±17.3 (5)	57.4±17.5 (5)	77.6±20.7 (5)	3.55±0.05 (8)
Epinephrine	10 µg	38.6± 2.7 (10)	30.3± 1.4 (10)	33.2± 3.9 (10)	35.1± 1.9 (10)	2.92±0.18 (8)[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.01$.

( ) = Number of rats in parentheses.

Table 3. Effects of different doses of dopamine on pituitary prolactin release *in vitro*.

Treatment (ng/ml)	ng prolactin released per mg of pituitary	Percent of control (average)
0 (controls)	884±68[a]	100.0
2	872±34	98.6
10	857±41	96.9
20	843±57	95.4
40	787±86	89.0
80	497±34[b]	56.2
160	440±14[b]	49.8
320	432±25[b]	48.9
640	425±18[b]	48.0

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.05$.

separate experiments a dose of 20 ng of norepinephrine significantly increased prolactin release by an average of 39% (Table 4). A dose of 10 ng also stimulated prolactin release by an average of 31%, but this increase was not significant because of the large standard errors. Doses of 200 or 500 ng of norepinephrine significantly inhibited prolactin release. A dose of 10 ng of epinephrine increased prolactin release by an average of 47%, whereas 400 or 1,000 ng markedly inhibited pituitary prolactin release.

4. Effects of presence of drugs in sample on prolactin radioimmunoassay

Dopamine, norepinephrine and epinephrine had no effect on the prolactin radioimmunoassay system when incorporated directly into the incubation tubes as shown in Figure 5. They did not alter the antigen-antibody reaction or change the binding capacity of the purified, un-labelled rat prolactin. Thus, the presence of these drugs in the radioimmunoassay system did not influence the results reported here. Alpha-methyl-para-tyrosine, a potent stimulator, and ergocornine, a potent inhibitor of pituitary prolactin release (Lu et al., 1970; Lu et al., 1971), had no effect on prolactin radioimmunoassay.

D. Conclusions

The present study demonstrates that an intracarotid or intraperitoneal injection of large doses of dopamine, norepinephrine or epinephrine had no effect on serum prolactin levels. The larger of the two doses of dopamine and epinephrine given

Table 4. Effects of different doses of norepinephrine and epinephrine on pituitary prolactin release in vitro.

Treatment (ng/ml)	ng prolactin released/mg pituitary		Percent of control (ave.)
	Experiment 1	Experiment 2	
Norepinephrine			
0 (controls)	884± 68[a]	651± 61	100.0
2	959± 87	744± 53	111.4
10	1,261±239[b]	775± 35[b]	130.8
20	1,163±103[b]	949± 58[b]	138.7
40	1,028± 71[b]	697± 62[b]	111.7
200	658± 28[b]	274± 36[b]	58.3
500	115± 17[b]	---	13.0
Epinephrine			
0 (controls)	884± 68	937±154	100.0
2	985±117[b]	1,021±139	110.2
10	1,226±100[b]	1,258±148	136.5
20	1,248±190	981±107	122.9
40	923±119[b]	926±105[b]	101.6
400	390± 27[b]	419± 32[b]	44.4
1,000	---	187± 24[b]	19.9

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.05$.

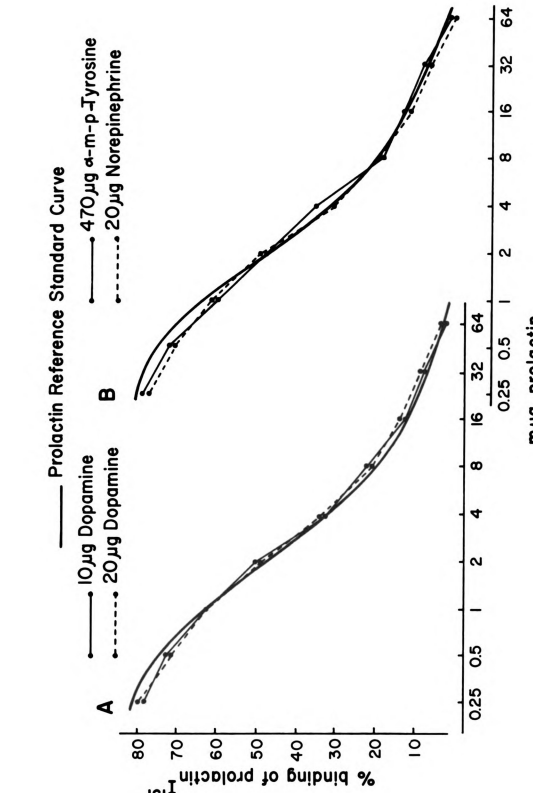


Figure 5. Rat Prolactin Reference Standard Curves Showing Lack of Effect of Drugs on Antigen (Prolactin)-Antibody Reaction.

intracarotidly produced small reductions in pituitary prolactin concentrations, but the significance of this is not clear since the serum prolactin values were not altered. Catecholamines have been reported not to cross the "blood-brain barrier" (Wurtman, 1970; Steinman et al., 1969), undoubtedly accounting for their lack of action on prolactin release. It will be shown in a subsequent experiment that precursors of catecholamines or inhibitors of catecholamine metabolism do cross the "blood-brain barrier" and do alter serum prolactin levels.

The in vitro experiments indicate that the effects of catecholamines on pituitary prolactin release in vitro are dose-dependent, with high doses producing inhibition, intermediate doses no effect, and low doses stimulation of prolactin release. The higher doses are within the range used by MacLeod (1969) and Birge et al. (1970) to demonstrate inhibition of prolactin release in vitro. The low stimulating doses (10-20 ng/ml) are approximately equal to the amounts of catecholamines reported to be present in the rat hypothalamus (Lippman, 1968; Donoso et al., 1967). The physiologic significance of the in vitro actions of catecholamines is not evident, since they inhibit prolactin release only at pharmacologic dose levels (Lu et al., 1970a). Catecholamines are not present in detectable amounts in pituitary protal blood (Wurtman, 1970), and failed to alter prolactin release when presented directly to the pituitary via portal

vessel infusion (Kamberi *et al.*, 1970b). Drugs which significantly stimulate pituitary prolactin release *in vivo* also profoundly inhibit prolactin release when incubated with rat pituitary *in vitro* (Lu *et al.*, 1970a). Thus, the effects of catecholamines on pituitary prolactin release *in vitro* do not necessarily reflect their actions *in vivo*.

II. Effects of Central Acting Drugs on Serum and Pituitary Prolactin Levels

A. Objectives

Inasmuch as catecholamines do not readily pass through the "blood-brain barrier" (Wurtman, 1970; Koelle, 1970; Innes and Nickerson, 1970), the present study was undertaken to determine whether other drugs known to enter the brain and to increase or decrease hypothalamic catecholamine levels could change serum and pituitary prolactin concentrations. The drugs used were L-DOPA, the immediate precursor of dopamine; three monoamine oxidase (MAO) inhibitors (pargyline, iproniazid, and Lilly compound-15641) that depress normal metabolism of catecholamines and therefore increase brain catecholamine activity; Methyl-DOPA, which competes for DOPA-decarboxylase to synthesize methyl-dopamine but reduces synthesis of dopamine and norepinephrine; Reserpine, which interfers with storage and/or induces depletion of catecholamines; chlorpromazine, a tranquilizer which blocks catecholamine activity at the post-synaptic receptor sites; Tyrosine

analogs (alpha-methyl-para-tyrosine and alpha-methyl-meta-tyrosine), which inhibit synthesis of catecholamines by blocking the enzyme tyrosine hydroxylase; And d-amphetamine, an amine releaser which inhibits amine uptake by nerve termines (McGeer, 1971).

B. Materials and Methods

1. Animals

Mature, 4- to 5-month-old, virgin female Sprague-Dawley rats, weighing 220-250 g each, were used in all experiments. Two complete estrous cycles of 4 to 5 days duration were followed on all female rats before they were injected with one of the drugs on the day of proestrus or diestrus as indicated in the experiments.

2. Drugs

The drugs used were L-DOPA (Hoffmann-La Roche, Inc., Nutley, New Jersey), pargyline hydrochloride (Abbott Labs., North Chicago, Illinois), iproniazid phosphate (Hoffmann-La Roche, Inc., Nutley, New Jersey), Lilly compound-15641 (N-2-o-Chlorophenoxy-ethyl-cyclopropylamine) (Lilly Research Labs., Eli Lilly and Co., Indianapolis, Indiana), methyl-DOPA (Merck, Sharp and Dohme Research Labs., Merck and Co., Rahway, New Jersey), reserpine (Nutritional Biochemicals Corp., Cleveland, Ohio and Ciba Pharmaceutical Co., Summit,

New Jersey), chlorpromazine hydrochloride (Research Labs., Smith, Kline and French Labs., Philadelphia, Pa.), alpha-methyl-para-tyrosine (Lederle Labs., American Cyanamid Co., Pearl River, New York), alpha-methyl-meta-tyrosine (Mann Research Labs., Inc., New York, N.Y.), d-amphetamine sulphate (Research Labs., Smith, Kline and French Labs., Philadelphia, Pa.).

3. Treatments

All drugs except L-DOPA, methyl-DOPA and tyrosine analogs were dissolved directly in 0.85% NaCl solution. The reserpine obtained from Ciba Pharmaceutical Co. was in a solution form (Serpasil, reserpine USP; 2.5 mg/ml). L-DOPA and methyl-DOPA were each dissolved in a warm solution of 0.5 N HCl to which 0.5 N NaOH was added a drop at a time to bring the pH to 2.8. To avoid oxidation and precipitation, L-DOPA and methyl-DOPA solutions were administered into rats immediately after dissolving of the drugs. Tyrosine analogs were first dissolved in 0.1 N NaOH and then adjusted to pH 10.0 by adding 1.0 N HCl a drop at a time. All the drug solutions were injected intraperitoneally into cycling female rats at 10 AM on the day of proestrus, unless otherwise stated in the experiments. The controls received injections of physiological saline or medium of pH 2.8 or 10.0.

Individual blood samples (0.5-0.7 ml) were collected by cardiac puncture under light ether anesthesia at 0, 0.5,

1, 2 and 4 hours after each treatment. A pre-treatment blood sample was taken from each rat prior to drug injection for comparison with subsequent blood samples. The rats were killed by decapitation after the last blood samples were collected. Serum was separated and anterior pituitary was homogenized according to the methods described under Materials and Methods. The sera and pituitary homogenates were kept frozen at -20°C until assayed.

The hypothalami of the rats treated with saline (controls), iproniazid, pargyline, L-DOPA, or pargyline and L-DOPA together, were quickly removed after the rats were killed. The hypothalami were preserved and later homogenized according to the methods described under Materials and Methods. Mature male rats, weighing 250-300 g each, were used as pituitary donors for the in vitro assay of hypothalamic extracts. Each anterior pituitary half was incubated with one hypothalamic equivalent in 2 ml medium 199 at a pH of 7.4. The media were assayed for prolactin at the end of 4 hours incubation. Prolactin in the medium was calculated in terms of ng released per mg of anterior pituitary tissue.

Since reserpine, chlorpromazine, and alpha-methyl-para-tyrosine inhibit catecholamine activity by acting on the brain via different mechanisms (Coppola, 1968), in a separate experiment it was considered of interest to compare the effects of L-DOPA, the immediate precursor of dopamine, in combination with reserpine, chlorpromazine, or alpha-methyl-

para-tyrosine on pituitary release of prolactin. In experiment I, a single intraperitoneal injection of reserpine, chlorpromazine, and/or L-DOPA was given to cycling female rats on the day of proestrus according to the time schedule shown in Table 8. A pre-treatment blood sample and subsequent blood samples before and after drug injection were collected for prolactin assay. The experiment was terminated by 1:00 PM. In experiment II, cycling female rats were treated with a first dose of 50 mg alpha-methyl-para-tyrosine at 10 AM and a second dose of 30 mg alpha-methyl-para-tyrosine at 6 PM on the day of diestrus to deplete hypothalamic catecholamines (Creveling et al., 1968). First blood samples were taken at 8 PM before a single dose of 25 mg L-DOPA was given to the rats. Subsequent blood samples were collected at 9 and 11 PM. Another group of female rats received a single dose of 25 mg L-DOPA at 8 PM on the day of diestrus was used as the controls.

4. Prolactin assay

Prolactin in individual serum samples and pituitary homogenates were assayed by radioimmunoassay. Each sample was assayed at 2 or 3 different dose levels, and the prolactin values were averaged and expressed in terms of a purified rat prolactin reference standard, NIAMD-rat prolactin-RP-1.

Sample mean and standard error of the mean were calculated for each drug treatment group. Student's "t" test

was used to determine the significance of differences in serum prolactin values between pre-treatment and post-treatment samples, and also for the differences in pituitary prolactin concentrations between different treatment groups.

C. Results

1. Effects of a single injection of drugs which increase hypothalamic catecholamines on serum and pituitary prolactin levels

The data in Table 5 show that a single intraperitoneal injection of L-DOPA reduced serum prolactin about 50% by 30 minutes or 1 hour after injection as compared to pre-treatment values. By 2 hours after injection serum prolactin values were reduced 60% and pituitary prolactin concentration was significantly increased. Pargyline produced smaller reductions in serum prolactin and no significant change in pituitary prolactin concentration. When both L-DOPA and pargyline were given together, there were greater decreases in serum prolactin values than when either drug was given alone. The combination produced no significant change in pituitary prolactin concentration. Lilly compound-15641 or iproniazid produced no effect on serum prolactin by 30 minutes or 1 hour after injection, but evoked about a 40-50% reduction by 2 hours after injection. Lilly compound-15641 but not iproniazid increased pituitary concentration

Table 5. Effects of a single intraperitoneal injection of drugs on serum and pituitary prolactin concentrations.

Treatment (7 rats/group)	Dose/ rat	Serum prolactin concentrations, ng/ml				Pit. prolactin concentrations, µg/mg AP
		Pre-treatment	30 min	1 hr	2 hr	
pH 2.8 medium (controls)	0.3 ml	39.7±4.1[a]	35.9± 3.0	36.6± 2.6	32.5± 2.4	5.17±0.10
L-DOPA	12 mg	43.5±3.2	21.3± 0.8[c]	22.5± 2.2[c]	16.7± 1.2[c]	6.04±0.40[b]
Methyl-DOPA	80 mg	51.1±5.5	238.8±21.3[c]	329.8±42.9[c]	246.0±47.0[c]	4.10±0.14[c]
Saline (controls)	0.5 ml	42.4±2.5	48.3± 6.7	48.2± 6.2	45.5± 2.9	4.66±0.14
Pargyline	15 mg	46.9±3.9	28.2± 1.8[c]	26.8± 2.7[c]	25.0± 2.8[c]	4.67±0.18
Pargyline + L-DOPA	15 mg +12 mg	37.4±3.3	16.7± 0.8[c]	15.9± 1.5[c]	17.5± 1.4[c]	5.31±0.05
Lilly Compound 15461	8 mg	52.0±7.2	52.2± 8.2	43.3± 6.3	31.5± 5.4[b]	5.81±0.22[c]
Iproniazid	40 mg	48.9±6.4	45.7±10.0	42.2± 9.5	22.4± 3.5[c]	4.44±0.18
d-Amphetamine	1.2 mg	35.7±1.7	142.1±31.5[c]	264.0±52.9[c]	239.0±61.3[c]	2.94±0.16[c]

[a]Mean and standard error of the mean.

[b]Significantly different from controls, $p < 0.05$.

[c]Significantly different from controls, $p < 0.01$.

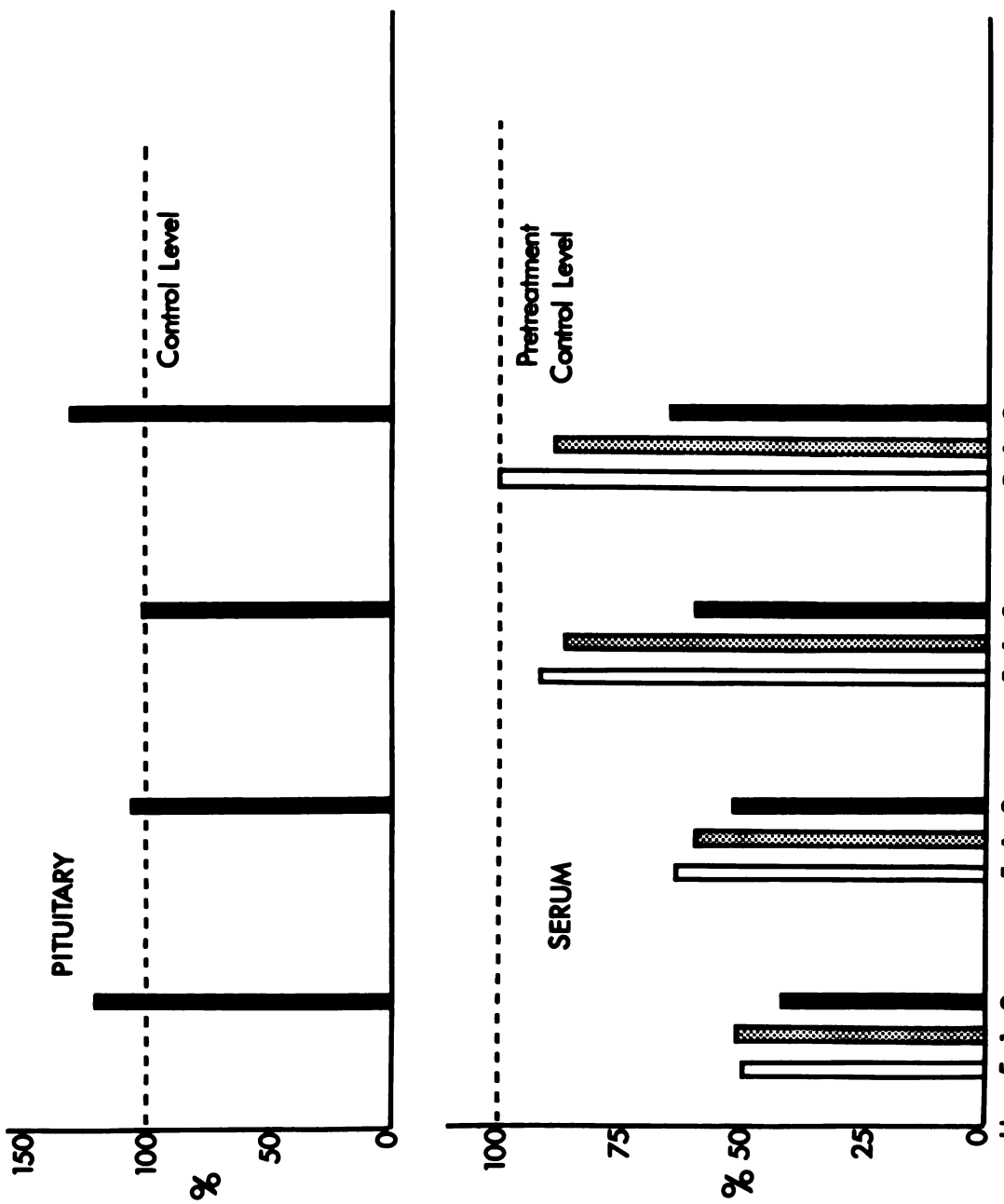


Figure 6. Effects of Drugs That Increase Hypothalamic Catecholamines on Serum and Pituitary Prolactin Concentrations.

of prolactin. Figure 6 shows the percentage changes in serum and pituitary prolactin values after administration of these drugs.

2. Effects of a single injection of drugs which increase hypothalamic catecholamines on PIF activity in the hypothalamus

The data in Table 6 show that the pituitary halves incubated with the hypothalami of rats treated with iproniazid, pargyline, L-DOPA, or pargyline and L-DOPA together, released less prolactin than that with the hypothalami of saline controls, indicating that more PIF activity was present in the hypothalamic extract from the drug-injected rats than in the controls. The combination of pargyline and L-DOPA was more effective than either alone for increasing hypothalamic PIF activity.

3. Effects of a single injection of drugs which decrease hypothalamic catecholamines on serum and pituitary prolactin levels

Table 5 shows that an injection of methyl-DOPA increased serum prolactin over pre-treatment levels about 5-fold by 30 minutes, about 6-fold by 1 hour, and about 5-fold by 2 hours after injection. Methyl-DOPA significantly reduced pituitary concentration of prolactin. A single injection of d-amphetamine increased serum prolactin over

Table 6. Effects of drugs on hypothalamic PIF activity.

Treatment (6 hypothalami per group)	Hypothalamic PIF activity (ng prolactin released per mg pit.)
Saline (controls)	618±43[a]
Iproniazid	558±34
Pargyline	483±37[b]
L-DOPA	463±32[c]
Pargyline + L-DOPA	367±14[c]

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.05$.

[c]Significantly different from the controls, $p < 0.01$.

pre-treatment values about 4-fold by 30 minutes, about 7-fold by 1 hour, and about 6-fold by 2 hours after injection. This drug reduced pituitary prolactin concentration by about 44%.

The data in Table 7 show that a single injection of reserpine or chlorpromazine produced marked increases in serum prolactin concentration when compared with pre-treatment values or with saline controls. Reserpine produced no effect on serum prolactin levels after one hour, a small increase after 2 hours, about 5-fold increase by 4 hours after injection, and reduced pituitary prolactin concentration by about half of that in the saline controls. Chlorpromazine raised serum prolactin about 4-fold by one hour after injection, about 7-fold by four hours after injection, and reduced pituitary prolactin to about 1/4 of that in the saline controls. A single injection of alpha-methyl-para-tyrosine more than doubled serum prolactin levels 30 minutes later, and increased serum prolactin values about 5-fold by 1 and 2 hours after injection. This drug reduced pituitary concentration of prolactin to about 1/3 of that in pH 10.0 medium controls. Alpha-methyl-meta-tyrosine increased serum prolactin about 8-fold over the pre-treatment values by 30 minutes after injection. Prolactin levels declined by 1 and 2 hours after injection but were still about four times greater than the pre-treatment values. The pituitary prolactin concentration was significantly increased by two hours

Table 7. Effects of a single intraperitoneal injection of drugs on serum and pituitary prolactin concentrations.

Treatment	Dose/ 200 g bw	Serum prolactin concentrations, ng/ml					Pit. prolactin concentrations. µg/mg AP
		Pre-treatment	30 min	1 hr	2 hr	3 hr	
Saline (controls)	0.6 ml	57.0± 7.5[a] (11)[c]		48.7± 7.4	40.6± 5.7	51.3± 9.1	4.24±0.34
Reserpine	2.0 mg	51.0±11.9 (9)		49.1± 9.5	80.1±18.9	280.6±48.7[b]	2.23±0.32[b]
Chlorpro-mazine	5.0 mg	48.2± 3.1 (10)		191.2±33.4[b]	220.8±43.5[b]	344.4±64.9[b]	1.03±0.11[b]
pH 10.0 medium (controls)	1.6 ml	41.2± 6.5 (5)	58.2± 8.4	38.2± 3.8	80.0±24.5		3.15±0.15
a-m-p-tyrosine	40 mg	43.9± 6.0 (5)	110.0±11.2[b]	189.6±13.7[b]	201.6±26.4[b]		1.03±0.06[b]
a-m-m-tyrosine	40 mg	36.8± 1.6 (11)	299.5±48.8[b]	160.7±17.6[b]	155.6±29.6[b]		5.06±0.65[b]

[a]Mean and standard error of the mean.

[b]Significantly different from controls, $p < 0.01$.

[c]( ) = Number of rats in parentheses.

Note: a-m-p-tyrosine = alpha-methyl-para-tyrosine.
a-m-m-tyrosine = alpha-methyl-meta-tyrosine.

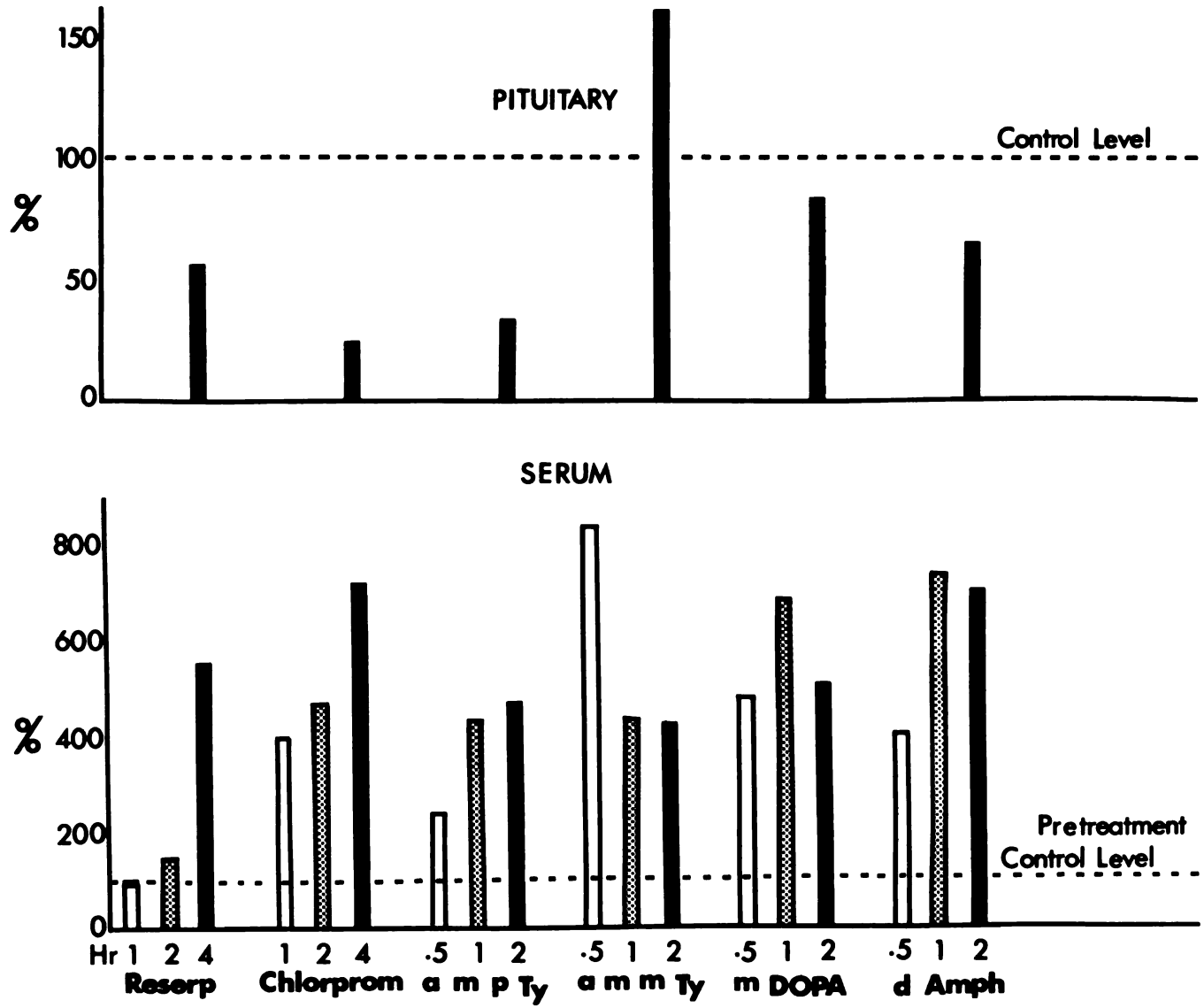


Figure 7. Effects of Drugs That Decrease Hypothalamic Catecholamines on Serum and Pituitary Prolactin Concentration.

after a single injection of alpha-methyl-meta-tyrosine. The percentage changes in serum and pituitary prolactin concentrations are shown in Figure 7.

4. Effects of L-DOPA on serum prolactin levels in rats pre-treated with reserpine, chlorpromazine or alpha-methyl-para-tyrosine

Table 8 shows that a single injection of L-DOPA markedly decreased serum prolactin levels by 1, 2 and 3 hours after injection as seen in previous experiments. On the other hand, reserpine raised serum prolactin about 6-fold by one hour, about 7-fold by 2 hours and about 8-fold by 3 hours after injection. Since a solution form of reserpine (Serpasil) was used, hence a quick action of drug on the pituitary release of prolactin was observed. Chlorpromazine elicited 6 to 9 folds increase in serum prolactin by 1, 2 and 3 hours after injection as seen in provious experiments. A single injection of L-DOPA within one hour after reserpine produced a transitory but highly significant reduction in serum prolactin levels. By 2 hours after L-DOPA, no decrease in serum prolactin was observed in reserpine-pretreated rats, indicating that the inhibition of pituitary prolactin release by L-DOPA after reserpine was short-lived. An injection of L-DOPA after chlorpromazine produced no significant reduction on serum prolactin as compared to pre-L-DOPA levels or the control values in rats treated with chlorpromazine alone.

Table 8. Effects of a single intraperitoneal injection of reserpine, chlorpromazine, and/or L-Dopa on serum prolactin levels in proestrous rats.

Treatment (6 rats/group)	Dose/ rat	Time of drug injection, and serum prolactin levels (ng/ml)			
		10:00 AM	11:00 AM	12:00 AM	1:00 PM
Saline (controls)	0.5 ml	57.0± 6.0[a] SA ↓	64.3± 6.3	69.8±13.8	76.8±11.5
L-Dopa	12 mg	53.5± 2.3 DOPA ↓	21.3± 1.0[b]	21.5± 0.8[b]	29.3± 5.3[b]
Reserpine	1.5 mg	55.0± 9.7 RSP ↓	361.5±22.0[b]	342.0±27.0[b]	415.8±37.5[b]
Chlorpromazine	4 mg	51.8± 6.3 CPZ ↓	343.5±28.5[b]	393.8±36.8[b]	423.5±30.8[b]
Reserpine + L-Dopa	1.5 mg 12 mg	62.5±10.8 RSP ↓	415.3±35.0[b] DOPA ↓	95.5±25.5	379.8±55.5[b]
Chlorpromazine + L-Dopa	4 mg 12 mg	42.8± 3.3 CPZ ↓	368.3±52.5[b] DOPA ↓	295.3±32.0[b]	395.0±22.8[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.01$.

Note: SA = saline; Dopa = L-Dopa; RSP = reserpine; CPZ = chlorpromazine; Dopa ↓ = time of L-Dopa given; and so forth.

The data in Table 9 indicate that a single injection of L-DOPA also produced marked reduction in serum prolactin in rats on the day of diestrus. Depletion of hypothalamic catecholamines by two successive doses of alpha-methyl-para-tyrosine resulted in a 10-fold increase in serum prolactin levels by 10 or 11 hours, and a 12-fold increase by 13 hours after the first injection. A single injection of 25 mg of L-DOPA within 2 hours after the second dose of alpha-methyl-para-tyrosine reduced serum prolactin about 75% by 1 hour after injection as compared to L-DOPA pre-treatment values. By 3 hours after L-DOPA injection the serum prolactin levels were reduced by 85%.

D. Conclusions

These results demonstrate that a single injection of L-DOPA, the immediate precursor of dopamine, produced a rapid decrease in serum prolactin and an increase in pituitary prolactin concentration. The ability of L-DOPA to raise brain catecholamine levels (Fuxe and Hokfelt, 1969; Innes and Nickerson, 1970; Koelle, 1970) is believed to be responsible for the decrease in pituitary prolactin release. The MAO inhibitors, pargyline, iproniazid, and Lilly compound-15641 (Fuller, 1968a; 1968b), all reduced serum prolactin levels significantly, presumably by interfering with the metabolism of catecholamines (Koelle, 1970) and thereby increasing their concentration in the hypothalamus. Pargyline was

Table 9. Effects of alpha-methyl-para-tyrosine and/or L-Dopa on serum prolactin levels in diestrous rats.

Treatment (6 rats/group)	Time and dose of drug injection, and serum prolactin levels (ng/ml)				
	10:00 AM	6:00 PM	8:00 PM	9:00 PM	11:00 PM
L-Dopa			28.7± 3.2[a] Dopa (25 mg)↓	16.4± 1.8[b]	14.1± 2.5[b]
AMPT	AMPT (50 mg)[c]↓	AMPT (30 mg)↓	306.0±13.8[b] MED (0.3 ml)↓	301.8±48.0	360.3±24.0
AMPT + L-Dopa	AMPT (50 mg) ↓	AMPT (30 mg)↓	295.8± 8.5[b] Dopa (25 mg)↓	65.3±14.0[d]	36.3± 5.0[d]

[a] Mean and standard error of the mean.

[b] Significantly different from the serum prolactin values at 8:00 PM, $p < 0.05$.

[c] ( ) = Dose per rat.

[d] Significantly different from the serum prolactin values at 8:00 PM, $p < 0.01$.

Note: AMPT = alpha-methyl-para-tyrosine.
AMPT ↓ = time of AMPT given; and so forth.
MED = pH 2.8 medium.

the most effective of the three MAO inhibitors used for decreasing serum prolactin, and when given together with L-DOPA, the reduction in serum prolactin was greater than when either drug was given alone, presumably because synthesis of catecholamines was enhanced and metabolism of catecholamines was reduced by the combination of these 2 drugs.

The large increase in serum prolactin and significant fall in pituitary prolactin produced by injection of methyl-DOPA is probably due to a reduction in brain catecholamines, since false neurotransmitters (methyl-dopamine and methyl-norepinephrine) instead of normal neurotransmitters (dopamine and norepinephrine) are synthesized by nerve cells in the presence of methyl-DOPA (Coppola, 1968; Innes and Nickerson, 1970). The significant increase in serum prolactin and depletion of pituitary prolactin by d-amphetamine presumably is due to its actions on release of norepinephrine and inhibition of dopamine and norepinephrine reuptake by nerve terminals (McGeer, 1971), although d-amphetamine may also inhibit MAO activity. Release of an amine from nerve endings and inhibition of reuptake could make catecholamines unavailable as neurotransmitters.

The present study also demonstrates that a single injection of reserpine, chlorpromazine, alpha-methyl-para-tyrosine or alpha-methyl-meta-tyrosine rapidly elevated serum prolactin levels. The suspension form of reserpine appeared to be less effective than chlorpromazine because of

slow absorption. When a solution form of reserpine was used in a later experiment, reserpine was shown to be as effective as chlorpromazine in stimulating pituitary release of prolactin. The increases in serum prolactin values evoked by reserpine, chlorpromazine and alpha-methyl-para-tyrosine were associated with a significant decline in pituitary prolactin concentration, whereas alpha-methyl-meta-tyrosine produced the largest rise in serum prolactin but also increased pituitary concentration of prolactin. This probably reflects stimulation of synthesis as well as release of prolactin from the pituitary by this tyrosine analog.

Reserpine, chlorpromazine and alpha-methyl-para-tyrosine decrease catecholamine activity by acting on the brain via different mechanisms (Coppola, 1968): reserpine interfers with storage and/or induces depletion of catecholamines; chlorpromazine inhibits catecholamine activity by blocking the post-synaptic receptor sites; alpha-methyl-para-tyrosine inhibits the synthesis of catecholamines by blocking the enzyme tyrosine hydroxylase at the rate-limiting step. The mechanisms of action of these three central acting drugs appears to be confirmed by the findings from the present study. Injection of L-DOPA produced a rapid and sustained inhibition of pituitary prolactin release after depletion of catecholamines by two doses of alpha-methyl-para-tyrosine. The rapid syntheses of dopamine and norepinephrine

by administered L-DOPA apparently by-passed the inhibitory step of tyrosine hydroxylase in converting tyrosine to L-DOPA. In contrast, L-DOPA produced only a transitory reduction in serum prolactin after reserpine, presumably because the strong and prolonged action of reserpine on catecholamine depletion. Reserpine quickly released the newly synthesized catecholamines from L-DOPA, thereby reducing the available catecholamines as functional neurotransmitters within the nerve terminals. Finally, injection of L-DOPA had no significant effect on serum prolactin levels in rats pre-treated with chlorpromazine. The apparent failure of L-DOPA to affect serum prolactin is possibly due to the blockade of receptor sites by chlorpromazine, thereby rendering neurotransmission impossible at the synaptic junctions even though catecholamines were available in the nerve endings.

In conclusion, the results of the present study are consistent with the hypothesis that hypothalamic catecholamines inhibit pituitary release of prolactin. Drugs known to enhance brain catecholamine activity produced a decrease in pituitary prolactin release, whereas drugs that reduce hypothalamic catecholamines increased pituitary prolactin release. The work by Kamberi et al. (1970) demonstrated increased PIF activity in the hypothalamo-pituitary portal blood after intraventricular injection of dopamine in rats. The present study further demonstrates that injection of iproniazid, pargyline, L-DOPA, or L-DOPA and pargyline

together, increased hypothalamic PIF activity and the combination of pargyline and L-DOPA was more effective than either alone. These observations suggest that the increase in hypothalamic catecholamine levels by these central acting drugs results in increased hypothalamic PIF activity, and this in turn inhibits prolactin release by the anterior pituitary. Thus, the hypothalamic catecholamines influence pituitary release of prolactin presumably by functioning as neurotransmitters between hypothalamic PIF and pituitary prolactin.

III. Stimulation of Pituitary Prolactin Secretion by Drugs that Depress Hypothalamic Catecholamines in Ovariectomized and Ovariectomized, Estrogen-Primed Rats

A. Objectives

Previous experiment demonstrated that central acting drugs including reserpine, chlorpromazine and tyrosine analogs evoked rapid release of pituitary prolactin in cycling rats (Lu et al., 1970b). Early work from our laboratory (Meites, 1962) also suggested that several pharmacological agents including reserpine and chlorpromazine stimulated pituitary prolactin secretion as evident on mammary secretion and/or lactation in estrogen-primed rats. The observed stimulation of mammary secretion by drugs may not be due solely to increased prolactin since release of ACTH by these drugs may also be involved in the mammary response (Meites, 1966).

Inasmuch as none of the early studies measured blood prolactin, the present study was undertaken to determine whether hormones and central acting drugs, some of which are known to depress hypothalamic catecholamines, could stimulate pituitary release of prolactin in ovariectomized and ovariectomized, estrogen-primed rats.

B. Materials and Methods

1. Animals

Mature, 3- to 4-month old, virgin female Sprague-Dawley rats, weighing 200-220 g each, were used in all experiments. The rats were bilaterally ovariectomized and later were treated with estrogen and/or drugs. Vaginal smears were followed on all rats for one week post-operation to check the completeness of ovariectomy, and no cornified vaginal epithelial cells were found.

2. Hormones and drugs

The hormones and drugs used were estradiol benzoate (Nutritional Biochemicals Corp., Cleveland, Ohio), hydrocortisone acetate (Merck, Sharp & Dohme, West Point, Pa.), acetylcholine bromide (K & K Labs., Inc., Plainview, N.Y.), chlorpromazine hydrochloride (Research Labs., Smith, Kline & French Labs., Philadelphia, Pa.), epinephrine chloride (Parke, Davis & Co., Detroit, Michigan), formalin (Fisher Scientific Co., Fair Lawn, N.J.), reserpine (Nutritional

Biochemicals Corp., Cleveland, Ohio), serotonin creatinine sulfate (Aldrich Chemical Co., Milwaukee, Wisconsin), alpha-methyl-para-tyrosine (Regis Chemical Co., Chicago, Illinois), and alpha-methyl-meta-tyrosine (Mann Research Labs., Inc., New York, N.Y.). Estradiol benzoate was suspended in corn oil and injected subcutaneously at dorsal skin of the post-cervical region. Hydrocortisone and all drugs except tyrosine analogs were dissolved directly in neutral PBS. Tyrosine analogs were dissolved in pH 10.0 medium as described in previous experiment. Hydrocortisone and all drug solutions were given by intraperitoneal injection.

3. Treatments

Seven days after ovariectomy, the rats were randomly divided into two groups for different treatments.

One group of rats was treated with daily injections of one of the nine drugs for 5 days as shown in Table 10. Pre-treatment blood samples were collected from the rats for comparison with post-treatment samples. Two subsequent blood samples were taken from the rats at one and 24 hours after the last injection of drug. The rats were killed by decapitation after the last blood samples were collected. Pituitaries were removed, weighed, and homogenized in neutral PBS. Sera were separated after centrifugation of blood samples. Both sera and pituitary homogenates were kept frozen at -20° C until assayed.

Another group of rats was first treated with estradiol benzoate (5 μg/day) for 5 days, and then given drug injections for 5 days (Table 11). Pre-treatment blood samples were collected from the rats 24 hours after the last injection of estradiol benzoate prior to the beginning of drug treatments. Blood samples were also collected at one and 24 hours after the last injection of drug before the rats were decapitated. Sera and pituitary homogenates were similarly prepared as shown above.

Inguinal mammary pads were removed, fixed in Bouin's, stained with hematoxylin, and rated for growth according to a standard method described under Materials and Methods.

4. Prolactin assay

Prolactin in individual serum samples and pituitary homogenates was measured by radioimmunoassay. Each sample was assayed at three different dose levels. The prolactin values were averaged and expressed in terms of a purified rat prolactin reference standard (HIV-8-C).

5. Statistics

Mean and standard error of the mean for pituitary and serum prolactin concentrations, anterior pituitary weights, and mammary gland growth ratings were calculated for each experimental group. Student's "t" test was used to determine the significance of differences between the control and experimental groups.

C. Results

1. Effects of ovariectomy and estrogen on pituitary prolactin release

Seven days after ovariectomy the serum prolactin levels were 27 ± 3 ng/ml, comparable to diestrous levels in cycling female rats (Amenomori et al., 1970). Injection for five days of estradiol benzoate produced a 4-fold (102 ± 5 ng/ml) increase in serum prolactin values. By 5 days after termination of estrogen injection, serum prolactin were significantly reduced (74 ± 16 or 57 ± 15 ng/ml; Table 11), but these levels were significantly higher than in the ovariectomized controls (Table 10). The pituitary prolactin concentrations were about 3-fold greater (2.44 ± 0.13 or 2.56 ± 0.23 µg/mg; Table 11) than that of the ovariectomized controls (0.74 ± 0.14 or 0.80 ± 0.16 µg/mg; Table 10).

2. Effects of multi-injections of drugs on pituitary prolactin release and mammary growth in ovariectomized rats

The data in Table 10 show that injections of saline or pH 10.0 medium for 5 days had no effect on serum prolactin in ovariectomized rats. Serum prolactin was increased about 3-fold over saline controls after 1 hour, and was slightly elevated by 24 hours after the last injection of chlorpromazine. Reserpine produced only small increases in

Table 10. Effects of multi-injections of drugs on pituitary prolactin secretion, anterior pituitary weight, and mammary growth in ovariectomized rats.

Treatment (6 rats/group)	Dose/ rat/day	Serum prolactin concentration, ng/ml* 1 hr	Serum prolactin concentration, ng/ml* 24 hr	Pit. prolactin concentration, µg/mg	Anterior Pituitary wt., mg	Average mammary gland growth rating
Saline, (controls)	0.5 ml	31± 3[a]	31± 1	0.74±0.14	9.2±0.4	2.8±0.4
Chlorpromazine	3 mg	116±18[c]	38± 3[b]	1.14±0.35	7.8±0.4	2.3±0.3
Reserpine	0.2 mg	37± 3	43± 4[c]	0.64±0.08	9.4±0.2	2.5±0.4
Acetylcholine	10 mg	----	38± 4	0.64±0.05	8.9±0.3	2.5±0.2
Serotonin	1 mg	----	38± 2[b]	0.63±0.02	9.2±0.4	3.0±0.3
Epinephrine	0.1 mg	----	38± 4	0.57±0.02	9.2±0.3	2.9±0.3
Hydrocortisone	2 mg	----	30± 3	0.81±0.10	9.0±0.6	3.5±0.3
Formalin, 10%	0.2 ml	----	41± 3[c]	0.92±0.15	9.2±0.4	3.1±0.4
pH 10.0 medium, (controls)	1.1 ml	33± 4	32± 5	0.80±0.16	9.6±0.6	2.3±0.2
a-m-p-tyrosine	20 mg	97±12[c]	51±12	1.32±0.14[c]	10.2±0.6	2.8±0.3
a-m-m-tyrosine	40 mg	56± 5[b]	42± 9	1.46±0.15[c]	10.9±0.3[b]	3.1±0.3[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the control, $p < 0.05$.

[c]Significantly different from the control, $p < 0.01$.

*Blood samples were taken at 1 and 24 hours after the last injection.

Note: a-m-p-tyrosine = alpha-methyl-para-tyrosine.
a-m-m-tyrosine = alpha-methyl-meta-tyrosine.

Table 11. Effects of multi-injections of drugs on pituitary prolactin secretion, anterior pituitary weight, and mammary growth in ovariectomized, estrogen-primed rats.

Treatment (6 rats/group)	Dose/ rat/day	Serum prolactin concentration, ng/ml* 1 hr	Serum prolactin concentration, ng/ml* 24 hr	Pit. prolactin concentration, µg/ml	Anterior Pituitary wt., mg	Average mammary gland growth rating
Saline (controls)	0.5 ml	74±16[a]	67±15	2.44±0.13	13.9±0.3	3.5±0.3
Chlorpromazine	3 mg	533±84[c]	109±21	2.03±0.14[b]	12.8±0.7	5.8±0.4[c]
Reserpine	0.2 mg	171±13[c]	143±27[b]	2.32±0.10	12.1±0.9	4.7±0.2[c]
Acetylcholine	10 mg	----	100±23	2.34±0.12	14.2±0.3	4.2±0.3
Serotonin	1 mg	----	54± 5	3.10±0.10[c]	11.5±1.2	4.2±0.3
Epinephrine	0.1 mg	----	94±19	2.54±0.20	12.5±0.7	4.5±0.4[b]
Hydrocortisone	2 mg	----	47± 5	3.35±0.31[c]	11.1±0.6	4.0±0.2
Formalin, 10%	0.2 ml	----	72±10	2.15±0.16	12.8±0.7	4.4±0.3[b]
pH 10.0 medium (controls)	1.1 ml	57±15	63±18	2.56±0.23	14.1±0.5	4.1±0.1
a-m-p-tyrosine	20 mg	263±34[c]	95±10	3.06±0.23	16.2±0.5[c]	4.4±0.1[b]
a-m-m-tyrosine	40 mg	274±86[b]	106±21	3.27±0.16	15.7±0.9	5.0±0.2[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the control, $p < 0.05$.

[c]Significantly different from the control, $p < 0.01$.

*Blood samples were taken at 1 and 24 hours after the last injection.

Note: a-m-p-tyrosine = alpha-methyl-para-tyrosine.

a-m-m-tyrosine = alpha-methyl-meta-tyrosine.

serum prolactin levels by 1 or 24 hours after the last injection of the drug. Serotonin or formalin evoked small increases in serum prolactin by 24 hours after the last injection. Acetycholine, epinephrine or hydrocortisone had no effect on serum prolactin values by 24 hours after the last injection. Alpha-methyl-para-tyrosine and alpha-methyl-meta-tyrosine produced a 2- and 3-fold increase respectively in serum prolactin over medium (pH 10.0) injected controls by 1 hour after the last injection. The serum prolactin values were only slightly elevated by 24 hours after the last injection of the tyrosine analogs. No significant difference in pituitary prolactin concentrations, anterior pituitary weights, or mammary growth was observed at the end of 5 days treatment with any of these drugs except the tyrosine analogs. Both alpha-methyl-para-tyrosine and alpha-methyl-meta-tyrosine significantly increased pituitary prolactin concentrations in the ovariectomized rats as compared to medium injected controls. Alpha-methyl-meta-tyrosine but not alpha-methyl-para-tyrosine increased anterior pituitary weights and stimulated mammary growth.

3. Effects of multi-injections of drugs on pituitary prolactin secretion and mammary growth in ovariectomized, estrogen-primed rats

Serum prolactin was increased about 7-fold over saline controls after 1 hour, and was slightly elevated 24

hours after the last injection of chlorpromazine. Reserpine produced a 2-fold increase in serum prolactin over the controls 1 or 24 hours after the last injection. Acetylcholine, serotonin, epinephrine, hydrocortisone or formalin had no effect on serum prolactin 24 hours after the last injection. Both tyrosine analogs evoked 5-fold increases in serum prolactin over the controls 1 hour after the last injection. There were small but significant increases in serum prolactin by 24 hours after the last injections of both tyrosine analogs. Chlorpromazine significantly reduced, whereas sero tonin and hydrocortisone increased pituitary prolactin concentrations. All the other 6 drugs had no effect on pituitary prolactin concentration. Alpha-methyl-para-tyrosine was the only drug which significantly increased anterior pituitary weight. Chlorpromazine, reserpine, epinephrine, formalin, and the 2 tyrosine analogs significantly stimulated mammary growth (Table 11).

D. Conclusions

The present study demonstrates that chlorpromazine, reserpine and tyrosine analogs, drugs known to depress hypothalamic catecholamine, stimulated pituitary release of prolactin in estrogen-primed ovariectomized rats more effectively than in non-estrogen-primed controls. The difference in responsiveness to the drugs may be due to much higher initial

pituitary concentrations of prolactin after estrogen treatment and to increased sensitivity of the pituitary-hypothalamic system.

Ovariectomy enhances norepinephrine synthesis by the hypothalamus (Wurtman et al., 1969; Anton-Tay and Wurtman, 1968; Anton-Tay et al., 1970), and leads to a slight increase in the steady-state concentration of the catecholamines (Coppola, 1968) and a marked acceleration in catecholamine turn-over rate (Anton-Tay and Wurtman, 1968). This would also lead to increased PIF release and reduced serum prolactin levels, as found here. It is possible that drugs known to depress catecholamines may not be so effective in suppressing hypothalamic catecholamine activity when the turn-over rate of the latter is initially accelerated. This also may account for the reduced effectiveness of chlorpromazine, reserpine and tyrosine analogs in stimulating pituitary prolactin release in ovariectomized rats. A reducation in hypothalamic catecholamine activity would be expected to depress hypothalamic PIF activity. Alpha-methyl-meta-tyrosine also is known to increase pituitary prolactin concentrations in cycling female rats (Lu et al., 1970b).

The significant reduction in pituitary prolactin concentration by chlorpromazine may reflect sustained stimulation of prolactin release by this drug. A rapid decrease in pituitary prolactin concentration by chlorpromazine also is seen in cycling female rats (Lu et al., 1970b). The

mechanism by which serotonin and hydrocortisone increase pituitary concentration of prolactin remains to be determined, although this was previously reported for hydrocortisone (Meites, 1966) and there is evidence that serotonin stimulates prolactin release (Kamberi et al., 1971a).

In most cases serum prolactin levels after drug administration were higher by one hour than after 24 hours, indicating rapid stimulation by the drugs of pituitary release of prolactin. Hence, the time of blood sampling after drug administration influences the results. In general, increased pituitary release of prolactin results in enhanced mammary growth in estrogen-primed rats. However, drugs which have no significant effect on prolactin release such as epinephrine and formalin also stimulate mammary growth, presumably due to enhanced release of ACTH by these pharmacological agents.

IV. Effects of L-DOPA on Serum Prolactin and Prolactin Release-Inhibiting Activity in Intact and Hypophysectomized, Pituitary-Grafted Rats

A. Objectives

Transplantation of anterior pituitary grafts underneath the kidney capsule of hypophysectomized rats resulted in sustained prolactin release (Chen et al., 1970), presumably due to removal of direct inhibition by the hypothalamus of prolactin release (Meites et al., 1963; Meites, 1970).

Previous experiment demonstrated that a single intraperitoneal injection of L-DOPA decreased serum prolactin and increased hypothalamic content of PIF in cycling female rats (Lu and Meites, 1971). It was considered of interest therefore, to determine the effects of L-DOPA on serum prolactin levels, hypothalamic PIF content, and serum prolactin release-inhibiting activity in hypophysectomized rats with or without an anterior pituitary graft and in intact female rats.

B. Materials and Methods

1. Animals

A group of mature hypophysectomized female Sprague-Dawley rats (average body weight = 193 g each) was grafted with a single anterior pituitary underneath the left kidney capsule 7 days after hypophysectomy. Mature cycling female rats of the same strain were used as pituitary donors. Another group of mature hypophysectomized female rats was grafted without a pituitary transplant. Mature male Sprague-Dawley rats (250-300 g each) were used as pituitary donors for *in vitro* assay of hypothalamic PIF and serum prolactin releasing activity. Mature cycling female rats of the same strain also were injected with saline or L-DOPA at 10 AM on the day of proestrus.

2. Treatments

Five days following pituitary-transplantation and 12 days after hypophysectomy, rats with or without an anterior pituitary graft were divided into 2 subgroups. After collection of a pre-treatment blood sample at 10 AM, each rat was given 0.5 ml physiological saline or 12 mg of L-DOPA by a single intraperitoneal injection. Subsequent blood samples were collected at 30 minutes, 1 hour and 2 hours after saline or L-DOPA administration, and the rats were killed after the last blood samples were collected. Blood samples from the intact rats were collected just before and 2 hours after L-DOPA injection. The pituitary fossa of each hypophysectomized rat was examined under magnification and found to be free of pituitary tissue. The hypothalami were removed, pooled in groups and kept frozen in 0.1 N HCl. The blood samples were centrifuged, and the sera were kept frozen until assayed as described under Materials and Methods.

3. In vitro assay of hypothalamic PIF activity

The pooled hypothalami from each treatment group were homogenized and extracted in 0.1 N HCl as described under Materials and Methods. Male pituitary-donor rats were killed by decapitation. Anterior pituitaries were removed and hemisected. After 30 minutes pre-incubation, each anterior pituitary half was placed into a culture tube

containing medium 199 (pH 7.4) and extract of one hypothalamic fragment in a total volume of 2.1 ml. Incubations were carried out in a Dubnoff metabolic shaker as described under Materials and Methods. At the end of 4-hour incubation, pituitary halves were removed and incubation media were kept frozen at -20°C until assayed.

4. In vitro assay of serum prolactin release-inhibiting activity

Individual serum samples were assayed for prolactin releasing activity by incubating with single anterior pituitary halves, as indicated above. A total of 0.5 ml serum was incorporated into 1.5 ml medium 199 (pH 7.4) in a culture tube containing one anterior pituitary half.

5. Prolactin assay

Prolactin in individual serum samples and incubation media were measured by radioimmunoassay. Each sample was assayed at three different dose levels, and the prolactin values were averaged and expressed in terms of the purified rat prolactin reference standard, NIAMD-rat prolactin-RP-1. The prolactin values reported in the serum incubation experiments (Table 12) were corrected for the amounts of serum prolactin added to the incubation medium.

Sample mean and standard error of the mean were calculated for each experiment group. Student's "t" test was

Table 12. Effects of a single iv injection of L-dopa on serum prolactin values.

Exp.	Treatment (dose/rat)	Serum prolactin conc., ng/ml: Pre-treatment (10 am)	30 min	1 hr	2 hr
		Hypothysectomized rats with 1 AP graft			
1.	Saline, 0.5 ml (control)	132±10[a] (3)	145±5 (3)		156±23 (6)
	L-Dopa, 12 mg	126± 9 (3)	75±6[b] (3)		68± 6[b] (6)
2.	Saline, 0.5 ml (control)	123±14 (12)		132±35 (12)	
	L-Dopa, 12 mg	118±13 (12)		58±17[b] (12)	
		Hypophysectomized rats with no AP graft			
3.	Saline, 0.5 ml (control)	13± 1 (12)		15± 2 (12)	
	L-Dopa, 12 mg	14± 2 (12)		14± 3 (12)	
		Intact, Proestrous rats			
4.	Saline, 0.5 ml (control)	51±13 (6)			68± 9 (6)
	L-Dopa, 12 mg	68±19 (6)			34± 6[b] (6)

[a]Mean ± standard error of mean.

[b]Significantly different from controls, $p < 0.05$.

( ) = No. of rats per treatment.

used to determine the significance of differences between the control and L-DOPA treated groups.

C. Results

1. Effects of hypophysectomy on body growth and serum prolactin levels

Seven days after hypophysectomy, average body weight of the hypophysectomized rats was 193 ± 2 g each. The final average body weight was 199 ± 5 g. No significant increase in body weight was observed over the 5-day period in the hypophysectomized rats with no pituitary transplant.

Twelve days after hypophysectomy, the serum prolactin values were about 14 ng/ml (Table 12) in terms of the NIAMD-rat prolactin-RP-1 reference standard. This is in good agreement with our previous report of serum prolactin values of up to 10 ng/ml in hypophysectomized rats when expressed in terms of HIV-8-C rat prolactin standard (Chen et al., 1970). The "prolactin" in hypophysectomized rats is believed to reflect non-specific binding by rat serum proteins to anti-rat prolactin antibody.

2. Effects of transplantation of a single anterior pituitary on body growth and serum prolactin levels in hypophysectomized rats

By the end of the 5-day period after a single anterior pituitary transplantation, body weights of the

hypophysectomized rats were significantly increased from an initial average of 193 ± 2 g to 209 ± 4 g each. The serum prolactin values were 132 ± 10 ng/ml which are about 2-fold higher than that of cycling female rats (51 ± 13 ng/ml) on the morning of proestrus day (Table 12).

3. Effects of L-DOPA on serum prolactin levels

The data in Table 12 and in Figure 8 show that a single intraperitoneal injection of saline (controls) had no effect on subsequent serum prolactin levels in hypophysectomized or hypophysectomized, pituitary-grafted rats (Experiments 1-3). L-DOPA decreased serum prolactin concentrations by 50-60% 30 minutes, 1 or 2 hours after injection when compared with pre-treatment values or saline controls (Experiments 1-2). L-DOPA produced no effect on serum prolactin levels in hypophysectomized rats with no pituitary transplant (Experiment 3). In intact cycling female rats, L-DOPA reduced serum prolactin values to about half of pre-treatment levels (Experiment 4).

4. Effects of L-DOPA on serum prolactin release-inhibiting activity

The data in Table 13 and in Figure 9 show that the serum from the hypophysectomized and intact female rats prior to treatment with L-DOPA did not differ in their effects on pituitary prolactin release *in vitro*. An injection of saline

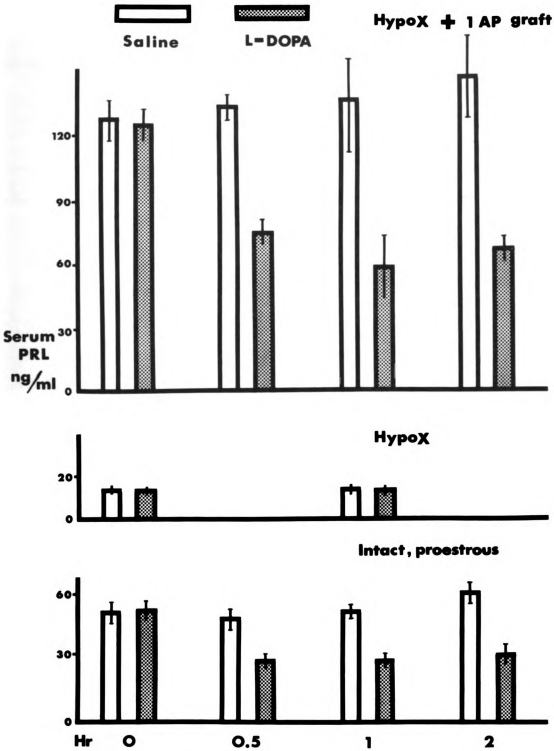


Figure 8. Effects of L-DOPA on Serum Prolactin Levels in Intact, Hypophysectomized, or Hypophysectomized, Pituitary-Grafted Female Rats.

Table 13. In vitro assay of PIF activity in serum and hypothalamus from rats given L-dopa.

Exp.	Treatment	Pre-treatment	Prolactin released (µg prolactin 30 min
Hypophysectomized rats with 1 AP graft			
1.	Saline, 0.5 mg (controls)	1.07±0.10[a] (3)	1.07±0.07(3)
	L-Dopa, 12 mg	1.13±0.03 (3)	0.87±0.17(3)
2.	Saline, 0.5 ml (controls)	1.41±0.10 (12)	
	L-Dopa, 12 mg	1.28±0.05 (12)	
Hypophysectomized rats with no AP graft			
3.	Saline, 0.5 ml (controls)	1.23±0.07 (12)	
	L-Dopa, 12 mg	1.16±0.05 (12)	
Intact, proestrous rats			
4.	Saline, 0.5 ml (controls)	1.24±0.14 (6)	
	L-Dopa, 12 mg	1.20±0.15 (6)	

[a]Mean ± standard error of mean.

[b]Significantly different from the controls, $p < 0.05$.

( ) = No. of rats per treatment.

in vitro after serum incubation released/mg AP)		Prolactin released in vitro after Incubation with hypothalamic extract (μg prolactin released/mg AP)	
Time after treatment		Time after treatment	
1 hr	2 hr	1 hr	2 hr
	1.30±0.13 (6)		
	0.77±0.03[b](6)		
1.28±0.11 (12)		0.42±0.04 (12)	
0.47±0.03[b](12)		0.28±0.03[b](12)	
1.34±0.15 (12)		0.48±0.05 (12)	
0.44±0.05[b](12)		0.32±0.04[b](12)	
	1.27±0.05 (6)		0.55±0.04 (6)
	0.64±0.03[b](6)		0.44±0.02[b](6)

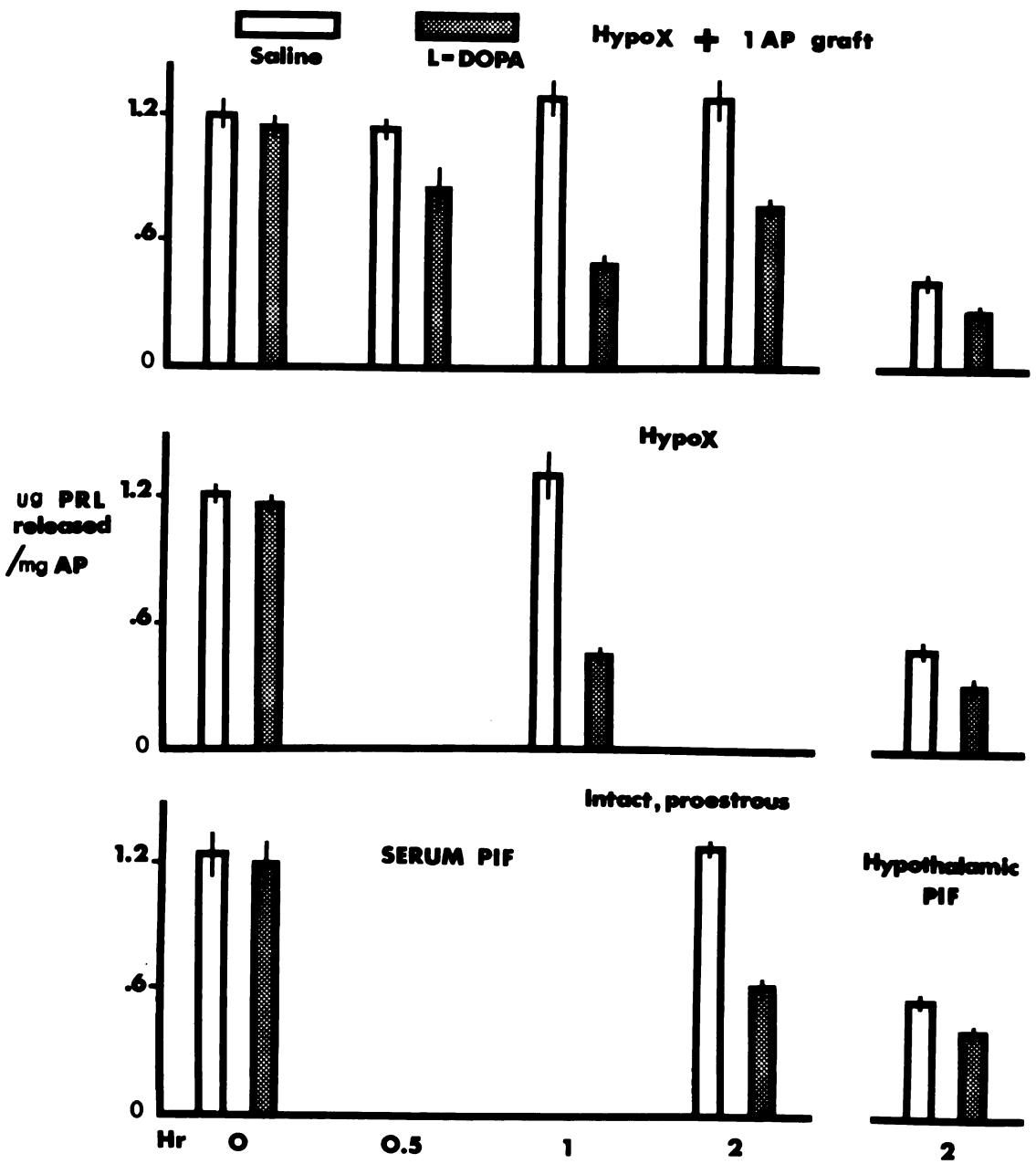


Figure 9. Effects of L-DOPA on Serum Prolactin Releasing Activity in Intact, Hypophysectomized, or Hypophysectomized, Pituitary-Grafted Female Rats.

(controls) had no effect on serum prolactin releasing activity in hypophysectomized or hypophysectomized, pituitary-grafted rats (Experiments 1-3). Experiment 1 shows that the incubated serum from the L-DOPA treated rats produced a small decrease in prolactin release by 30 minutes and a significant decrease of 40% by 2 hours after L-DOPA injection as compared to pre-treatment or saline control values. Experiment 2 shows that the serum from the L-DOPA treated rats produced a 65% decrease in prolactin release when incubated with pituitary as compared to saline controls or pre-treatment values. The serum of hypophysectomized rats with no pituitary transplant (Experiment 3) given L-DOPA also elicited a 65% decrease in prolactin release by incubated pituitary. Experiment 4 shows that the serum from L-DOPA treated intact proestrous rats reduced prolactin release by almost 50%. All experiments show that injection of L-DOPA produced prolactin release-inhibiting activity in the serum.

5. Effects of L-DOPA on hypothalamic PIF activity

The data in Table 13 and in Figure 9 also show that L-DOPA increased hypothalamic PIF content by 35% in hypophysectomized or hypophysectomized, pituitary-transplanted female rats by 1 hour after L-DOPA injection. L-DOPA produced a 20% increase in hypothalamic PIF activity in intact female rats by 2 hours after the injection. All experiments show that L-DOPA administration increased hypothalamic PIF content.

D. Conclusions

The present study demonstrates that transplantation of a single anterior pituitary underneath the kidney capsule results in increased body weight and sustained prolactin release in hypophysectomized rats. Early work from our laboratory (Meites and Kragt, 1964) demonstrated that transplantation of a single pituitary graft induced body weight gains in immature hypophysectomized female rats. As a result of removal of direct hypothalamic inhibition by pituitary transplantation, stimulation of prolactin turn-over rate produces an increase in serum prolactin and a decrease in prolactin concentration (or content) in the pituitary graft (Lu et al., 1971).

L-DOPA administration reduces serum prolactin levels in hypophysectomized rats with an anterior pituitary transplant and in intact rats, and this is associated with an increase in hypothalamic PIF content and the appearance of prolactin release-inhibiting activity in the serum. It is probable, therefore, that in the present study L-DOPA inhibited pituitary prolactin release both by increasing PIF activity in the hypothalamus, and by producing prolactin release-inhibiting activity in the serum.

The prolactin release-inhibiting activity in the serum after L-DOPA may represent increased synthesis and release of hypothalamic PIF into the systemic blood. The

possibility that some L-DOPA remained in the serum and directly inhibited pituitary prolactin release remains to be studied, although L-DOPA is rapidly metabolized.

V. Effects of Serotonin, Melatonin, 5-Hydroxytryptophan and Tryptophan on Serum Prolactin and Hypothalamic and Serum Prolactin Releasing Activity

A. Objectives

Kamberi et al. (1970a, 1971a) reported that an injection of serotonin or melatonin into the third ventricle of rats increased prolactin and decreased LH and FSH levels in the blood. However, Lu et al. (1970b) observed that a single intraperitoneal injection of serotonin did not alter serum and pituitary prolactin concentrations in cycling female rats. Inasmuch as serotonin, likes catecholamines, does not readily pass through the "blood-brain barrier" (Douglas, 1971), the present study was undertaken to determine whether precursors of serotonin known to enter the brain and to increase brain serotonin concentrations (Fernstrom and Wurtman, 1971) could change serum prolactin levels in the rat. The precursor substrates used were L-tryptophan and the immediate precursor 5-hydroxytryptophan. The effects of serotonin and melatonin on serum prolactin values also were assessed in cycling female rats. In an attempt to clarify the mechanism of action by which 5-hydroxytryptophan stimulates prolactin release, its effects on

serum prolactin and hypothalamic and serum prolactin releasing activity were assessed in hypophysectomized and hypophysectomized, pituitary-grafted rats.

B. Materials and Methods

1. Animals

Mature, 4- to 5-month-old, virgin female Sprague-Dawley rats, weighing 220-250 g each, were used in the experiments. Two complete estrous cycles of 4 to 5 days duration were followed on all female rats before they were injected with one of the drugs on the day of proestrus.

A group of mature hypophysectomized female Sprague-Dawley rats (average body weight = 196 g each) were grafted with a single anterior pituitary (AP) underneath the left kidney capsule 7 days after hypophysectomy. Mature cycling female rats of the same strain were used as pituitary donors. Another group of mature hypophysectomized female rats were grafted without an AP transplant. Mature male Sprague-Dawley rats (250-300 g each) were used as pituitary donors for *in vitro* assay of hypothalamic and serum prolactin releasing activity.

2. Drugs

The drugs used were L-tryptophan, DL-5-hydroxytryptophan, serotonin (5-hydroxytryptamine) creatinine sulfate,

and melatonin (N-acetyl-5-methoxytryptamine). All compounds were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. L-tryptophan and 5-hydroxytryptophan were each dissolved in slightly alkalized saline to which 0.5 N HCl was added a drop at a time to bring the pH to 6.7. Melatonin was first dissolved in a small amount of absolute alcohol and then diluted with physiological saline to contain 0.3% ethanol. Serotonin was directly dissolved in 0.85% NaCl for intravenous administrations.

3. Treatments

All drug solutions were injected intraperitoneally into cycling female rats via the caudal veins at 11 AM on the day of proestrus. The control rats received injections of saline-ethanol solution or pH 6.7 medium. Individual blood samples (0.5-0.7 ml) were collected by cardiac puncture under light ether anesthesia at 30 minutes, 1 hour and 2 hours after each treatment. A pre-treatment blood sample was taken from each rat prior to drug injection for comparison with subsequent blood samples. Since injections of drugs were made while the rats were anesthetized lightly under ether, the pre-treatment blood sample was collected 30 minutes prior to drug injection to avoid prolonged ether treatment.

Seven days following pituitary-transplantation and 14 days after hypophysectomy, rats with or without an AP

graft were given 25 mg of 5-hydroxytryptophan or 0.6 ml pH 6.7 medium by a single injection via the caudal veins at 10:30 AM. A pre-treatment blood sample was taken from each rat at 10 AM. Subsequent blood sample was taken 60 minutes after drug injection. The rats were killed by decapitation after the post-treatment blood samples were collected. The pituitary fossa of each hypophysectomized rat was examined under magnification and found to be free of pituitary tissue. The hypothalami were removed, polled in groups and kept frozen in 0.1 N HCl at -20° C until assayed *in vitro*.

The blood samples were kept in a refrigerator at 4° C for 4 hours before they were centrifuged. The serum was separated and preserved as described under Materials and Methods.

4. *In vitro assay of hypothalamic prolactin releasing activity*

The pooled hypothalami from each treatment group were homogenized and extracted in 0.1 N HCl as described under Materials and Methods. Male pituitary-donor rats were killed by decapitation. Anterior pituitaries were removed and hemisected. After 30 minutes pre-incubation, each AP half was placed into a 5-ml culture tube containing medium 199 and extract of one hypothalamic fragment in a total volume of 2.15 ml. Incubations were carried out in a Dubnoff metabolic shaking incubator as described under Materials and

Methods. At the end of 4-hour incubation, AP halves were removed and incubation media were kept frozen at -20°C until assayed for prolactin activity.

5. In vitro assay of serum prolactin releasing activity

Individual serum samples were assayed for prolactin releasing activity by incubating with single AP halves. A total of 0.5 ml serum was incorporated into 1.5 ml medium 199 (pH 7.4) in a culture tube containing one AP half.

6. Prolactin assay

Prolactin in individual serum samples and incubation media were measured by radioimmunoassay. Each sample was assayed at two different dose levels, and the prolactin values were averaged and expressed in terms of the purified rat prolactin reference standard, NIAMD-rat prolactin-RP-1. The prolactin values reported in the serum incubation experiments (Table 15) were corrected for the amounts of serum prolactin added to the incubation medium.

C. Results

1. Effects of a single intravenous injections of L-tryptophan, 5-hydroxytryptophan, serotonin, and melatonin on serum prolactin levels in cycling female rats

The data in Table 14 show that a single intravenous injection of saline-ethanol solution or medium of pH 6.7

had no effect on subsequent serum prolactin values. The prolactin levels in the serum were slightly elevated by 1 PM on the day of proestrus. Administration of L-tryptophan produced no effect on serum prolactin by 30 minutes and 1 hour, and evoked a small increase by 2 hours after injection. This increase in serum prolactin was not statistically significant due to the large standard error. By contrast, a single injection of 5-hydroxytryptophan, the immediate precursor of serotonin, raised serum prolactin about 9-fold by 30 minutes, about 6-fold by 1 hour, and about 2-fold by 2 hours after injection as compared to control rats received medium of pH 6.7. Intravenous injection of serotonin produced no significant changes in serum prolactin levels after 30 minutes, 1 and 2 hours, when compared with pre-treatment or saline-ethanol-control values. An injection of melatonin produced no changes in serum prolactin by 30 minutes, and elicited a 2-fold increase by 1 hour and a 3-fold increase by 2 hours after injection.

2. Effects of 5-hydroxytryptophan administration on serum prolactin levels in hypophysectomized and hypophysectomized, pituitary-grafted rats

Table 15 shows that the serum prolactin values were about 14 ng/ml in hypophysectomized rats (Experiment 1). This was in good agreement with previous reports on serum prolactin values of 10-14 ng/ml in hypophysectomized rats

Table 14. Effects of a single intravenous injection of tryptophan, 5-hydroxytryptophan, serotonin and melatonin on serum prolactin values.

Treatment (8 rats/group)	Dose/ rat	Serum prolactin concentrations, ng/ml			
		Pre-treatment (10:30 AM)	30 min (11:30 AM)	1 hr (12:00 AM)	2 hr (1:00 PM)
pH 6.7 medium (control)	0.6 ml	42.7± 9.9[a]	51.6±10.2	69.8±13.8	118.2±35.0
L-tryptophan	30 mg	49.6± 8.3	101.8±39.1	84.9±26.7	210.4±49.9
5-hydroxytryptophan	30 mg	44.8± 5.4	436.8±53.3[c]	364.0±41.2[c]	227.5±29.4[b]
Saline-ethanol (control)	0.5 ml	52.5± 4.8	56.5± 5.5	64.3± 6.3	85.6±15.2
Serotonin	0.5 mg	45.5±12.8	48.5± 6.5	44.8±11.6	61.0±10.6
Melatonin	3 mg	40.8± 9.2	72.6±19.4	139.6±40.6[b]	242.0±63.8[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the control, $p < 0.05$.

[c]Significantly different from the control, $p < 0.01$.

Table 15. Effects of a single intravenous injection of 5-hydroxytryptophan on serum prolactin values, and prolactin-releasing activity in the serum and hypothalamus of hypophysectomized and hypothysectomized, pituitary-grafted rats.

Treatment (5 rats/group)	Dose/ rat	Serum prolactin conc., ng/ml	
		Time after treatment	
		Pre-treatment (10 AM)	60 min (11:30 AM)
Experiment 1: Hypothysectomized rats with no AP graft			
pH 6.7 medium (control)	0.6 ml	14± 1[a]	12± 1
5-Hydroxytrayptophan	25 mg	13± 1	15± 2
Experiment 2: Hypophysectomized rats with 1 AP graft			
pH 6.7 medium (control)	0.6 ml	135±12	156±54
5-Hydroxytryptophan	25 mg	153±17	298±55[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the control, $p < 0.05$.

Prolactin released *in vitro* after serum incubation; µg prolactin released/mg AP		Prolactin released *in vitro* after incubation with hypothalamic extract (µg prolactin released/mg AP)
Time after treatment		Time after treatment
Pre-treatment	60 min	60 min
1.20±0.09	1.33±0.14	0.51±0.07
1.40±0.18	1.38±0.16	0.51±0.03
1.51±0.15	1.35±0.17	0.43±0.03
1.40±0.21	1.65±0.16	0.47±0.03

(Chen et al., 1970; Lu and Meites, 1972). The "prolactin" in hypophysectomized rats is believed to reflect non-specific binding by rat serum proteins to anti-rat prolactin antibody. An injection of pH 6.7 medium had no effect on subsequent serum prolactin levels in hypophysectomized or hypophysectomized, pituitary-grafted rats (Experiments 1-2). An injection of 5-hydroxytryptophan increased serum prolactin concentrations by about 95% 1 hour after injection into hypophysectomized rats with a pituitary graft (Experiment 2) when compared with pre-treatment values or controls 1 hour after injection of pH 6.7 medium. It can be seen that 5-hydroxytryptophan had no effect on serum prolactin levels in hypophysectomized rats with no AP graft (Experiment 1).

3. Effects of 5-hydroxytryptophan administration on serum and hypothalamic prolactin releasing activity

The data in Table 15 show that the sera from hypophysectomized and hypophysectomized, pituitary-grafted rats prior to treatment with 5-hydroxytryptophan or pH 6.7 medium did not differ in their effects on pituitary prolactin release in vitro (Experiments 1-2). An injection of 5-hydroxytryptophan had no effect on serum prolactin releasing activity in hypophysectomized or hypophysectomized, pituitary-grafted rats (Experiments 1-2).

Extracts of hypothalami from hypophysectomized rats treated with 5-hydroxytryptophan did not differ in their

effects on prolactin release when compared with medium-control values (Experiment 1). In hypophysectomized, pituitary-grafted rats, administration of 5-hydroxytryptophan did not significantly alter hypothalamic prolactin releasing activity. The hypothalami from hypophysectomized and hypophysectomized, pituitary-grafted rats did not differ in their effects on pituitary prolactin release *in vitro* (Experiments 1-2). These experiments show that administration of 5-hydroxytryptophan did not change hypothalamic and serum prolactin releasing activity in hypophysectomized rats with or without an AP graft.

D. Conclusions

These results demonstrate that a single injection of 5-hydroxytryptophan, the immediate precursor of serotonin, produced a rapid increase in serum prolactin in cycling female rats and in hypophysectomized rats with one AP transplant. The ability of 5-hydroxytryptophan to raise brain serotonin concentration (Fernstrom and Wurtman, 1971) is believed to be responsible for the stimulation of pituitary prolactin release. Another precursor, tryptophan (one step prior to 5-hydroxytryptophan) of serotonin produced only a small but insignificant rise in serum prolactin values. The two major biochemical pathways for tryptophan oxidation to 5-hydroxytryptophan and to kynurenine (White *et al.*, 1968) apparently account for the lesser ability of L-tryptophan

to increase serum prolactin, although intraperitoneal injection of L-tryptophan was reported to increase barin serotonin concentration rapidly in rats (Fernstrom and Wurtman, 1971). Injection of melatonin also produced significant increases in serum prolactin values in cycling female rats, confirming the observations of Kamberi et al. (1971a) that an injection of melatonin into the third ventricle of rats elevated serum prolactin values. However, the results of the present and previous report (Lu et al., 1970b) do not substantiate the finding of Kamberi et al (1971) that serotonin itself can stimulate pituitary release of prolactin. This could be due to the different routes of serotonin administration. Systemically administered serotonin apparently does not pass through the "blood-brain barrier" in significant amounts (Douglas, 1971).

Administration of 5-hydroxytryptophan also significantly stimulated prolactin release by the pituitary graft in hypophysectomized rats 1 hour after injection. The increase in pituitary release of prolactin by 5-hydroxytryptophan could be due to suppression of PIF or stimulation of PRF activity in the hypothalamus. However, there was no detectable change in hypothalamic and serum prolactin releasing activity observed after 5-hydroxytryptophan administration under these experimental conditions. The in vitro assay method used in the present study may not be adequate to detect PRF activity in the hypothalamus and its release into the systemic circulation.

VI. Direct Inhibition by Ergocornine of Pituitary Prolactin Release

A. Objectives

Recent work from our laboratory (Nagasawa and Meites, 1970; Wuttke et al., 1971) demonstrated that ergocornine reduced pituitary and serum prolactin concentrations. Ergocornine also was shown to increase hypothalamic PIF content (Wuttke et al., 1971), suggesting that it acted at least in part via the hypothalamus. It was of interest therefore to determine whether ergocornine could also act directly on the pituitary to inhibit prolactin release.

Estrogen stimulates pituitary prolactin release in vivo (Meites et al., 1963; Nagasawa et al., 1969; Amenomori et al., 1970; Chen and Meites, 1970) and in vitro (Nicoll and Meites, 1962; Ratner et al., 1963 Nicoll and Meites, 1964) both by a direct action on the pituitary and via the hypothalamus by reducing PIF content (Ratner and Meites, 1964). It was of interest, therefore, to see whether ergocornine could counteract the stimulatory effect of estrogen on pituitary prolactin release. The effects of ergocornine and/or estrogen were assessed in ovariectomized rats, in hypophysectomized-ovariectomized rats with a single anterior pituitary transplant, in intact cycling female rats, and on pituitary tissue incubated in vitro.

Wuttke and Meites (1971) reported that injections of ergocornine for 1 or 3 estrous cycles inhibited luteolysis

of corpora lutea in cycling rats, presumably due to inhibition of the normal rise in blood prolactin on the days of proestrus and estrus. In the present study, it was considered of interest to see whether injections of ergocornine into cycling rats for 4-5 weeks could interfere with the estrous cycle by inducing accumulation of corpora lutea in the ovaries.

B. Materials and Methods

1. Animals

Mature, 3- to 4-month-old, virgin female Sprague-Dawley rats, weighing 200-220 g, or 4- to 5-month-old female rats weighing 225-240 g each, were bilaterally ovariectomized and later injected with estrogen and/or ergocornine. Intact, 3- to 4-month-old, cycling female rats of the same strain were given daily injections of ergocornine for 4-5 weeks. Mature, hypophysectomized, female rats of the same strain, weighing 190-210 g each, were ovariectomized and transplanted with a single anterior pituitary underneath the left kidney capsule 7 days after hypophysectomy. Mature male Sprague-Dawley rats, weighing 250-300 g each, served as pituitary donors for the transplantation studies and for the *in vitro* incubation experiments.

2. Drugs and Hormones

Ergocornine methanesulfonate (Sandoz Ltd., Basel, Switzerland) was first dissolved in a small amount of absolute alcohol and then diluted with physiological saline to make up a suspension containing 0.3% alcohol. Estradiol benzoate (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in corn oil and injected subcutaneously in a volume of 0.3 ml under the cervicodorsal skin, and ergocornine was injected intraperitoneally in a volume of 0.5 ml. Ovine prolactin (NIH-P-S 8) was dissolved in slightly alkalized 0.85% NaCl at a concentration of 1 mg/0.4 ml.

3. Treatments

Estrogen and/or ergocornine were administered to the experimental rats as shown in Tables 16, 17, and 18. Blood samples were collected from each rat at the end of each experiment before the rats were killed by guillotine. Inguinal mammary pads were excised from each animal and prepared for whole mount evaluation as described under Materials and Methods. The anterior pituitaries were removed, weighed, and homogenized. Blood samples were centrifuged, and the sera were separated. Both the sera and pituitary homogenates were kept frozen at -20° C until assayed as described under Materials and Methods.

4. Ovarian histology

Intact, cycling female rats were killed at the end of the 4- or 5-week treatment with saline, ergocornine, or ergocornine plus prolactin. The ovaries were removed, cleaned, weighed, fixed in Bouin's fluid, sectioned at 10 μ each, and stained with hematoxylin and eosin for microscopic examination. The number of copora lutea were counted on three sections taken from the longest axis of individual ovaries from all rats of each group, and averaged.

5. Pituitary incubations

Each anterior pituitary half was placed in a culture tube containing 2 ml of medium 199 at a pH of 7.4. Incubations were carried out in a Dubnoff metabolic shaker as described under Materials and Methods. After 30 minutes preincubation, the medium was removed and replaced with 2 ml of fresh medium 199 containing one of the following amounts of reagents: 0.5, 1.0 or 2.0 μg of estradiol (Nutritional Biochemicals Corp., Cleveland, Ohio); 0.2 or 2.0 μg of ergocornine; 0.5 μg estradiol and 0.2 μg ergocornine; 1.0 μg estradiol and 0.2 μg ergocornine; 2.0 μg estradiol and 0.2 μg ergocornine. Each reagent was first dissolved in a small amount of absolute alcohol and incorporated into medium 199 to a concentration of 0.3% alcohol. Six incubation tubes were used for each treatment. The control tubes contained only 2 ml of medium 199 with 0.3% alcohol. After 12-hour

incubation, the anterior pituitary halves were removed from the medium, weighed and homogenized. Both the incubation media and pituitary homogenates were kept frozen at -20°C until assayed as described under Materials and Methods.

6. Prolactin assay

Prolactin in individual serum samples, pituitary homogenates and incubation media was measured by radioimmunoassay. Each sample was assayed at 3 different dose levels. The prolactin values were averaged, and expressed in terms of the purified rat prolactin reference standard, H-10-10-B.

Mean and standard error of the mean for prolactin values in anterior pituitary, serum and incubation media were calculated for each experimental group, and subjected to analysis of variance. Ovarian weights, anterior pituitary weights, number of corpora lutes, and mammary gland growth ratings were similarly analyzed. Duncan's new multiple range test (Duncan, 1955) was used to determine the significance of differences between experimental groups.

C. Results

1. Effects of estradiol benzoate and ergocornine in ovariectomized rats

It can be seen (Table 16, Experiment 1; and Figure 10) that daily injections of 5 (Group 2) or 10 μg estradiol benzoate (Group 4) for five days, beginning two days after

ovariectomy of three- to four-month-old rats, produced a 3- to 4-fold increase in pituitary prolactin concentration and a 6- to 8-fold increase in serum prolactin levels as compared with the ovariectomized controls (Group 1). A dose of 0.1 mg ergocornine partially counteracted the stimulatory action of estrogen on pituitary and serum prolactin levels (Group 3 and 5), although the latter counteraction was not statistically significant. Each dose of estradiol benzoate increased anterior pituitary weight by about 50%, but injections of ergocornine completely blocked this increase by the estrogen. Estradiol benzoate also signigicantly stimulated mammary growth in the ovariectomized rats, and ergocornine partially blocked this action of estrogen.

Beginning on the ninth day after ovariectomy, four- to five-month-old rats were treated as shown in Table 16, Experiment 2. Daily injections for five days of 5 (Group 2) or 10 μg estradiol benzoate (Group 4) significantly increased pituitary and serum prolactin concentrations. Ergocornine partially but significantly counteracted these stimulatory effects of estrogen (Groups 3 and 5). Ergocornine also partially inhibited the ability of estrogen to increase anterior pituitary weight. The values reported in these rats are not strictly comparable to those in Experiment 1 since these rats were older initially and injections were begun nine instead of two days after ovariectomy.

Table 16. Effects of estradiol benzoate (EB) and ergocornine methanesulfonate (ERG) on pituitary and serum prolactin concentrations, pituitary weight and mammary growth in ovariectomized rats.

Treatment	Av. AP weight, mg	Av. AP prolactin conc., µg/mg AP	Av. serum prolactin, ng/ml	Av. mam. growth rating (1 to 6)
		Experiment 1		
1. Controls (8)	7.4±0.3*[a]	1.0±0.1[a]	15.3± 1.2[a]	2.1±0.2[a]
2. EB, 5 µg (8)	11.5±0.4[b]	3.9±0.2[b]	95.0±12.3[b]	4.3±0.1[b]
3. EB, 5 µg + ERG, 0.1 mg (8)	7.5±0.3[a]	3.2±0.2[c]	70.1± 5.4[b]	3.1±0.2[c]
4. EB, 10 µg (8)	11.9±0.3[b]	4.5±0.3[d]	127.9±21.6[c]	4.4±0.1[b]
5. EB, 10 µg + ERG, 0.1 mg (8)	7.5±0.4[a]	3.4±0.2[c]	103.8±11.9[c]	3.4±0.1[c]
		Experiment 2		
1. Controls (4)	9.4±0.9*[a]	0.7±0.1[a]	21.5± 3.4[a]	
2. EB, 5 µg (4)	15.8±0.1[b]	2.5±0.1[b]	73.5± 5.7[b]	
3. EB, 5 µg + ERG, 0.3 mg (4)	11.0±0.1[c]	1.3±0.1[b]	35.1± 3.6[c]	
4. EB, 10 µg (4)	15.5±0.5[b]	2.1±0.1[d]	80.1± 7.8[b]	
5. EB, 10 µg + ERG, 0.3 mg (4)	11.9±0.4[d]	1.5±0.1[c]	38.3± 1.4[c]	

*Mean and standard error of mean.

[a,b,c,d]Values with different superscripts are significantly different ($p < 0.05$) from each other.

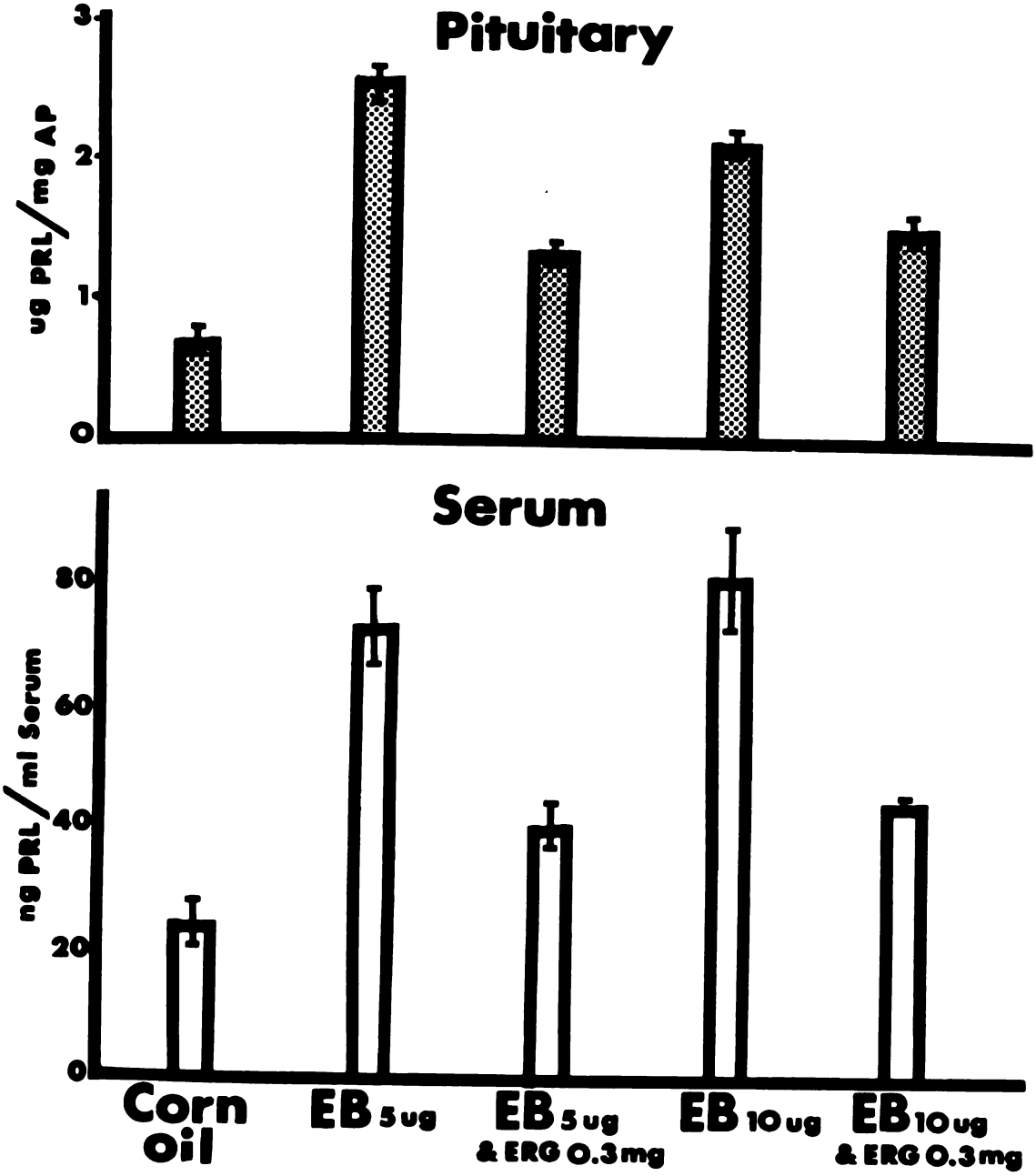


Figure 10. Effects of Estradiol Benzoate (EB) and Ergocornine Methanesulfonate (ERG) on Serum and Pituitary Prolactin Concentrations in Ovariectomized Rats.

2. Effects of estradiol benzoate and ergocornine in hypophysectomized-ovariectomized rats with a single anterior pituitary transplant

Beginning on the second day after ovariectomy and pituitary transplantation, the hypophysectomized rats were divided into four groups and treated for five days as shown in Table 17 and Figure 11. The animals were killed 24 hours after the last injection (between 12:00 noon and 1:00 PM) and each pituitary graft was removed from the kidney capsule, weighed, and homogenized in neutral PBS. The pituitary fossa of each rat was examined under magnification, and found to be free of pituitary tissue.

The data in Table 17 show that control rats (Group 1) bearing one anterior pituitary graft had serum prolactin levels as high as those of rats on the morning of proestrus or estrus (Amenomori et al., 1970; Chen et al., 1970). Ergocornine at a dose of 0.1 mg/day (Group 2) markedly depressed serum prolactin concentration, decreasing it only to 1/4 of the control values (Group 1). Daily injections of 5 μg estradiol benzoate for five days (Group 3) significantly increased pituitary and serum prolactin and stimulated mammary growth, whereas ergocornine partially counteracted these effects of estrogen (Group 4). Ergocornine alone partially but significantly decreased the weight of the anterior pituitary graft, but had no effect on pituitary weight in the estrogen-treated hypophysectomized-ovariectomized rats.

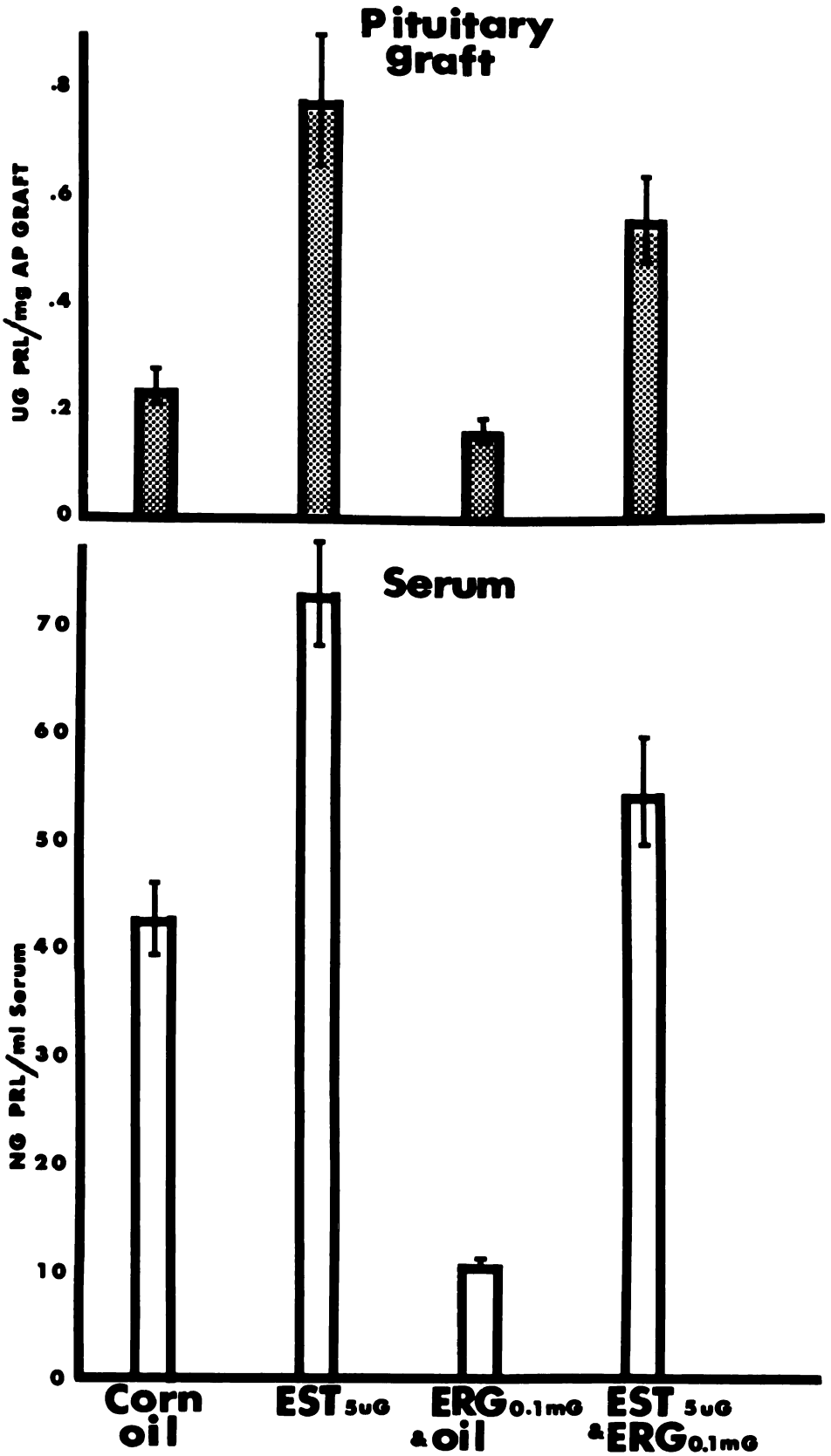
Table 17. Effects of estradiol benzoate (EB) and ergocornine methanesulfonate (ERG) on pituitary and serum prolactin concentrations, pituitary weight and mammary growth in hypophysectomized-ovariectomized rats with a pituitary transplant.

Treatment (8 rats/group)	AP weight, mg	AP prolactin concentration, µg/mg AP	Serum, prolactin, ng/ml	Mammary growth rating (1 to 6)
1. Corn oil (controls)	4.5 0.6*[a]	0.2 0.0[a]	43.4 3.6[a]	1.5 0.1[a]
2. ERG, 0.1 mg	3.8 0.3[b]	0.2 0.0[a]	10.9 0.3[b]	1.1 0.1[b]
3. EB, 5 µg	4.4 0.3[a]	0.8 0.1[b]	73.3 5.1[c]	1.9 0.1[c]
4. EB, 5 µg + ERG, 0.1 mg	4.5 0.5[a]	0.6 0.1[c]	54.7 5.1[d]	1.5 0.1[a]

*Mean and standard error of mean.

[a,b,c,d]Values with different superscripts are significantly different ($p < 0.05$) from each other.

Figure 11. Effects of Estradiol Benzoate (EST) and Ergocornine Methanesulfonate (ERG) on Serum and Pituitary Prolactin Concentrations in Hypophysectomized-Ovariectomized, Pituitary-Grafted Rats.


Pituitary
Graft
UG PRL/mg AP GRAFT
0
.2
.4
.6
.8
Serum
NG PRL/ml Serum
0
10
20
30
40
50
60
70
Corn oil
EST 5uG
ERG 0.1mG & oil
EST 5uG & ERG 0.1mG

Microscopic examination of trichrome-stained sections of anterior pituitary tissue from ergocornine-treated rats indicated that this drug reduced the number and granulation of acidophils.

3. Effects of daily injections of ergocornine with or without prolactin during 7 estrous cycles on the ovaries

A total of 10 cycling female rats were each injected intraperitoneally with 0.15 mg of ergocornine per rat at 12:00 noon daily, beginning on the last day of diestrus prior to the expected day of proestrus and continuing for 7 estrous cycles thereafter. Estrous cycles were followed on all female rats by examining vaginal smears daily. At 2:00 PM on the day of proestrus and again at 10:00 AM on the day of estrus during the last cycle, 5 of the 10 rats were each given a single dose of 1 mg prolactin by subcutaneous injections. A group of 5 control cycling rats were each given daily injections of saline-ethanol solution during the same period as the above rats. The rats were killed during diestrus of the last cycle.

The data in Table 18 show that daily injections of ergocornine significantly increased ovarian weight as compared with the controls. On the other hand, 2 injections of prolactin on the days of proestrus and estrus during the last cycle completely prevented any increase in ovarian weight. The number of corpora lutea per ovarian section

Table 18. Effects of ergocornine methanesulfonate (ERG) and prolactin during 7 estrous cycles on ovaries.

Treatment (5 rats/group)	Dose/day	Body wt. gm	Ovarian wt. mg/pair	Corpora lutea, no./ovarian section
Saline-ethanol (controls)	0.5 ml	275±2[#a]	82.9±4.8[a]	5.75±0.41[a]
ERG	0.15 mg	257±3[b]	120.3±8.8[b]	9.75±1.05[b]
ERG + prolactin	0.15 mg 1.0 mg	263±2[b]	92.1±3.2[a]	6.13±0.77[a]

[#]Mean and standard error of the mean.

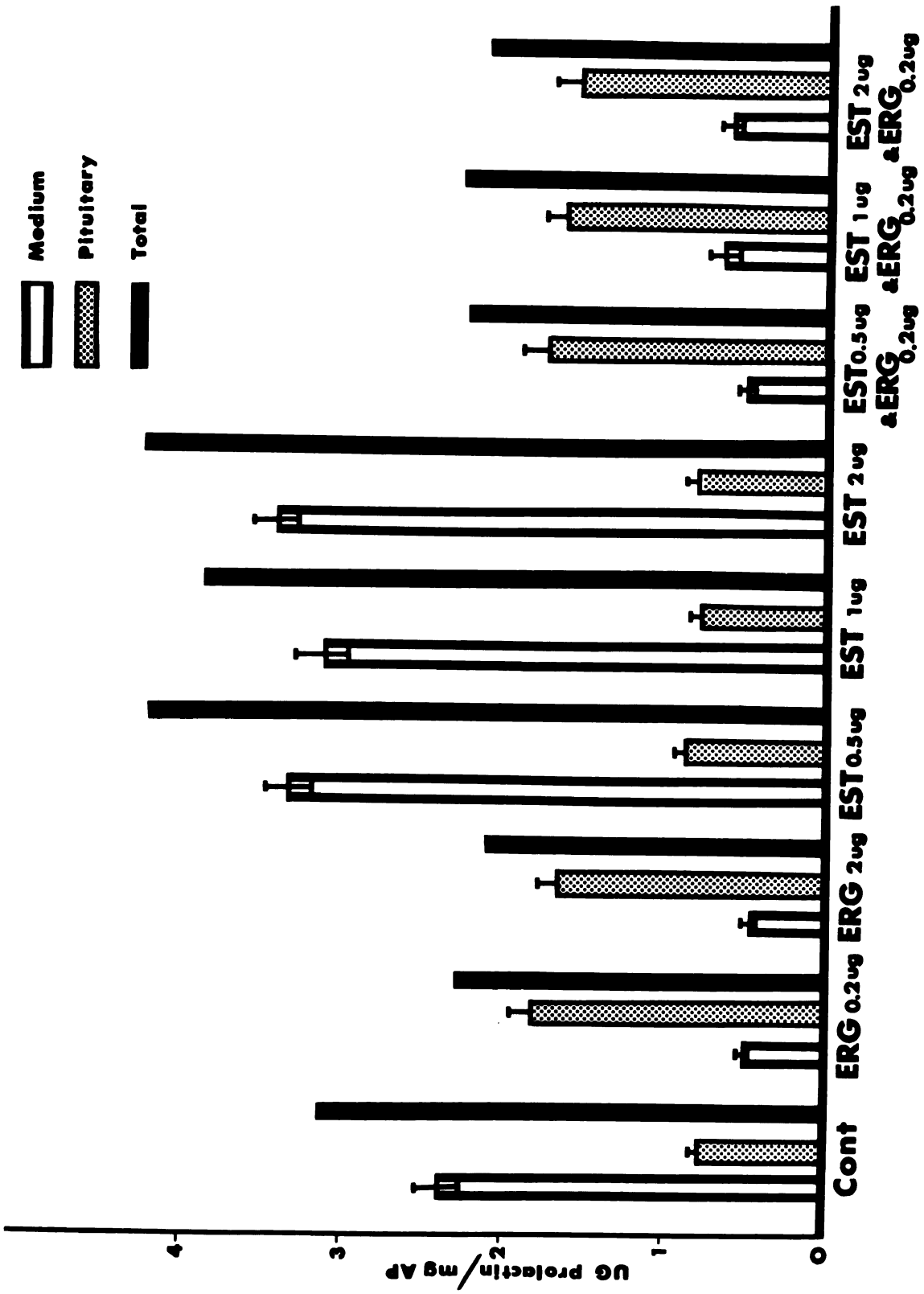
[a,b]Values with different superscripts are significantly different ($p < 0.05$) from each other.

in the ergocornine-treated rats was significantly greater than that in the controls or rats given both ergocornine and prolactin. This appears to be due to retention of the previous as well as the most recent crop of corpora lutea. During the 4-5-week period, rats of all 3 experiment groups exhibited normal estrous cycling of 4-5 days duration. Rats given ergocornine alone or ergocornine and prolactin weighed significantly less than the controls at the end of 7-cycle treatment.

4. In vitro effects of estradiol and ergocornine on pituitary release of prolactin

The data in Figure 12 show that estradiol, in doses ranging from 0.5 to 2.0 µg/anterior pituitary half, increased pituitary release of prolactin by 30 to 40% without producing changes in pituitary prolactin concentration at the end of 12 hours of incubation. Ergocornine alone (0.2 or 2.0 µg/anterior pituitary half) inhibited pituitary prolactin release by about 80%, and, when incubated together with estradiol, it completely blocked estrogen stimulation of prolactin release. It can be seen in every case that inhibition of prolactin release by ergocornine was accompanied by a significant increase in pituitary concentration of prolactin. When compared with the controls, it is apparent that more total prolactin was recovered from the incubation

Figure 12. Effects of Ergocornine Methanesulfonate (ERG), Estradiol (EST) or both on Pituitary Prolactin Concentration and Release *In Vitro*.


Medium
Pituitary
Total
UG Prolactin/mg AP
0
1
2
3
4
Cont
ERG 0.2ug
ERG 2ug
EST 0.5ug
EST 1ug
EST 2ug
EST 0.5ug &ERG 0.2ug
EST 1ug &ERG 0.2ug
EST 2ug &ERG 0.2ug

system when the pituitaries were incubated with estradiol, whereas less total prolactin was recovered when the pituitaries were incubated with ergocornine.

D. Conclusions

The present work demonstrates that ergocornine can inhibit pituitary release of prolactin, and can counteract the stimulatory effect of estrogen on prolactin release. When the pituitary is removed from direct hypothalamic control, as by grafting it underneath the kidney capsule (Chen et al., 1970) or by incubating it in vitro (Meites et al., 1961), prolactin release is increased. The results of the present study suggest that ergocornine directly inhibits prolactin release by the transplanted pituitary and by the pituitary incubated in vitro.

In ovariectomized rats, injections of estradiol benzoate increase both pituitary and serum prolactin concentrations. Ergocornine can partially counteract the stimulatory action of estrogen on pituitary prolactin release, and can partially or completely prevent the ability of estrogen to increase pituitary size.

In hypophysectomized, ovariectomized rats with a single anterior pituitary transplant, ergocornine suppresses and estradiol benzoate stimulates prolactin release. Ergocornine partially counteracts the estrogen stimulation of prolactin release by the pituitary graft. Inhibition by

ergocornine of prolactin release by the pituitary transplant is believed to be due mainly to its direct action on the pituitary.

In intact, cycling female rats, injections of ergocornine for 7 cycles do not interfere with the normal estrous cycle, indicating that pituitary secretion of FSH and LH was not critically impaired. It is apparent that the inhibition by ergocornine of luteolysis of corpora lutea is due solely to suppression of prolactin release by this ergot drug, since injections of prolactin on the days of proestrus and estrus completely prevented any increase in ovarian weight in the ergocornine-treated rats.

During a 12-hour incubation period, the presence of estradiol in the medium can stimulate pituitary release of prolactin. Inasmuch as incorporation of ergocornine alone into the incubation medium inhibits prolactin release from the anterior pituitary halves, it can be assumed that its action on pituitary tissue is responsible for its suppression of estrogen stimulation. The data also suggest that ergocornine inhibits prolactin synthesis in vitro, since less total prolactin is recovered when ergocornine is present in the incubation system.

VII. Secretion of Prolactin in Rats Bearing A Pituitary Mammotropic Tumor; Inhibition by Ergot Drugs of Tumor Growth and Prolactin Release

A. Objectives

We recently reported that ergocornine, an ergot derivative, significantly inhibited prolactin secretion by a direct action on the pituitary (Lu et al., 1971). We also found that ergocornine could prevent estrogen from increasing the size of pituitary and secretion of prolactin in ovariectomized rats and hypophysectomized, ovariectomized rats with a pituitary graft. The Furth pituitary mammotropic tumor (MtT.W 15) of rats is known to secrete large amounts of prolactin and GH (Furth, 1961). The present study was undertaken to measure the change in serum prolactin levels in relation to growth of transplanted pituitary tumors in rats, and to see whether ergot drugs can inhibit the growth of pituitary tumors and reduce secretion of prolactin.

B. Materials and Methods

1. Drugs

The ergot drugs used were ergocornine methanesulfonate (Sandoz Ltd., Basle, Switzerland), 2-Br-(a)-ergocryptine mesylate (Sandoz Ltd., Basle, Switzerland), ergonovine

maleate (Eli Lilly and Co., Indianapolis, Indiana), and Lilly compound-55327 (dl-N-9, 10-Didehydro-6-methyl-8a-ergolinyl-formamide) (Eli Lilly and Co., Indianapolis, Indiana). All drugs were first dissolved in a small amount of absolute alcohol and then incorporated into 0.85% NaCl solution to contain 1% ethanol.

2. Treatment

Fifty-day-old inbred female rats of the Wistar-Furth strain were given transplants of Furth pituitary mammotropic tumor (MtT.W 15) subcutaneously in order to induce growth of the tumors as described under Materials and Methods. When the tumors attained a size of 2-3 cm in diameter (7-9 weeks after transplantation), the rats were divided uniformly into 5 groups and given local subcutaneous injections, daily, as follows: (a) 0.05 mg of Lilly compound-55327; (b) 0.2 mg of ergocornine for the first week and 0.1 mg of ergocornine for the subsequent weeks; (c) 0.2 mg of ergonovine; (d) 0.3 mg of ergocryptine; (e) 0.2 ml of 1% saline-ethanol solution per 100 g of body weight.

Individual body weights and tumor diameter were recorded weekly. Mean tumor diameter (average of the two largest diameters) in each rat was measured with calipers (Fisher Scientific Co., Fair Lawn, New Jersey) and blood samples were collected while the animal was under light ether anesthesia. At the end of 3 weeks, rats given daily

injections of ergocornine were killed. The pituitary tumors were removed, fixed in Bouin's fluid, sectioned and stained with Masson's trichrome stain for microscopic examination.

Seventy five days after transplantation, another group of rats with transplanted MtT.W 15 pituitary tumors were tested to determine the effects of ergot drugs on serum prolactin. The rats were divided into subgroups according to the size of tumors (Tables 19 and 20). After a pre-treatment blood sample was taken and the tumor diameter was measured from each rat at 11:00 AM, a single intraperitoneal injection of Lilly compound-55327 or saline-ethanol solution was given to the rat. Subsequent blood samples were collected at 1, 2 and 3 hours after injection.

3. Prolactin assay

Prolactin in individual serum samples was measured by radioimmunoassay. Serum was diluted with neutral phosphate buffer saline to a 1: 10 working concentration for the assay. Each diluted serum sample was assayed at three different dose levels (20-100 µl). Prolactin values were averaged, and expressed in terms of the purified rat prolactin reference standard, NIAMD-rat prolactin-RP-1.

Mean and standard error of the mean for serum prolactin values, mean tumor diameter, and body weight were calculated for each experiment group. Student's "t" test

was used to determine the significance of difference between the control and experimental groups, and between the pre-treatment and post-treatment values as well.

C. Results

1. The size of tumor and serum prolactin levels in rats bearing transplanted pituitary tumors

Sixty days after transplantation, a group of rats bearing small, palpable, but not measurable pituitary tumors had an average body weight of 180 ± 18 each and a mean serum prolactin concentration of 99 ± 26 ng/ml.

Table 19 shows the correlation between the body weights, the size of tumors, and serum prolactin levels in rats bearing transplanted pituitary tumors 75 days after transplantation. One, 2 or 3 MtT.W 15 tumors were found in each rat. The body weights ranged from 210 to 373 g each, mean tumor diameter from 8 to 98 mm, and prolactin concentrations in the serum from 280 to 22,500 ng/ml. In general, rats with larger body size and/or tumor size had higher concentrations of prolactin in the blood. A similar trend of correlation also was shown in Table 20 (the pre-treatment serum prolactin values) and in Table 21 (the saline-ethanol controls). The number of tumors per rat and the two largest tumor diameters rather than the mean tumor diameter was closely correlated with the prolactin concentrations in the blood.

Table 19. Body weights, tumor size and serum prolactin concentrations in rats bearing pituitary mammotropic tumor transplants at 75 days after transplantation.

Body wt., gm	Tumor diameters,[a] mm x mm	Mean tumor diameter, mm	Serum prolactin conc., ng/ml
One tumor/rat			
310	35 x 28	32	20,250
314	42 x 24	33	15,040
352	51 x 33	42	12,250
301	44 x 23	33	11,270
267	26 x 21	23	10,250
332	47 x 19	33	8,275
260	37 x 17	27	3,755
242	17 x 11	14	2,695
256	24 x 11	17	1,035
224	14 x 11	12	785
236	21 x 11	16	545
223	10 x 7	8	385
215	12 x 10	11	350
210	10 x 6	8	280

Two tumors/rat

305	37 x 19 + 27 x 19	51	18,750
364	39 x 27 + 20 x 19	52	16,620
373	50 x 29 + 41 x 22	71	16,250
352	42 x 24 + 21 x 20	53	15,000
312	49 x 26 + 23 x 13	45	13,300
332	45 x 31 + 29 x 15	60	11,120
305	38 x 23 + 20 x 11	46	8,600
285	32 x 15 + 20 x 13	40	5,380
260	21 x 19 + 21 x 12	36	2,660
227	22 x 11 + 15 x 11	29	2,360

Three tumors/rat

295	34 x 18 + 29 x 21 + 22 x 19	71	22,500
343	44 x 22 + 40 x 25 + 39 x 27	98	21,050

[a]The two largest tumor diameters.

2. Effects of a single injection of Lilly compound-55327 on serum prolactin levels in rats bearing transplanted pituitary tumors

Table 20 shows that rats bearing MtT.W 15 pituitary tumors of different sizes had wide ranges of prolactin values in the blood (pre-treatment values). Injection of 1% saline-ethanol solution into these rats had no effect on subsequent serum prolactin levels as compared with pre-treatment values. In 4 groups of rats bearing different sizes of tumors, a single intraperitoneal injection of Lilly compound-55327 significantly reduced serum prolactin levels by 30 to 60% by 1, 2 and 3 hours after injection. In all 4 groups of rats, the per cent inhibition of serum prolactin by 2 or 3 hours was higher than by one hour after injection, indicating sustained inhibition by this ergot drug on prolactin release from the pituitary tumors.

3. Effects of daily injections of Lilly compound-55327 on growth of pituitary tumors and secretion of prolactin in rats during 7 weeks

Since previous experiments demonstrated that this ergot drug significantly reduced serum prolactin levels in rats bearing transplanted pituitary tumors, another experiment was undertaken to determine whether this ergot drug could inhibit the growth of pituitary tumors and suppress the secretion of prolactin by daily injections. Rats bearing

Table 20. Effects of a single injection of ergot drugs on serum prolactin levels in rats bearing transplanted pituitary tumors of different size.

Treatment (dose/100 g BW)	Mean tumor diameter, mm	No. of rats	Serum prolactin concentrations, ng/ml			
			Pre-treatment	1 hour	2 hours	3 hours
Saline-ethanol, 0.2 ml (controls)	49± 4[a]	2	14,166± 835	14,000± 500	13,265± 595	11,785± 665
	30± 3	2	6,015±2,260	5,600±2,025	5,800±2,330	5,695±2,680
	16± 2	2	1,865± 830	1,845± 945	1,900± 965	1,943±1,053
Lilly compound-55327, 80 µg	67±19	3	21,267± 658	14,280±1,148[c]	13,740±1,936[b]	12,230± 665[c]
	30± 3	3	12,220±1,446	6,710± 607[b]	5,812±1,365[b]	5,087±1.946[b]
	33± 4	2	2,510± 150	1,265± 295[c]	1,160± 100[c]	985± 45[c]
	10± 2	2	315± 35	195± 40[b]	126± 48[b]	173± 54[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the pre-treatment values, $p < 0.05$.

[c]Significantly different from the pre-treatment values, $p < 0.01$.

transplanted pituitary tumors of 2-3 cm in diameter were given Lilly compound-55327 or saline-ethanol solution continuously for 7 weeks.

Table 21 and Figure 13 show that the initial body weights, mean tumor diameters and serum prolactin concentrations between the control and experimental groups were not statistically different. It is apparent that the control rats continued to gain body weights, and showed increases in size of tumors and serum levels of prolactin. During a period of 7 weeks, the body weights, mean tumor diameters and serum prolactin concentrations were increased about 2-, 3-, and 6-fold respectively in the controls. Daily injections of Lilly compound-55327 produced inhibition of tumor growth and prolactin release. The mean tumor diameters of the ergot-treated rats were significantly less than in the controls by 6 and 7 weeks after injections, and by one week post-treatment. The serum prolactin concentrations in rats given the ergot drug were lower than in the controls during most of the 7 weeks, although the values were not statistically different by 5 and 7 weeks after injections because of the large standard errors. At the dose given, this ergot drug had no effect on body weight gains as compared with the saline-ethanol controls.

Table 21. Effects of subcutaneous injections of Lilly compound-55327 on body weights, tumor growth and serum prolactin levels in rats bearing transplanted pituitary tumors.

Treatment (7 rats/group)	Pre-treatment	Weeks of treatment 1	2	3
Body weight, g				
Saline-ethanol (controls)	228±10[a]	263± 6	294±12	321±9
Lilly compound -55327	234± 9	262±11	282± 7	300±6
Mean tumor diameter, mm				
Saline-ethanol (controls)	23.9±3.5	31.0±4.2	37.5±5.5	46.1±7.5
Lilly compound -55327	21.5±4.6	27.5±4.3	33.1±4.7	40.0±5.2
Serum prolactin concentrations, ng/ml				
Saline-ethanol (controls)	1,831±533	----	3,176±688	4,225±802
Lilly compound -55327	1,038±476	----	1,462±463[b]	2,143±564[b]

[a]Mean and standard error of the mean.

[b]Significantly different from the controls, $p < 0.05$.

Note: Dose of drug used: 1% saline-ethanol 0.2 ml, or Lilly compound-55327 50 µg per 100 g body weight.

Weeks of treatment				One week post-treatment
4	5	6	7	
349±12	369±22	394±9	416±13	435±11
322± 8	345± 9	361±7	384± 7	414± 9
55.5±4.1	63.4±5.1	72.5±8.3	81.5±4.6	91.0±7.6
44.8±8.5	49.2±5.9	52.7±3.1[b]	56.5±3.2[b]	62.5±6.4[b]
6,764±1,241	7,544±1,138	8,910±1,017	11,281±1,917	13,541±1,987
3,595± 931	5,371± 770	6,327± 861[b]	7,853±1,378	9,749±1,782

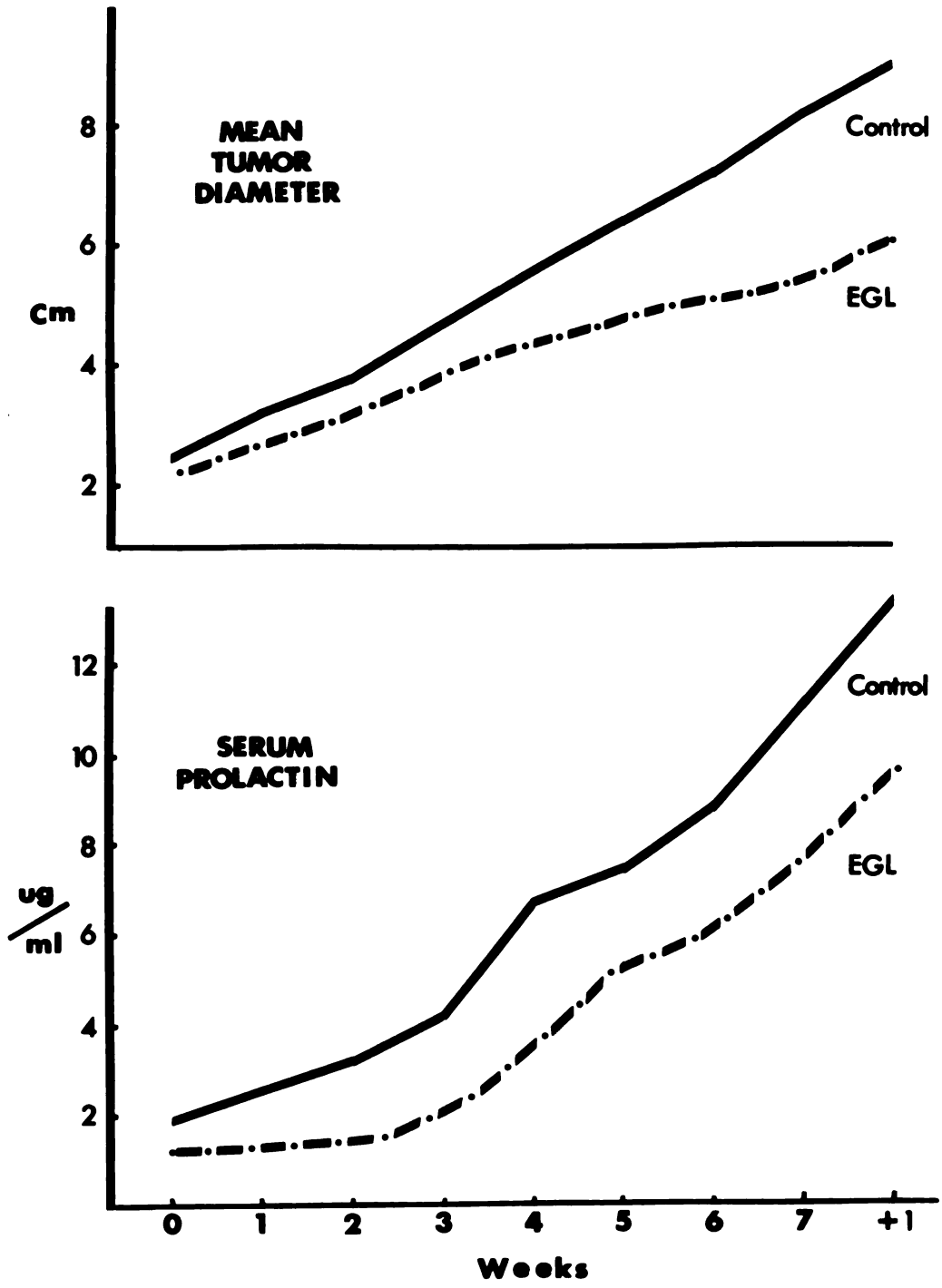


Figure 13. Effects of Lilly Compound-55327 (EGL) on the Growth of MtT. W 15 Pituitary Tumors and Serum Prolactin in Rats.

4. Effects of daily injections of ergot drugs on the growth of pituitary tumors during 5 weeks

Figure 14 shows that injections of ergocornine significantly reduced the mean tumor diameter, whereas in the control group the pituitary tumors continued to increase in size. The mean tumor diameter was reduced by 15% in rats given ergocornine; in control rats there was a 58% gain in the size of tumors. Ergocornine treatment was terminated at the end of 3 weeks. Microscopic examination revealed that the transplanted pituitary tumor from control rats consisted of numerous cells of different size with prominent nuclei and many mitotic figures (Figure 15, A). Tumors from rats treated with ergocornine consisted of relatively few, separated, large cells with absent or pycnotic nuclei (Figure 15, B).

At the dose given, ergocryptine produced a small but insignificant inhibition of pituitary tumor growth by the end of 5 weeks treatment. Ergonovine significantly but not completely inhibited tumor growth. There was a 78% increase in mean tumor diameter in the controls, but only a 28% gain in tumor size in rats treated with ergonovine.

At the doses given, none of the 3 ergot drugs had any significant effect on body weight, although necrosis of the tail was observed in every rat injected with ergocornine or ergonovine.

Figure 14. Effects of Three Ergot Drugs on the Growth of MtT W 15 Pituitary Tumors in Rats. ECP = ergocryptine; ENV = ergonovine; ERG = ergocornine.

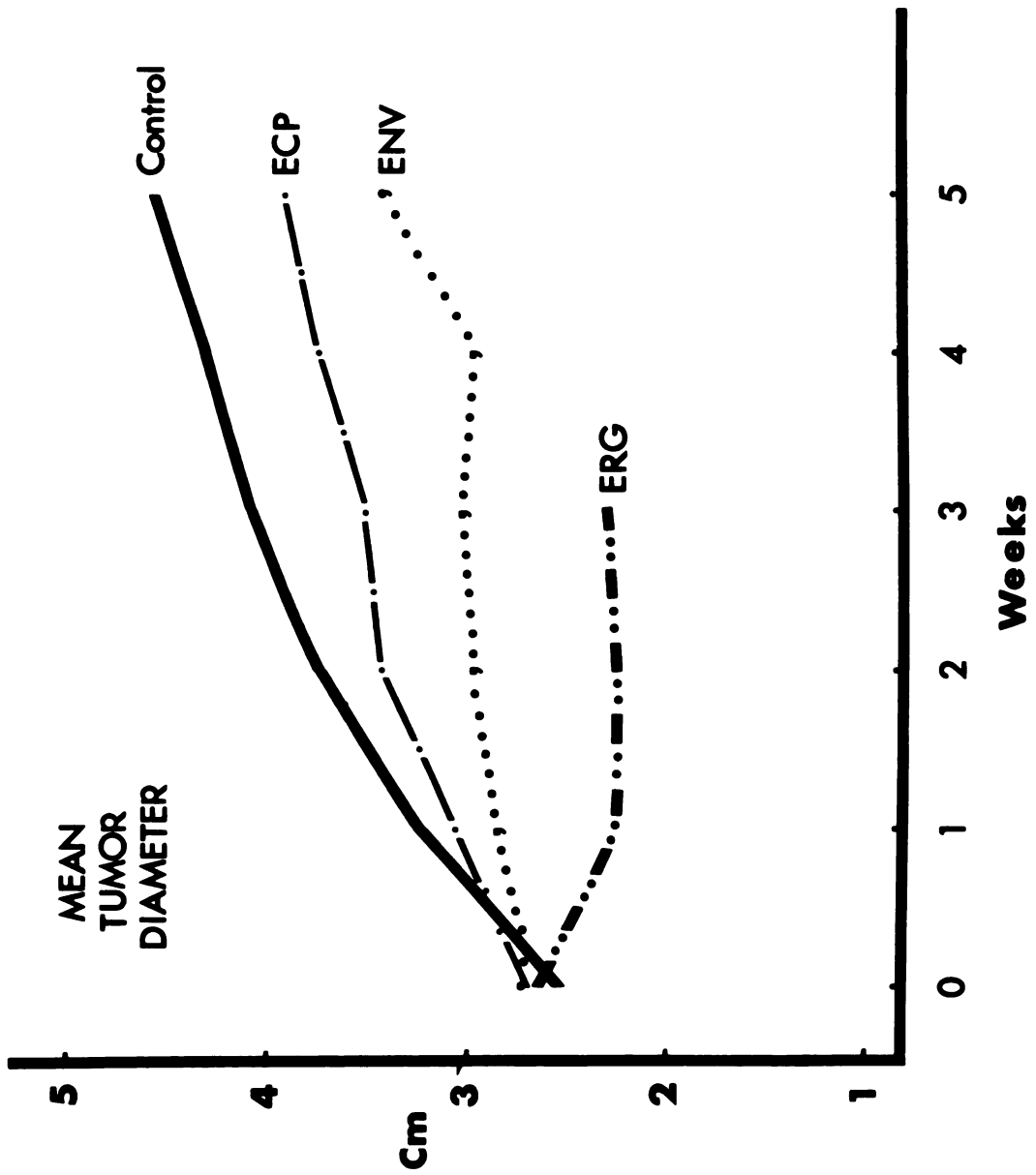
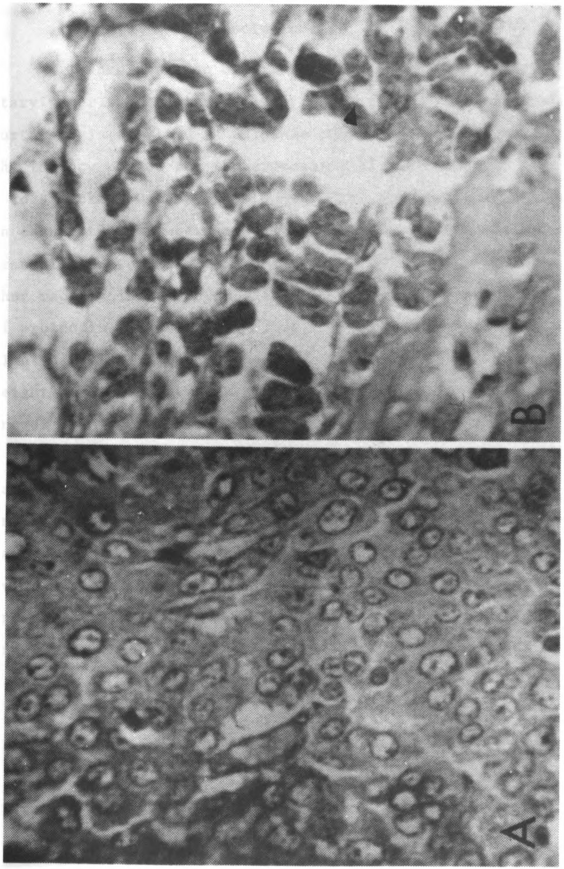

MEAN
TUMOR
DIAMETER
Control
ECP
ENV
ERG
Cm
5
4
3
2
1
0
1
2
3
4
5
Weeks

Figure 15. (A) Section from a transplanted pituitary tumor from a control rat showing cells of diverse size with prominent nuclei and several mitotic figures. (B) Section from a transplanted pituitary tumor from a rat treated with ergocornine, showing large, separated cells with few pycnotic nuclei (X 680).


B
A

D. Conclusions

Transplantation of a suspension of MtT. W 15 pituitary tumor subcutaneously into inbred female rats of Wisatr-Furth strain resulted in palpable tumors in 100% of the rats. The latency period for the appearance of tumors ranged from 3 to 8 weeks. One, 2 or 3 tumors of palpable size were found in each rat by 10 weeks after transplantation. Once the transplanted tumors attained a size of 2-3 cm in diameter, they rapidly increased in size and released enormous amounts of prolactin (GH as well). Thus, the rats bearing transplanted MtT. W 15 tumors show a 2-fold increase in body weight, a 3-fold increase in mean tumor diameter and a 6-fold increase in serum prolactin during a period of 7 weeks.

The number and size of the MtT. W 15 tumors in each rat were closely related to the concentration of prolactin in the blood. Rats with larger tumors had higher serum prolactin values and body weight. These observations provide evidence that the transplanted pituitary tumors gradually increase their secretion of prolactin and GH with increase in growth, thereby promoting the gain in body weight of the rats.

A single injection of an ergot derivative, Lilly compound-55327, produced a highly significant reduction in serum prolactin concentrations in rats bearing transplanted MtT. W 15 tumors. Daily injections of ergocornine, and to a lesser extent, ergonovine and Lilly compound-55327,

significantly inhibited growth of pituitary tumors. Ergocryptine was not effective at the dose given. Associated with the tumor regression induced by ergocornine, there was pycnosis or loss of nuclei, and disappearance of cells of the tumor tissue. Since a single or daily multiple injection of Lilly compound-55327 significantly reduced the capacity of the pituitary tumor to secrete prolactin, the present study suggests that major action of the ergot drugs was exerted directly on the tumor to inhibit its growth and suppress secretion of prolactin.

VIII. In Vivo and In Vitro Effects of Synthetic Pyro-Glutamyl-Histidyl-Proline Amide (TRH) on Pituitary Prolactin Release in Rats

A. Objectives

Tashjian et al. (1971) reported that synthetic thyrotropin-releasing hormone, pyro-glutamyl-histidyl-proline amide (TRH), increased prolactin and decreased GH release when added to cultures or short-term incubations of clonal cells from rat pituitary tumors. These workers also reported that clonal strains of rat pituitary tumor cells showed a significant increase in prolactin and a decrease in GH release when extracts of rat hypothalamus, cerebral cortex, kidney or liver were added to the culture medium

(Tashjian et al., 1970). This raises a question about the specificity of the reported effects of TRH on prolactin and GH release by rat pituitary tumor cells in vitro.

Thyroid hormones have been well known to influence rat pituitary prolactin release. Addition of thyroxine or triiodothyronine to a culture system increased pituitary prolactin release (Nicoll and Meites, 1962, 1963, 1964). Thyroidectomy or thiouracil administration greatly reduced pituitary prolactin concentration (McQueens-Williams, 1935; Meites and Turner, 1947), whereas injections of thyroid hormones restored or even increased pituitary prolactin levels (Chen and Meites, 1969). Blood prolactin levels were not measured in these studies.

It was considered of interest, therefore, to determine the in vitro effects of synthetic TRH on release of prolactin by normal and hypothyroid rat pituitary tissue and pituitary tumor tissue, and to assess the in vivo effects of TRH injections on pituitary and serum concentrations of prolactin in intact and thyro-parathyroidectomized rats.

B. Materials and Methods

1. Animals and hormones

Mature male Sprague-Dawley rats, weighing 250-280 g each, were used for both in vivo and in vitro experiments. Two groups of rats were thyro-parathyroidectomized 6 days

prior to TRH or saline treatment, as shown in Table 25, and were given 1% calcium lactate solution throughout the experimental period. Two groups of rats were thyro-parathyroidectomized and later were treated with or without TSH as indicated in Table 26. Another two groups of rats were thyro-parathyroidectomized and later their AP's were incubated with or without TRH in vitro as shown in Table 23.

Synthetic TRH, in solution or crystalline form, was kindly provided by Dr. M. S. Anderson of Abbott Lab., North Chicago, Illinois, and by Merck, Sharp and Dohme Research Labs., Rahway, New Jersey. TRH was directly incorporated into medium 199 for incubation, and dissolved in 0.85% saline for intravenous administration. This TRH produced a log dose-response curve when injected into mice and assayed for serum TSH by the method of McKenzie (1958) (Quadri and Meites, unpublished.) TSH solution was prepared by dissolving ovine NIH-TSH-S 6 (2.47 IU/mg) in 0.85% saline in a concentration of 2.0 IU/ml.

2. Pituitary incubations

Male donor rats were decapitated by guillotine and the anterior pituitaries (AP) were removed and placed in a petri dish over a moistened filter paper. The AP's were hemisected and weighed. Each AP half was placed in a 5-ml culture tube containing 2 ml of medium 199 at a pH of 7.4. The incubations were carried out as described under Materials

and Methods. Synthetic TRH in amounts of 3 or 30 ng were incorporated into the incubation medium. After 4, 8 and 12 hours of incubation, 100 µl of medium was removed from each tube and frozen at -20° C until assayed for prolactin. In some experiments, media were sampled for assay after 1, 2, 4 and 6 hours of incubation. The incubated AP halves were removed from the medium and weighed at the end of incubation. Pituitary tumor tissue was dissected from an inbred female Wistar-Furth rat carrying a MtT. W 15 pituitary tumor transplant. The tumor tissue was cut into 5- or 6-mg pieces, and was incubated with TRH in medium 199 as described above.

3. Prolactin assay

Blood samples (0.5-1.0 ml) were collected from the rats by cardiac puncture under light ether anesthesia, except in the thyro-parathyroidectomized rats with jugular cannula. The animals were killed by guillotine and the AP's were removed, weighed, homogenized and later assayed for prolactin.

Prolactin in individual serum samples, AP homogenates and incubation media was measured by the standard radioimmunoassay. Each sample was assayed at 2 different dose levels, and the prolactin values were averaged and expressed in terms of a purified rat prolactin reference standard, NIAMD-rat prolactin-RP-1. Mean and standard error of the mean for

prolactin values in AP, serum and incubation media were calculated for each experimental group. AP, thyroid and adrenal weights were similarly analyzed. Student's "t" test was used to determine the significance of difference between experimental groups, and also differences between pre-treatment and post-treatment samples.

C. Results

1. In vitro effects of TRH on prolactin release by normal rat pituitary and by rat pituitary tumor tissue

It can be seen (Table 22, Experiment 1) that prolactin release into the medium by control pituitary tissue increased progressively during 12 hours of incubation. Addition of 3 or 30 ng of TRH to the incubation medium did not change the rate of prolactin release.

The data in Experiment 2 show that control pituitary tumor tissue released about 3 times as much prolactin into the incubation medium during the first 4 hours as normal pituitary on a per mg basis. During the subsequent 4 or 8 hours of incubation, only a relatively small increase in prolactin release was observed. Addition of 3 or 30 ng TRH did not alter release of prolactin by the pituitary tumor tissue.

Table 22. Effects of synthetic TRH on prolactin release by normal rat pituitary and by rat pituitary tumor tissue *in vitro*.

Treatment (6 tubes/group)	Incubation period (hr)		
	4	8	12
Exp. 1: Incubation of TRH with normal rat pituitary tissue (ng prolactin released/mg AP)			
Control	604±93[a]	874±87	1,181±52
TRH (3 ng)	594±84	907±68	1,207±67
TRH (30 ng)	621±97	891±87	1,101±93
Exp. 2: Incubation of TRH with rat pituitary-tumor tissue (µg prolactin released/mg tumor tissue)			
Control	1.87±0.18	2.02±0.12	2.54±0.17
TRH (3 ng)	1.79±0.26	1.95±0.14	2.74±0.12
TRH (30 ng)	1.86±0.19	2.14±0.94	2.74±0.30

[a]Mean ± SEM.

2. In vitro effects of TRH on prolactin release by hypothyroid rat pituitary

Anterior pituitaries were removed from male rats 5 weeks after thyroprarthyroidectomy. Each AP half was incubated in medium 199 as above. The data in Table 23 show that AP halves from hypothyroid rats released only about 50% as much prolactin as released by AP halves from normal rats (see Table 22, Experiment 1). Addition of 3 or 30 ng of TRH to the incubation medium increased prolactin release by about 30% by the AP halves from thryo-parathyroidectomized rats after 4 hours of incubation, but had no effect on prolactin release by the end of 8 or 12 hours of incubation. It was also found that the weights of AP halves from thyroparathyroidectomized rats were significantly higher than the AP halves from intact rats (4.92 ± 0.15 vs. 3.55 ± 0.19 mg/AP half). It will be seen in subsequent experiments that thyroparathyroidectomy results in significant reduction in pituitary concentration of prolactin (see Tables 25 and 26).

3. Effects of a single intravenous injection of TRH on serum prolactin levels in intact male rats

After a pre-treatment blood sample was collected at 11 AM, a single injection of TRH was given to normal male rats by the tail vein. Subsequent blood samples were removed 15 and 30 minutes after TRH administration. The data in Table 24 (Experiment 1) show that TRH did not alter serum prolactin levels.

Table 23. Effects of synthetic TRH on prolactin release by hypothyroid rat pituitary *in vitro*.

Treatment (6 tubes/group)	Incubation period (hr)		
	4	8	12
Incubation of TRH with hypothyroid rat pituitary tissue (ng prolactin released/mg AP)			
Control	273±22[a]	456±49	549±54
TRH (3 ng)	341±18[b]	475±59	556±47
TRH (30 ng)	345±17[b]	503±34	559±52

[a]Mean and standard error of the mean.

[b]Significantly different from the control, $p < 0.05$.

Table 24. Effects of a single iv injection of synthetic TRH on serum prolactin levels.

Treatment (6 rats/group)	Serum prolactin levels (ng/ml)		
	Pre-treatment	15 min	30 min
Exp. 1: Injection of TRH via caudal vein			
Saline (0.3 ml)	25±2[a]	22±5	27±6
TRH (7.5 µg)	23±4	25±4	27±7
Exp. 2: Infusion of TRH via carotid cannula			
(1) Infusion at 30 min after cannulation			
TRH (5 µg)	8.3±0.7	8.5±0.7	6.7±0.6
(2) Infusion at 24 hr after cannulation			
TRH (5 µg)	41±7	48±6	39±8

[a]Mean ± SEM.

In Experiment 2, rats with carotid cannulae were infused with a single dose of TRH. A pre-infusion blood sample was collected 30 minutes after cannulation under sodium pentobarbital anesthesia, and 5 μg of TRH in 0.3 ml of saline followed by 0.2 ml of saline rinse were infused into each rat via the cannula. Subsequent blood samples were taken at 15 and 30 minutes after TRH infusion. The data in Experiment 2 show that sodium pentobarbital depressed serum prolactin levels as reported previously by our laboratory (Wuttke and Meites, 1970), and that infusion of 5 μg of TRH directly into a carotid artery did not change serum prolactin values. Twenty-four hours after cannulation, serum prolactin was elevated to levels significantly higher than intact controls. This increase presumably is due to the effect of sodium pentobarbital (Wuttke et al., 1971) and the stress from chronic cannulation (Neill, 1972). Infusion of TRH did not alter prolactin values.

4. Effects of multi-injections of TRH on pituitary prolactin release in intact and thyro-parathyroidectomized male rats

Since the foregoing experiments provided no evidence for a direct stimulatory action by TRH on pituitary prolactin release, another experiment was undertaken to determine whether TRH could increase pituitary prolactin secretion by stimulation of the TRH-thyroid system. Intact and thyro-parathyroidectomized male rats were injected via the caudal

vein with saline or 50 μg TRH daily for 6 days. On the 7th day blood samples were collected and the rats were killed. The pituitaries, thyroids and adrenals were removed and weighed. The data in Table 25 and Figure 16 show that thyro-parathyroidectomy resulted in a significant reduction in pituitary prolactin concentration and in a small but insignificant fall in serum prolactin. Injections of TRH into intact rats produced a significant increase in pituitary and serum prolactin concentration as compared to thyro-parathyroidectomized rats treated with or without TRH. Injections of TRH into intact rats also resulted in a significant increase in pituitary prolactin and produced a small but insignificant elevation in serum prolactin values as compared to intact control rats. TRH injections or thyro-parathyroidectomy had no significant effect on the weights of the pituitary, adrenals or thyroid under these experimental conditions.

5. Effects of multi-injections of TSH in thyro-parathyroidectomized male rats on pituitary prolactin release

Since injections of TSH were reported to deplete hypothalamic TRH content in thyro-parathyroidectomized rats (Motta, 1970), the effects of multi-injections of TSH on pituitary and serum prolactin concentrations were measured in thyro-parathyroidectomized rats. Beginning on the 15th day after thyro-parathyroidectomy, half of the rats were given

Table 25. Effects of iv injections of synthetic TRH on pituitary and serum prolactin concentration.

Exp.	Treatment (6 rats/group) (dose/day)	Ant. pit. (wt., mg)	Pituitary prolactin, (µg/mg AP)	Serum prolactin (mg/ml)
A.	Intact, saline (0.3 ml) (controls)	9.1±0.3[a]	1.15±0.06	24±5
B.	Thyroparathyroidectomized, saline (0.3 ml)	10.2±0.1[b]	0.71±0.17[b]	17±4
C.	Intact, TRH (50 µg)	9.2±0.3	1.41±0.07[b]	31±4
D.	Thyroparathyroidectomized, TRH (50 µg)	10.6±0.7	0.79±0.12[c]	16±3[c]

[a]Mean ± SEM

[b]Significantly different from A (intact, saline controls), $p < 0.05$.

[c]Significantly different from C (intact, TRH 50 µg), $p < 0.01$.

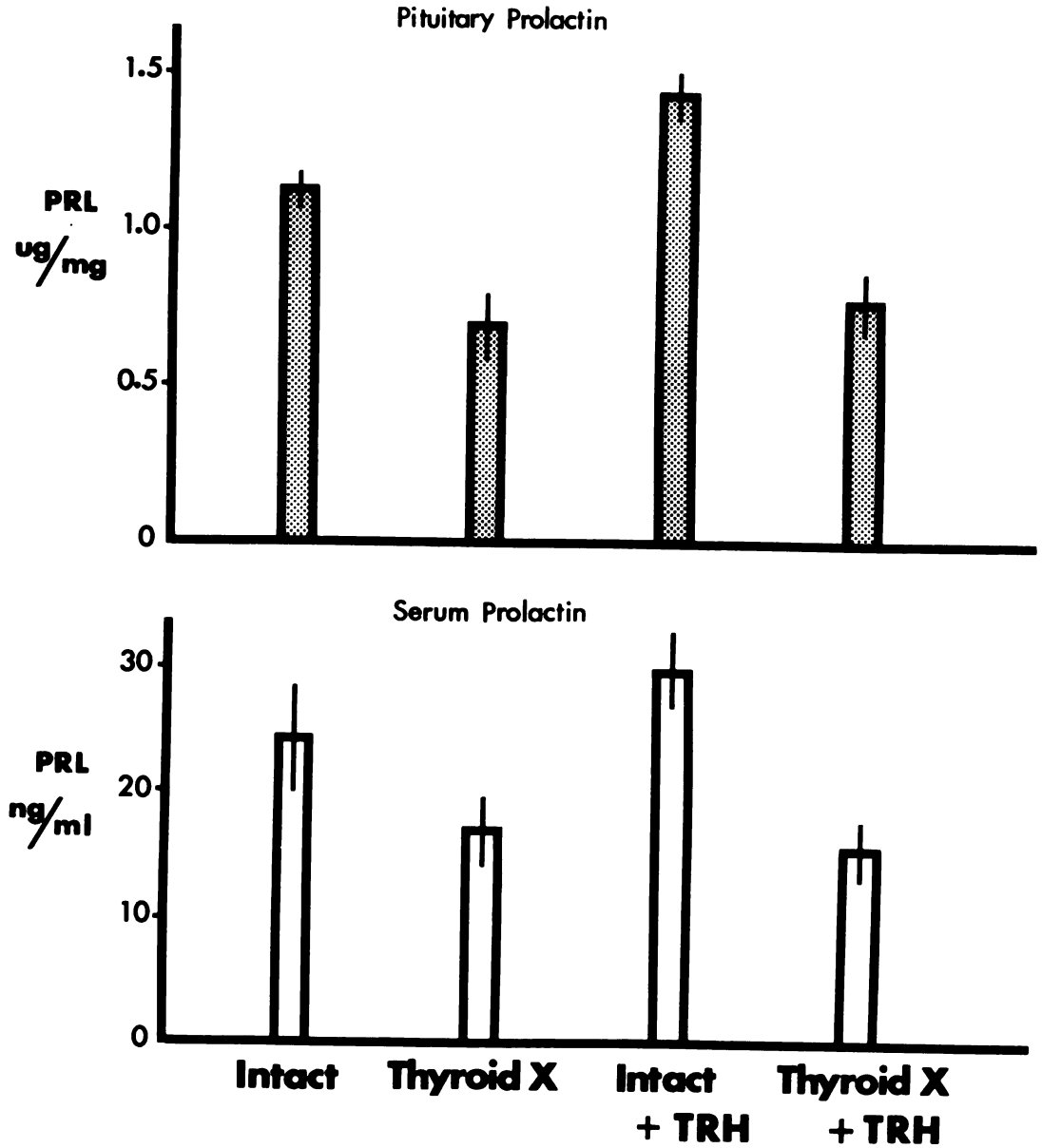


Figure 16. Effects of Multi-Injections of TRH on Pituitary and Serum Prolactin Concentrations in Intact and Thyro-Parathyroidectomized Male Rats.

a daily subcutaneous injection of 0.5 IU TSH/100 g body weight for 14 days, and the other half were given physiological saline. A group of intact male rats was given saline injections and served as controls. The rats were killed by guillotine at the end of 2 weeks treatment, and blood samples were collected from the trunk. Anterior pituitaries were removed, weighed, homogenized, and later assayed for prolactin. The data in Table 26 show that thyro-parathyroidectomy resulted in a significant increase in anterior pituitary weight and in a marked reduction in pituitary prolactin concentration when compared with the intact controls. Serum prolactin concentrations were not significantly reduced in the thyro-parathyroidectomized as compared to the control rats. TSH injections in thyro-parathyroidectomized rats had no effect on pituitary or serum prolactin values.

D. Conclusions

The *in vitro* results in the present study provide no evidence that synthetic TRH directly stimulates prolactin release from the normal male rat pituitary or from rat pituitary tumor tissue. However, it was observed that addition of TRH to an incubation medium produced a 30% increase in prolactin release by pituitary halves from thyro-parathyroidectomized rats. An increase in prolactin and a decrease in GH release was observed by Tashjian *et al.* (1971) when TRH was added to a culture or incubation system

Table 26. Effects of TSH injection and/or thyro-parathyroidectomy on anterior pituitary weight and pituitary and serum prolactin concentrations.

Treatment (6 rats/group)	Dose/day	Ant. pit. (wt. mg)	Pit. PRL, (µg/mg AP)	Serum PRL, (ng/ml)
Intact, saline (controls)	0.3 ml	8.2±0.3[a]	0.94±0.04	35±8
Thyroparathyroidectomized, saline	0.3 ml	10.8±0.3[b]	0.28±0.02[c]	24±5
Thyroparathyroidectomized, TSH	0.5 IU/100 g bw	10.3±0.5[b]	0.31±0.03[c]	24±3

[a]Mean ± SEM

[b]Significantly different from the controls, $p < 0.01$.

[c]Significantly different from the controls, $p < 0.001$.

containing clonal cells from rat pituitary tumor. It is possible therefore, that individual pituitary cells as well as the pituitary of hypothyroid rats may react somewhat differently than normal rat pituitary tissue to synthetic TRH. The physiological significance of these observations in the rat is difficult to assess.

The in vivo results show that multi-injections of TRH into intact rats increased pituitary prolactin concentration significantly and produced a small but insignificant rise in serum prolactin values. TRH had no effect on pituitary or serum prolactin levels in thyro-parathyroidectomized rats. This suggests that TRH acted via the TSH-thyroid system to increase pituitary and serum prolactin values in the rat. The observation that multi-injections of TSH had no effect on prolactin release in thyro-parathyroidectomized rats, suggests that its reported ability to deplete hypothalamic TRH content in thyro-parathyroidectomized rats (Motta, 1970) did not alter prolactin release. It appears therefore, that neither an increase from exogenously administered TRH nor a decrease in endogenous TRH in thyro-parathyroidectomized rats can influence prolactin release. A single injection or infusion of TRH into intact rats also failed to alter serum prolactin value by 15 or 30 minutes after injection. Multi-injections of TRH were necessary to produce even the limited increase in pituitary prolactin concentration observed in

this study. These results indicate that synthetic TRH is not a specific releaser of prolactin in the rat (Lu et al., 1972).

Thyro-parathyroidectomy resulted in significant increase in pituitary weight and a marked reduction in pituitary prolactin concentration. Inasmuch as serum prolactin levels were not significantly lowered by thyro-parathyroidectomy, it appears that pituitary synthesis was decreased to a greater extent than release of prolactin, or that the metabolism of circulating prolactin was so reduced by thyro-parathyroidectomy that it remained longer in the circulation.

GENERAL DISCUSSION

The data presented in this thesis indicate that pituitary release of prolactin is under dual control by the hypothalamus, and also can be influenced by systems outside the hypothalamus. There is little doubt that pituitary prolactin release is mainly under the control of PIF, but probably is also influenced by the PRF in the hypothalamus. Hypothalamic catecholamines depress whereas serotonin stimulates prolactin release. The catecholamines promote PIF release and serotonin may induce PRF release. Several drugs and hormones also can influence prolactin release by a direct action on the pituitary. Thus, it was found that estrogen stimulated, and ergocornine (ERG) inhibited prolactin release by a direct action on the incubated rat pituitary *in vitro*. As a result of ERG inhibition of prolactin release, accumulation of prolactin within the pituitary tissue was observed.

Estrogen increased both pituitary and serum prolactin levels *in vivo*, indicating stimulation of both synthesis and release of pituitary prolactin. Estrogen was shown to increase the number and granulation of pituitary acidophils (the putative prolactin cells) (Gersten and Baker, 1970). Ovariectomy resulted in a significant decrease in both pituitary and serum prolactin values, presumably due to a marked

acceleration of catecholamine turnover rate and increased PIF release as a result of lack of estrogen. Ovariectomy leads to a decreased sensitivity of the hypothalamo-pituitary system in response to changes in hypothalamic catecholamine activity. Thus, pituitary release of prolactin in ovariectomized rats is stimulated less by central acting drugs which decrease catecholamine levels in the hypothalamus than in intact female rats. Estrogen decreases PIF activity in the hypothalamus (Ratner and Meites, 1965). These results suggest that estrogen stimulates prolactin secretion by acting both on the pituitary and via the hypothalamus.

When ERG was administered in vivo, it counteracted the stimulatory action of estrogen on prolactin secretion, and inhibited prolactin release by a direct action on the pituitary graft underneath the kidney capsule in hypophysectomized rats. When ergot drugs, particularly ERG, were given to rats bearing pituitary "mammotropic" tumor transplants, they produced a rapid decrease in serum prolactin and induced tumor regression. There was loss of nuclei and disappearance of cells in the pituitary tumor tissue after ERG treatment, suggesting that ERG acted directly on the pituitary tumor tissue to reduce its capacity to secrete prolactin. ERG also was shown to reduce the number and granulation of acidophils within the pituitary graft in hypothysectomized rats with a single pituitary transplant. It was reported that ERG increased hypothalamic PIF activity

(Wuttke et al., 1971), suggesting that ERG inhibits prolactin release by acting both on the pituitary directly and via the hypothalamus. Inasmuch as ERG inhibits prolactin release from incubated pituitary tissue in vitro, from transplanted pituitary and from pituitary tumor in vivo, it can be assumed that inhibition of prolactin release by ERG is mainly due to its direct action on the pituitary.

The inhibition by ERG of estrogen stimulation of pituitary prolactin release does not necessarily indicate that both agents act on the same pituitary sites. Since ERG produced a 80% inhibition of prolactin release by incubated pituitary in vitro as compared to control pituitary, and resulted in accumulation of prolactin within the pituitary tissue, this strongly suggests that the major action of ERG is on the cell membrane to block the normal release of prolactin. Since ergot drugs are well known for their ability to induce vasoconstriction of peripheral blood vessels, they may similarly inhibit the blood supply to the pituitary prolactin cells.

It was first shown by Nagasawa and Meites (1970) that ERG inhibited mammary tumor growth in the rat. Microscopic examinations revealed that ERG prevented the extensive proliferation of epithelial tissue usually seen in mammary tumor tissue (Lu and Meites, unpublished). ERG has been shown to be a useful agent for specific inhibition of prolactin release, since at the doese used they inhibited

only prolactin but not gonadotropins. Thus, daily injections of ERG into cycling female rats increased ovarian weight and number of corpora lutea by partially or completely preventing luteolysis of old corpora lutea (Wuttke and Meites, 1971). However, ERG did not interfer with normal estrous cycles during 5 weeks of treatment.

This thesis presents additional evidence that biogenic amines, particularly catecholamines and indoleamines, in the hypothalamus and possibly the pineal play important roles in the control of pituitary prolactin release. It long has been debated whether catecholamines have any effect on pituitary secretion of prolactin and other hormones. A survey of earlier literature indicates that inconsistent results and contradictory conclusions were reported. Insofar as prolactin is concerned, these discrepancies could be due to the different pharmacological effect of catecholamines and their rapid metabolism in vivo and in vitro, insensitivity of pigeon crop bioassay for prolactin, inability to measure biogenic amines in the hypothalamus, etc. As shown in this thesis, pituitary prolactin concentration and serum prolactin values often go in opposite directions shortly after stimulation of prolactin release. Measures of pituitary prolactin levels only frequently led to the wrong conclusions. Prolactin concentration in the systemic blood seems to represent the most reliable index of pituitary prolactin release. Previously, however, it was impossible

to detect changes in prolactin in the blood until a specific radioimmunoassay for rat prolactin was developed. Evidence also was presented here that the duration of prolactin release or inhibition of release depends on the nature of the drug and the route of drug administration. Altered serum prolactin levels may return to normal within a few hours following drug injection. Unless sequential blood samples are measured by radioimmunoassay, investigators can easily miss the optimal inhibition of prolactin release by drugs. Erroneous conclusions also were drawn from testing the effects of catecholamines *in vitro*. The *in vitro* effects of catecholamines on pituitary prolactin release appear to be of doubtful physiologic significance, since high doses inhibited and small doses stimulated prolactin release. These amines also are rapidly degraded to form compounds which can exert powerful pharmacological action on cell membrane metabolism. Conclusions from *in vivo* experiments also may be invalid, since systemically administered catecholamines do not pass through the "blood-brain barrier" in amounts sufficient to reach the hypothalamus. Only precursors of catecholamines (tyrosine or L-DOPA) or monoamine oxidase inhibitors (i.e. pargyline, iproniazid, etc.) can reach the brain.

A single injection of L-DOPA, the immediate precursor of dopamine, evoked a rapid decrease in serum prolactin and an increase in pituitary prolactin concentration,

indicating inhibition of pituitary release of prolactin. Injections of three monoamine oxidase (MAO) inhibitors, pargyline, iproniazid, and Lilly compound-15641, also significantly reduced serum prolactin vlaues. It is significant that inhibition of prolactin release by L-DOPA and by the MAO inhibitors was associated with increased PIF activity in the hypothalamus. Kamberi et al. (1970) demonstrated that a single injection of dopamine into the third ventricle of rats increased PIF activity in the pituitary portal blood, whereas direct infusion of catecholamines into a single portal vessel had no effect on pituitary release of prolactin. From these and other related studies, the concept evolved that hypothalamic catecholamines inhibit pituitary prolactin release by increasing synthesis and release of PIF by the hypothalamus.

In view of the finding that L-DOPA inhibited mammary tumor growth in the rat (Quadri and Meites, unpublished), and induced cessation of persistent lactation and caused resumption of menstral cycles in patients with Forbes-Albright syndrome (Turkington, 1971), it can be assumed that L-DOPA acts mainly via a central mechanism to inhibit prolactin and stimulate gonadotropin release by the pituitary. Evidence is presented in this thesis that a single injection of methyl-DOPA, d-amphetamine, reserpine, chlorpromazine, and tyrosine analogs, drugs known to reduce hypothalamic catecholamine levels, evoked a rapid release

of prolactin by the pituitary. Other workers have reported that reserpine (Ratner and Meites, 1965) and perphenazine (Danon et al., 1963), a compound related to chlorpromazine, decreased hypothalamic PIF activity. It seems probable that methyl-DOPA, d-amphetamine and the two tyrosine analogs used here can reduce PIF activity in the hypothalamus.

It is noteworthy that hypothalamic catecholamines inhibit prolactin but stimulate gonadotropin release. Prolactin secretion often goes in an opposite direction to gonadotropin secretion under many physiological states. Prolactin is relatively high during late pregnancy, in postpartum lactation, and in old rats, whereas LH and FSH are relatively low during these conditions. In cycling female rats, prolactin and gonadotropins reach a peak at approximately the same time, on the late afternoon of proestrus (Wuttke and Meites, 1970; Gay et al., 1970). It remains to be determined, however, to what extent hypothalamic catecholamines participate in the control of these hormonal peaks, since catecholamines are stimulatory to pituitary release of gonadotropins but inhibitory to prolactin release. It is possible that the direct stimulatory action of estrogen on the pituitary may override catecholamine inhibition of prolactin release, while catecholamines stimulate the release of gonadotropins. Another possible explanation is that the rise of these three hormones is the result of direct stimulation by estrogen on the pituitary without

participation of catecholamines. Estradiol has been shown to directly induce LH release by the rat pituitary *in vitro* (Piacsek and Meites, 1966). It would be interesting to see whether an injection of L-DOPA prior to the time of the prolactin surge on the late afternoon of proestrus can reduce or prevent the prolactin rise. There is also the possibility that serotonin may participate in this process.

This thesis presents evidence that serotonin and its product, metatonin, have stimulatory effects on pituitary release of prolactin. A single intraperitoneal injection of 5-hydroxytryptophan, the immediate precursor of serotonin, evoked a rapid and profound elevation in serum prolactin in cycling female rats. 5-Hydroxytryptophan also increased prolactin release in hypophysectomized, pituitary-grafted rats. Serotonin given by systemic injection was ineffective on prolactin release. Kamberi *et al*. (1971a) reported that a single injection of serotonin or melatonin into the third ventricle of rats increased prolactin in the blood, but they did not measure changes in PIF activity in the pituitary portal blood. This thesis presents evidence that injection of 5-hydroxytryptophan does not change PIF nor induce PRF activity in the hypothalamus or in the systemic blood by our standard *in vitro* method. It is possible, however, that this *in vitro* method may not be adequate to detect the appearance of the presumed PRF in the hypothalamus and its release into the systemic circulation. It would be

of interest to see whether the serum from these drug injected rats could increase prolactin release *in vivo*.

Results presented in this thesis provide no proof that synthetic thyrotropin-releasing hormone (TRH) is a specific releaser for prolactin in the rat. TRH injection has no effect on serum prolactin in intact and thyro-parathyroidectomized male rats. TRH increased prolactin concentration in the pituitary when it was injected daily in relatively high dose for 6 days into intact male rats. This apparent stimulation of prolactin secretion by TRH was due to activation of the TSH-thyroid system, since no effect was observed in thyro-parathyroidectomized rats. TRH did not increase prolactin release when incubated with normal rat pituitary or rat pituitary tumor tissue *in vitro*. TRH slightly increased prolactin release by about 30% by pituitary tissue from long-term hypothyroid rats. The relation of these observations in the rat to reports that a single injection of TRH can produce a rapid rise in blood prolactin concentration in humans (Bowers *et al.*, 1971; Jacobs *et al.*, 1971), monkeys (Knobil, personal communication), and cattle (Convey *et al.*, 1972) is not clear. In view of the rapidity with which an injection of TRH elevates blood prolactin levels in these mammalian species, and the evidence that TRH is even more effective in raising blood prolactin in hypothyroid than in euthyroid humans, it appears doubtful that TRH acts via the TSH-thyroid system to elevate blood

prolactin in human subjects. It long has been known that the rat is relatively hyperthyroid as compared to other species (Meites, 1949, 1950), and thyroid hormones can increase pituitary prolactin levels in the rat. The observations that hypothyroid human subjects are more responsive to TRH to increase prolactin than hyperthroid humans may be analogous to what occurs in the relatively hyperthyroid rat. Thus, the failure of TRH to stimulate prolactin release by the rat pituitary may be due to its relative hyperthyroid state. It remains to be determined how TRH evokes a rapid rise in blood prolactin in primates and in cattle. Synthetic TRH may be related chemically to the presumed PRF molecule.

The relation of biogenic monoamines, ergot drugs, and estrogen to pituitary release of prolactin can be summarized in a diagram as shown in Figure 17.

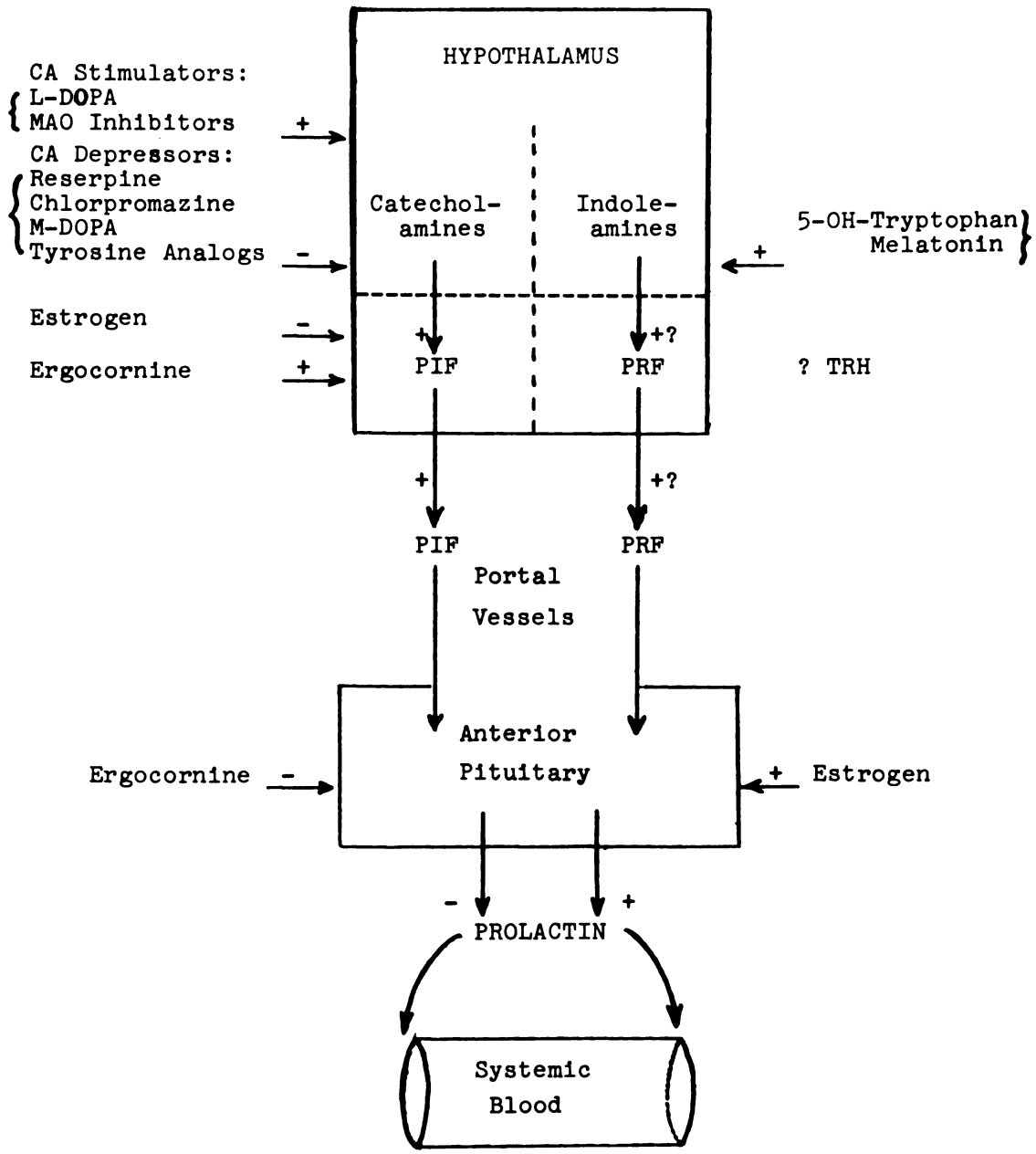


Figure 17. Diagram Showing the Relation of Biogenic Monoamines, Ergot Drugs, and Estrogen to Pituitary Prolactin Release in Rats.

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Wuttke, W., M. Gelato and J. Meites. 1971. Mechanisms of pentobarbital actions on prolactin release. Endocrinology. 89:1191-1194.

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APPENDIX

CURRICULUM VITAE AND LIST OF PUBLICATIONS

CURRICULUM VITAE

NAME: LU, Kuew-Hsiung (John)

DATE OF BIRTH: September 16, 1937

PLACE OF BIRTH: Miaoli, Taiwan, China

NATIONALITY: Chinese (Permanent resident of U.S.A.)

MARITAL STATUS: Married SEX: Male

PRESENT ADDRESS: Department of Physiology
Michigan State University
East Lansing, Michigan 48823

FUTURE ADDRESS: Department of Physiology
School of Medicine
The University of Pittsburgh
Pittsburgh, Pennsylvania 15213

HOME ADDRESS: 144 Wen-Fa Road
Miaoli, Taiwan
Republic of China

EDUCATION:

Degree	Year	Institution	Major Field of Study
B.S.	1956-1962	National Taiwan Normal University	Biology
M.S.	1965-1967	National Taiwan University	Physiology
None	1967-1968	Purdue University	Endocrinology
Ph.D.	1968-1972	Michigan State University	Neuroendrocinology

HONORS:

(a) Recipient of the National Fellowship for Natural Science Students (Taiwan), 1957-1961.
(b) Recipient of the NCSD Research Fellowship, the National Council on Science Development (Taiwan), 1965-1967.
(c) Elected Associated Member of Sigma Xi, 1970.
(d) Sigma Xi Graduate Student Research Merit Award, 1971.
(e) Elected Full Member of Sigma Xi, 1972.

POSITIONS HELD:

(a) Post-doctoral Fellow, The University of Pittsburgh, October, 1972.
(b) Teaching Assistant in Physiology and Research Assistant in Neuroendocrinology, Michigan State University, 1969-1972.
(c) Teaching Assistant in Biological Sciences, Michigan State University, 1968-1969.
(d) Research Assistant in Endocrinology, Purdue University, 1967-1968.
(e) Teaching Assistant in Zoology and Physiology, National Taiwan Normal University, 1963-1965.
(f) High School Teacher in Biology, Taiwan, 1961-1962.

TALKS PRESENTED AT SCIENTIFIC MEETINGS:

Meetings	Year	Topic
56th Annual Meeting of Federation of American Societies for Experimental Biology. Atlantic City, N.J.	1972	TSH-releasing hormone effects on prolactin release by rat pituitary *in vivo* and *in vitro*.
76th Annual Meeting of Michigan Academy of Science. East Lansing, Michigan.	1972	Inhibition of prolactin secretion and stimulation of PIF release by L-DOPA in rats.
55th Annual Meeting of Federation of American Societies for Experimental Biology. Chicago, Illinois.	1971	Direct inhibition by ergocornine of pituitary prolactin secretion.

54th Annual Meeting of Federation of American Societies for Experimental Biology. Atlantic City, N.J.	1970	In vivo and in vitro effects of drugs on prolactin release by the rat pituitary.

RESEARCH PUBLICATIONS:

1. Lu, K. H. 1967. Force-feeding in the hypophysectomized immature rat. Thesis for the Degree of Master of Science. National Taiwan University.

2. Philpott, J. E., M. X. Zarrow, H. Deneberg, K. H. Lu, R. W. Fuller and J. M. Hunt. 1969. Phenethanolamine N-methyl transferase and adrenal activity in the neonatal rat. Life Sciences 8(1):367-371.

3. Lu, K. H., Y. Koch, Y. Amenomori, C. L. Chen, and J. Meites. 1970. In vivo and in vitro effects of drugs on prolactin release by the rat pituitary. Federation Proceedings 29(2):579.

4. Lu, K. H., Y. Amenomori, C. L. Chen and J. Meites. 1970. Effects of central acting drugs on serum and pituitary prolactin levels in rats. Endocrinology 87:667-672.

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

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

7. Lu, K. H. and J. Meites. 1971. Inhibition by L-DOPA and monoamine oxidase inhibitors of pituitary prolactin release: Stimulation by methyldopa and d-amphetamine. Proc. Soc. Expt. Biol. Med. 137:480-483.

8. Lu, K. H., Y. Koch and J. Meites. 1971. Direct inhibition by ergocornine of pituitary prolactin secretion. Federation Proceedings 30(2):474.

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

10. Quadri, S. K., K. H. Lu and J. Meites. 1972. Ergot-induced inhibition of pituitary tumor growth in rats. Science 176:417-418.

11. Gelato, M. C., K. H. Lu and J. Meites. 1972. Inhibition of luteolysis by iproniazid during the estrous cycle in rats. Program 5th Annual Meeting, The Society for the Study of Reproduction, East Lansing, pp. 80.

12. Meites, J., K. H. Lu, W. Wuttke, C. W. Welsch, H. Nagasawa, and S. K. Quadri. 1972. Recent studies on functions and control of prolactin secretion in rats. Recent Progress in Hormone Research. 28:471-516.

13. Lu, K. H., K. H. Kortright, C. J. Shaar and J. Meites. 1972. TSH-releasing hormone effects on prolactin release by rat pituitary in vivo and in vitro. Federation Proceedings, 31(2):221.

14. Lu, K. H. and J. Meites. 1972. Effects of L-DOPA on serum prolactin and prolactin inhibiting activity in intact and hypophysectomized, pituitary-grafted rats. Endocrinology :868-872.

15. Lu, K. H., C. J. Shaar, K. H. Kortright and J. Meites. 1972. Effects of synthetic TRH on in vitro and in vivo prolactin release in the rat. Endocrinology 91: (December).