This is to certify that the

thesis entitled

IDENTIFICATION OF RADIOIMMUNOASSAYABLE LUTEINIZING HORMONE IN CEREBROSPINAL FLUID OF PONY MARES: EFFECTS OF SEASON AND HORMONAL TREATMENT

presented by

Robert G. Allen, Jr.

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Husbandry

R H Douglas

Major professor

Date 9/20/79

IDENTIFICATION OF RADIOIMMUNOASSAYABLE LUTEINIZING HORMONE IN CEREBROSPINAL FLUID OF PONY MARES: EFFECTS OF SEASON AND HORMONAL TREATMENT

By

Robert G. Allen, Jr.

A THESIS

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

MASTER OF SCIENCE

Department of Animal Husbandry

1979

ABSTRACT

IDENTIFICATION OF RADIOIMMUNOASSAYABLE LUTEINIZING HORMONE IN CEREBROSPINAL FLUID OF PONY MARES: EFFECTS OF SEASON AND HORMONAL TREATMENT

By

Robert G. Allen, Jr.

Three experiments were designed to examine the presence of luteinizing hormone (LH) in plasma and cerebrospinal fluid (CSF) of pony mares.

In Experiment I, CSF and plasma were collected at various times during the estrous cycle of pony mares. Experiment II examined LH in CSF and plasma of seasonally-anovulatory mares following administration of pituitary extract or GnRH. Experiment III examined levels of LH present in CSF and plasma of seasonally-anovulatory mares following GnRH administration with or without steroid pretreatment. LH was immunoassayable in CSF in all experiments. Plasma LH in Experiment I was higher at ovulation that at day 2 of estrus or day 10 post-ovulation. Pituitary extract and GnRH (Experiment II) caused similar plasma LH concentrations; no difference was observed in CSF LH levels. Plasma LH was elevated ($p < .05$) following GnRH

when preceeded by progesterone alone or estradiol alone in Experiment III. Progesterone pretreatment caused elevation ($p < .05$) in LH levels in CSF following GNRH.

DEDICATION

To my parents, without whose emotional, supportive, and financial sustenance, my educational pursuits would have been a burden, rather than an enjoyable academic challenge.

ACKNOWLEDGMENTS

Dr. Robert H. Douglas: Many thanks to Bob, who provided constant stimulation and helped shape my desires; whose relationship as a friend will, hopefully, continue for many years to come.

Dr. R. F. Nacheiner and Dr. James Ireland, along with Dr. Douglas, whose input and aid as my committee members helped coalesce my studies into this finished product.

Beth Hershman, Pat Burns and Pat Melrose, special thanks for allowing me to pick their brains for invaluable information, both professional and personal.

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vi
Chapter	
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	2
Cerebrospinal Fluid	2
The Blood-Brain and Blood-CSF Barrier	3
Crossing the Blood-CSF Barrier	5
Ventricular Functional Anatomy	7
Hypothalamic-Hypophyseal Portal Vasculature	10
Relation of Ependyma to Portal Vasculature	14
Gonadotropins in Hypothalamus and Median Eminence	15
Intraventricular Administration of Releasing Hormones and Their Effects	17
Changes in Ventricular Morphological Structure with Reproductive State	18
III. MATERIALS AND METHODS	22
Experiment I--LH Levels During the Estrous Cycle	23
Experiment II--Exogenously Induced LH Surges in Seasonally-Anovulatory Pony Mares	24
Experiment III--Steroid Pretreatment Effects on GnRH-Induced Release of LH in Plasma and CSF	24

Chapter Page

IV. RESULTS 28

Experiment I--LH Levels During the Estrous Cycle 28
Statistics for Experiments II and III . 30
Experiment II--Exogenously Induced LH in Seasonally-Anovulatory Pony Mares 30
Experiment III--Steroid Pretreatment Effects on GnRH Induced Release of LH in Plasma and CSF 34

V. DISCUSSION 38

LITERATURE CITED 47

LIST OF TABLES

Table		Page
1.	Experiment 1--Plasma and Cerebrospinal Fluid (CSF) Levels of LH on Day 2 of Estrus, Day of Ovulation and Day 10 Post-Ovulation in Mares	23
2.	Experiment II--Plasma and Cerebrospinal Fluid (CSF) Concentrations of LH Following Injection of Equine Pituitary Extract or GnRH in Seasonally-Anovulatory Mares .	25
3.	Experiment II	31
4.	Experiment III	32

LIST OF FIGURES

Figure		Page
1.	Experiment III	26
2.	Experiment I--Plasma (upper graph) and CSF (lower graph) levels following sampling at day 2 of estrus, day of ovulation and day 10 post-ovulation	29
3.	Plasma (lfet) and CSF (right) concentrations of LH in seasonally-annovulatory pony mares following treatment with saline (control), pituitory extract (375 Fevold-Hisawanits SC) or GnRH (1 mg SC) (Experiment II)	33
4.	Plasma (left) and CSF (right) concentrations of LH following GnRH or saline injection	35

CHAPTER I

INTRODUCTION

The key neuro-endocrine centers responsible for controlling reproductive cyclicity in the mammal reside primarily in the hypothalamic and circumventricular areas of the third ventricle of the brain. The predominant medium for hormonal communication between the gonads and the centers, as well as for communication between the centers themselves is considered to be the vascular system. However, since these control centers are bathed by cerebrospinal fluid (CSF) recent studies have been directed toward examining the role of CSF, if any, in endocrine communication.

The studies discussed herein were conducted to test the thesis that in the female equine, radioimmunoassayable luteinizing hormone (LH) is present in CSF at the level of the cisterna magna and that the concentrations of LH present in plasma are reflected in CSF.

CHAPTER II

REVIEW OF LITERATURE

Cerebrospinal Fluid

The hypothalamus, median eminence and pineal are bathed by the cerebrospinal fluid (CSF) which fills the brain-ventricular system. CSF is formed principally by the choroid plexus which lines the ventricular system. The primary site of origin of CSF is the choroid plexus of the lateral ventricles. CSF then passes into the third ventricle, combines with that secreted by the third ventricle, then into the fourth ventricle where more fluid is added. CSF then passes into the cisterna magna, ultimately reaches the arachnoid villi and empties into the venous sinuses where it is transported into the systemic circulation (39).

At the present time there is little question that CSF is a secretory product (23, 25). This is based not only on differences in the ionic composition of this fluid as compared to an ultrafiltrate of plasma, but also on the characteristics of sodium movement between blood and the fluid within the ventricular system (2, 27, 28).

CSF has a chemical composition different from that of a protein-free filtrate of plasma, the concentrations of glucose and most amino acids being much less in the CSF than in the plasma. A similar situation prevails where ions are concerned, especially K, Cl and Na (29).

The Blood-Brain and Blood-CSF Barrier

As pointed out, constituents of cerebrospinal fluid are not the same as those of the extracellular fluid elsewhere in the body. It is generally accepted that barriers called blood-cerebrospinal and blood-brain barriers exist between the blood and the cerebrospinal fluid and the blood and brain fluid, respectively. The barrier concept arose from some of the early experiments published by Goldmann (35, 36). In his first experiment (35), Goldmann observed that the acidic dye trypan blue was distributed widely throughout the body when given intravenously, but the brain and spinal cord remained virtually unstained. In his second experiment (36), Goldmann injected the dye into the CSF and found definite pharmacological symptoms that had been lacking after intravenous administration. Goldmann also observed no restraint on the passage of trypan blue out of the brain capillaries into the interstitium of the choroid plexus although passage onwards into the CSF was restrained. It is now recognized, however, that many substances in plasma do

cross the blood-brain-CSF barriers and enter the CSF. These barriers are more complex than a simple restraint on passage from blood to brain. Quantitatively, the blood-brain barrier is reflected in variable rates of net flux of substances from blood into the tissue of the brain; rates that are governed by multiple factors including the molecule size, charge, protein binding and lipid solubility of the substance (33, 69, 5, 6).

Numerous studies were designed to examine the different permeability of brain capillaries as opposed to muscle capillaries. Because a characteristic feature of the brain capillary is its close investment by glial cells, the low permeability of the blood-brain barrier has been attributed to this glial covering. However, Brightman (16) demonstrated that large electron microscope tracers, such as ferritin, could pass between the gaps between the glial cell processes. This shifted emphasis toward the involvement of the capillary endothelium, although studies showed no morphological differences between the capillaries of skeletal muscle and those of the brain (17). However, Reese and Karnovsky (84) showed that whereas the intercellular chefts of muscle capillaries allowed the passage of horseradish peroxidase (MW 40,000), this electron-microscope marker was unable to pass through the clefts in brain capillaries. The formation of tight junctions,

sealing the intercellular clefts, constituted the morphological feature that lay at the basis of the restricted permeability (17). Oldendorf (70) supports this view, attributing the special features of the blood-brain barrier, namely specificity for individual hexoses and amino acids, including sterospecificity and active transport of anions,to the capillary endothelial cells.

Crossing the Blood-CSF Barrier

The CSF was hypothesized as a possible medium for transfer of metabolic requirements to the brain. The essential amino acids were found to penetrate the blood-CSF barrier much more readily than nonessential amino acids (5, 6) and glucose was found to selectively cross the blood-brain barrier (26); later, it was demonstrated that the isolated choroid plexus could accumulate the glucose analog, 3-methylglucose (24).

Later studies emphasized substances that may control the hypothalamic-hypophyseal axis and their possible presence in the CSF. These would include the steroids responsible for part of the feedback control of hypothalamic function and many of the peptide and glycoprotein hormones. Ondo et al. (73) examined the presence of numerous radioactive substances in hypophyseal portal blood following intraventricular administration. Corticosterone-4-^{14}C, ovine luteinizing homone-^{131}I (Ovine LH),

bovine hemoglobin-^{131}I, and Na-^{131}I served as the marker substances. These substances were injected into the third ventricle of anesthetized rats via a glass cannula, and radioactivity measured in pituitary stalk vessels. Within 30 minutes after injection, an appreciable quantity of Na, ovine LH and corticosterone was detected in pituitary stalk vasculature, suggesting those substances crossed from CSF to the brain. Bovine hemoglobin crossed to a lesser extent. Luteinizing hormone-releasing hormone (LRH) was also found to cross from CSF into pituitary portal blood (7), as were prostaglandins (10).

Studies have also been conducted examining transfer from systemic circulation into CSF. An experiment by Kumar and Knowles (53), using ovariectomized monkeys, indicated that following injection of tritiated 17-β estradiol systemically, radioactivity was detected in CSF and in tanycytes. Marynick et al. (62, 63) injected tritiated estrogen, testosterone, cortisol and 17-hydroxyprogesterone intravenously into rhesus monkeys and monitored for presence of label in CSF. In all cases, the label was detected in CSF following injections.

Douglas (30) reported that plasma levels of both estradiol 17-β and progesterone were reflected in CSF of pony mares. Plasma and CSF concentration

were higher during late diestrus than during early diestrus and plasma and CSF concentrations of estradiol-17β were higher during estrus than during diestrus. The concentration of hormone in CSF relative to the concentration in plasma averaged 10% for progesterone and 80% for estradiol-17β. Similarly, when exogenous testosterone was injected intramuscularly in pony geldings (66), the increase in plasma concentration of testosterone was accompanied by a similar increase in CSF. Average concentration of testosterone was approximately 55% of the concentration in plasma.

LRH and thyrotropin-releasing hormone (TRH) have been detected in the CSF of rats (44) and prolactin found in human CSF in patients without endocrine pathologies (3) and in rats (18). Adrenocorticotrophic hormone (ACTH) has also been detected in human CSF (1).

Ventricular Functional Anatomy

In order to fully examine the role that CSF might play in regulation of endocrine function, it is important to study the cellular lining of the ventricules. The majority of the ependymal cells that line the upper wall of the third ventricle are of the "classical" variety, i.e., cuboidal epithelial cells. Such cells are usually rather uniform in size, arranged in rows and densely packed. Typical ependymal cells usually possess kinocilia

and microvilli and appear to form a continuous wall (15). Scanning electron microscopy (SEM) of rhesus monkey ventricular sections have revealed a myriad of information. Coates (21) observed that the lateral walls of the third ventricle are not completely flat, but rather, have regions of ridges and grooves that are especially conspicuous in two areas: one originates in the area of the interventricular foramen and runs down to the optic chiasma, while the other follows the contour of the mammillary bodies to the cerebral aqueduct. At low magnification the surface of the lateral walls appears highly textured. The textured appearance of the lateral walls is the result of a dense carpet of cilia that extends anteriorly to the lamina terminalis where it stops abruptly and is continuous posteriorly into the ciliated cerebral aqueduct. The number of ciliated cells on the lateral wall decreases ventrally, creating a transition zone as the nonciliated ventral floor is approached. Clumps of cilia on the lateral walls are so close that it is difficult to observe surface morphology below the level of the cilia. It is generally believed that cilia aid circulation of CSF (53, 56, 90). Observations of the third ventricle of rabbits reveal that small color-dyed particulate matter applied at the interventricular foramen is swept down from the interventricular foramen toward the floor

of the third ventricle by wave-like activity of cilia (Coates, unpublished). The absorption capacities of typical ependymal cells are well established. Polloy and Davson (80) demonstrated that modified ependymal cells within the choroid plexus absorb CSF. Klatzo (48), in cats, demonstrated fluorescein-labeled albumen movement through the ependymal layer of the third ventricle, especially in the region of the hypothalamic nuclei.

Within the lower walls of the third ventricle, particularly in the boundaries of the infundibular recess, and the floor of the ventricle, is found an entirely different variety of ependymal cell: the tanycyte (also referred to as specialized ependymal cell of the third ventricle). In the rat, the ependymal lining of the lower wall of the third ventricle becomes increasingly irregular in the vicinity of the infundibular recess. The orderly arrangement of typical ependymal cells gives way to multiple layers of tanycytes, irregular in form and variable in size. Many of these cells are goblet-shaped and send bulbous protrusions into the ventricular lumen. Also extending from these cells are long filimentous structures that are often irregular in shape and diameter (67). Bleier (11) examined the various forms of the basal processes of tanycytes and their distribution within the wall of the third ventricle. He states,

> The ependymal processes assume a variety of forms, lengths and calibers. They may be slender and smooth, beaded or knobby. Some divide or have branches that may be slender and perpendicular to the main process, or short and tortuous; others appear to be branched.

The floor and lower lateral walls of the third ventricle of mature monkeys are incredibly rich in surface specializations including microvilli, blebs and an assortment of polymorphous small surface protrusions. Knigge and Scott (49) have suggested that in this region microvilli may function in absorption-transport of hypothalamic releasing factors from the CSF to the portal vasculature, thus influencing the pituitary gland.

Hypothalamic-Hypophyseal Portal Vasculature

Passage of substances from CSF into portal vasculature, and from portal vasculature into CSF has been demonstrated in numerous species. In order to understand the direction of flow of substances in portal vasculature, it is important to review some of the most recent literature. Bergland and Page (9) and Page and Bergland (76, 77) conducted a series of experiments examining the angio-architecture of the pituitary-median eminence complex by examining corrosion casts of injected specimens with the scanning electron microscope. Species examined were mice, rats, rabbits, sheep, dogs and rhesus monkeys. In all species studied the arterial supply was restricted to the neurohypophyseal capillary bed. A single inferior

hypophyseal artery arises from the cavernous portion of each internal carotid artery to supply the infundibular process (neural lobe). A series of superior hypophyseal arteries form a ring about, and supply, arterial blood to the infundibulum (median eminence). A middle hypophyseal artery, of variable origin, connects to the infundibular stem or process [formerly designated trabecular artery (97), loral artery (65), or artery to the infundibular stem (58)]. In all species studied, a confluent capillary network united the components of the neurohypophysis (median eminence, infundibular stalk and infundibular process). This reticulum of capillaries extending from the infundibulum to the infundibular process is supplied with arterial blood from above through the superior hypophyseal arteries, and from below through inferior hypophyseal arteries. An external plexus of interconnecting capillaries forms the outer shell of median eminence casts. This plexus is supplied by superior hypophyseal arteries, is continuous with the capillary bed of the infundibular stalk, and is drained by long portal vessels descending to the pars distalis. An internal plexus of capillaries projects into the bowl formed by external plexus capillaries. The internal plexus is supplied by vessels of the external plexus, is continuous posteriorly with the capillary bed of the infundibular stalk, and is drained by long portal vessels descending to the pars

distalis. Within the infundibular process lies a capillary bed continuous with the capillary bed of the infundibular stalk. Between the capillary bed of the infundibular process and that of the adenohypophysis lies an avascular cleft. Short portal vessels bridge the cleft to connect the capillary beds of the pars distalis and infundibular process. Page and Bergland found no evidence of lateral hypophyseal veins draining into the cavernous sinus from the adenohypophysis [in contrast to Wislocki (94)]. Rather, all of the veins draining the adenohypophsis were found at the sulcus separating the pars distalis from the infundibular process. Each of these veins joined a neurohypophyseal vein and the two together extended to the carvernous sinus. These veins were designated confluent capillary veins. No connections from the adenohypophysis to the carotid arteries were found. The presence of a confluent capillary bed within the neurohypophysis, its location between two sites of arterial supply and its angio-architecture suggests that the direction of blood flow between median eminence and infundibular process may be reversible. Extirpation of the posterior lobe produced venous stasis within the pars distalis (93). Szentogathai (92) suggested that adenohypophyseal blood may drain into the neurohypophyseal capillary bed. Green and Harris (38) observed flow going toward the pars distalis in long portal vessels on the

ventral aspect of the pituitary, whereas Szentagothai and Torok noted blood going from the pars distalis in short portal vessels on the dorsal aspect of the pituitary. Oliver et al. (71) demonstrated retrograde blood flow in the infundibular stalk. They cannulated a single long portal vessel and measured LH, TSH, PRL, ACTH, alpha-MSH and vasopressin in portal plasma. LH, TSH, PRL and ACTH levels in portal blood from anterior lobectomized rats were greatly depressed, alpha-MSH concentrations were decreased moderately, and vasopressin levels were unaffected. After removal of the posterior lobe, the concentrations of LH and TSH in portal plasma did not change but those of PRL, ACTH, alpha-MSH and vasopressin decreased markedly, suggesting vascular communication between adenohypophysis and neurohypophysis and transfer from stalk vessels draining the posterior pituitary into long portal vessels supplying the anterior pituitary. Page et al. (75) have suggested that blood reaching the base of tanycytes contains high concentrations of both hypothalamic and adenohypophyseal secretions. As further evidence that CSF may be a medium through which releasing factors are carried, deafferentation of the medial basal hypothalamus, presumed to destroy the TSH hypophysiotrophic neurones decreased pituitary thyrotropin secretion but a low level of function was maintained (37).

Relation of Ependyma to Portal Vasculature

In the mouse, rat, rabbit and cat, ependymal cells were found to extend numerous basal processes into all cell groups and areas of the hypothalamus (12). Within the hypothalamic area, the greatest density of processes originated from tanycytes situated in the lower half of the ventricle; most of these processes extended into the region of the tuber cinerum and the dorsomedial, ventromedial and arcuate nuclei. Many of the processes appear to end on neurons and capillaries (87, 52). Around vessels of the hypothalamus, many terminations take the form of end-feet, networks or loops. The basal processes of the tanycytes in the infundibular region may branch into two or more filaments, which in turn subdivide into small fibers with enlarged end-feet. These end-feet frequently terminate on or in close proximity to capillaries of the pituitary portal system. Brawer (13) was able to trace the basal processes of tanycytes in the region of the arcuate nucleus to terminations on the basement membranes of capillaries within the nucleus. In a fine structure study of the ependyma in the recessus infundibularis of the guinea pig, Wittkowski (96) observed an abundance of axons in close proximity to tanycytes, many of which formed synapse-like contacts (axotanycytic synapses). In an earlier study (41), ^{3}H-dopamine was

infused intraventricularly in rats. Five minutes following infusion, heavy labeling of tanycyte processes in the midcentral median eminence could be demonstrated. These processes are observed to course through the palisade zone and to routinely terminate in large numbers on the portal perivascular space. Axotanycytic synapses have also been reported in the rat by Kobayashi and Ishii (54) and by McArthur (64). That substances can pass from the CSF into hypophyseal portal blood has been mentioned previously (73).

The interposition of tanycytes between two diverse anatomical sites, i.e., the apical surface exposed to and bathed in CSF of the third ventricle, the other, distally situated basal processes making intimate contact with subjacent neural and vascular elements, is an anatomical arrangement that may permit two-way transport of substances between the CSF and portal blood (67).

Gonadotropins in Hypothalamus and Median Eminence

LRH and TRH, when injected intraventricularly, cause the release of LH and TSH, respectively. How these releasing factors enter the portal system, if that is the mechanism, is under investigation presently. When the medial preoptic area was stimulated in proestrous rats, the LRH concentration in portal blood increased (82). In the equine, LRH and corticotropin releasing hormone were

found distributed among electron-dense granules in axons contained in the median eminence (42). LRH was also found in median eminence of rats. Pelletier et al. (79) and Naik (68) demonstrated that specialized ependymal cells (tanycytes) bordering the third ventricle in rats showed varied immunohistochemical reactions for LRH during different phases of the estrous cycle. Estrus, metestrus and early diestrus phases showed very weak immunofluorescent LRH in the specialized ependyma of the infundibular recess, while immuno-electron microscopic observations showed pleomorphic LRH granules in the specialized ependyma during late diestrus and proestrus stages. Zimmerman et al. (91) demonstrated by immunoperoxidase bridge technique the presence of GnRH in the arcuate nuclei of the hypothalamus and throughout tanycytes of the median eminence. Joseph et al. (45) demonstrated TRH in the median eminence of rats. The concentration of median eminence TRH decreased with hypophysectomy or with ovariectomy. Growth hormone-inhibiting hormone was detected in granules in rat median eminence (79). Silverman et al. (91) showed that in in vitro culture, the median eminence accumulated and transported the non-utilizable amino acid, α-amino-isobutyric acid (AIB). The aforementioned studies further support the hypothesis that tanycytes may provide a communicative channel between CSF and portal blood.

Intraventricular Administration of Releasing Hormones and Their Effects

The releasing hormones LRH and TRH have both been found in the CSF and these levels have been shown to be affected by different experimental conditions (44). These experiments lend support to the hypothesis that the CSF-ventricular system may be involved in a brain-pituitary relationship; it may serve, in part, as a route whereby hypothalamic hormones are delivered to the pituitary.

Kendall et al. (47) showed that a large dose of TRH (300 ng) injected intraventricularly was equally effective in elevating TSH in plasma concentrations as TRH injected intravenously. Gordon et al. (37) injected TRH into the third ventricle and into the venous system and compared plasma TSH concentrations. Using a dose of 50 ng, they found that the TRH injected into the ventricles caused a slight, but significant, elevation in plasma TSH concentrations 5 minutes post-injection, but intravenous administration of TRH was more effective than injection into the third ventricle (i.e., fivefold increase in plasma TSH concentration following systemic injection of TRH compared to ventricular administration).

Another series of experiments examined intraventricular administration of LRH and its effect on plasma LH concentrations. Ondo et al. (73) found that LRH injected into the third ventricle of rats caused significant

increase in the plasma concentration of LH, indicating increased LH release. Increased release was evident 10 minutes following injection. LRH injected into the cisterna magna also caused an increase in plasma LH concentration, but a delay of 90 to 120 minutes was seen, suggesting that the LRH first may have entered the systemic circulation before reaching the anterior pituitary. Ben-Jonathan et al. (7) examined the efficacy of intraventricularly and intravenously administered LRH in releasing LH which was measured in pituitary stalk portal blood. Ten to fifteen minutes after 125 ng LRH injected intraventricularly, LH was detected in portal blood; plasma levels of LH increased in proportion to the dose of LRH. Intravenously administered LRH stimulated LH release slightly faster than did LRH given intraventricularly, but the effect of intraventricular LRH injections lasted much longer. Plasma LH following intravenous administration of 125 ng TRH peaked at 20 minutes post-injection (200 ng/ml) while 120 minutes following 125 ng TRH intraventricularly, plasma LH had not yet peaked (>450 ng/ml).

Changes in Ventricular Morphological Structure with Reproductive State

The ventricular anatomical structure is seen to change with the introduction of various substances and with changes in reproductive state. Structural

modification of the ependymal surface in response to the presence of epinephrine or dopamine was reported by Schecter and Weiner (88). In control rats, the floor of the third ventricle was mostly smooth-surfaced with few microvilli or other irregularities. In the lateral recess, bleb-like protrusions were numerous and the surface was accordingly more irregular. Within five minutes of intraventricular injection of epinephrine or dopamine, the ependymal surface of the ventricular floor erupted with many protruding processes, thereby changing from the smooth area normally present into a highly irregular one. Such structural modifications were not observed in the roof of the lateral recess or in nearby areas following amine administration.

To examine the effect of reproductive state on ventricular morphology, Coates and Davis (22) examined the changes occurring in estrous and anestrous ewes. SEM of the third ventricle of estrous ewes revealed a profusion of microvilli and small protrusions covering the surfaces of the ependymal cells. Regions showing this change were the nonciliated portion of the ventral part of the lateral walls and the nonciliated floor, especially near the mouth of the infundibularis. Similar regions from anestrus ewes showed many more bare-surfaced cells. The pattern seemed to be greater numbers of cells with

bare surface zones scattered in among microvillus cells in anestrous ewes. Comparisons to estrogen- and progesterone-treated anestrous ewes showed an over-all intermediate appearance between estrous and anestrous animals.

In the adult female monkey, the morphology of the tanycytes was found to vary with the stage of the menstrual cycle (52). The ependyma of the lower lateral walls and floor of the third ventricle showed increasing bleb-like protrusions and microvilli during the preovulatory and post-ovulatory stages, while during menstruation the apical surfaces were reminiscent of those seen in juvenile animals--few microvilli and cytoplasmic projections. Following ovariectomy, apical projections were almost completely lost. Knowles and Kumar (52) also observed a double-layer arrangement of tanycytes present in males. With ovariectomy in females, a space, similar to that observed in males, developed between the two strata of ependymal cells. Administration of estrogen considerably reduced, but did not eliminate, the space that developed between ependymal layers following removal of the ovaries. In males, following castration, apical projections did not appear to be greatly altered, although they were slightly decreased in size. Also, the double-layer arrangement of tanycytes was lost. A single injection of testosterone was sufficient to restore the

space. That the morphology of ependymal cells may be altered with changes in reproductive state has also been suggested by observations in the skunk (40) and in the ferret (43). SEM observations of third ventricles from ovariectomized and ovariectomized gonadal steroid-treated rabbits and ewes (20, 19) and rats (14) during different stages of the estrous cycle also suggest that there are differences in surface morphology of the lower part of lateral walls and ventral floor which seem to be related to sexual status. There do seem to be species differences, however, with extremes of large membranous blebs and widespread rich microvillous development being exhibited by monkeys and more subtle changes occurring in the rat, with changes shown by rabbits and ewes somewhere in between.

CHAPTER III

MATERIALS AND METHODS

A series of three experiments was designed to examine the presence of luteinizing hormone (LH) in plasma and cerebrospinal fluid (CSF) of pony mares. The first experiment utilized cycling pony mares from which plasma and CSF samples were taken. The second experiment examined LH concentrations in CSF and plasma of seasonally-anovulatory mares following administration of a commercial equine pituitary extract (Pitropin) or GnRH. The third experiment examined the levels of LH present in CSF and plasma of seasonally-anovulatory mares following GnRH administration with or without steroid pretreatment. LH was quantified according to the method of Whitmore, Wentworth and Ginther (95). For all the experiments blood and CSF were collected at approximately the same time. Blood was collected by venipuncture from the jugular vein and CSF was collected acutely at the level of the cisterna magna with a 4.5 inch Beckton-Dickinson, 22-gauge spinal needle. Plasma was harvested following refrigeration of the blood sample and CSF was frozen immediately after collection.

Experiment I--LH Levels During the Estrous Cycle

Four cycling pony mares of mixed breeding and age were utilized in this experiment. Plasma and CSF were collected from each pony mare on day two of estrus (2E), day of ovulation (Ov), and day ten post-ovulation (10 PO). To determine the stage of estrous cycle, each mare was teased with a pony stallion every day until signs of estrus were evident. Beginning with the first day of estrus, the ovaries of each pony mare were palpated daily, per rectum, until the day of ovulation. On each day of sample collection, 20cc of blood and 10cc of CSF were taken. Table 1 illustrates the collection regimen. Statistics for this experiment were calculated for a double split-plot design with repeat measure.

TABLE 1.--Experiment 1--Plasma and Cerebrospinal Fluid (CSF) Levels of LH on Day 2 of Estrus, Day of Ovulation and Day 10 Post-Ovulation in Mares

Days	Plasma	CSF
2 of Estrus	4[a]	4
Ovulation	4	3
10 Post-Ovulation	4	4

[a]Each mare was sampled on day 2 estrus, day of ovulation and day 10 post-ovulation for both plasma and CSF.

Differences within a source were calculated using orthogonal contrasts for data with heterogeneous variance and a statistical outlier.

Experiment II--Exogenously Induced LH Surges in Seasonally-Anovulatory Pony Mares

A total of 12 seasonally-anovulatory pony mares of mixed breeding and age were randomly assigned to three treatment groups (n=4/group). Group 1 was injected subcutaneously (sc) with 5cc of saline and served as vehicle controls. Group 2 was injected sc with 375 Fevold-Hisaw units of a commercially available equine pituitary extract (Pitropin, Biological Specialties, Middleton, Wis.). Group 3 was injected with 1 mg of synthetic GnRH (Beckman Biologicals; Palo Alto, Calif.). The dosages used were based on preliminary trials in which plasma levels of LH appeared to reach approximately ovulatory levels within one hour following injection of 375 units of Pitropin or 1 mg of GnRH. Blood (10 ml) and CSF (3 ml) were collected at 0 (before injections) and at one, two, three and twenty-four hours following administration of treatment (Table 2). Blood collected was refrigerated and CSF immediately frozen.

Experiment III--Steroid Pretreatment Effects on GnRH-Induced Release of LH in Plasma and CSF

This experiment was designed in order to examine the effect that steroid pretreatment may have on LH

TABLE 2.--Experiment II--Plasma and Cerebrospinal Fluid (CSF) Concentrations of LH Following Injection of Equine Pituitary Extract or GnRH in Seasonally-Anovulatory Mares

Group	Source	
	Plasma	CSF
Control	4[a]	4
Equine Pituitary-Extract[b]	4	4
GnRH[c]	4	4

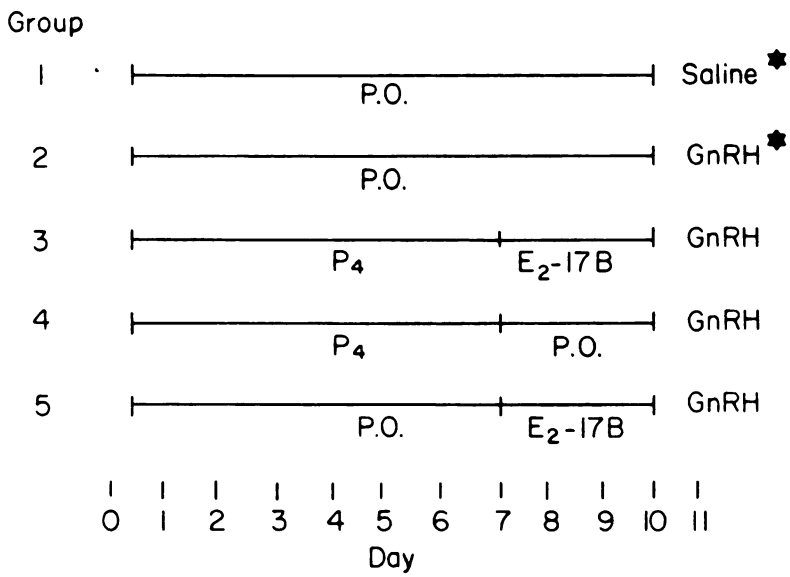
[a]Plasma and CSF were collected at 0, 1, 2, 3, and 24 hours after injection of saline (controls), equine pituitary-extract or GnRH from four mares in each group.

[b]Equine pituitary-extract (PITROPIN) = 375 fevold-hisaw units/injection (SC).

[c]Gonadotropin releasing hormone = 1 MG/INJ (SC).

concentrations in plasma and CSF following GnRH injection. A total of 25 seasonally-anovulatory pony mares of mixed breeding and age were randomly assigned to five treatment groups of five mares each. The experimental regimen is shown in Figure 1. Each group received either a steroid or vehicle pretreatment for 10 days followed by treatment with GnRH on day 11. Group 1 received 10 days of daily injections of 5cc peanut oil (used as vehicle for steroids), followed by injection with 10cc saline (vehicle for GnRH) on day 11. In all mares and treatment groups, injections were administered sc, once daily. Group 2 was injected

Effects of Gonadal Steroid Pretreatment on Plasma and Cerebrospinal Fluid (CSF) Levels of LH Following GnRH Injection In Seasonally-Anovulatory Mares.



Peanut Oil (P.O.) - 5cc /inj.
Saline - 5 cc /inj.
GnRH - 1mg /inj.
Progesterone (P_4) - 100 mg/inj.
Estradiol - 17 B (E_2-17B) - 400μg/inj.

* Plasma & CSF Samples Were Then Taken at 0,1,3,24 hrs. After Saline or GnRH.

Figure 1.--Experiment III.

for 10 days with peanut oil (5cc/injection), followed by injection of 1mg of GnRH on day 11. Group 3 was injected for 7 days with 100mg progestrone (P_4)/day, followed by 3 days of 400µg estradiol-17β (E_2-17β)/day, followed by injection of 1mg GnRH on day 11. Group 4 was injected for 7 days with 100mg P_4/day, followed by 3 days of 5cc peanut oil/day, followed by injection of 1mg GnRH on day 11. Group 5 was injected for 7 days with 5cc peanut oil/day, followed by 3 days of 400µg E_2-17β/day, followed by injection of 1mg GnRH on day 11. On day 11 blood (10 ml) and CSF (3ml) were collected at time zero (prior to saline or GnRH) and at one, three, and twenty-four hours following treatment. Blood was refrigerated and CSF frozen immediately following collection.

CHAPTER IV

RESULTS

Experiment I--LH Levels During the Estrous Cycle

Results for this experiment are illustrated in Figure 2 for plasma (upper graph) and CSF (lower graph). As can be seen there is an increase in LH concentration in plasma seen on the day of ovulation. This peak mean value of 8.4 ng/ml was significantly different ($p < .05$) than either the second day of estrus (6.1 ng/ml) or day ten post-ovulation (3.6 ng/ml). This is consistent with the literature on plasma LH concentrations at the time of ovulation in the equine. The lower graph illustrates LH concentrations in CSF collected at the cisterna magna. LH is detectable and measurable on all three days of collection, with mean values on day two of estrus, day of ovulation and day ten post-ovulation of 1.1 ng/ml, 1.5 ng/ml and 1.4 ng/ml, respectively. Although these values are not significantly different from each other, they indicate that LH is detectable in CSF (values were significantly greater than the sensitivity of the assay). In one mare a peak value of 9.7 ng/ml on the day of ovulation was detected; values for this mare on day two of estrus and day 10 post-ovulation were 1.5 ng/ml and

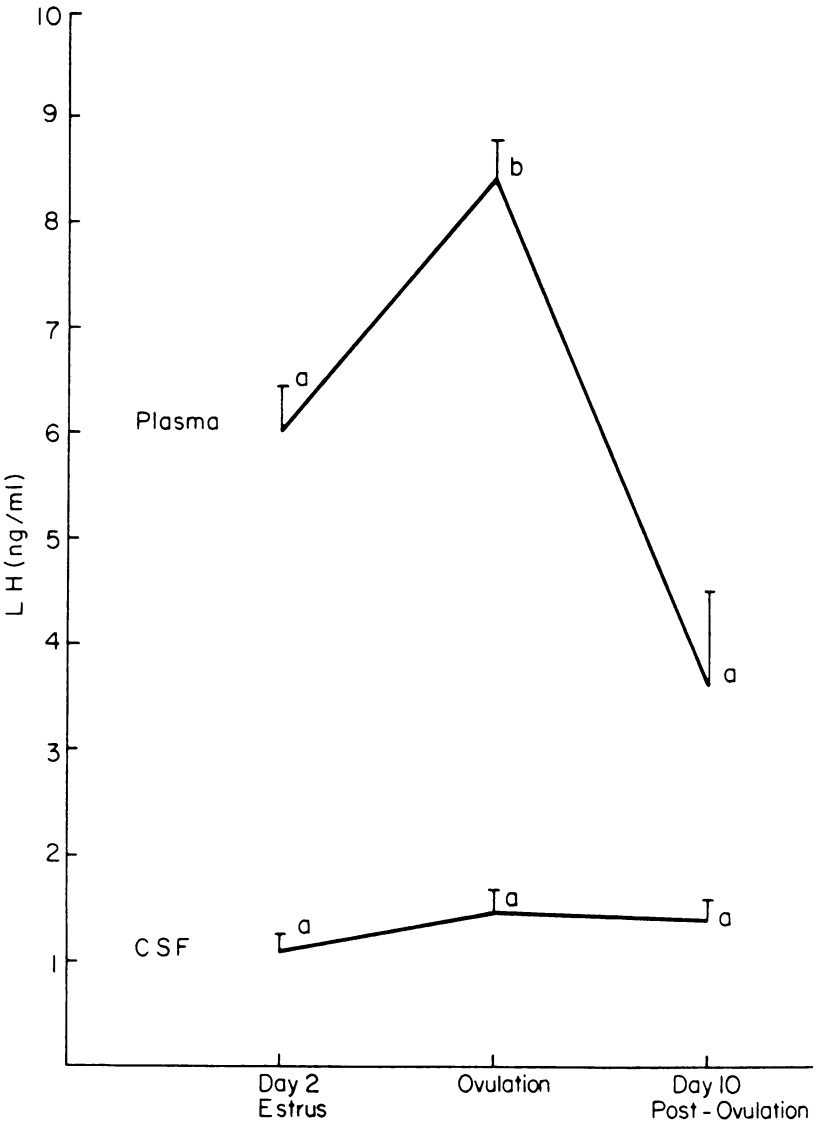


Figure 2.--Experiment 1--Plasma (upper graph) and CSF (lower graph) levels following sampling at day 2 of estrus, day of ovulation and day 10 post-ovulation. Differing superscripts (lower case letters) denote significant difference ($P < .05$) within a source.

1.1 ng/ml, respectively. The peak value of 9.7 ng for this mare was found to be a statistical outlier and was eliminated from data which were statistically analyzed for differences among days.

Statistics for Experiments II and III

LH data for Experiments II and III were analyzed by a double split plot analysis of variance. The model components and levels of significance are shown in Table 3 (Experiment II) and Table 4 (Experiment III). Significant three way interactions (Treatment X Source X Hour) were present in both experiments and apparent differences in pre-treatment concentrations of LH among different treatment groups within each experiment also were present. It was felt that the most meaningful biological interpretations of these data would result from comparisons among hours within each source for each treatment group.

Experiment II--Exogenously Induced LH in Seasonally-Anovulatory Pony Mares

This experiment examined entry of LH into the CSF following an exogenously administered LH surge (pituitary extract) or an induced endogenous LH surge (GnRH). Plasma and CSF concentrations of LH are shown in Figure 3. Plasma concentrations of LH in the control group (saline) did not change ($p > .05$) over any of the sampling times studied. In the group receiving pituitary extract,

TABLE 3.--Experiment II--Analysis of Variance Table

Source	df	Sum of Squares	Mean Square	F Value	Significance Level
T	2	29.9	15.0	3.2	0.1
M(T)	13	60.8	4.7		
H	4	9.4	2.3	2.8	0.5
H*T	8	4.2	0.5	0.6	NS
H*M(T)	52	42.9	0.8		
S	1	0.2	0.2	0.1	NS
S*T	2	17.7	8.8	6.3	0.1
S*H	4	6.0	1.5	1.1	NS
S*H*T	8	21.6	2.7	1.9	0.1
S*M(T)	9	29.6	3.3	2.9	0.05
S*H*M(T)	32	43.3	1.4		

T = Treatment; NS = not significant; M = Mare; H = Hour; S = Source

Model: $y = T + M(T) + H + H \times T + H \times M(T) + S + S \times T + S \times H + S \times H \times T + S \times M(T) + S \times H \times M(T)$.

TABLE 4.--Experiment III--Analysis of Variance Table

Source	df	Sum of Squares	Mean Square	F Value	Significance Level
T	4	8.9	2.2	0.5	NS
M(T)	20	88.0	4.4		
H	3	24.5	8.2	16.7	.005
H*T	12	9.0	0.8	1.5	NS
H*M(T)	60	29.6	0.5		
S	1	56.5	56.5	194.8	.005
S*T	4	1.0	0.2	0.8	NS
S*H	3	14.0	4.7	16.1	.005
S*H*T	12	7.9	0.7	2.3	.01
S*M(T)	20	72.2	3.6	12.4	.005
S*H*M(T)	53	15.4	0.3		

T = Treatment; M = Mare; H = Hour; S = Source; NS = Not Significant

Model: Y = T + M(T) + H + H x T + H x M(T) + S + S x T + S x H + S x H x T + S x M(T) + S x H x M(T).

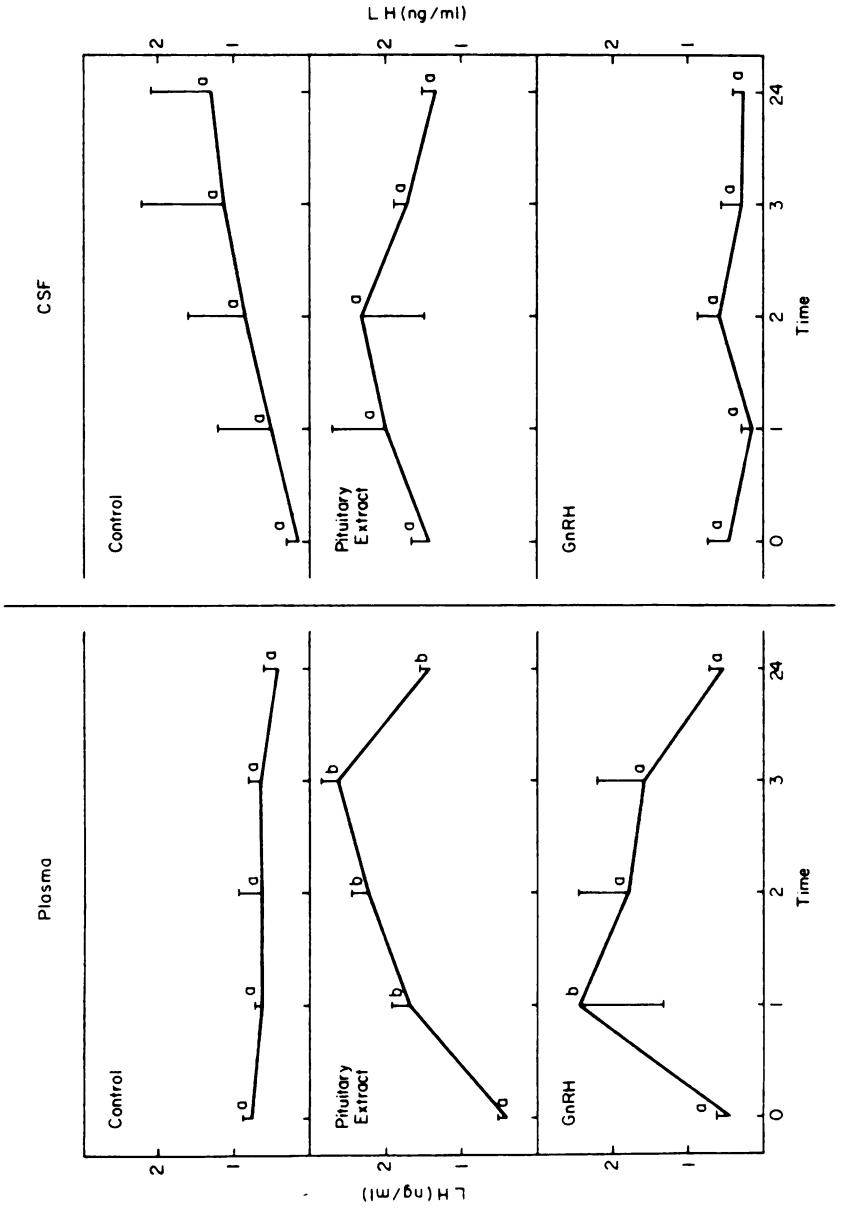


Figure 3.--Plasma (left) and CSF (right) concentrations of LH in seasonally-anovulatory pony mares following treatment with saline (control), pituitary extract (375 Fevold-Hisaw units SC) or GnRH (1mg SC) (Experiment II). Differing superscripts denote a significant difference ($P < .05$) within a treatment source over time.

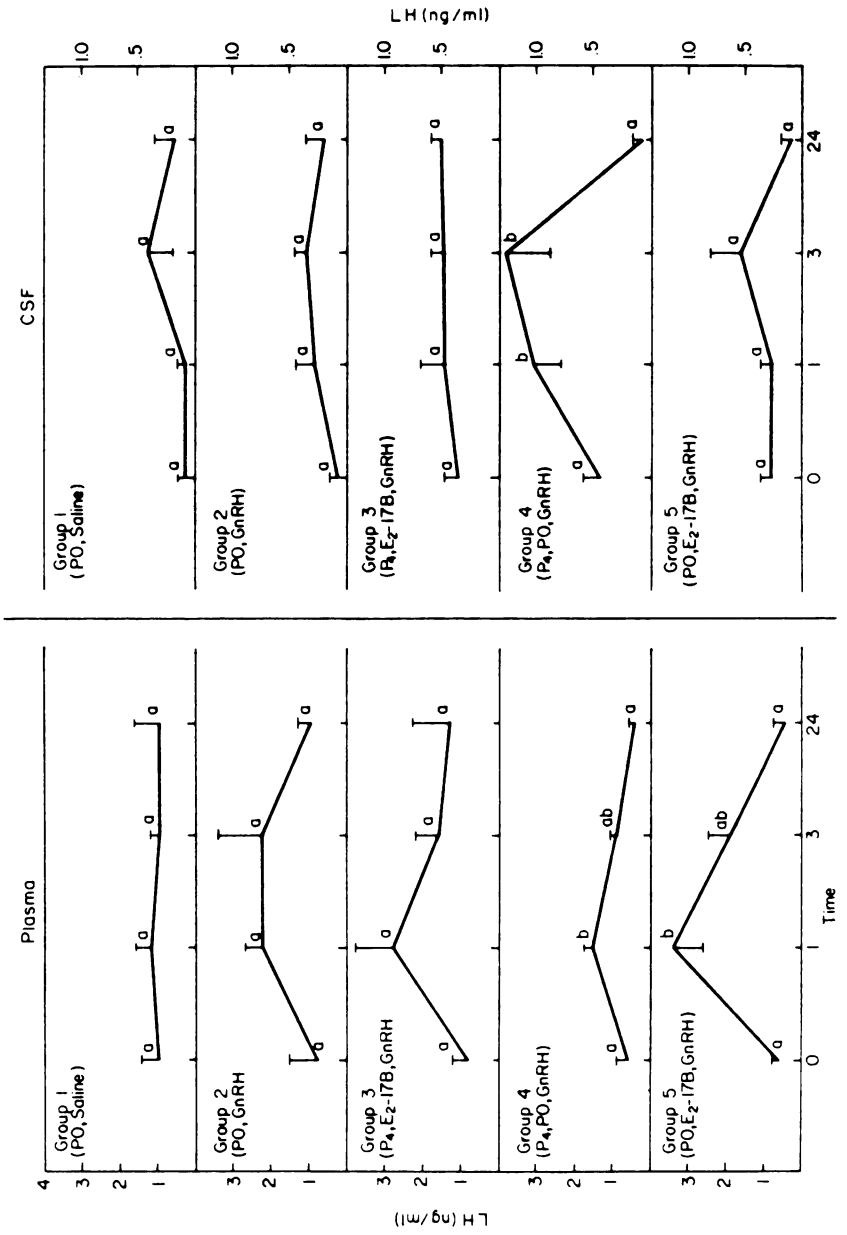
however, plasma LH concentrations at 1, 2, 3, and 24 hours post-injection were all significantly greater ($p < .05$) than pretreatment levels (time zero). Mean plasma concentrations in the GnRH group was elevated at 1 hour following injection ($p < .05$). Although concentrations at two and three hours post-injection appear to be elevated, they were not significantly different from pretreatment values. LH was detectable in CSF in all three treatment groups, although there were no significant differences from pretreatment levels observed in any of the three treatment groups.

These data indicate that in the seasonally-anovulatory pony mare, a significant increase in plasma LH, induced by exogenous treatment, was not associated with change in CSF levels of LH.

Experiment III--Steroid Pretreatment Effects on GnRH Induced Release of LH in Plasma and CSF

Figure 4 depicts changes over time (0, 1, 3 and 24 hours) in plasma and CSF, following treatment with saline (controls) or GnRH. In controls, plasma LH concentrations at 1, 3 and 24 hours were not different from the pretreatment sample (0 hr). In group two (PO, GnRH) and in group three (P4, E2-17β, GnRH) the concentration of plasma LH at 1 hr tended ($P < .1$) to be greater than the pretreatment level but levels at 3 and 24 hours were

Figure 4.--Plasma (left) and CSF (right) concentrations of LH following GnRH or saline injection. Groups 1 and 2 received 10 days of peanut oil (PO) injections preceeding treatment with saline (Group 1) or GnRH (Group 2) on day 11. Groups 3 and 4 received 7 days of progesterone injections (P_4) then 3 days of ether estradiol 17β (E_2-17β) to Group 3 or PO (Group 4) before treatment with GnRH on day 11. Group 5 received 7 days of PO, 3 days of E_2-17β then GnRH on day 11. Differing superscripts denote a significant difference ($p < .05$) within a treatment source over time.


Plasma
CSF
LH (ng/ml)
Time
Group 1 (PO, Saline)
Group 2 (PO, GnRH)
Group 3 (P4, E2-17B, GnRH)
Group 4 (P4, PO, GnRH)
Group 5 (PO, E2-17B, GnRH)

not significantly different. However, ingroups four (P4, PO, GnRH) and five (PO, E_2-17β, GnRH) a significant increase ($P < .05$) in plasma LH occurred at 1 hour but values at 3 and 24 hours were not different ($P > .05$) from pretreatment concentrations.

Concentrations of LH in CSF were not affected by treatment in any group except group four (P4, PO, GnRH). Levels of LH were significantly elevated ($P < .05$) at 1 and 3 hours after treatment with GnRH but were not different from pretreatment values by 24 hours.

Pretreatment of seasonally-anovulatory pony mares with progesterone for seven days followed by GnRH treatment on the fourth day following the last progesterone injection, resulted in significantly increased concentrations of LH in both plasma and CSF. These results demonstrate that the concentration of radioimmunoassayable LH in CSF of pony mares can be altered by progesterone.

CHAPTER IV

DISCUSSION

Recently studies have shown substances with molecular weights in excess of 25,000 (prolactin, prealbumen and β-lipoprotein) to be present in the CSF of either humans or rats (3, 4, 32). The results of the three experiments detailed in this thesis have shown immunoassayable LH to be present in CSF, as well as in plasma, in seasonally-anovulatory and seasonally-ovulatory pony mares, and that the concentration of LH in CSF is affected by administration of exogenous vs. endogenously-induced LH and by steroid pretreatment followed by GnRH injection.

In Experiment II the dosages of Pitropin and GnRH, as described, produced similar plasma LH levels, although CSF LH concentrations differed greatly for the two treatments. LH levels in CSF for the group receiving Pitropin reached similar levels to those observed in the plasma. Peak plasma value of 2.63 ng/ml was observed 3 hours post-injection, while a peak concentration of 2.3 ng/ml was observed in CSF 2 hours post-injection. Porter et al (82) observed that the infusion of LH into a hypophysial portal vessel causes release of LH from the anterior pituitary, indicating that LH may have a positive feedback effect

upon LH release, which may explain similar levels observed in plasma and CSF in the treatment group receiving pituitary extract. Another possibility may be that in seasonally-anovulatory mares there is little restriction to the passage of exogenous LH into CSF.

The group receiving GnRH showed LH concentrations in CSF 10-20% of levels observed in plasma, quite different than results obtained with Pitropin. Studies with anovulatory ewes and ovariectomized monkeys have shown that the ventricular ependymal cells under these conditions are inactive with minimal surface protrustions (22, 52). Rhesus monkeys at time of menstruation also indicated surface morphology of the third ventricle to contain few microvilli and cytoplasmic projections (52). Pelletier et al. (79) and Naik (68) demonstrated that specialized ependymal cells bordering the third ventricle in rats showed varied immunohistochemical reactions for LRH during different phases of the estrous cycle. The animals used in this experiment were seasonally-anovulatory, suggesting an inactive ventricular morphology, which may explain the increase observed in plasma LH without an accompanying increase in CSF LH concentration.

How LH, or other large protein hormones, might reach the ventricular system has been the goal of numerous researchers. Passage of substances from the ventricular system into the systemic and portal circulation and

its reverse, from circulatory system into CSF, has been demonstrated in a number of species (7, 53, 62, 63, 66, 73). Brightman (16) found that ferritin could pass between the gaps between glial cell processes of brain capillaries. The possibility exists that LH detected in the CSF may have entered via or between tanycytes connecting pituitary portal vasculature with CSF of the third ventricle, and that the stage of estrous cycle determines characteristic features of the ventricular lining, and therefore passage of substances between CSF and the vascular system. Evidence for vascular connection between adenohypophysis, neurohypophysis and hypothalamus (9) and observations that anterior pituitary hormones are present in portal vasculature (72) support the presence of LH in portal vasculature adjacent to CSF of the third ventricle. Löfgren in 1959 (60) and later Duvernoy et al (31) noted that capillary loops of the pituitary portal system are arranged in a wide network just underneath the ependyma. Transfer of substances from CSF into portal vasculature and its reverse from portal vasculature into CSF (61, 62, 72, 10, 7); the presence of releasing hormones in ependyma of the median eminence (42, 78, 68); and the observation that tanycytes which line the extreme anterior floor of the third ventricle send terminals to the capillaries of the organosum vascularis of the lamina terminalis (59), help support the concept that the

tanycytes, particularly in the median eminence, may function as a means of endocrine communication.

As a means of examining the LH levels present in cycling mares, we undertook the experiment whereby CSF and plasma were collected from cycling pony mares on day 2 of estrus, day of ovulation, and day 10 post-ovulation, as described. Again as in seasonally-anovulatory mares, LH is detectable in the CSF on all 3 days of collection. Results showed that there was a tendency for LH to rise in CSF on the day of ovulation, but no significant difference was observed. It is unfortunate that only 4 mares were utilized per group, as random error can affect results greatly. One mare did reach a peak of 9.75ng/ml on the day of ovulation, but this value was removed as an outlier in the statistical analysis. Changes in ventricular ependymal morphology in estrous ewes (21) and in female rhesus monkeys (52) have shown consistent morphological changes with stage of the estrous cycle. Repetition of this experiment with a larger number of mares as treatment group would be beneficial to determine whether or not entrance of LH into CSF also correlates with changes in morphological structure associated with stage of the estrous cycle.

Experiment III was designed to examine the effects that steroid pretreatment to seasonally-anovulatory mares

might have on LH levels in CSF and plasma following GnRH administration. Coates and Davis (22) observed changes in ventricular morphology in estrous, anestrous and estrogen-progesterone treated ewes utilizing scanning electron microscopy. Surface morphology of ependymal cells in the third ventricle of ewes treated with estrogen and progesterone were intermediate in appearance between that of estrous and of anestrous ewes. The steroid pretreatment with estrogen and progesterone in Experiment III can only attempt to mimic changes during the normal cycle. We found that a significant elevation of LH levels in CSF and plasma occurred in the group given progesterone pretreatment followed by GnRH injection (Group 4). Interestingly, the group receiving both progesterone and estrogen pretreatment (Group 3) and the group receiving estrogen alone (Group 5) did not show significant changes following treatment with GnRH in CSF, but the group receiving estradiol pretreatment alone did show a significant elevation in plasma following GnRH administration. This is consistent with literature concerning the positive effect of estradiol on LH concentrations in plasma following GnRH administration. GnRH did not cause a significant change in either plasma or CSF LH concentrations when no steroids were administered as pretreatments, although there was a tendency for LH to rise in plasma.

In the group receiving GnRH preceeded by peanut oil (PO), there is a three-fold increase in plasma LH from pretreatment level to 1 hour post-injection. This increase is similar to that observed in Experiment II following GnRH. Because of the large amount of animal variation, this result is not significant, although it mimics the increase seen in cycling pony mares at day of ovulation compared to day 10 post-ovulation. Again, in group 3 of Experiment III, a 3.5-fold difference at 1 hour post-injection of GnRH compared to pretreatment levels with P_4 and E_2-17β pretreatment is observed, although again this result is not significant. Failure to cause a significant increase in plasma LH with GnRH suggests that a larger dose of GnRH should have been administered.

The result that we find most interesting is the observation that progesterone pretreatment results in a significant rise in LH in the CSF following GnRH treatment and that this elevation is still evident 3 hours post-treatment. A similar rise was also seen in plasma LH concentrations; a significant increase at 1 hour following GnRH treatment, but not at 3 hours. This result seems to be contrary to previous literature pertaining to the effect on LH levels in plasma when GnRH is preceeded by progesterone pretreatment. If indeed these results are real, then progesterone, or lack of progesterone following elevation somehow is affecting either the LH

released by GnRH or the ability of GnRH to cause release of LH.

The possibility exists that progesterone may have an affinity for tanycytes overlying the median eminence and affects permeability of LH from portal vasculature into CSF or possibly affects the efficacy of GnRH in causing LH release. Kumar and Knowles (57) observed that, following systemic injection of tritium-labeled estradiol-17β into ovariectomized monkeys, radioactivity was detected in the CSF and in tanycytes. Consistent with the CSF-steroid transport hypothesis is the observation that most estrogen-concentrating neurons are in nuclei contiguous with the ventricular system (92).

Knowles and Kumar (52) observed bulbous structures in the ependymal cells (tanycytes) bordering the third ventricle during the early pre-ovulatory period in the rhesus monkey. They observed that these bulbous structures persisted in the post-ovulatory phase also, but almost disappeared in ovariectomized monkeys. These authors implicated that the changes of the bulbous ependymal lining found in tanycytes may be due to the level of estrogen in the CSF. Naik (68) has shown stored LH-RH in the CSF of the optic and infundibular recesses. Naik has localized many LH-RH nerve endings in this region, very close to the ependymal surface in contact with the CSF.

There is sufficient morphological evidence of cyclic secretion by these nerve endings into the CSF in females. There are numerous observations concerning tanycyte secretion into the third ventricle (51, 52, 55, 85, 89). Ependymal cells along the ventricular surface of the median eminence have numerous microvilli and marginal and surface folds which seem to entrap CSF containing certain material, and pass it to the portal blood of the median eminence through their long branching ependymal processes. Immunohistochemical LH-RH localization studies (68) by light and fluorescent microscopy show a clear cyclic accumulation of the LH-RH positive material in the CSF of the infundibular recess. This material reaches its peak during late diestrus and early proestrus phases, immediately after which it starts declining and is almost absent in late proestrus. Cyclic reduction of the stainable material from the ventricular lumen during the proestrus phase shows the transport of this material through the specialized ependyma to the portal blood. It seems possible that LH-RH from some of the areas of the circumventricular organs is transported efficiently to the infundibular recess through the CSF to be further carried by the ependymal cells to the median eminence portal system, and that cyclic changes in ventricular ependyma may affect entrance of releasing factors into portal

vasculature, and that LH detected in CSF may play a part in feedback control of the hypothalamus. Following cessation of progesterone pretreatment in Group 4 on day 7, there is a three-day span before treatment with GnRH on the eleventh day. The decrease in plasma progesterone may mimic the drop normally observed during late diestrus in cycling mares, and may sensitize receptors for GnRH as LH normally begins to rise during this time.

These experiments have provided stimulus to continue examining the role that CSF might play in the role of hypothalamic-hypophyseal regulation of reproductive function. The presence of LH in CSF, and the ability of hormonal changes to alter LH levels, provides enough impetus to continue study in this area.

LITERATURE CITED

LITERATURE CITED

1. Allen, J.D.; J. W. Kendall; R. McGilura and C. Vancura. 1974. Immunoreactive ACTH in cerebrospinal fluid. J. Clin. Endocrinology 38:586-593.

2. Ames, A. 3rd; M. Sakanoue and S. Endo. 1964. Na, K, Ca, Mg & Cl concentrations in choroid plexus fluid and cisternal fluid compared with plasma ultrafiltrate. J. Neurophysiol. 27:672-681.

3. Assies, J.; A. P. M. Schellekens and J. L. Touber. 1978. Prolactin in human cerebrospinal fluid. J. Clin. Endocrinol. Metab. 46:576-586.

4. Assies, J.; A. Schellekens and J. L. Touber. 1978. Protein hormone in CSF: evidence for retrograde transport of PRL from the pituitary to the brain in man. Clin. Endocrinol. 8: 487-491.

5. Banos, G.; P. M. Daniel; S. R. Moorhouse and O. E. Pratt. 1973. The influx of amino acids into the brain of the rat in vivo: the essential compared with some non-essential amino acids. Proc. R. Soc. B. 183:59-70.

6. Banos, G.; P. M. Daniel; S. R. Moorhouse; and O. E. Pratt. 1975. The requirements of the brain for some amino acids. J. Physiol. 246:539-548.

7. Ben-Jonathan, N.; R. S. Mical and J. C. Porter. 1974. Transport of LRF from CSF to hypophysial portal and systemic blood and the release of LH. Endocrinology 95:18-25.

8. Bergland, R. M.; S. L. Davis and R. B. Page. 1977. Pituitary secretes to brain. Lancet, Aug. 6:276.

9. Bergland, R. M. and R. B. Page. 1978. Can the pituitary secrete directly to the brain? Affirmative anatomical evidence. Endocrin. 102:1325.

10. Bito, L. Z.; H. Davson and J. R. Hollingswort. 1975. Facilitated transport of prostaglandins across the blood-cerobrospinal fluid and blood-brain barriers. J. Physiol. 256: 273-285.

11. Bleier, R. 1971. The relations of ependyma to neurons and capillaries in the hypothalamus: a golgi-cox study. J. Comp. Neurol. 142: 142-439.

12. Bleier, R. 1972. Structural relationship of ependymal cells and their processes within the hypothalamus; in Brain-Endocrine Interaction. Median Eminence: Structure and Function, Int. Symp. Munich. 1971. (Basel: Karger, 1972) 306-318.

13. Brawer, J. 1972. The fine structure of the ependymal tanycytes at the level of the arcuate nucleus. J. Comp. Neurol. 145:25.

14. Brawer, J. R.; P. S. Lin and C. Sonnenschein. 1974. Morphological plasticity in the wall of the third ventricle during the estrous cycle in the rat: a scanning electron microscopic study. Anat. Rec. 179:481-490.

15. Brightman, M. and S. Palay. 1963. The fine structure of ependyma in the brain of the rat. J. Cell Biol. 19:415.

16. Brightman, M. W. 1965. The distribution within the brain of ferritin injected into cerebrospinal fluid compartments. II. Parenchymal distribution. Am. J. Anat. 117:193-220.

17. Burgess, A. and M. B. Segal. 1970. Morphological changes associated with inhibition of fluid transport in the rabbit choroid plexus. J. Physiol. 208:88-91P.

18. Clemens, J. A. and B. D. Sawyer. 1974. Identification of prolactin in cerebrospinal fluid. Exp. Brain Res. 21:399-402.

19. Coates, P. and S. L. Davis. 1975. Response of ependymal tanycytes in sheep third ventricle to ovariectomy, estrogen and progesterone: scanning electron microscopy (SEM). Anat. Rec. 181:333-334 (Abstr.).

20. Coates, P. W. 1976. Scanning electron microscopy (SEM) of rabbit third ventricle after ovariectomy and estrogen or progesterone treatment. Anat. Rec. 184:580 (Abstr.).

21. Coates, P. 1977. The third ventricle of monkeys. Cell Tiss. Res. 177:307-316.

22. Coates, P. and S. L. Davis. 1977. The sheep third ventricle: scanning electron microscopy of estrous, anestrous and estrogen-progesterone-treated anestrous ewes. Biol. of Reprod. 17:567-573.

23. Coxon, R. V. 1968. Cerebrospinal fluid transport. Prog. Brain Res. 29:135-146.

24. Csaky, T. Z. and B. M. Rigor. 1968. The choroid plexus as a glucose barrier. Prog. Brain Res. 29:147-154.

25. Cserr, H. F. 1971. Physiology of the choroid plexus. Physiol. Rev. 51:273-311.

26. Davson, H. 1956. Physiology of the Ocular and Cerebrospinal Fluids. London: Churchill.

27. Davson, H. and M. Pollay. 1963. Influence of various drugs on the transport of ^{131}I and PAH across the cerebrospinal fluid-blood barrier. J. Physiol. (Lond.) 167:239-246.

28. Davson, H. and M. B. Segal. 1970. The effects of some inhibitors and accelerators of sodium transport on the turnover of ^{22}Na in the cerebrospinal fluid and the brain. J. Physiol. (Lond.) 209:131-153.

29. Davson, H. 1976. The blood-brain barrier. J. Physiol. 255:1-28.

30. Douglas, R. H. 1976. Progesterone and estradiol in cerebral spinal fluid. J. of An. Sci. 43: 282 (Abst.).

31. Duvernoy, H.; J. Koritke and G. Monnier. 1971. Sur la vascularization du tuber posterieur chez l'homme et sur les relations vasculaires tubero-hypophysaires. J. Neurovisc. Rel. 32:112-142.

32. Flegenhauer, K. 1974. Proteinsize and cerebrospinal fluid composition. Klinische. Wochenschrift, 52:1158-1164.

33. Fishman, R. A. and N. P. Christy. 1965. Fate of adrenal corticol steroids following intrathecal injection. Neurology 15:1.

34. Ginther, O. J. and B. C. Wentworth. 1974. Effect of a synthetic gonadotropin-releasing hormone on plasma concentrations of luteinizing hormone in ponies. Am. J. Vet. Res. Vol. 35, No. 1.

35. Goldmann, E. E. 1909. Die aussere und innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Farbung." Beitr Klin Chir. 64:192-265.

36. Goldmann, E. E. 1913. Vitalfarbung am Zentralnervensystem. Abh preuss Akad. Wiss, Phys-Math Kl. I, 1-6.

37. Gordon, J. H.; J. Ballinger and S. Reichlin. 1972. Plasma thyrotropin responses to thyrotropin-releasing hormone after injection into the third ventricle, systemic circulation, median eminence and anterior pituitary. Endocrinology 91:696-701.

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

39. Guyton, Arthur C. 1976. Textbook of Medical Physiology. 5th Ed. Philadelphia: Saunders.

40. Hagendoorn, J. 1965. Seasonal changes in the ependyma of the third ventricle of the skunk. Anat. Rec. 151:453.

41. Horstmann, E. 1954. Die faserglia des selachiergehirns. Z. Zellforsch. 39:588-617.

42. Ishii, S.; M. Wada and Y. Oota. 1975. Identification of neurosecretory granules carrying adenohypophyseal hormone-releasing hormone, in Brain-Endocrine Interaction II. The Ventricular System, 2nd Int. Sym. Shizuoka, 1974: 70-79.

43. Jones, C. 1967. Changes in specialized ependyma of the ferret in relation to the oestrous cycle. Thesis for B. Sc., Birmingham University.

44. Joseph, S. A.; S. Sorrentino, Jr. and D. K. Sundberg. 1975. Releasing hormones LRF and TRF in the cerebrospinal fluid of the third ventricle in Brain-Endocrine Interaction II. The Ventricular System. 2nd Int. Sym., Shizuoka, 1974: 306-312.

45. Joseph, S. A.; D. E. Scott; S. S. Vaola; K. M. Knigge and G. Krobisch-Dudley. 1974. Localization and content of thyrotropin-releasing factor in median eminence of the hypothalamus. Acta Endocrin. Kbh. 74:215-225.

46. Kendall, J.; Y. Grimm and G. Shimshak. 1969. Relation of cerebrospinal fluid circulation to the ACTH-suppressing effects of corticosteroid implants in the rat brain. Endocrinol. 85:200.

47. Kendall, J. W.; L. H. Rees and R. Kramer. 1971. Thyrotropin releasing hormone (TRH) stimulation of thyroidal radioiodine release in the rat: comparison between intravenous and intraventricular administration. Endocrinology 88:1503.

48. Klatzo, I.; J. Miquel; P. Ferriz; J. Prokop and D. Smith. 1964. Observations on the passage of the florescein labeled serum proteins (FLSP) from the cerebrospinal fluid. J. Neuropath. exp. Neurol. 23:18.

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

50. Knigge, K. M. and S. A. Joseph. 1973. Thyrotropin-releasing factor (TRF) in cerebrospinal fluid of the third ventricle of rats. Acta Endocr. Copenh. 76:209-213.

51. Knowles, F. 1969. Ependymal secretion, especially in the hypothalamic region. J. Neurovisc. Relat. Suppl. 9, 97-110.

52. Knowles, F. and T. Kumar. 1969. Structural changes, related to reproduction, in the hypothalamus and in the pars tuberalis of the rhesus monkey. Philosoph. Trans. Royal Soc. London. 256:357.

53. Knowles, F. 1972. Ependyma of the third ventricle in relation to pituitary functions. Progr. Brain Res. 38:255-270.

54. Kobayashi, H and I. Ishii. 1968. The median eminence as storage site for releasing factors and other biologically active substances, in Progress in Endocrinology, Proc. Third Int. Congr. Endocrinol., Mexico. 548-554.

55. Kobayashi, H; T. Matsui and S. Ishii. 1970. Functional electron microscopy of the hypothalamic median eminence. Int. Rev. Cytol. 29:281-381.

56. Kozlowski, G. P.; D. E. Scott and G. K. Dudley. 1973. Scanning electron microscopy of the third ventricle of sheep. Z. Zelforsch 136:169-176.

57. Kumar, T. and F. Knowles. 1967. A system linking the third ventricle with the pars tuberalis of the rhesus monkey. Nature 215:54.

58. Landsmeer, J. M. F. 1951. Vessels of the rats hypophysis. Acta. Anat. 12:82.

59. Leveque, T. F. in Knigge, K. M.; D. E. Scott and A Weindl (eds.). Brain-Endocrine Interaction: Median Eminence: Structure and Function, S. Karger, Basel, Switzerland, 1972, p. 304.

60. Lofgren, F. 1959. New aspects of the hypothalamic control of the adenohypophysis. Acta. Morph. Scand. 2:220-229.

61. Marynick, S. P.; W. W. Hoveno, II; M. H. Ebert and D. L. Loriaux. 1976. Studies on the transfer of steroid hormones across the blood-cerebrospinal fluid barrier in the rhesus monkey. Endocrinology 99:400-405.

62. Marynick, S. P.; G. B. Smith; M. H. Ebert and D. L. Loriau. 1977. Studies of the transfer of steroid hormones across the blood-cerebrospinal fluid barrier in the rhesus monkey. II. Endocrinology 101:562-567.

63. Mayhew, I. G. 1975. Collection of cerebrospinal fluid from the horse. Cornell Vet. 65:500-511.

64. McArthur, N. 1970. Ultrastructural changes in the ependymal cells, tanycytes and pituicytes of the distal infundibulum in newborn and adult male rats following castration and treatment with cyporterone acetate. Dissertation, U. of Missouri, Columbia, Missouri.

65. McConnell, E. M. 1953. The arterial blood supply of the human hypophysis cerebri. Anat. Rec. 115:175.

66. Melrose, P. A. and Douglas R. H. 1977. Testosterone in blood and CSF of geldings following exogenous administration of testosterone. Amer. Soc. Anim. Sci. Meeting. (Abstr.)187.

67. Mitchell, J. A. 1975. Functional anatomy of the hypothalamus: possible role of the ependyma in Hypothalamic Hormones 1975. E. S. E. Hafez and J. R. Reed (eds.). Ann Arbor Science Publishers.

68. Naik, D. V. 1976. Immuno-histochemical localization of LH-RH during different phases of estrous cycle of rat, with reference to the preoptic and arcuate neurons, and the ependymal cells. Cell Tiss. Res. 173:143.

69. Oldendorf, W. H. 1971. Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. Am. J. Physiol. 221:1629-1639.

70. Oldendorf, W. H. 1973. Stereospecificity of blood-brain barrier permeability to amino acids. Am. J. Physiol. 224:967-969.

71. Oliver, C.; J. L. Charvet; J. L. Codaccioni; J. Vague and J. C. Porter. 1974. TRH in human CSF. Lancet 1:873.

72. Oliver, C.; R. S. Mical and J. C. Porter. 1977. Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinol. 101:598.

73. Ondo, J. G.; R. S. Mical and J. C. Porter. 1972. Passage of radioactive substances from CSF to hypophysial portal blood. Endocrinology 91:1239-1246.

74. Oxender, W. D.; P. A. Noden and M. C. Pratt. 1977. Serum luteinizing hormone, estrus and ovulation in mares following treatment with prostaglandin $F_{2\alpha}$ and gonadotropin-releasing hormone. Am. J. Vet. Res. 38:No. 5.

75. Page, R. B.; B. L. Munger and R. M. Bergland. 1976. Scanning microscopy of pituitary vascular casts: the rabbit pituitary portal system revisited. Am. J. Anat. 146:273.

76. Page, R. B. and R. M. Bergland. 1977. Pituitary vasculature, in the Pituitary, Academic Press, Inc., New York, San Francisco, London.

77. Page, R. B. and R. M. Bergland. 1977. The neurohypophyseal capillary bed: anatomy and arterial supply. Amer. J. Anat. 148:345.

78. Pelletier, G.; F. Labrie; A. Arimura and A. V. Schally. 1974. Electron microscopic immunohistochemical localization of growth hormone-release inhibiting hormone (Somatostatin) in rat median eminence. Am. J. Anat. 140:445-450.

79. Pelletier, G.; R. Leclere and F. Labrie. 1974. Immunohistochemical localization of LH-RH and neurophysin in the rat hypothalamus and neurohypophysis. 56th Annual Meeting of Endocrine Society. 129:147 (Abstr.).

80. Pollay, M. and H. Davson. 1963. The passage of certain substances out of the cerebrospinal fluid. Brain 86:137.

81. Pollay, Michael. 1975. Formation of cerebrospinal fluid. J. Neurosurg. 42:665-673.

82. Porter, J. C.; N. Ben-Jonathan; C. Oliver; R. L. Eskay and A. J. Winters. 1976. Interrelationship of CSF, hypophysial portal vessels and hypothalamus and their role in the regulation of anterior pituitary function. Neuroend. Reg. Fert., Int. Sump., Simla, 71-79.

83. Rall, D. P. in LaDu, B. N.; H. G. Mandel and E. L. Way (eds.). Fundamentals of Drug Metabolism and Drug Disposition, Williams and Wilkins, Baltimore, 1971, p. 76.

84. Reese, T. S. and M. J. Karnovsky. 1967. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell Biol. 34:207-217.

85. Rodriguez, E. M. 1969. Ependymal specializations, I. Fine structure of the neural (internal) region of the toad median eminence, with particular reference to the connections between the ependymal cells and the subependymal capillary loops. Z. Zellforsch. 102:153-171.

86. Rodriguez, E. M. 1970. Morphological and functional relationships between the hypothalamoneurohypophyseal system and cerebrospinal fluid, in Aspects of Neuroendocrinology. W. Bargmann, B. Scharres (eds.). Berlin-Heidelberg-New York. pp. 352-365.

87. Rodriguez, E. M. 1972. Comparative and functional morphology of the median eminence, in Brain-Endocrine Interaction, K. M. Knigge, D. E. Scott and A. Weinde (eds.) Karger, Basel. pp. 319-334.

88. Schecter, J. and R. Weiner. 1972. Ultrastructural changes in the ependymal lining of the median eminence following the intraventricular administration of catecholamine. Anat. Rec. 172:643.

89. Scott, D. E. and K. M. Knigge. 1970. Ultrastructural changes in the median eminence of the rat following deafferentation of the basal hypothalamus. Z. Zellforsch. 105:1-32.

90. Scott, D. E.; G. P. Kozlowski and M. N. Sheridan. 1974. Scanning microscopy in the ultrastructural analysis of the mammalian cerebral ventricular system. Int. Rev. Cytol. 37: 349-388.

91. Silverman, A. J.; K. M. Knigge and W. A. Peck. 1972. Transport capacity of median eminence. I. Amino acid transport. Neuroend. 9:123-132.

92. Stumpf, W. 1972. Hypophysiotropic neurons in the periventricular brain: topography of estradiol-concentrating neurons, in Steroid Hormones and Brain Function. C. Sawyer and R. Gorski (eds.), Los Angeles: University of California Press, 1972. pp. 215-227.

93. Szentagothai, J; B. Flerko; B. Mess and B. Halasz. 1962. Hypothalamic control of the anterior pituitary. Akademiai Kiado, Budapest, p. 81.

94. Torok, B. 1964. Structure of the vascular connections of the hypothalamo-hypophyseal region. Acta. Anat. 59:84.

95. Whitmore, H. L.; B. C. Wentworth and O. J. Ginther. 1973. Circulating levels of luteinizing hormone during the estrous cycle of mares based on radioimmunoassay. Amer. J. Vet. Res. 34:631.

96. Wislocki, G. M. 1938. The vascular supply of the hypophysis cerebri of the rhesus monkey and man. Proc. Assoc. New. Ment. Dis. 17:48.

97. Wittkowksi, W. 1967. Zur ultrastruktur der ependymalen tanyzyten und pituizyten sowie ihre synaptische verknüpfung in de neurohypophyse des meerschweinchens. Acta. Anat. 67:338.

98. Xuereb, G. P.; M. Prichard and P. M. Daniel. 1954. The arterial supply and venous drainage of the human hypophysis cerebri. Q. J. Exp. Physiol. 39:199.

99. Zimmermann, E. A.; K. C. Hsu; M. Ferin and G. P. Kozlowski. 1974. Localization of gonadotropin-releasing hormone (GnRH) in the hypothalamus of the mouse by immunoperoxidase technique. Endocrinol. 95:1-8.