THE INFLUENCE OF THE PINEAL PEPTIDE ARGININE VASOTOCIN ON REPRODUCTIVE ADENO-HYPOPHYSEAL HORMONES

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CHAPTER I

INTRODUCTION

The epiphysis cerebri or pineal gland has been a subject of interest and speculation for over 2000 years. The ancient Greeks were the first to characterize the pineal gland describing it as a cone-shaped organ. In western civilization, Herophilos of Alexandria (325-280 B.C.) was the first to develop a theory concerning the function of the pineal gland. He held that the gland functioned as a tap regulating the stream of "spiritus" from the third and fourth ventricles of the brain. This view was held for over twenty centuries and was expanded on by the philosopher Descartis (1596-1650). He regarded the pineal gland as the seat of the soul, especially the seat of imagination, consciousness and memory. However, shortly after publication Descartis' philosophies were refuted by several other philosophers including Stensen and Kant. Interest in the mammalian pineal gland then waned until the early twentieth century.

In the early twentieth century, several investigators concluded that the pineal gland was probably a rudimentary organ which might have functional significance in early phylogenetic stages but had no importance in mammals. The only importance attributed to this gland was its use as a point of orientation by the radiologists as a result of the electron dense concretions found in the pineal.

Fortuitously, Heubner in 1898 described a boy suffering from a pinealoma who exhibited symptoms of premature puberty (Kappers, 1976). Several years later, this syndrome was named puberta praecox or genitosomia praecox (Marburg, 1913, 1930). Clinical symptoms included a pinealoma, premature development of the primary and secondary sexual organs and was found to occur mainly in boys. Its etiology was believed to be hypopinealism due to pineal degeneration or to destruction of specific pineal parenchyma. The existence of hyperpinealism has also been described (Berkeley, 1926; Kappers, 1976). This syndrome is characterized by excessively slow and incomplete sexual development with a secondary general adiposity. Both hyperpinealism and hypopinealism have been described in recent literature (Mincer et al., 1976; Kubo et al., 1977).

As early as 1910, ablation experiments in rats (Thieblot, 1965) supported the clinical evidence relating the pineal gland with the reproductive system. These experiments demonstrated the appearance of precocious secondary sexual characteristics, augmentation of testicular weight and development of the surface of the seminiferous tubules. Further evidence, beginning in the 1930's and 1940's (Fischer, 1938; Fischer, 1943) and continuing into the 1970's (Wurtman et al., 1959; Milcu et al., 1963; Reiss, 1963; Soffer, 1965; Orts et al., 1974a, 1975b; Ebels et al., 1970; Cheesman and Fariss, 1970b; Ota et al., 1975) have demonstrated the antireproductive properties of pineal extracts. Regardless of the chemical nature of the compound(s) eliciting the effects, all have demonstrated the ability of these compounds to inhibit the reproductive system either at the gonad, pituitary or hypothalamus. Two of these antireproductive compounds have been

isolated from the pineal and identified. Both the indoleamine melatonin (Lerner and Takahaski, 1960; Wurtman and Axelrod, 1965) and the peptide arginine vasotocin (AVT) (Milcu et al., 1963; Cheesman, 1970a) have numerous antireproductive properties attributed to them, although both are still controversial after two decades of research.

This thesis will deal with the influence of the purported pineal hormone AVT on two of the reproductive hormones of the anterior pituitary. The effects of AVT on circulating levels of luteinizing hormone (LH) and prolactin in rats and on LH and prolactin release from hemisectioned pituitaries in in vitro incubation will be discussed.

In summary, the reproductive effects of the pineal gland have been manifested clinically in such diseases as puberta praecox or hyperpinealism. Many ablation and replacement experiments have reinforced the observed clinical relationship between the pineal gland and the reproductive system and have further demonstrated the potent antireproductive properties of pineal extracts. This thesis will examine the relationship of the pineal peptide AVT and the adenohypophysial hormones LH and prolactin.

CHAPTER II

REVIEW OF THE LITERATURE

Modern research concerning the pineal gland was stimulated by Kitay and Altschule (1954) who reviewed the previous literature and concluded that although much of the literature was confusing and contradictory, one could establish a statistical relationship between the pineal gland and gonadal function.

Wurtman and others (Wurtman et al., 1959, 1961; Reiss et al., 1963a, 1963b) in the late 1950's and early 1960's established a relationship between the pineal gland, pineal secretions, photoperiod and the reproductive system. This seemingly simple relationship has become an elusive enigma to interpret.

Relationship Between Pineal and Photoperiod

The relationship between the pineal gland and photoperiod has been a subject of intense research since Kitay and Altschules (1954) rekindled the interest in pineal physiology. Traditionally, the relationship between photoperiod and melatonin synthesis has been used as an index of pineal-photoperiod relationship.

It was determined that the indoleamine 5-hydroxytryptamine (serotonin) was first converted to N-acetylserotonin by the enzyme serotonin-N-acetyltransferase (NAT) (Weissbach, 1960). N-acetylserotonin was then found to be converted to melatonin by the

enzyme hydroxyindole-O-methyltransferase (HIOMT) (Axelrod and Weissbach, 1961). The relationship between photoperiod and melatonin synthesis was established when a decrease in pineal serotonin was observed during darkness and elevated levels observed during periods of light exposure (Quay, 1963). Subsequently, an increase in N-acetylserotonin (Klein and Weller, 1973) and melatonin concentration both within the pineal gland (Lynch, 1971) and in the blood (Pehlam et al., 1972) during the night has been observed.

Present dogma suggests that darkness is the stimulus for the release of norepinephrine from the pineal adrenergic endings (Deguchi and Axelrod, 1972). This catecholamine then acts via beta-adrenergic receptors to stimulate adenylate cyclase (Weiss and Costa, 1967) which results in an increase in cyclic AMP (Strada et al., 1972). This increase in cyclic AMP then stimulates an increase in NAT (Deguchi and Axelrod, 1972). The enzyme HIOMT might also increase in activity during periods of darkness although there is disagreement concerning such a rhythm (Reiter, 1976d). Whatever the case, the end result is an increase in melatonin synthesis and release during periods of darkness.

Anatomical studies corroborate the relationship between the eye and the pineal gland. Photic input into the pineal, as determined by HIOMT activity, originates from the inferior accessory optic tract (Moore et al., 1967; Moore et al., 1968). Leaving the main optic tract caudal to the optic chiasm, it terminates in the medial terminal nucleus of the inferior accessory optic tract (Hayhow et al., 1960). The tracts between the nucleus of the inferior accessory optic tract and the preganglionic fibers to the superior cervical ganglia have not

been traced. Extending from the superior cervical ganglia of the sympathetic chain the tracts then innervate the pineal. Evidence for this connection is supported by studies which demonstrate that bilateral superior cervical ganglionectomy rendered pineal HIOMT activity unable to respond to changes in the photoperiod (Wurtman et al., 1964; Reiter and Sorrentino, 1971; Eichler and Moore, 1971; Moore and Rapport, 1971). Additional evidence to demonstrate the existence of these tracts has been supplied by Moore and colleagues (1968) who demonstrated that destruction of the primary optic tracts at the level of the lateral geniculate bodies does not effect pineal HIOMT activity while destruction of the inferior accessory optic tracts within the medial forebrain bundle eliminates pineal HIOMT response to darkness.

Physiological Effects of Photoperiod on Reproduction

There are numerous physiological effects of photoperiod on the reproductive system. As early as 1894, Cook (N.Y.J. Obstet. Gynecol., editorial) reported that Eskimo women cease menstruation for 4 months during the long period of darkness in the winter months (Reiter et al., 1975). More recent reports also confirm this early observation. Women with irregular menstrual cycles subjected to a period of constant light of 3 to 4 days near the time of ovulation resulted in menstrual flow in 2 weeks (Dewan, 1967; Dewan and Rock, 1969).

In the adult female rat, long term exposure to constant light is known to produce the phenomenon known as persistent estrous (Browman, 1937; Fiske, 1941; Daane and Parlow, 1971). This phenomenon is characterized by a chronic vaginal cornification, enlarged ovaries,

failure to ovulate, a slight elevation in LH and Follicle Stimulating Hormone (FSH) and presumably an increase in circulating estrogens. In the immature female rat, constant light accelerates puberty (Fiske, 1941; Piácsek and Streur, 1975); however, the younger the rat the longer it takes to observe the phenomenon of persistent estrous (Takeo et al., 1975).

The opposite effects on reproductive physiology are observed in rats exposed to either long dark periods or constant darkness. Fiske (1941) demonstrated that young female rats exposed to short photoperiod start estrous cycles at an older age. The effects of photoperiod on the estrous cycle seem to depend on the age and strain of the rat. Adult rats kept in continuous darkness for 80 days exhibited longer cycles with more diestrous smears (Jöchle, 1956). Whereas, Wistar rats placed in constant darkness or blinded as adults will exhibit complete anestrous in 20% of the cases (Hoffmann, 1969).

Testicular atrophy has also been observed in response to a decreased photoperiod when prepubertal rats were used as an experimental model (Reiter, 1968; Takahashi et al., 1971). When rats are maintained on a short photoperiod of 2 hours of light daily and compared with rats maintained on a long photoperiod of 23 hours of day light, an 11% reduction in testis weight is observed in the rats on the short photoperiod (Kinson and Peat, 1971). This testicular hypotrophy disappears after 4 weeks while a decrease in prostate growth continued up to 7 weeks. After 4 weeks of reduced lighting an 85% reduction in testosterone from testicular venous blood was observed.

The reproductive effects of alterations in the photoperiod can be nicely reversed by either pinealectomy or the administration of pineal

extracts. Jöchle (1956) demonstrated that the administration of bovine pineal extracts to cycling female rats would prevent the persistent vaginal estrous produced by constant light. Furthermore, Wurtman and colleagues (1961) demonstrated that constant light and pinealectomy increased ovarian weights while rats in constant darkness exhibited smaller ovaries. The increase in ovarian weight produced by constant light was prevented by bovine pineal extracts. It has also been reported that pineal glands from rats maintained in constant light contain no antireproductive material as these extracts failed to inhibit compensatory ovarian hypertrophy (COH) in mice (Reiter et al., 1975). This observation was supported by Hoffmann and Cullin (1975) who reported that pinealectomy does not influence the persistent estrous induced by constant light. However, it should be noted that under normal photoperiods, the pineal from day-killed rats is just as capable of reducing COH as that from night-killed rats (Reither et al., 1975d). Thus, there is good evidence to suggest that the functional status of the pineal is at least partially dependent upon environmental lighting. This relationship as well as the relationship between lighting and reproductive physiology has led many to investigate the relationship between the pineal gland and reproductive physiology.

Antireproductive Properties of the Pineal Gland

Most research concerning the antireproductive properties of the pineal gland has implicated either the gonads or the pituitary as target organs. Some researchers have reported a direct effect of the pineal gland on the pituitary. Thiebolt (1965) reported that pinealectomy

resulted in an increase in the number of pituitary basophils and presumably hyperactivity of the pituitary; however, others have reported no effect of pinealectomy on pituitary morphology (Wragg, 1967). Wurtman (1959) reported that pinealectomy caused a significant increase in pituitary weight while the administration of protein free bovine pineal extracts resulted in a decrease in pituitary weight when compared to control animals.

Indolamines

Further examination has yielded fruitful information concerning the influence of the pineal gland or its extracts on the pituitary content and circulating levels of tropic hormones. Fraschini and colleagues (1968) found that pinealectomy results in an increase in pituitary LH content and presumably release as indicated by an increase in the weight of the testis, ventral prostate and seminal vesicle. They also determined that implants of either pineal tissue or melatonin into the median eminence or midbrain reticular substance of castrate rats resulted in a reduction of pituitary LH stores. Melatonin was also found to reduce plasma LH levels. However, when this compound was implanted into the pituitary it was observed to have no effect on LH. This was interpreted as indicating that pineal indoles modify pituitary function via the hypothalamus. Motta and colleagues (1967) also observed that high doses of melatonin significantly reduced prostate and seminal vesicle weight while having no apparant affect on testis weight.

Recent evidence has also suggested that melatonin can affect gonadotropin secretion. In adult male rats, melatonin given in high

doses subcutaneously for 6 days was found to significantly reduce serum LH levels and decrease seminal vesicle and ventral prostate weight (Moguilevsky et al., 1976). They further demonstrated that simultaneous treatment with melatonin and clomiphene (a substance which is believed to stimulate the hypothalamus to release luteinizing hormone releasing hormone (LRH) resulted in an inhibitory effect on serum LH. They also found that melatonin given with exogenous LRH did not modify the pituitary's response to LRH. This was interpreted as indicating that melatonin acts on the hypothelamus to inhibit LH release. However, the results from pituitary glands studied in organ culture indicate that melatonin, serotonin, and 5-methoxytryptamine are capable of reducing the pituitary's response to LRH in vitro (Martin et al., 1977). The indoleamime 5-hydroxytryptophol has also been reported to decrease pituitary LH content (Fraschini et al., 1971). Interestingly, when administered systemically, 5-hydroxytryptophol does not affect pituitary or serum LH levels.

Apparently melatonin is able to affect only LH synthesis and release from the anterior pituitary (Reiter and Fraschini, 1969) while pinealectomy has been shown to affect both LH and FSH (Fraschini, 1969). Recently, serotonin containing nerve cells associated with LRH production have been discovered in the arcuate and ventromedial nucleus (Smith and Kappers, 1975). The fact that pinealectomy reduced the number of serotonin containing neurons in these nuclei, coupled with the fact that serotonin inhibits both the synthesis and release of LRH in the hypothalamus (Moszkowska et al., 1973), has led Smith and Kappers (1975) to postulate that certain pineal compounds stimulate the

production of serotonin in neurons in the hypophysiotropic area of the hypothalamus which then inhibit the production of LRH.

Although there seems to be much evidence that would suggest that melatonin is an antireproductive compound, several have suggested that melatonin is actually progonadotropic in the rat and hamster. Reiter and colleagues (1975a, 1975b, 1975c, 1976a, 1976b, 1976c, 1977a, 1977b) have demonstrated that melatonin implants can reverse the effects of blinding and anosmia in both the hamster and the rat when given late in the photoperiod to an animal with an intact pineal. They found that melatonin restored the levels of both LH and prolactin that had been previously reduced by blinding and pinealectomy. It has also been reported that melatonin implants can block the inhibitory action of a partially purified pineal antigonadotropin on compensatory ovarian hypertrophy in mice (Orts et al., 1975a). Tamarkin et al. (1977) suggested that exogenous melatonin may be either potentiated or inhibited by the presence of the pineal or its antireproductive compounds depending on the time of injection.

The indoleamine melatonin is also known to directly affect the gonads. As early as 1963, Wurtman and colleagues (1963) found that microgram amounts of melatonin was capable of decreasing both ovarian weight and the incidence of estrus. They found that a single injection of melatonin decreased the incidence of estrus among rats exposed to constant light. However, others have not been able to find an effect of microgram quantities of melatonin in the immature or mature rat on gonadal weight or the estrous cycle (Ebels and Prop, 1965). Interestingly, they did find that melatonin would slightly reduce the light

induced depletion of lipids in the pineal. More recently, it has been determined that melatonin will selectively inhibit the incorporation of ^{125}I-LH into the ovaries of rats while not effecting ^{125}I-FSH incorporation into the ovaries (Trentini et al., 1976). This was interpreted as indicating that melatonin directly prevents the stimulatory action of LH on the ovary.

Perhaps one of the most important effects of melatonin is the inhibition of the conversion of steroid precursors to androgens (Ellis, 1969). It was determined that the conversion of androstenedione to testosterone was the point of indole interference. Apparently the inhibition by melatonin is mostly non-competitive and involves the suppression of 17 B-hydroxysteroid dehydrogenase, 17 α-hydroxylase and C-17-C-20 lyase systems (Ellis, 1972).

Unknown Peptides

Other laboratories have reported the existence of several unidentified pineal peptides. Benson and his colleagues (Benson et al., 1971; Benson et al., 1972) have demonstrated that a melatonin free compound(s) was significantly more potent than melatonin in blocking COH in mice. They have also demonstrated the ability of this compound(s) to inhibit the postcastration rise in serum LH in the mature male rat (Orts et al., 1974a), delay vaginal opening time and reduce the incidence of constant ertrus in female rats exposed to constant light (Orts et al., 1974b). Orts and colleagues (Orts et al., 1975b) have demonstrated the ability to these compound(s) to block pregnancy, reduce the pre-ovulatory surge of LH (Orts et al., 1977b) and reduce serum LH and testosterone in the male rat (Orts, 1977a). Benson and

colleagues (Rosenblum et al., 1976; Benson et al., 1976) have reported a difference between AVT and their partially purified pineal extract, observed by both differential localization and chemical means.

Others have reported the presence of pineal polypeptides with antireproductive properties. Ebels, Moszkowska and Scimama (1970) isolated a compound which inhibits in vitro the secretion of FSH from the anterior pituitary and also inhibits the hypophysiotropic hormones (Citharel et al., 1973). A somewhat similar extract of bovine pineals has also been isolated which inhibits ovulation that has been induced with pregnant mares serum (PMS) and human chorionic gonadotropin (HCG) (Ota et al., 1975).

While most of these putative pineal polypeptides have been demonstrated to be free of the pineal indole melatonin, most have not demonstrated either the presence or absence of AVT in their extracts. Biochemical isolation and identification needs to be accomplished before any definitive statements can be made concerning the existence of several new pineal antigonadotropins.

Arginine Vasotocin

Identification, localization and release. Being the most ubiquitous and probably the oldest of the active neuropeptides, the octapeptide arginine vasotocin (AVT) is found in all major vertebrate groups from the cyclostomes to the mammals (Sawyer, 1968). This peptide was first tentatively isolated and identified in the bovine pineal using bioassays which take advantage of its pressor and oxytocic activities (Milcu et al., 1963). More specifically, these included the rat pressor, rat uterus, hen oxytocic and frog bladder bioassay. It

was found that magnesium enhanced the oxytocic activity of this peptide while oxytocin had greater activity without magnesium. Based on this observation they determined the peptide they had isolated was not oxytocin. They also found extremely high frog bladder and hen oxytocic activity with low rat pressor and oxytocic potencies. This constituted good evidence the peptide was not arginine vasopressin (AVP). Chromatography revealed a single spot with the same Rf value as AVT. Treatment with trypsin was found to destroy the pineal peptide's biological activity, thus supplying additional evidence that the peptide was not oxytocin as oxytocin is unaffected by tryptic digestion. The existence of a disulfide bond in the pineal peptide was also demonstrated by reduction with sodium thioglycolate, which also inactivated the pressor and oxytocic activity. Based on these observations, Milcu and colleagues determined that the peptide was AVT.

Using very similar biological and chemical assays, Pavel (1965) isolated a polypeptide from pig pineal glands with biological, enzymatic and chromatographic characteristics not significantly different from those of synthetic lysine vasotocin. The difference between bovine and porcine vasotocin is consistent with the fact that bovine neurohypophyseal extracts contain arginine vasopressin, whereas pig extracts contain lysine vasopressin.

The biochemical isolation and characterization of AVT was finally accomplished in 1970 (Cheesman and Fariss, 1970a; Cheesman, 1970b). Using amino acid analysis and mass spectometry, AVT was identified in the bovine pineal gland.

Immunocytochemical evidence has also suggested the presence of a distinct population of cells in the rat pineal gland which contains AVT

(Bowie and Herbert, 1976). These cells were found to be distributed diffusely throughout the gland and appeared very irregular in form with extensive perivascular and intercellular processes. These processes were frequently found cupped around neighboring cells.

Radioimmunoassay data indicate that the pineal gland of the rat contains 22.4 uU AVT/gland (Rosenbloom and Fisher, 1974, 1975a, 1975b). The rabbit subcommissural organ was also reported to have radioimmunoassayable levels of AVT. Both the pineal and the subcommissural organ of the younger animal contains more AVT than the adult (Rosenbloom and Fisher, 1975b). Recent observations using immunofluorescence techniques have also suggested the presence of AVT in the fetal pituitary gland (Swaab et al., 1976).

Subsequently, Pavel (1971) has attempted to identify the structures in the bovine pineal that contain this peptide. It was determined that in the adult only the pineal stalk exhibited frog bladder activity while both the fetal stalk and gland exhibited high activity. Assuming that the frog bladder assay reflects the presence of AVT, activity is found only in those regions of the pineal that contain secretory ependymal cells. Since the entire fetal pineal is formed by secretory ependymal cells whereas, in the adult the only ependymal cells are those found in the pineal stalk which constitutes the pineal recess of the third ventricle (Anderson, 1965), this would strongly suggest that AVT is liberated from the pineal by ependymosecretion.

Further investigations have confirmed the existence of AVT in the pineal glands of fetal, neonatal and adult male rats (Pavel et al., 1975b). The AVT content of the male rat pineal markedly decreases from

the fetal to adult animal and was correlated with the regression of pineal secretory ependymal cells during development. Pavel has subsequently isolated a polypeptide released from cultured pineal ependymal cells (rat fetuses aged 17 to 19 days post-coitum) that exhibited hydroosmotic, antidiuretic and rat uterine activity that could not be distinguished from synthetic AVT (Pavel et al., 1977). Paper chromatography revealed Rf values (Rf 0.15-0.35) not significantly different from synthetic AVT but significantly different from the fast moving region corresponding to synthetic oxytocin (Rf 0.55-0.65) used as a standard. The total amount to AVT released from these cultured cells was about 40 times greater than the amount contained in non-incubated pineal glands of the same age. This was interpreted as strongly suggesting de novo synthesis of AVT.

Very similar results were seen when human fetal pineal glands (male, age 95-115 days) were cultured (Pavel, 1973a). The ratio of hydroosmotic to antidiuretic activity of the pineal peptide was found to be 181 with that of synthetic AVT to be 195. The hydroosmotic and antidiuretic activities were destroyed by tryptic digestion, oxidative inactivation by tryosinase and reductive inactivation by sodium thioglycollate. The total amount of AVT-like peptide released into the culture media was ten times greater than the amount contained in the nonincubated pineal glands of the same age. Again Pavel interpreted this as indicating de novo synthesis of AVT.

Several investigators have found evidence of a neurophysin-like protein in the pineal which is presumably associated with AVT. Reinhay and colleagues (1974) found two neurophysin-like proteins in bovine pineal extracts. They appeared to be associated with a biologically

and immunologically active neuropeptide similar to AVT. Subsequently, Legros et al. (1976) has also identified immunologically active neurophysin and AVT from human fetal pineal glands. However, the AVT was not attached to the neurophysin, suggesting a rapid enzymatic degradation of the precursor molecule.

A vasotocin-like peptide has also been identified in the cerebrospinal fluid (CSF) of both humans and cats (Pavel, 1970; Pavel and Coeulescu, 1972). The biological activity of the CSF peptide differed significantly from those of the known human neurohypophysis (as assayed by antidiuretic, oxytocic and frog bladder activities) but were not significantly different from synthetic AVT. In the cat, intraventricular injections of 0.85 M NaCl stimulated the release of AVT into the third ventricle. The physiological significance of this remains to be elucidated.

Pavel (1973b) has also demonstrated that injections of melatonin (100 ug/kg of body weight) into the third ventricle of cats or intravenous injections of melatonin (5 ug/kg body weight) stimulated the release of a vasotocin-like peptide into the third ventricle. Melatonin by either route of administration had no effect on AVP or oxytocin release into the third ventricle. Pavel concluded that melatonin acts not as a neuroendocrine transducer, but as an intermediate step that facilitates AVT release.

Along with melatonin and saline, Goldstein and Pavel (1977) have reported that LRH, thyrotropin releasing hormone (TRH) and somatotropin release inhibitory factor (SRIF) are capable of releasing AVT into the third ventricle of the cat when injected into the carotid artery. Oxytocin and saline had no effect on AVT release when administered similarly.

Recently, a diurnal rhythm of vasotocin has been reported in the male rat pineal (Calb et al., 1977). It was determined that the pineal content of AVT was highest at noon and lowest around midnight. They also determined that animals exposed to constant light contained a diminished pineal content of AVT while those exposed to constant dark had enhanced pineal AVT content. Pavel interpreted this as indicating a decrease in AVT synthesis with constant light and an increase in synthesis with constant dark. This was interpreted as indicating that AVT synthesis and release was enhanced during the night.

Antireproductive Properties of Arginine Vasotocin in the Immature Animal. The physiological importance of AVT has been the subject of investigation in only a few laboratories over the last decade. In 1966, Pavel was one of the first to examine the antireproductive properties of synthetic AVT and to compare it with a peptide isolated from the pineal (Pavel and Petrescu, 1966). Immature female mice received PMS along with either a purified pineal peptide or synthetic AVT (subcutaneously) for three days and were necropsied on the fourth day. The data demonstrated that the stimulatory action of PMS on mice uteri and ovaries was inhibited by both the pineal peptide and synthetic AVT. Pavel concluded that the inhibitory principle from the bovine pineal gland was identical to AVT and that both can directly effect the ovary.

Based on this and similar research, several laboratories have investigated the importance of AVT in the immature animal. Vaughan and colleagues (1976a) reported that single daily subcutaneous injections of AVT on days one through five after birth resulted in significantly elevated testis and accessory organ weights at 30 and 60 days of age.

By 90 days of age, the gonad and accessory organ weight in the control animals were equivalent to that of the AVT-treated groups. This was interpreted as indicating a possible early maturation of the hypothalamaco-hypophysial-gonadal axis after AVT injection, although this interpretation and experiment remains to be confirmed.

In contrast, 1 ug AVT was injected daily for three consecutive days in immature (25 day old) male mice. Upon autopsy 24 hours after the last injection, growth of the ventral prostate, seminal vesicles and coagulating glands were significantly inhibited (Vaughan et al., 1974b).

The administration of AVT to PMS and/or HCG treated immature mice (Vaughan et al., 1976a; Vaughan et al., 1976c) or immature rats (Smith et al., 1972) resulted in a decrease in ovarian and uterine weights in the mice and a reduction in both the mean number of ova shed and the mean ovarian weight in the rat. AVT may also inhibit the growth of the gonads and accessory organs when immature animals are not challenged with exogenous gonadotropins (Vaughan et al., 1974a; Vaughan et al., 1974b).

In another experiment, Vaughan and colleagues (1975a) examined the time course of uterine and ovarian growth in the immature mouse after HCG stimulation and the effect of AVT on this stimulus. Female mice, 21 days old, received a single injection of 0.25 IU HCG intraperitoneally (IP). They were then divided into groups and each group received AVT (1 ug) at 0,12,24,36,48, and 60 hours post-HCG administration. Controls received Ringers lactate at the appropriate times. Representative animals from each group were necropsied at 0,12,24,36, 48,60 and 72 hours. The results indicated a diurnal rhythm in

ovarian weight with HCG treatment. AVT treatment blocked this rhythm during the last 2 days. The initial increase in uterine weight was not blocked although latter phases of growth were significantly inhibited.

Since a single injection of HCG causes estrogen release and uterine growth in the immature mouse within a few hours, these results were interpreted as indicating that the action of AVT probably occurred at either the hypothalamus where it may modify LRH release or at the pituitary where it may modulate synthesis and/or release of the gonadotropins. This explanation was favored over a peripheral action of AVT because of its inability to block the initial growth of the ovaries and uteri.

Recently the effect of AVT on PMS induced ovulation in immature female rats has been studied in somewhat more detail (Johnson et al., 1978). They found that if AVT is given every two hours beginning on the day preceding ovulation, the rise in plasma FSH is advanced and the magnitude of the LH peak is enhanced. However, there was a significant inhibition of ovarian and uterine weights. This was interpreted as indicating that AVT had a direct effect on the ovary. It was also found that if AVT is given to PMS-treated female rats which are then sacrificed prior to the afternoon surge of prolactin, a significant elevation of prolactin is observed (Johnson, 1978).

Antireproductive Properties of Arginine Vasotocin in the Mature Animal. The administration of 2 ug AVT to pregnant mice resulted in delayed parturition and death of seven out of eight litters by the morning following delivery (Vaughan et al., 1976a, 1976b). The causative factors involved are not understood. Also, if AVT was

administered to normal cycling female mice, the estrous cycle was prolonged with the treated animals spending more time in diestrus. When paired with fertile males, these mice mated as soon as they came into estrus; however, AVT treatment inhibited fertile mating. Of those that became pregnant, all carried their litters to term and delivered as many pups per litter as the control animal (no injections were given during pregnancy).

Moszkowska and Ebels (1968) incubated half-anterior pituitaries with synthetic AVT and the corresponding control halves with no treatment. The resulting medium was then bioassayed for FSH. They found that both the medium incubated with AVT and the medium mixed with AVT after the incubation would result in a decrease in uterine weight. They interpreted this as indicating that AVT acts on the gonads or the gonadotropic hormone(s) and not on the secretion of the anterior hypophysis in vitro.

It has also been demonstrated that AVT can inhibit COH in a dose related fashion in adult mice after intraperitoneal injection and after a single intraventricular injection (Vaughan et al., 1976a). Reduction of the disulfide bond of AVT by mercaptoethanol not only destroyed AVT's ability to inhibit COH, but actually resulted in hypertrophied ovaries larger than the control animals. Along with AVT, the peptides AVP, oxytocin, 4-leucine vasotocin (4-leu-AVT), and lysine vasopressin (LVP) were tested for their ability to reduce COH in both their unreduced and reduced form. AVT, AVP, 4-leu-AVT, and LVP in the unreduced form inhibited COH while AVT, AVP and LVP in the reduced form enhanced ovarian hypertrophy. Vaughan interpreted this as indicating that a basic amino acid in position 8 of the sidechain

and a closed disulfide bond are necessary for the antigonadotropic activity while a basic sidechain (basic amino acid in position 8) and an open ring is progonadotropic.

Pavel also examined the influence of AVT on COH in mice (Pavel et al., 1973c). He found that 0.5 uU AVT administered into the third cerebral ventricle completely inhibited COH when measured 8 days later. Comparable results were found when 25 uU AVT were administered IV or 50 uU (0.1 ng) were given IP. From this, Pavel postulated that AVT inhibits COH by acting on gonadotropin(s) or on the synthesis or release of LRH. Similar results were seen when human fetal pineal extracts were administered to mice (Pavel et al., 1973/1974). They received 0.5 ml of the extract IP and the results observed were identical with 50 uU (0.0001 ug) of synthetic AVT in inhibiting COH. When compared to melatonin, 100 g of the indole was needed to inhibit COH to the same degree. Treatment of both human fetal pineal extracts and synthetic AVT with trypsin, tyrosinase, and sodium thioglycollate completely destroyed their COH inhibitory effects.

Pavel further reasoned that if AVT has a physiological significance in inhibiting anterior pituitary hormones, it should be able to overcome the effects of pinealectomy; more specifically on pituitary prolactin content as measured by the intradermal pigeon crop sac assay (Pavel et al., 1975a). He found that 0.0002 pg AVT (-120,000 molecules) injected into the third ventricle of the mouse, on the day of pinealectomy, prevented the increase in pituitary prolactin which occurs 7 days after pinealectomy. This response appears to be dose dependent as 2 pg AVT reduced the prolactin content below that of the sham-operated animals. This is believed to represent the lowest concentration reported so far

for a biological substance able to produce an endocrine effect.

Because of the similarity between the terminal tripeptide of both AVT and LRH, several laboratories have investigated the possibility that AVT can competitively inhibit LRH receptor sites or stimulate LH release at the level of the pituitary. Vaughan and colleagues (1976a) found that LRH + AVT significantly augmented the accumulation of LH in the media of half anterior pituitaries (HAP) incubated for 5 hours when compared to their control halves. This seemingly progonadotropic response is very difficult to explain. Possible explanations offered included the opening of the disulfide bond or the liberation of the terminal tripeptide. The terminal tripeptide (Pro-Arg-Gly (NH_2)) is known to potentiate the activity of FSH and LH on rat ovaries (De La Lastra et al., 1973).

Using anterior pituitaries from intact male rats and estrogen-progesterone primed female rats as models, it was determined (Vaughan et al., 1975b) that both LH and prolactin were released from AVT treated half anterior pituitaries into culture medium. The terminal tripeptide of AVT, Pro-Arg-Gly (NH_2), failed to elicit a change in the release of LH and prolactin. These results are very difficult to explain and the author only offers the explanation that high levels of prolactin are frequently found in cases of primary hypogonadism in human males and rats.

The same laboratory has also demonstrated that the intravenous administration of AVT to either estrogen-progesterone or estrogen-progesterone primed pinealectomized male rats will stimulate prolactin release in vivo (Vaughan et al., 1976d). This was accomplished in an acute preparation with plasma levels of prolactin measured within 20

minutes of AVT administration. Based on this and the previous experiment, the authors have proposed that AVT is an extrahypothalamic prolactin releasing factor.

Subsequently, Blask and fellow researchers (1976) have observed that intravenous administration of AVT to estrogen-progesterone primed male rats will release prolactin 10 minutes after injection. They also observed that prolactin secretion from hemipituitaries incubated with haloperidol, propranolol or haloperidol plus AVT was unaffected (Blask et al., 1977). However, if hemipituitaries were incubated with propranolol plus AVT, a significant reduction in prolactin release was observed. This was interpreted as indicating that AVT has a B-adrenergic type receptor on the lactotrophs of the anterior pituitary.

In contrast, it was recently concluded that AVT does not effect the secretion of FSH or LH by rat anterior pituitary cells in a monolayer cell culture (Demoulin et al., 1973). They cultured anterior pituitary cells from 14 adult male rats in Dulbecco Modified Eagles Minimum Essential Medium for 2 days. Synthetic AVT was then added in concentrations ranging from 10^{-7} to 10^{-18} M and the incubation was continued for three days, at which time the glands were washed and then incubated for another 6 hours with AVT in the same concentrations, with or without LRH at a final concentration of 10^{-8} M. Their results indicated that AVT effected neither the basal rate nor the LRH stimulated release of either gonadotropin. They further hypothesized that if AVT is involved in the regulation of gonadotropin secretion, the site of action is probably the hypothalamus. In agreement with this hypothesis, Vaughan (1978) demonstrated that AVT was capable of blocking the post-castration rise of serum LH and FSH. She also determined, using a

bioassay, that the hypothalami from the AVT treated rats contained significantly more LRH.

Others have found that AVT, when given intravenously to unanesthesized proestrus rats at a rate of 1.0 ug/hour for 3 hours, was capable of suppressing the preovulatory surge of LH and prolactin but had no effect on the concurrent surge of FSH (Cheesman et al., 1977a, 1977b). Furthermore, they found no effect on the tonic level of any of the aforementioned hormones. Additionally, it was found that ovulation was also inhibited in those animals treated with AVT. Investigating the mechanisms involved, they determined that picogram quantities of AVT injected intraventricularly will eliminate the preovulatory surge of LH in 33% of the rats (Osland et al., 1977). AVT was also found to have no effect on the prostaglandin E_2 (PGE_2)-induced release of LH or the LH release associated with the electrochemically stimulated medial preoptic area. They concluded that AVT probably inhibits the LH surge at the medial preoptic level or higher centers.

Summarizing the data just presented proves to be a difficult task. There appears to be two major theories concerning the release of AVT. It would appear that Pavel and his colleagues believe that AVT is most important in the fetal animal. He has demonstrated that AVT is synthesized in the fetal pineal gland and is secreted from the pineal gland by ependymal secretory cells into the third ventricle. In the adult, he has demonstrated its existence only in the pineal stalk. After AVT enters into the CSF, it then exhibits an effect on the hypothalamus or pituitary leading ultimately to one of several antireproductive responses such as inhibiting COH or uterine weight. He has suggested that AVT might stimulate prolactin release.

The second theory may be attributed to Reiter and colleagues (1976e). They postulate that the pineal polypeptides are secreted by pineal secretory cells--which may be either pinealocytes, glial cells or neurons--conjugated to carrier proteins in much the same way that vasopressin and oxytocin are bound to neurophysin I and II. These peptides are exocytotically extruded into the extracellular space along with considerable exocytotic debris. In a method similar to that of the posterior pituitary, the blood Ca^{++} is then exchanged with the polypeptide hormone resulting in the release of the hormone into the blood stream. The by-products of this process, the carrier protein complex and the exocytotic debris then calcify and form the commonly known corpora arenacea or pineal sand. Once into the blood stream the peptide can then exhibit its antireproductive properties. They have postulated that one of these properties is affecting the release of prolactin from the pituitary, thus qualifying AVT as an extrahypothalamic prolactin releasing factor. According to the same group of researchers, AVT also appears to affect both the gonads and the hypothalamus.

There is evidence to support both theories of AVT secretion. Whichever the case, it appears that once secreted, AVT can affect several levels of the hypothalamic-pituitary-gonadal axis.

CHAPTER III

MATERIALS AND METHODS

In order to determine if AVT has an effect on reproductive endocrinology, three different experimental approaches were used in this study. They included the *in vivo* administration of the peptide, anterior pituitary incubations and pituitary homogenate uptake of ^{125}I-LRH in the presence or absence of AVT. The parameters observed in the first two experimental approaches were changes in LH and prolactin concentrations. The response observed in the last approach was the ability of pituitary tissue to take up ^{125}I-LRH.

Animals

The animals used in this experiment were of the Sprague-Dawley strain and were Charles River's CD rats obtained from Charles River, Inc. They were maintained in the Oklahoma State University facilities in an ambient temperature of 23^{o}C and in a diurnal lighting of 14 hours light and 10 hours dark (14L:10D) with the daily photoperoid beginning of 0800 hours. All animals received food and water *ad libitum*.

The castrate rats used in the pituitary incubations experiments were castrated at 35 days of age while the castrate rats used in the ^{125}I-LRH uptake experiments were castrated at 28 days of age. All castrate rats were allowed 30 days to recover from the surgery. All surgery was performed under aseptic conditions using ether anesthesia.

Those castrate male rats receiving estrogen-progesterone (E-P) priming received one injection of the steroids subcutaneously 72 hours prior to the incubations or the ^{125}I-LRH uptake studies. Each rat received 50 ug 17B-estradiol (Sigma, No. E-8875) and 25 mg progesterone (Sigma, No. 95C-0320) in 0.5cc corn oil as a vehicle. This is a modification of Ramirez and McCann (1963) method for estrogen-progesterone priming rats for testing LRH releasing activity.

In Vivo Administration of Peptide

Thirty male rats 115 days of age, were utilized in this experiment and were divided into 5 equal groups. Rats in groups A and B received 0.1 ml of saline at 1100 hours each day for 5 days. Groups C, D and E received 0.1, 1.0 or 10.0 ug AVT, respectively. The AVT was administered in 0.1 cc of saline at 1100 hours each day for 5 days. In all cases the diluent and the AVT were injected subcutaneously.

On the sixth day of the experiment, the rats were anesthetized with urethane (78 mg/100 g bw) given intraperitoneally. The right jugular vein was then exposed and cannulated with "intramedic" polyethylene tubing P.E. 50 (I.D. 0.023", O.D. 0.038") which was previously filled with heparinized (50 units/cc) saline. The rats were packed with moist gauze and kept warm throughout the experiment.

At 1100 hours a pre-injection (time 0) blood sample was collected from each rat. Immediately thereafter, the rats in Groups B, C, D and E received 100 ng LRH in 0.1 cc of saline through the venous catheter which was then rinsed with 1 cc of heparinized (10 units/cc) saline. Rats in Group A received 0.1 cc of saline containing no LRH. Additional blood samples from each animal were collected at 30, 60, 120

and 180 minutes post-injection. The samples of 30, 60 and 120 minutes were collected by the venous catheter while the 180 minutes samples were collected by decapitation. After all but the final blood sample, 1 cc of heparinized (10 units/cc) saline was added as a volume replacement. All blood samples were then centrifuged, the plasma collected frozen at -20°C until analyzed for LH and prolactin.

Pituitary Incubations

A modification of the procedure developed by Mittler and Meites (1966) for pituitary incubations was used for all experiments. The rats were lightly anesthesized with ether and decapitated. The skull cap and brain were removed exposing the pituitary gland. Upon removal of the pituitary gland, the posterior lobe was dissected from the anterior lobe and discarded. The anterior pituitary was then hemisectioned with each of the half anterior pituitaries (HAP) weighed and immediately placed into separate incubation flasks. Each flask had been previously coated with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and contained 2 ml of incubation media consisting of Krebs-Ringer bicarbonate with 200 mg percent glucose (KRBG, Appendix A). The HAP were then preincubated for 30 minutes at the end of which each HAP was removed and placed into a fresh BSA-coated flask containing 2 ml of media. The HAP were then incubated for either 4 or 6 hours with gassing every 30 minutes with 95% O_2-5% CO_2. Both preincubation and incubation were performed at 37°C with continuous shaking (American Optical Water Bath Shaker) at a rate of 60 cycles per minute. After termination of the incubation both the

incubation media and aqueous extracts of the HAP were stored frozen at -20°C until assayed for LH or prolactin.

Using this procedure, one HAP served as a control and was incubated with KRBG containing varying concentrations of LRH. The corresponding HAP then served as experimental tissue and was incubated with KRBG and LRH along with varying concentrations of AVT. (Appendix B describes the LRH and AVT in more detail). Anterior pituitaries from rats under various steroid environments were utilized for the pituitary incubations.

Pituitary Incubation I

In this experiment the HAP utilized were obtained from either 100 day old intact male or 100 day old estrogen-progesterone primed castrate male rats. In either case, the 6 control HAP received no treatment while their corresponding halves were incubated with 1 ng/ml LRH. Each HAP was incubated for 6 hours.

Pituitary Incubation II

Intact male rats 100 days of age served as pituitary donors in this experiment. The HAP from each of ten animals were incubated in each of 4 treatment groups. All HAP were incubated with 1 ng/ml LRH while the media of the experimental halves also contained AVT in log concentrations from 0.01 to 10 ng/ml. The experiment was terminated after 6 hours.

Pituitary Incubation III

Pituitaries were obtained from 125 day old intact male rats. Six pairs of HAP were incubated in each treatment group. All HAP were incubated with 10 ng/ml LRH while the experiment HAP were also incubated with 1,10 or 100 ng/ml AVT in their incubation media. The pituitaries were incubated for 6 hours.

Pituitary Incubation IV

In this experiment castrated male rats served as pituitary donors. All rats were 60 days of age upon sacrifice. The control HAP were incubated with 1 ng/ml LRH while the corresponding halves were incubated with 1 ng/ml LRH and log concentrations of AVT ranging from 0.1 to 100 ng/ml. There were 6 pairs of HAP per treatment group. After 4 hours of incubation the experiment was terminated.

Pituitary Incubation V

The design of this experiment was the same as that of pituitary incubation IV except that estrogen-progesterone primed castrate male rats served as pituitary donors.

Pituitary Incubation VI

Intact female rats 100 days of age served as pituitary donors. These rats served as donors regardless of the stage of the estrous cycle. All HAP were incubated with 1 ng/ml LRH while the experimental halves were also incubated with log concentrations of AVT ranging from 1 to 1000 ng/ml. Five pairs of HAP were incubated in each treatment group. All HAP were incubated for 6 hours.

Pituitary Incubation VII

In this experiment 10 intact female rats 100 days of age in various stages of the estrous cycle served as pituitary donors. Control HAP were incubated in media with the experimental HAP also incubated with 1 ng/ml AVT. All pituitaries were incubated for 6 hours.

Pituitary Incubation VIII

Ten intact male rats 100 days of age served as pituitary donors in this experiment. All HAP were incubated with 1 ng/ml LRH. The experimental halves were also incubated with 1 ng/ml AVP. In the second part of this experiment, 10 intact male rats of the same age as those previously discussed served as pituitary donors. Control HAP were incubated in media only while experimental halves were incubated with 1 ng/ml AVP. All pituitaries in this experiment were incubated for 6 hours.

Pituitary Homogenate Uptake of ^{125}I-LRH

The ability of AVT to affect the uptake of ^{125}I-LRH in a pituitary homogenate was examined in both intact male and estrogen-progesterone primed castrate male rats using the techniques of Pedroza et al. (1977). All rats utilized were 60 days of age. The rats were lightly anesthesized with ether and rapidly decapitated. The anterior pituitaries were collected, pooled and homogenized in Hepes buffer (Sigma, No. H-3375) (pH 7.2) with 0.5 mg/ml Bactracin (Sigma, No. B-0125). The homogenate was then placed into tubes such that each tube contained

the equivalent of one pituitary in 0.5 ml of buffer. The tubes also contained varying concentrations of LRH or AVT ranging from 1 to 1000 mM in 10 ul of buffer. Each tube also contained 4.5 nM ^{125}I-LRH (440 uCi/ug) in 25 ul of buffer. The LRH was iodinated using the lactoperoxidase method of Nett et al. (1973). Tubes without pituitary homogenate but containing 535 ul buffer served as blanks. All tubes were allowed to incubate for 30 minutes at 4^{o}C. The incubation was stopped by centrifugation at 7500 rpm for 15 minutes at 4^{o}C. The pellet of homogenate was washed 2 times with Hepes buffer and radioactivity of the pellet was counted in a gamma counter (Nuclear-Chicago). All values were corrected by substracting blank values.

Radioimmunoassay of LH and Prolactin

The radioimmunoassay (RIA) of rat LH was performed in duplicate by the double antibody RIA procedure as described by Niswender et al. (1968). The anti-LH antibody (anti-ovine LH antibody, pool No. 15) was kindly supplied by Dr. G. Niswender. The purified LH for iodination (LH I-4) was supplied by Dr. A. F. Parlow through the NIAMD Rat Pituitary Hormone Distribution Program. The LH concentrations were expressed in terms of NIAMD Rat LH RP-1 having a biological potency of 0.03 x NIH LH-S1 (OAAD assay). The assay was determined to have a within assay variation of 7.5% and a between assay variation of 10.3% (Appendix C contains parallel inhibition curves for LH RIA).

The RIA for rat prolactin was performed in duplicate using the double antibody RIA procedure as described by the NIAMD rat prolactin kit provided by the NIH Rat Pituitary Hormone Distribution Program. The antiserum utilized was NIAMD anti-rat prolactin S-4 while the

antigen for iodination provided was NIAMD rat prolactin I-2. The prolactin concentrations were expressed in terms of NIAMD rat prolactin RP-1 with a biological potency of 11 IU/mg (mouse deciduoma assay). The assay was determined to have a within assay variation of 6.8% and a between assay variation of 18.4%. (Appendix D contains parallel inhibition curves for prolactin RIA).

The RIA for both LH and prolactin were determined from incubation media which had been previously diluted with phosphate buffered saline (PBS, pH 7.4). The results of all assays were calculated on a programmable Ollivete P652 computer. This computer was programmed to construct a standard curve directly from the assay standards and also to correct the data for non-specific binding.

Enzyme Determination

In order to determine the viability of the HAP after incubation, enzyme analysis was performed with "Dow Diagnostic Kit" for serum glutamic oxalacetic transaminase (GOT). This test is based on ultraviolet spectral detection of NADH changes which are proportional to GOT activity (Amador and Wacker, 1962). Media from anterior pituitaries that were incubated for 2, 4, or 6 hours with oxygen were compared as well as media incubated for 6 hours without oxygen.

Histological Preparations

In order to determine the amount of cellular degeneration after incubation, the HAP were fixed in 10 percent buffered formalin for at least 48 hours. They were subsequently embedded, sectioned and stained with hematoxylin-eosin. Two HAP that were incubated for 6

hours without oxygen and to fresh pituitary tissue. Parameters examined included the appearance of nucleus and connective tissue stroma.

Statistical Analysis

The pituitary incubation experiments were analyzed using the paired t-test (Batson, 1956). This test measures the variance of the mean differences. The t value was then calculated as the mean difference divided by the standard error. The in vivo administration of peptides experiment was analyzed using the one-way analysis of variance, the Newman-Keul test and the paired t-test (Steele and Torrie, 1960; Zivin and Bartko, 1976). All hormone values are expressed as mean $\pm$ standard error. The ^{125}I-LRH uptake studies were analyzed using the one-way analysis of variance and the student t-test (Steele and Torrie, 1960).

CHAPTER IV

RESULTS

Enzymatic and Histological Determinations

The results of the GOT assay as depicted in Figure 1 indicate a gradual but non-significant release of the enzyme from the anterior pituitary over the 6 hour incubation period. In contrast, when HAP were incubated for 6 hours under the same conditions without oxygen, a much larger release of GOT was observed. When comparing the GOT levels after 6 hours of incubation, the enzyme levels more than doubled in the media from those glands incubated without oxygen.

The results of the histological examination indicate that the integrity of incubated pituitary tissue does differ from that of freshly fixed tissue (Table I). However, significantly $P < .001$) more pyknotic nuclei and loose connective tissue were observed in those glands incubated for 6 hours without oxygen when compared to those incubated for 6 hours with oxygen.

In Vivo Administration of Peptide

Table II illustrates the in vivo effect of LRH and AVT on LH release. The only significant effect observed at time 0 was with group C. These rats contained significantly ($P < .05$) less LH than those in group B. The initial withdrawal of blood from the rats in group A (those receiving neither LRH nor AVT) resulted in a small non-

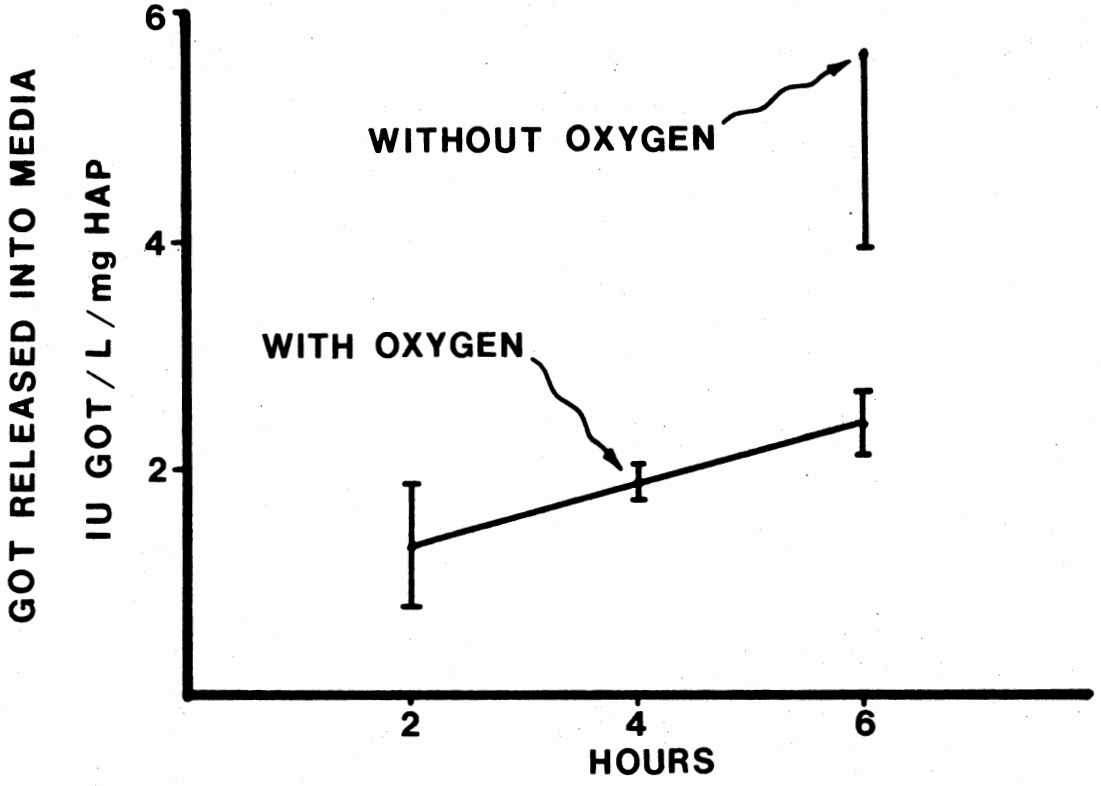

Figure 1. Enzyme Analysis of Media From Rat Half Anterior Pituitaries Incubated With and Without Oxygen. (n = 3)

TABLE I

HISTOLOGICAL COMPARISON OF RAT PITUITARY TISSUE INCUBATED IN THE PRESENCE OR ABSENCE OF OXYGEN WITH FRESH TISSUE

Treatment	Number of Pyknotic Nuclei[1]
Fresh	6 ± 1
Incubated with oxygen	18 ± 1[2]
Incubated without oxygen	270 ± 23[2,3]

[1]Number of pyknotic nuclei observed in a 10 x 10 grid at X430 power, mean $\pm$ S.E. ($n = 3$).

[2]Significantly ($P < .001$) more pyknotic nuclei than in fresh tissue.

[3]Significantly ($P < .001$) more pyknotic nuclei than in tissue incubated with oxygen.

TABLE II

EFFECT OF IN VIVO ADMINISTRATION OF AVT ON LRH STIMULATED LH RELEASE IN MALE RATS

Time[1] (Minutes)	GROUP A	B	C	D	E	F^{11}
			AVT (ug)			
	0	0	0.1	1	10	
			LRH (100 ng)			
0	11 ± 2[2]	15 ± 4	7 ± 1[6]	13 ± 3	10 ± 2	9.31[3]
30	89 ± 37	632 ± 114[7,9]	500 ± 101[7,9]	384 ± 113[7,8]	357 ± 66[8]	4.88[4]
60	37 ± 9[6]	86 ± 7[8]	56 ± 5[6,10]	48 ± 15[6]	58 ± 9[9]	4.02[5]
120	16 ± 4	23 ± 4	34 ± 15	20 ± 6	18 ± 2[8]	0.81
180	13 ± 3	18 ± 4	17 ± 5	18 ± 10	14 ± 1	0.15

TABLE II (Continued)

[1]Time after LRH administration.

[2]Expressed as ng/ml (n = 6), mean ± S.E.

[3]Probability < .005 that all means are from the same population.

[4]Probability < .010 that all means are from the same population.

[5]Probability < .025 that all means are from the same population.

[6]Significantly (P < .05) less than group B.

[7]Significantly (P < .05) greater than group A.

[8]Significantly (P < .05) greater than time 0 sample.

[9]Significantly (P < .01) greater than time 0 sample.

[10]Significantly (P < .001) greater than time 0 sample.

[11]F values as determined by analysis of variance.

significant increase in LH at both 30 and 60 minutes. These values returned to basal levels by 120 minutes where they remained until termination of the experiment. Serum LH values of the rats in group B (those receiving LRH only) significantly ($P < .01$) increased from a pre-injection nadir of 15 $\pm$ 4 ng/ml at time 0 to a zenith of 632 $\pm$ 114 ng/ml within 30 minutes after the administration of LRH. This constitutes over a 40 fold increase in LH. These values were still significantly ($P < .05$) elevated at 60 minutes but returned to basal levels by 120 minutes. Similar results were observed in groups C, D and E (those rats receiving 0.1, 1 and 10 ug AVT per day, respectively). Significant increases were observed in these groups within 30 minutes after the administration of LRH. These values remained elevated at 60 minutes but returned to basal levels by either 120 or 180 minutes.

However, when the degree of responsiveness to LRH was compared among the various treatment groups, a decrease in LRH stimulated LH release was observed in those groups that received AVT. Those animals receiving the greater amount of AVT exhibited the smallest response of LRH-induced LH release. At 30 minutes, group E (10 ug AVT per day) was the only treatment group not releasing significantly more LH than group A. Groups B, C and D all released significantly ($P < 0.05$) more LH than did those in group A. A similar pattern of LH release was observed at 60 minutes. Significantly ($P < 0.05$) less LH was observed in groups C and D when compared to group B. The LH values in groups C and D returned to basal levels by 120 minutes while group E returned to basal levels at 180 minutes.

The effect of AVT on prolactin release in this experiment is summarized in Table III. No significant effect on prolactin release was

TABLE III

EFFECT OF IN VIVO ADMINISTRATION OF AVT ON PROLACTIN RELEASE IN MALE RATS

Time	GROUP A	B	C	D	E	F^2
	AVT (ug) 0	0	0.1	1	10	
	LRH (100 ng)					
0	30 ± 3[1]	30 ± 3	36 ± 7	37 ± 6	27 ± 4	0.79
30	45 ± 6	37 ± 1	35 ± 3	49 ± 8	58 ± 22	0.61
60	39 ± 2	40 ± 5	32 ± 3	45 ± 4	51 ± 9	1.60
120	35 ± 5	39 ± 6	45 ± 7	31 ± 3	44 ± 8	0.78
180	36 ± 4	31 ± 3	31 ± 7	42 ± 10	35 ± 1	0.82

[1]Expressed as ng/ml (n = 6), mean ± S.E.

[2]F values as determined by analysis of variance.

observed at time 0. In group A, the initial withdrawal of blood resulted in a slight but non-significant increase in prolactin release at 30 minutes which returned to basal levels by 60 minutes. A similar response was observed in treatment group E where a slight but non-significant increase was observed at 30, 60 and 120 minutes. No effect of LRH on prolactin release was observed in group B. In groups C, D and E AVT had no affect on prolactin release.

Pituitary Incubations

Pituitary Incubation I

The results of pituitary incubation I demonstrate the ability of LRH to release LH from HAP. This data indicates that 1 ng/ml LRH significantly released LH from anterior pituitaries taken from both intact ($P < .01$) and estrogen-progesterone primed castrate male ($P < .02$) rats (Tables IV and VI). Interestingly, LRH also significantly ($P < .05$) released prolactin from estrogen-progesterone primed castrate male rat pituitaries but not from the intact male HAP (Table IV and Table VI). In the intact male HAP, glandular concentrations of both LH and prolactin were unaffected by LRH (Table V). The LH concentration in HAP taken from estrogen-progesterone primed castrate male rats was significantly ($P < .001$) reduced (Table VII). The prolactin concentration in HAP from these rats was unaffected (Table VII).

Pituitary Incubation II

Pituitary incubation II examined the effect of logrithmic doses of AVT (0.01 ng/ml to 10 ng/ml) on the LRH (1 ng/ml) stimulated LH

TABLE IV

EFFECT OF LRH ON LH AND PROLACTIN RELEASE FROM INTACT MALE HALF ANTERIOR PITUITARIES

Treatment	LH[1]	Prolactin[1]
Control	1.98 ± 0.37	0.87 ± 0.18
LRH[2]	3.32 ± 0.52[3]	1.07 ± 0.26

[1]Expressed in ug/ml media/mg HAP, mean ± S.E. (n = 6).

[2]1 ng/ml.

[3]$P < .01$ vs. control.

TABLE V

EFFECT OF LRH ON LH AND PROLACTIN CONCENTRATIONS OF INTACT MALE HALF ANTERIOR PITUITARIES

Treatment	LH[1]	Prolactin[1]
Control	17.63 ± 4.15	0.85 ± 0.21
LRH[2]	18.09 ± 2.40	0.74 ± 0.15

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 6).

[2]1 ng/ml.

TABLE VI

EFFECT OF LRH ON LH AND PROLACTIN RELEASE FROM ESTROGEN-PROGESTERONE PRIMED CASTRATE MALE RAT HALF ANTERIOR PITUITARIES

Treatment	LH[1]	Prolactin[1]
Control	7.96 ± 1.81	0.53 ± 0.06
LRH[2]	13.82 ± 1.54[3]	0.76 ± 0.10[4]

[1]Expressed in ug/ml media/mg HAP, mean ± S.E. (n = 6).

[2]1 ng/ml.

[3]P < .02 vs. control.

[4]P < .05 vs. control.

TABLE VII

EFFECT OF LRH ON LH AND PROLACTIN CONCENTRATIONS OF ESTROGEN-PROGESTERONE PRIMED CASTRATE MALE RAT HALF ANTERIOR PITUITARIES

Treatment	LH[1]	Prolactin[1]
Control	161.99 ± 25.62	3.27 ± 0.46
LRH[2]	107.94 ± 20.46[3]	2.15 ± 0.23

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 6).

[2]1 ng/ml.

[3]P < .001 vs. control.

release and prolactin release from HAP taken from intact male rats. As indicated in Figure 2, AVT was observed to have no significant effect on LRH stimulated LH release. Similarly, AVT was found to have no significant effect on prolactin release from these HAP (Figure 3).

Table VIII depicts the corresponding LH and prolactin concentration of HAP after incubation. In the group of HAP incubated with 0.01 ng/ml AVT, a significant ($P < .05$) reduction in immunoassayable LH was observed. However, at higher concentrations of AVT no significant effect was observed although the data would indicate a similar trend. In contrast, a significant ($P < .02$) increase in prolactin concentration was observed in those glands incubated with 0.01, 0.1 and 1 ng/ml AVT (Table VIII).

Pituitary Incubation III

Results similar to those in pituitary incubation II were observed when the concentration of LRH in the incubation media was increased to 10 ng/ml. AVT was without effect on LRH stimulated LH release (Figure 4). Prolactin was also unaffected by the AVT concentrations utilized in this experiment (Figure 5).

Gland concentrations of LH after incubation were not significantly affected by the AVT treatment (although the HAP incubated with AVT all contained less LH (Table IX)). However, the prolactin concentrations of HAP incubated with 10 ng/ml AVT were significantly ($P < .02$) reduced (Table IX).

Pituitary Incubation IV

In this experiment, the effects of the removal of testicular

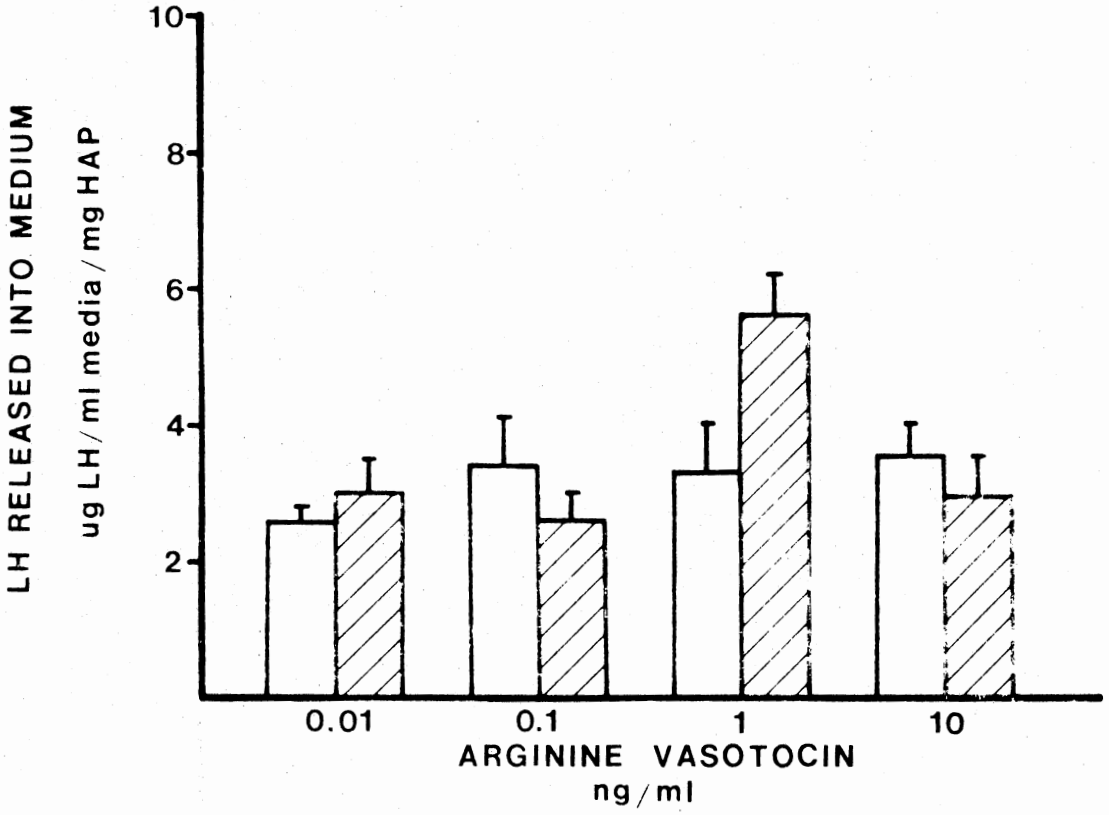

Figure 2. Effect of AVT on LRH Stimulated LH Released Into Media From Intact Male Rat Half Anterior Pituitaries. All HAP treated with 1 ng/ml LRH while cross-hatching indicates those also incubated with AVT. Expressed as mean $\pm$ S.E. (n = 10).

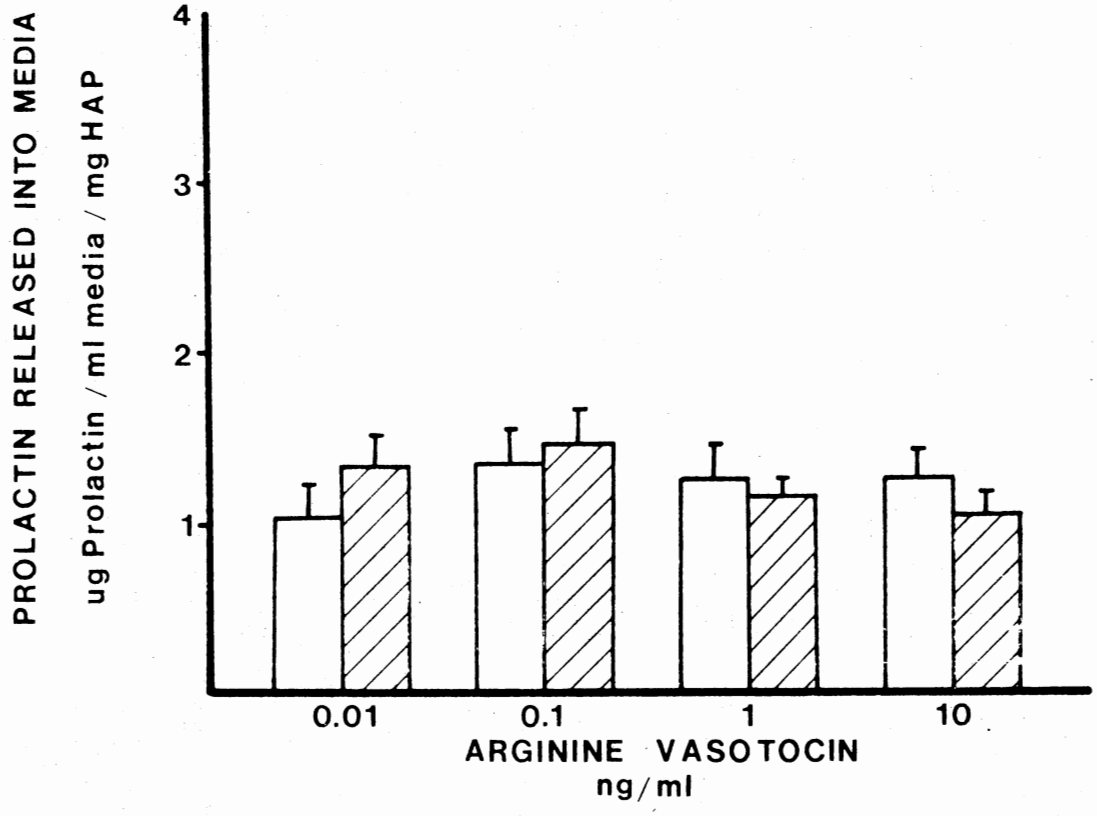

Figure 3. Effect of AVT on Prolactin Released Into Media From Intact Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean $\pm$ S.E. (n = 10).

TABLE VIII

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS OF LRH STIMULATED HALF ANTERIOR PITUITARIES FROM INTACT MALE RATS

Treatment[2]	LH[1]	Prolactin[1]
LRH	18.26 ± 2.58	0.71 ± 0.12
LRH + 0.01 ng/ml AVT	13.79 ± 1.77[3]	0.98 ± 0.08[4]
LRH	18.90 ± 4.31	0.82 ± 0.14
LRH + 0.1 ng/ml AVT	16.03 ± 2.92	1.09 ± 0.19[4]
LRH	21.37 ± 2.29	0.73 ± 0.13
LRH + 1 ng/ml AVT	19.62 ± 3.22	1.15 ± 0.09[4]
LRH	17.42 ± 2.39	0.97 ± 0.24
LRH + 10 ng/ml AVT	14.65 ± 1.90	0.90 ± 0.14

[1] Expressed in ug/mg HAP, mean ± S.E. (n = 10).

[2] All HAP treated with 1 ng/ml LRH alone or with indicated concentrations of AVT.

[3] $P < .05$ vs. corresponding control.

[4] $P < .02$ vs. corresponding control.

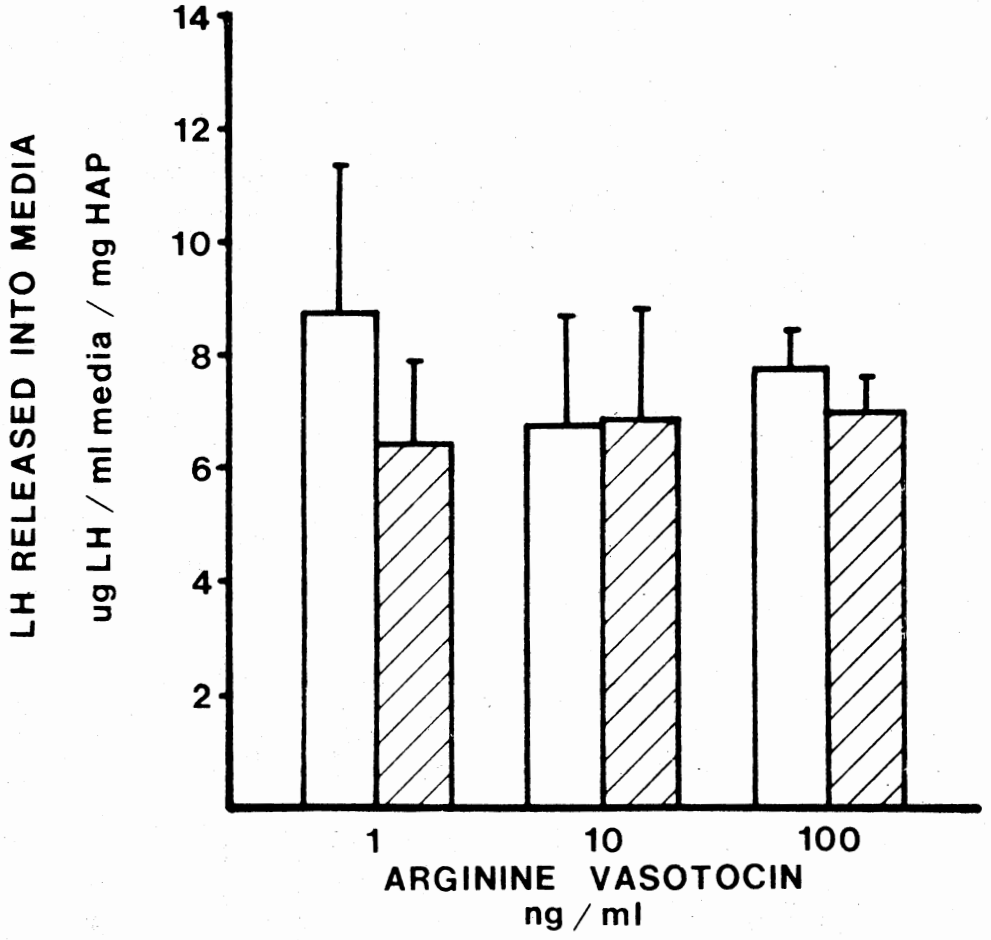

Figure 4. Effect of AVT on LRH Stimulated LH Released Into Media From Intact Male Rat Half Anterior Pituitaries. All HAP treated with 10 ng/ml LRH while cross-hatching indicates those also incubated with AVT. Expressed as mean $\pm$ S.E. (n = 6).

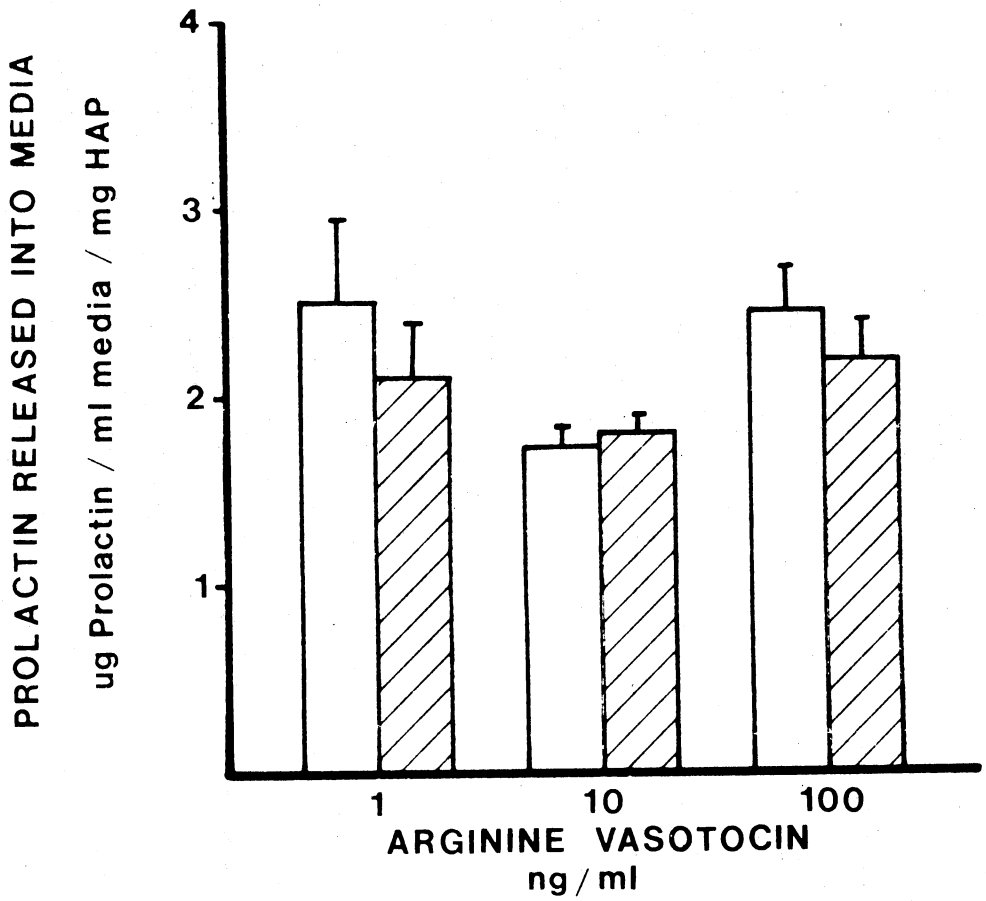

Figure 5. Effect of AVT on Prolactin Released Into Media From Intact Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean $\pm$ S.E. (n = 6).

TABLE IX

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS OF LRH STIMULATED HALF ANTERIOR PITUITARIES FROM INTACT MALE RATS

Treatment[2]	LH[1]	Prolactin[1]
LRH	24.64 ± 5.41	1.55 ± 0.28
LRH + 1 ng/ml AVT	22.52 ± 6.72	1.51 ± 0.26
LRH	20.42 ± 5.47	2.03 ± 0.09
LRH + 10 ng/ml AVT	18.67 ± 3.98	1.61 ± 0.10[3]
LRH	25.97 ± 6.12	1.46 ± 0.33
LRH + 100 ng/ml AVT	19.91 ± 3.57	1.02 ± 0.08

[1] Expressed in ug/mg HAP, mean ± S.E. (n = 6).

[2] All HAP treated with 10 ng/ml LRH alone or with the indicated concentrations of AVT.

[3] $P < .02$ vs. corresponding control.

steroids on the influence of AVT on LH and prolactin were examined. The results of this experiment were similar to those observed in the intact male rat. It was determined that AVT in log doses from 0.1 to 100 ng/ml had no effect on the LRH stimulated LH release from pituitaries obtained from castrate male rats (Figure 6). AVT was also observed to have no effect on prolactin release in this experiment (Figure 7).

In contrast, the HAP incubated with LRH and 0.1 ng/ml AVT were observed to contain significantly ($P < .01$) less LH than the corresponding halves incubated with LRH (Table X). The LRH-treated HAP receiving 1, 10 and 100 ng/ml AVT also contained less LH although this difference was not statistically significant. The HAP treated with 0.1 or 100 ng/ml AVT were also observed to contain significantly ($P < .05$ and $P < .01$, respectively) more prolactin than their corresponding control halves (Table X).

Pituitary Incubation V

In this experiment, the effect of AVT on LH and prolactin was examined in an animal model known to be sensitive to LRH. It was found that AVT in concentrations of 0.1 and 10 ng/ml was observed to significantly ($P < .01$ and $P < .05$, respectively) reduce the LRH stimulated LH release when compared to the corresponding control halves (Figure 8). A non-significant reduction of LH release was also observed at 1 and 100 ng/ml AVT. As with the previous incubations, AVT was found to have no significant effect on prolactin release in this animal model (Figure 9).

Although no statistically significant reduction in gland LH concentration was observed, the data indicates a non-significant reduction

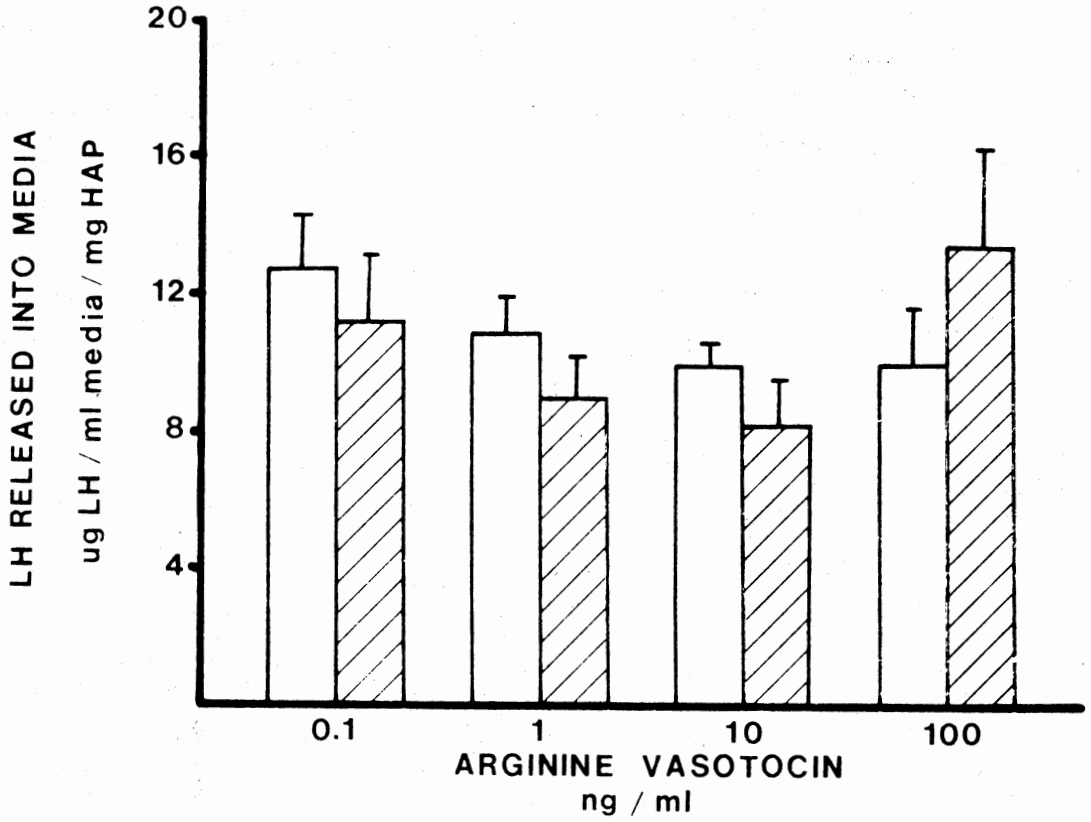

Figure 6. Effect of AVT on LRH Stimulated LH Released Into Media From Castrate Male Rat Half Anterior Pituitaries. All HAP treated with 1 ng/ml LRH while cross-hatching indicates those also incubated with AVT. Expressed as mean $\pm$ S.E. (n = 6).

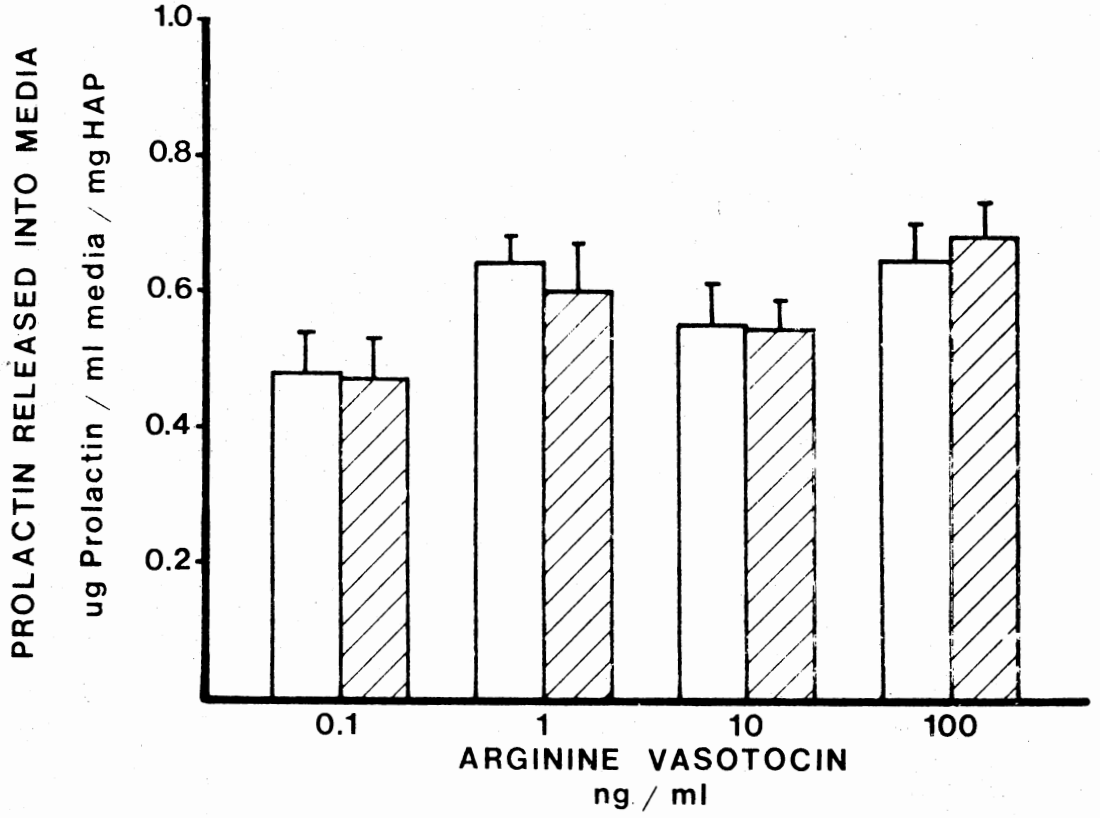

Figure 7. Effect of AVT on Prolactin Released Into Media From Castrate Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean $\pm$ S.E. (n = 6).

TABLE X

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS OF LRH STIMULATED HALF ANTERIOR PITUITARIES FROM CASTRATE MALE RATS

Treatment[2]	LH[1]	Prolactin[1]
LRH	103.97 ± 4.95	0.83 ± 0.08
LRH + 0.1 ng/ml AVT	65.92 ± 6.82[3]	1.09 ± 0.11[4]
LRH	91.91 ± 4.87	1.33 ± 0.32
LRH + 1 ng/ml AVT	87.58 ± 9.89	1.71 ± 0.32
LRH	96.60 ± 6.68	1.55 ± 0.15
LRH + 10 ng/ml AVT	79.50 ± 8.75	1.31 ± 0.17
LRH	105.82 ± 8.06	1.21 ± 0.19
LRH + 100 ng/ml AVT	97.03 ± 7.05	1.56 ± 0.21[3]

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 6).

[2]All HAP treated with 1 ng/ml LRH alone or with the indicated concentrations of AVT.

[3]$P < .01$ vs. corresponding control.

[4]$P < .05$ vs. corresponding control.

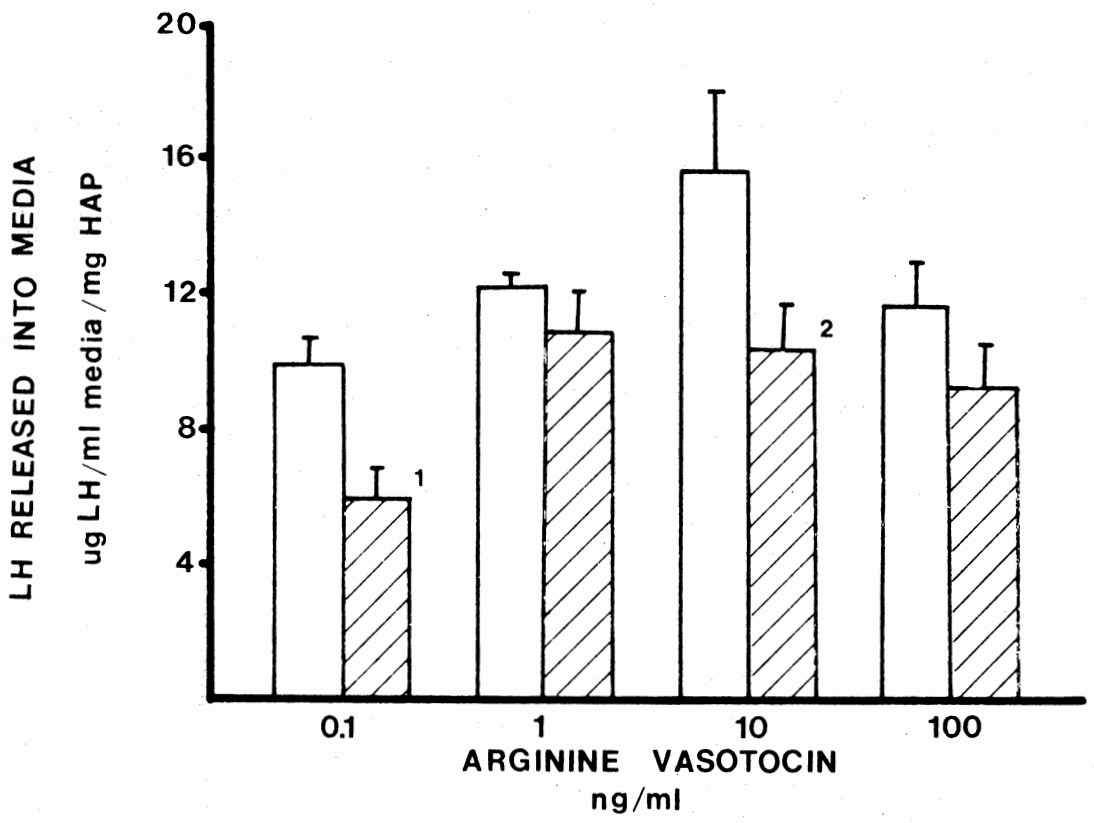

Figure 8. Effect of AVT on LRH Stimulated LH Released Into Media From Estrogen-Progesterone Primed Castrate Male Rat Half Anterior Pituitaries. All HAP treated with 1 ng/ml LRH while cross-hatching indicates those also incubated with AVT. Expressed as mean ± S.E. (n = 6).

[1] $P < .01$ vs. control.

[2] $P < .05$ vs. control.

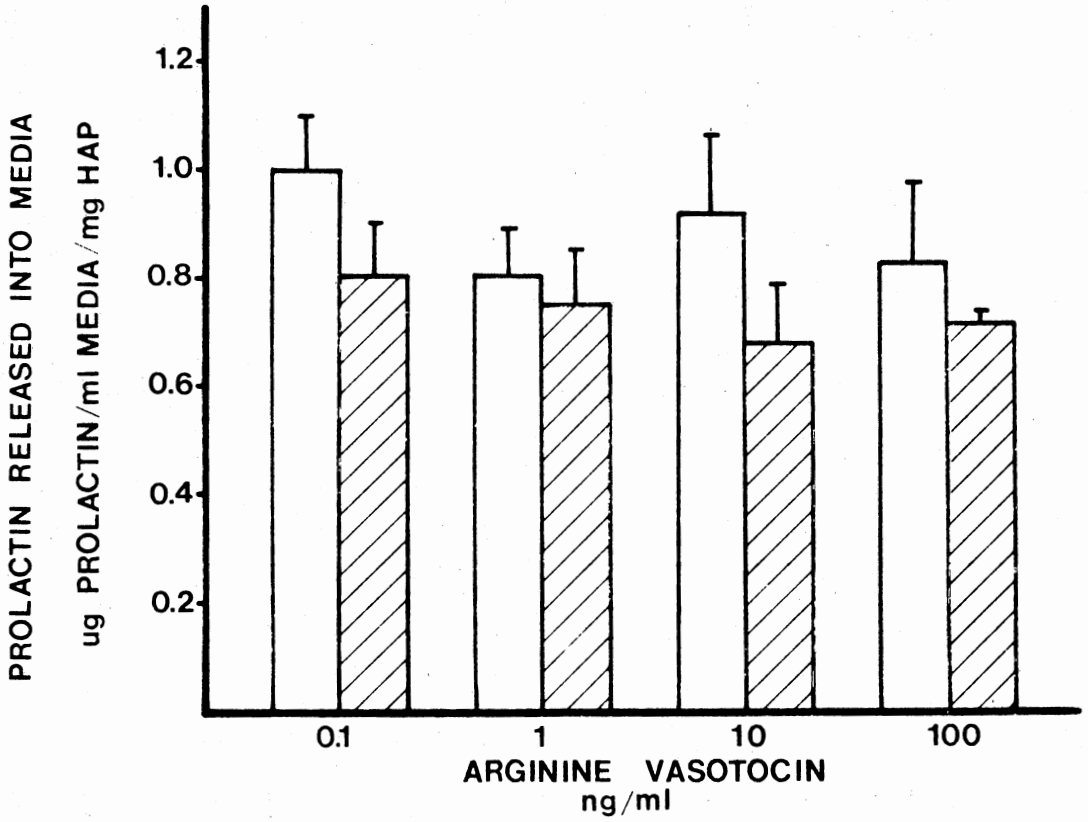

Figure 9. Effect of AVT on Prolactin Released Into Media From Estrogen-Progesterone Primed Castrate Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean $\pm$ S.E. (n = 6).

in those HAP incubated with 0.1, 1 and 10 ng/ml AVT (Table XI). An increase in prolactin concentration was observed in those glands receiving 10 and 100 ng/ml AVT with the increase being significant ($P < .01$) in those HAP receiving 100 ng/ml AVT (Table XI).

Pituitary Incubation VI

The effect of AVT on LRH stimulated LH release from intact female HAP was examined in this experiment. The results from this experiment indicate that AVT in concentrations of 10 and 100 ng/ml was capable of significantly ($P < .02$ and $P < .05$, respectively) reducing the LRH stimulated LH release (Figure 10). A non-significant reduction in LH release was also observed in those groups receiving 1 and 1000 ng/ml AVT. Again, AVT in the concentrations used in this experiment had no effect on prolactin release from the HAP of these intact female rats (Figure 11).

As with the previously described pituitary incubations, HAP incubated with LRH and AVT contained less LH than the control halves (Table XII). This was statistically significant ($P < .01$) in those HAP incubated with 1000 ng/ml AVT. Prolactin concentrations of these HAP were unaffected by the concentrations of AVT utilized in this experiment (Table XII).

Pituitary Incubation VII

The effect of AVT without LRH stimulation in the intact female rat was examined in this experiment. Figure 12 illustrates no significant effect of 1 ng/ml AVT on LH released from intact female HAP.

TABLE XI

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS OF LRH STIMULATED HALF ANTERIOR PITUITARIES FROM ESTROGEN-PROGESTERONE PRIMED CASTRATE MALE RATS

Treatment[2]	LH[1]	Prolactin[1]
LRH	177.46 ± 25.29	3.91 ± 0.26
LRH + 0.1 ng/ml AVT	135.13 ± 4.93	3.75 ± 0.31
LRH	151.70 ± 26.11	4.23 ± 0.56
LRH + 1 ng/ml AVT	140.43 ± 10.31	4.27 ± 0.62
LRH	147.45 ± 12.08	3.50 ± 0.51
LRH + 10 ng/ml AVT	135.69 ± 15.98	4.13 ± 0.54
LRH	162.34 ± 23.08	2.72 ± 0.27
LRH + 100 ng/ml AVT	161.49 ± 22.16	3.72 ± 0.26[3]

[1] Expressed in ug/mg HAP, mean ± S.E. (n = 6).

[2] All HAP treated with 1 ng/ml LRH alone or with the indicated concentrations of AVT.

[3] $P < .01$ vs. corresponding control.

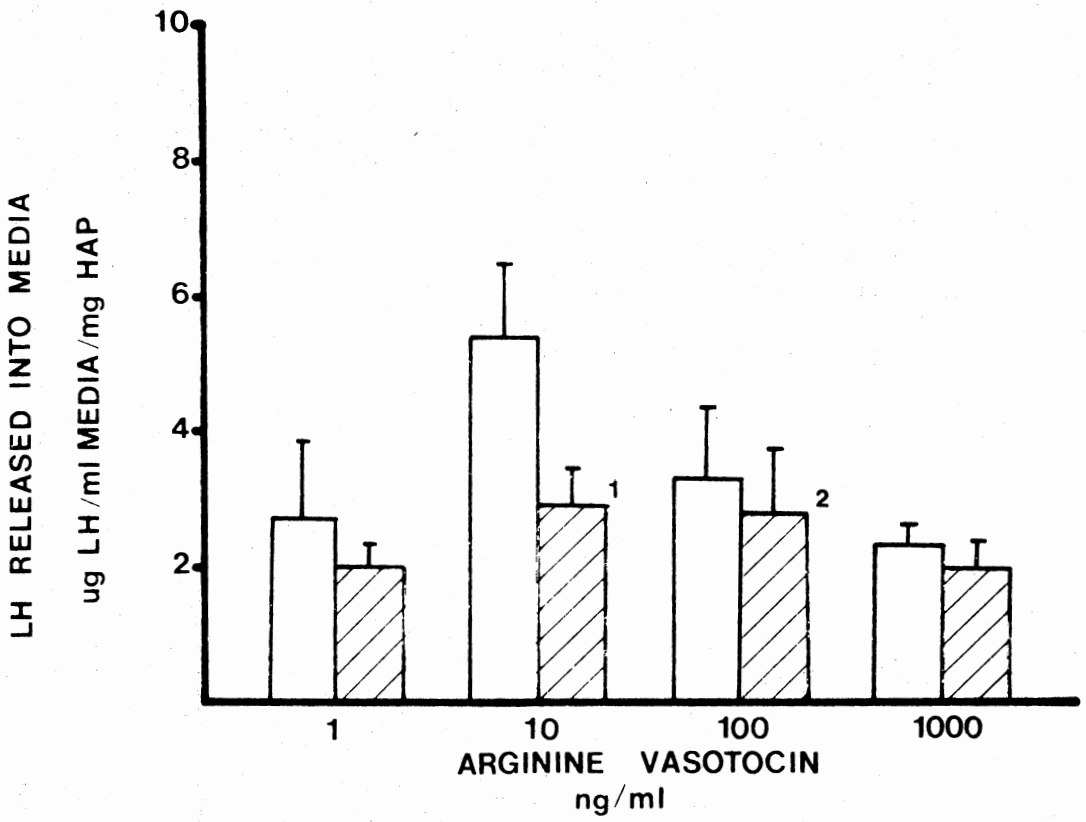

Figure 10. Effect of AVT on LRH Stimulated LH Released Into Media From Intact Female Rat Half Anterior Pituitaries. All HAP treated with 1 ng/ml LRH while cross-hatching indicates those also incubated with AVT. Expressed as mean ± S.E. (n = 5).

[1] $P < .02$ vs. control.

[2] $P < .05$ vs. control.

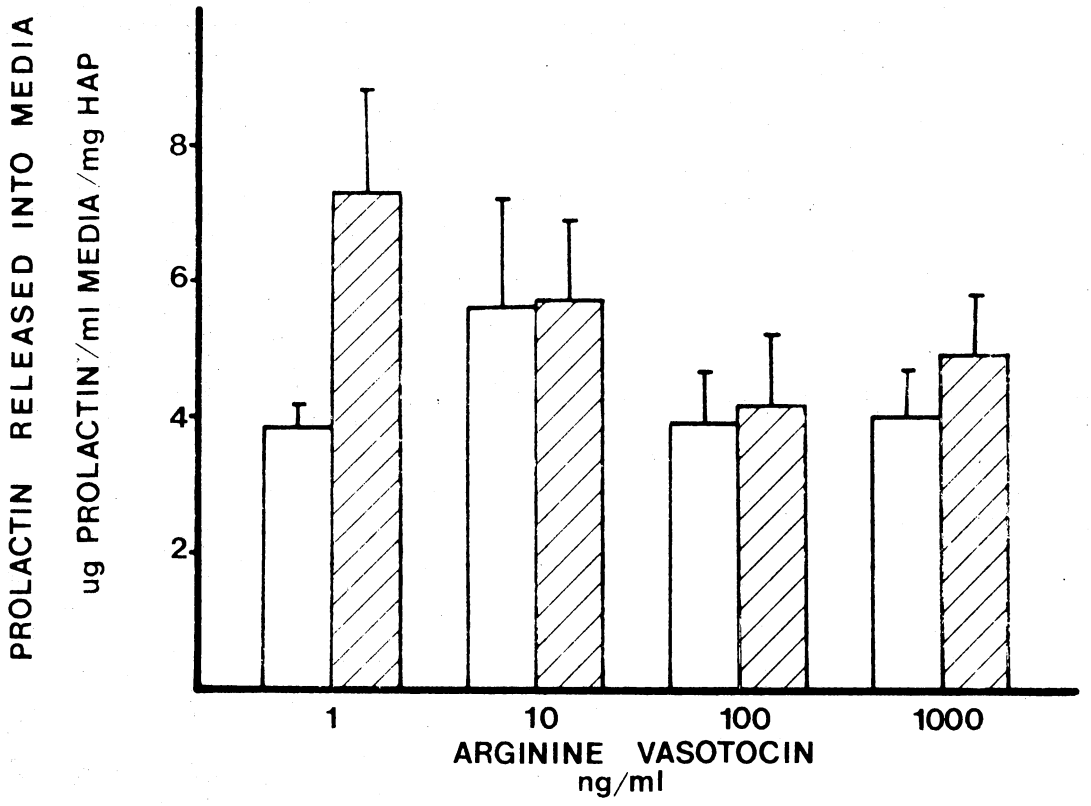

Figure 11. Effect of AVT on Prolactin Released Into Media From Intact Female Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean $\pm$ S.E. (n = 5).

TABLE XII

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS
OF LRH STIMULATED HALF ANTERIOR PITUITARIES
FROM INTACT FEMALE RATS

Treatment[2]	LH[1]	Prolactin[1]
LRH	9.60 ± 1.71	2.09 ± 0.21
LRH + 1 ng/ml AVT	7.59 ± 1.09	1.93 ± 0.14
LRH	8.71 ± 1.63	1.63 ± 0.10
LRH + 10 ng/ml AVT	8.46 ± 1.37	1.50 ± 0.05
LRH	8.07 ± 1.72	1.98 ± 0.28
LRH + 100 ng/ml AVT	7.00 ± 1.57	2.14 ± 0.33
LRH	9.26 ± 2.25	1.51 ± 0.14
LRH + 1000 ng/ml AVT	5.50 ± 1.48[3]	1.60 ± 0.09

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 5).

[2]All HAP treated with 1 ng/ml LRH alone or with the indicated concentrations of AVT.

[3]$P < .01$ vs. corresponding control.

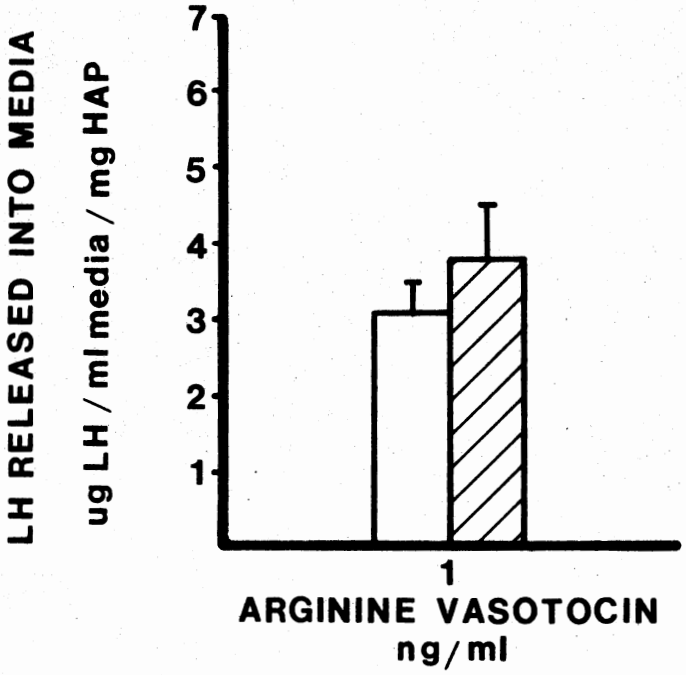

Figure 12. Effect of AVT on LH Released Into Media From Intact Female Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean ± S.E. (n = 10).

Prolactin release was also unaffected by the concentration of AVT used in this experiment (Figure 13).

Similarly, AVT did not significantly effect either the LH or prolactin concentrations of these HAP when compared to their corresponding control halves (Table XIII).

Pituitary Incubation VIII

In an attempt to determine whether the effects of AVT were specific, the effects of AVP on LH release in both non-stimulated and LRH stimulated intact male HAP were examined. As depicted in Figure 14, no significant effect of 1 ng/ml AVP was observed on LH release from AVP treated halves when compared to non-treated control halves. AVP was also found to be without effect on prolactin release in these HAP (Figure 15). Similarly, AVP (1 ng/ml) had no effect on the LRH stimulated LH release from these rats (Figure 16). However, AVP was found to significantly ($P < .02$) inhibit the release of prolactin from the HAP incubated with LRH (Figure 17).

The concentrations of LH and prolactin in intact male HAP incubated with AVP reveal that while AVP had no effect on LH concentration, prolactin was significantly ($P < .01$) reduced in those HAP incubated with AVP (Table XIV). However, when those glands were incubated with LRH and AVP no significant effect on LH or prolactin concentrations was observed (Table XV).

Pituitary Homogenate Uptake of ^{125}I-LRH

The ability of anterior pituitary homogenates obtained from intact male rat pituitaries to bind LRH is depicted in Figure 18. LRH in

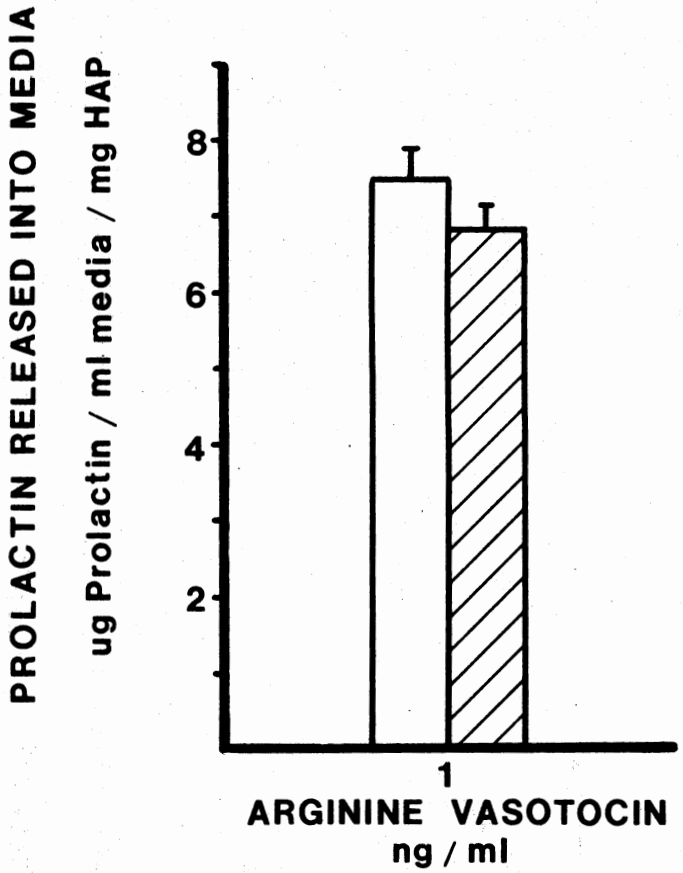

Figure 13. Effect of AVT on Prolactin Released Into Media From Intact Female Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVT. Expressed as mean ± S.E. (n = 10).

TABLE XIII

EFFECT OF AVT ON LH AND PROLACTIN CONCENTRATIONS OF HALF ANTERIOR PITUITARIES FROM INTACT FEMALE RATS

Treatment[2]	LH[1]	Prolactin[1]
Control	31.35 ± 8.40	2.35 ± 0.40
AVT[2]	26.09 ± 7.56	2.42 ± 0.42

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 10).

[2]1 ng/ml.

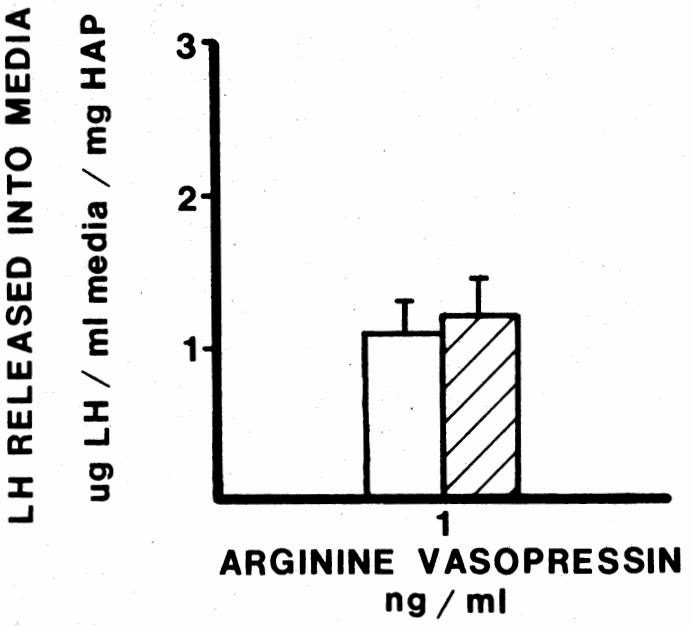

Figure 14. Effect of AVP on LH Released Into Media From Intact Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVP. Expressed as mean ± S.E. (n = 10).

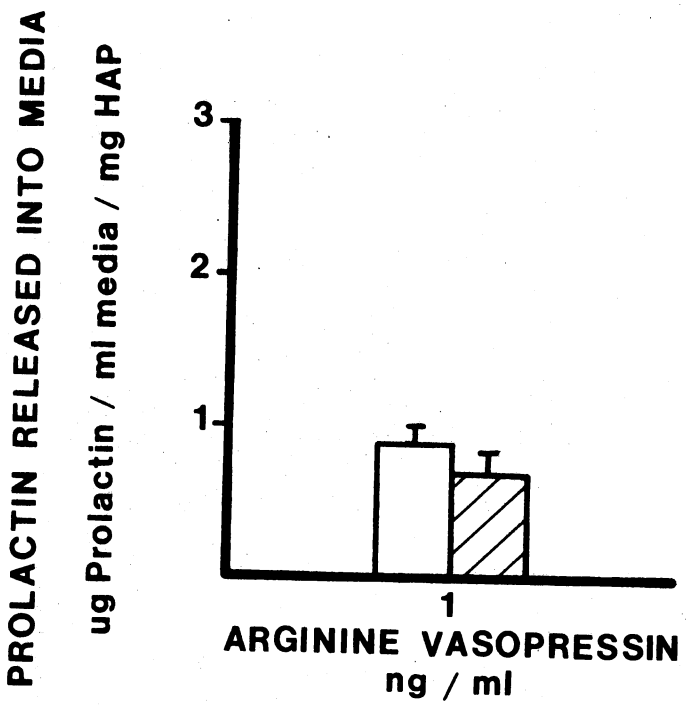

Figure 15. Effect of AVP on Prolactin Released Into Media From Intact Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVP. Expressed as mean ± S.E. (n = 10).

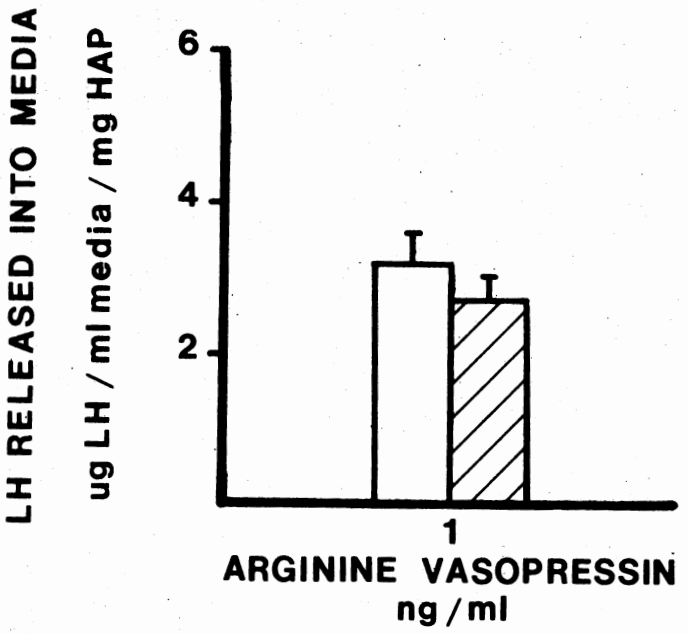

Figure 16. Effect of AVP on LRH Stimulated LH Released Into Media From Intact Male Rat Half Anterior Pituitaries. All HAP incubated with 1 ng/ml LRH while cross-hatching indicates those also incubated with AVP. Expressed as mean ± S.E. (n = 10).

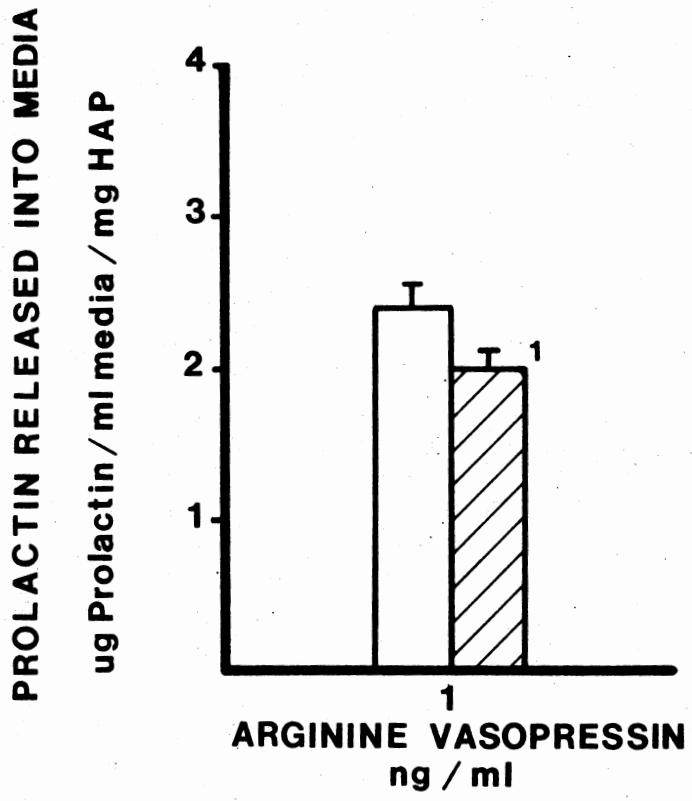

Figure 17. Effect of AVP on Prolactin Released Into Media From Intact Male Rat Half Anterior Pituitaries. Cross-hatching indicates those HAP incubated with AVP. Expressed as mean ± S.E. (n = 10).

[1] $P < .02$ vs. control.

TABLE XIV

EFFECT OF AVP ON LH AND PROLACTIN CONCENTRATIONS
OF HALF ANTERIOR PITUITARIES
FROM INTACT MALE RATS

Treatment	LH[1]	Prolactin[1]
Control	24.34 ± 4.03	4.44 ± 0.38
AVP[2]	25.02 ± 3.15	3.32 ± 0.30[3]

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 10).

[2]1 ng/ml.

[3]$P < .01$ vs. control.

TABLE XV

EFFECT OF AVP ON LH AND PROLACTIN CONCENTRATIONS OF LRH STIMULATED HALF ANTERIOR PITUITARIES FROM INTACT MALE RATS

Treatment	LH[1]	Prolactin[1]
LRH[2]	27.52 ± 6.70	1.83 ± 0.24
LRH[2] + 1 ng/ml AVP	35.54 ± 6.09	1.47 ± 0.31

[1]Expressed in ug/mg HAP, mean ± S.E. (n = 10).

[2]1 ng/ml.

concentrations of 10 and 50 nM was found to significantly ($P < .01$ and $P < .05$) displace ^{125}I-LRH from the anterior pituitary homogenates. Maximal displacement was found to occur at 100 nM LRH with no additional displacement observed at 1000 nM LRH. Figure 19 demonstrates the inability of AVT to displace the ^{125}I-LRH from the pituitary homogenate. Only at very high concentrations was AVT found to be able to non-significantly displace the ^{125}I-LRH from the pituitary tissue.

The ability of LRH to displace ^{125}I-LRH from pituitary tissue obtained from estrogen-progesterone primed castrate male rats is depicted in Figure 20. All results were analyzed using the one-way analysis of variance and the student t-test. In contrast to those results observed in the intact male pituitary homogenate, AVT was found to biphasically facilitate the binding of ^{125}I-LRH to the pituitary tissue (Figure 21). This increased binding was found to be significant ($P < .01$) at 50 nM AVT. However, the addition of 100 and 1000 nM AVT was found to no longer enhance the binding of ^{125}I-LRH. These values were not significantly different from those homogenates receiving only the ^{125}I-LRH.

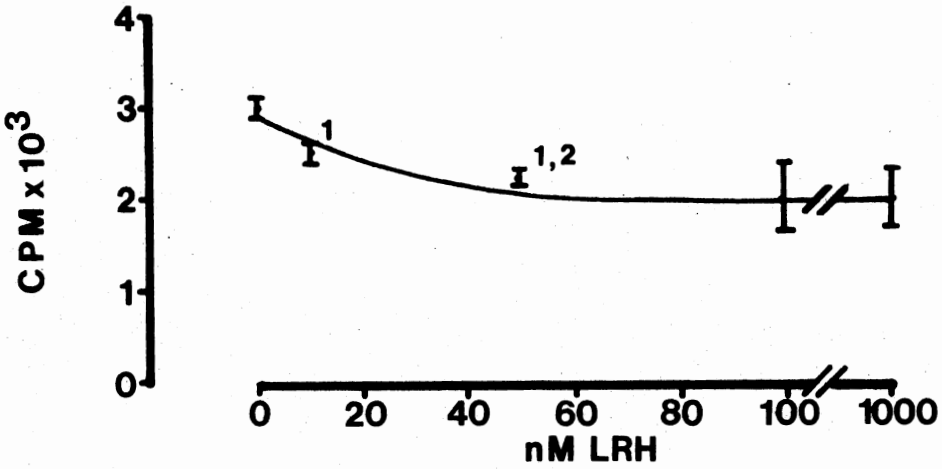

Figure 18. Displacement of ^{125}I-LRH From Intact Male Rat Pituitary Homogenate by LRH. Expressed as mean ± S.E. (n = 3).

$^{1}P < .01$ vs. 0 nM LRH

$^{2}P < .05$ vs. 10 nM LRH

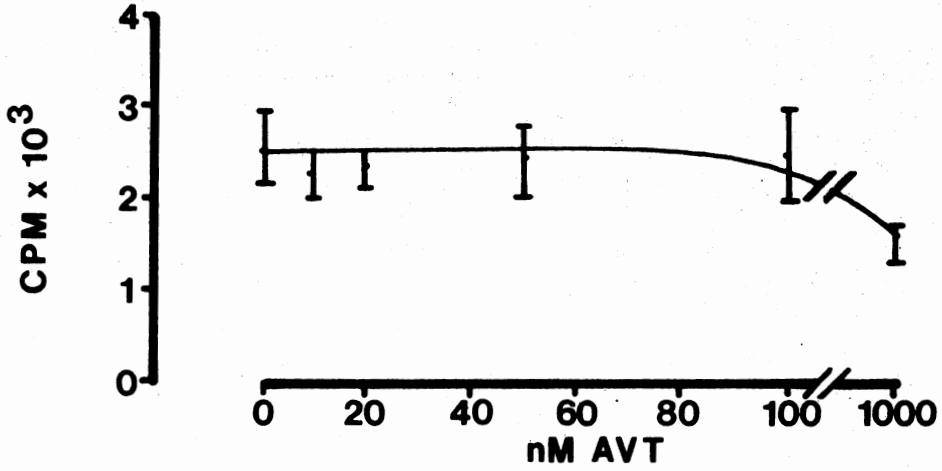

Figure 19. Displacement of ^{125}I-LRH From Intact Male Rat Pituitary Homogenate by AVT. Expressed as mean ± S.E. (n = 3).

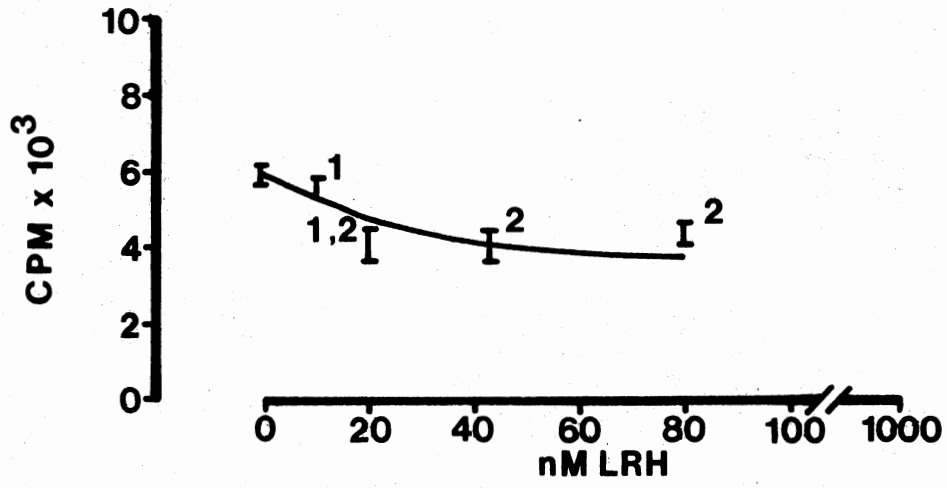

Figure 20. Displacement of ^{125}I-LRH From Estrogen-Progesterone Primed Castrate Male Rat Pituitary Homogenate by LRH. Expressed as mean ± S.E. (n = 3).

$^{1}P < .05$ vs. 0 nM LRH

$^{2}P < .01$ vs. 10 nM LRH

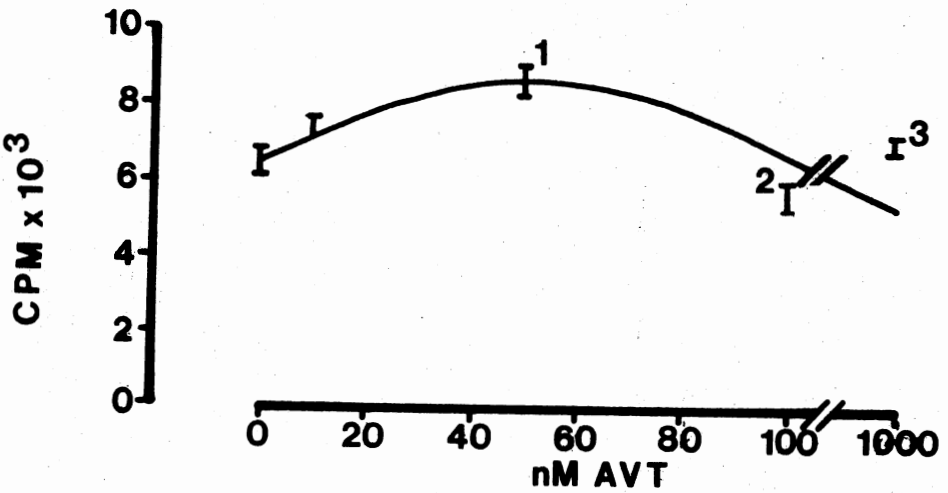

Figure 21. Displacement of ^{125}I-LRH From Estrogen-Progesterone Primed Castrate Male Rat Pituitary Homogenate by AVT. Expressed as mean ± S.E. (n = 3).

$^{1}P < .01$ vs. 0 nM AVT $^{2}P < .01$ vs. 50 nM AVT

$^{3}P < .02$ vs. 50 nM AVT

CHAPTER V

DISCUSSION

Much confusion exists concerning the site(s) of action of the antireproductive properties of arginine vasotocin. Some investigators have suggested that AVT exerts its antireproductive properties on the pituitary gland (Pavel et al., 1973c; Vaughan et al., 1975b) while others have suggested an effect on the gonads (Pavel and Petrescu, 1966; Moszkowska and Ebels, 1968; Johnson et al., 1978). Discrepancies exist between those investigating the influence of AVT on the pituitary (Vaughan et al., 1975b; Demoulin et al., 1977). This confusion may in part be due to different steroid environments in animal models used in the various experiments. This work examined the effect of AVT on LRH stimulated LH release and prolactin release from the pituitary of the adult rat. These parameters were examined under various steroid environments offered by several different animal models.

In Vivo Administration of Peptide

An attempt to determine the effect of chronic AVT treatment on the pituitary's responsiveness to LRH in male rats was undertaken and indicated that the LRH-induced rise of serum LH was modulated by this pineal peptide. LRH administration caused an increase in serum LH concentrations in all groups of animals within 30 minutes of administration. In most groups LH concentration returned to basal

levels by 120 minutes. This type of a response to an LRH stimulus is in agreement with Debeljuk et al. (1974) who found a significant increase in LH within 20 minutes of the intravenous administration of the peptide in intact male rats. Greeley et al. (1978) also observed a significant increase in LH after LRH administration in intact male rats. They found the LH levels in most rats were returning to basal levels within 80 minutes. This is also in agreement with the response observed in this experiment.

However, in rats receiving increasing concentrations of AVT, a decrease in LH released in the response to the challenge of LRH was observed. The 30 minute response in those rats receiving 0.1 or 1.0 ug AVT per day was significantly ($P < .05$) greater than those rats receiving neither AVT nor LRH while the response in the group receiving 10 ug AVT per day was not significantly greater than those which received neither AVT nor LRH. This was interpreted as indicating that 10 ug AVT per day reduced the pituitary's sensitivity to LRH so that LRH could no longer stimulate a significant release of LH over the control rats. While the 30 and 60 minute LH levels in the groups receiving 0.1 and 1.0 ug AVT were lower than those not receiving AVT, this reduction was significant ($P < .05$) only at the 60 minutes post-injection period. All values had returned to base levels by either 120 or 180 minutes.

This decrease in the pituitary's sensitivity to LRH may be explained by a direct or indirect effect on the LRH receptor. It is possible that AVT was competing for and occupying the LRH receptors in such a way that LRH could not bind to its receptors or once LRH was bound to its receptor site it could not elicit a biological

response. Several synthetic inhibitory analogues of LRH, including desHis^{2}-D-Leu^{6}-LRH, D-Phe^{2}-D-Leu^{6}-LRH and D-Phe^{2}-D-Phe^{6}-LRH have been observed to inhibit LH release for 4 hours after administration in the immature rat (Schally et al., 1976). As much as 500 ug was required to elicit this response. Thus, it is possible to inhibit LH release with inhibitory analogues that are believed to compete with LRH for LRH binding sites on the gonadotrope plasma membranes (Spona, 1974a). Inasmuch as LRH and AVT contain the same C-terminal amino acid content it may be that AVT acts as an inhibitory analogue of LRH. This type of action of AVT would have required a gradual release of AVT from the subcutaneous injections each day since large concentrations of synthetic analogues are required over a shorter period of time than that utilized in the present study. The rate of absorption from these injections was not measured.

As previously mentioned, a second possibility is that AVT directly affects either the LRH receptor or the cellular mechanism of action of LRH in such a way to render LRH incapable of eliciting a biological response. It is interesting to note that Cheesman et al. (1977a, 1977b) found that the intravenous and intraventricular administration of AVT will result in a suppression of the preovulatory surge of LH. It is possible that AVT was affecting the action of endogenous LRH on the pituitary although they concluded otherwise since the AVT failed to affect the LH levels in either diestrous or ovariectomized female rats. However, they failed to account for the differing steroid environments that exist in each of these animal models.

Another possible explanation for the antigonadotropic action of

AVT may be a direct effect of the pineal peptide on the testis and thereby indirectly affecting the response of the pituitary to LRH. By affecting steroid synthesis, AVT could alter the ratio of testosterone to estrogen and progesterone. It has been shown that in 2-month old castrate rats a combination of testosterone (1.5 mg) and 17B-estradiol (0.05 mg) given subcutaneously in oil resulted 48 hours later in a decreased LH responsiveness to 100 ng LRH given intravenously (Debeljuk et al., 1974). The same laboratory has also shown that low doses of estradiol benzoate will in itself depress the pituitary's response to LRH in the intact male rat (Debeljuk et al., 1972). Kulkarni et al. (1977) also reported that testosterone proprionate in combination with estrogen suppressed the facilatory affect of estradiol-17B on the LH response to LRH in a dose-related manner. Assuming AVT affects steroid synthesis, data has been reported that may explain the pituitary's altered responsiveness to LRH.

The results in this work are of particular interest as Milcu and colleagues (1975) reported that daily injections of a bovine pineal polypeptide for 3 or 6 days to intact male rats resulted in a decrease of testosterone both in serum and testicular tissue. The extraction procedure used to isolate this peptide was identical to that used by Milcu et al. (1963) when AVT was first tentatively identified in the bovine pineal gland. These results were interpreted as meaning that the pineal gland plays an inhibitory role at the level of testosterone synthesis.

Based on these observations it is possible the daily administration of AVT decreased testosterone, thus altering the testosterone to estrogen and progesterone ratios in such a way that a combination of

these steroids rendered the pituitary less responsive to exogenous LRH. A change in LH concentrations at time 0 might have been expected as a result of the altered steroid environment. In the group receiving 0.1 ug AVT a slight but significant ($P < .05$) decrease in pre-injection LH was observed when compared to controls. However, according to a recent study by Steinberger et al. (1977), changes in plasma steroids such as a decrease in testosterone induced by chronic administration of estradiol benzoate need not result in a significant change in plasma LH levels. Therefore a change in the concentration of circulating steroids might have occurred during AVT treatment without affecting a large change in plasma LH levels. An effect of AVT on gonadal steroids is also suggested by the reports of Vaughan et al. (1976d). Acute intravenous administration of AVT to estrogen-progesterone primed male rats resulted in no effect on serum LH concentrations, thus suggesting the necessity of chronic AVT treatment and LRH stimulation. These results together with the results of the present study suggest the effect of AVT in this experiment may have been directed toward gonadal steroid biosynthesis.

The daily administration of AVT had no significant effect on prolactin release in this experiment (Table III). This is at variance with the data of Vaughan et al. (1976d) who reported that AVT will release prolactin in a dose dependent fashion. However, major differences in experimental design may explain the difference in the results. They used an acute preparation involving the intravenous administration of AVT to either intact or pinealectomized, estrogen-progesterone primed male rats. Blood was collected for only 20 minutes after the administration of AVT. It was determined that prolactin levels in

most instances had peaked at 10 minutes and were returning to basal levels by 20 minutes. Other important differences include the use of a different route of administration of AVT and the use of a different animal model. These observations imply the effect of AVT on prolactin release is very short lived. Therefore, it is not surprising that AVT was observed to have no effect on prolactin release in this work. However, Cheesman et al. (1977b) found that the intravenous administration of AVT resulted in a suppression in the proestrous surge of prolactin. Differences in experimental design and animal models may also account for the discrepancies between this study and the results obtained by Cheesman and colleagues.

In Vitro Experiments

One of the major advantages of any in vitro techniques is the ability to control many of the variables found in vivo. In an attempt to more closely control the steroid environment, a modification of the in vitro pituitary incubation technique of Schally et al. (1972) and Mittler and Meites (1966) was used for part of the in vitro experiments in this study. Using this technique, one HAP served as a control while its corresponding half served as the experimental tissue.

Perhaps the major disadvantage with this technique is the potential for cellular degeneration to occur during the incubation period. In an attempt to determine the degree of degeneration occurring, GOT levels were measured in the media of incubated pituitaries. There was linear accumulation of GOT over the 6 hours incubation period, which would suggest a relatively constant release of GOT from the HAP and would argue against an increase in cellular degeneration

had not occurred although the assumption has to be made that a small degree of degeneration was occurring in those glands incubated with oxygen.

Light microscopic examination supported the conclusions drawn from the enzyme determinations. Those HAP incubated for 6 hours with oxygen contained significantly ($P < .001$) more pyknotic nuclei than fresh tissue. However, pituitaries incubated for 6 hours without oxygen also contained significantly ($P < .001$) more pyknotic nuclei than the fresh glands or those incubated for 6 hours with oxygen. A 15 fold increase in pyknotic nuclei was observed in those glands incubated without oxygen when composed to those incubated with oxygen. An increase in loose connective tissue was also observed in those glands incubated without oxygen. These results along with the enzyme determinations may be interpreted as indicating a slow cellular degeneration was occurring over the 6 hour incubation.

The functional status of the pituitary incubation technique was demonstrated by the ability of LRH to stimulate LH release in both the intact and estrogen-progesterone primed castrate male rat. In both models, 1 ng/ml LRH was found to significantly ($P < .01$ and $P < .02$, respectively) stimulate LH release into the media. This action of LRH is in agreement with accepted dogma (Schally et al., 1972; Schally et al., 1973). Using this technique, LRH was found to have no effect on prolactin release from HAP obtained from intact male rats. However, LRH was found to significantly ($P < .05$) release prolactin from the estrogen-progesterone primed castrate male rat HAP. This is believed to be the first report of such activity.

Incubation of HAP with LRH was found to have no effect on gland concentrations of either LH or prolactin in the intact male rat HAP. The gland concentration of LH in LRH treated HAP from estrogen-progesterone primed castrate male rat pituitaries was significantly ($P < .001$) less than control halves. No effect was observed on prolactin gland concentration. These differences in LH concentrations may be explained both by an increase in synthesis of LH and an increase in sensitivity of the pituitary to LRH stimulation as a result of the estrogen-progesterone prime. Present doctrine suggests that estrogen exerts a dual effect on LH. It is believed that short term administration of estrogen results in a decrease in the pituitary's ability to release LH while long term administration results in both an increase in LH synthesis and increased responsiveness of the pituitary to LRH stimulation (Cooper et al., 1974; Vilchez-Martinez et al., 1974). The data just described agree with accepted dogma concerning long term administration of estrogen.

The effect of AVT on LRH stimulated LH release in HAP obtained from intact male rats was examined in pituitary incubations II and III. No significant effect of various concentrations of AVT with either 1 or 10 ng/ml LRH on LH release was observed in these incubations. This would indicate that the steroid environment of the intact male was not conducive for AVT to exhibit antigonadotropic properties. This is in agreement with the report of Demoulin et al. (1977) in which AVT was found to have no effect on either basal LH secretion or LRH stimulated LH release from cultured pituitary cells obtained from intact male rats. However, this is in disagreement with Vaughan et al. (1975b) who reported that AVT was capable of releasing LH from HAP of intact

male rats. However these HAP in the latter study were not LRH stimulated and were incubated for only 3 hours. It is possible that either or both of these differences in experimental design may have resulted in the observations to be at variance with each other.

AVT was also observed to have no effect on prolactin secretion in pituitary incubation II and III (Figures 3 and 5). Again, this is in disagreement with the results of Vaughan and colleagues (1975b). They reported that various concentrations of AVT (100 ng/ml to 10 ug) promoted prolactin release from HAP in a dose related fashion. In pituitary incubation II, the concentrations of AVT used were log doses from 0.01 to 10 ng/ml while in pituitary incubation III log doses from 1 to 100 ng/ml AVT were used. Therefore, the concentrations of AVT utilized in this work were, for the most part, less than those used by others. This might explain the difference in results observed.

Removal of testicular steroids by castration also resulted in no significant effect of AVT on either LRH stimulated LH release (Figure 6) or on prolactin release from incubated pituitaries (Figure 7). However, replacement of the testicular steroids with estrogen and progesterone in the animals resulted in a decrease in the amount of LRH stimulate LH released into the media by those HAP incubated with AVT (Figure 8). This inhibition was significant ($P < .02$ and $P < .05$, respectively) with 0.1 and 10 ng/ml AVT. Prolactin release was not significantly affected in the pituitaries obtained from these rats (Figure 9).

These results indicate that estrogen and/or progesterone are required for AVT to exhibit an antigonadotropic action. It has been

reported that the effects of estrogen on LH appears to be dependent on several factors. The time course of administration of the steroid is important as a biphasic effect is observed (Jackson, 1973; Libertum et al., 1974; Apfelbaum and Taleisnik, 1976). It appears that short term administration of estrogen results in a decreased sensitivity of the pituitary to LRH while long term administration results in an enhanced sensitivity to LRH. Liu and Jackson (1977) have also reported that in vitro a) estrogen and LRH stimulated the incorporation of radioactive glucosamine into LH while having no effect on radioactive amino acid incorporation into LH; b) estrogen and LRH stimulate the release of newly synthesized LH (synthesis involving both protein and carbohydrate elongation); c) both estrogen and LRH act synergistically to enhance radioactive incorporation of glucosamine into LH while only estrogen enhances glucosamine uptake and incorporation into total protein, and; d) estrogen potentiates the effects of LRH on the release of immunoreactive LH and of glucosamine and amino acid labeled LH.

Little has been reported concerning the effects of progesterone on LRH stimulated LH release. Howland (1976) found that progesterone potentiates the LRH stimulated LH release in ovariectomized testosterone propionate treated female rats. Debeljuk et al. (1974) reported that in the intact rat 17-a-hydroxyprogesterone and pregnandiol did not modify the pituitary's response to LRH. However, in the castrate male rat 20-a-dihydroprogesterone or pregnandione slightly inhibited the LRH stimulated LH release. When progesterone was administered with estradiol an augmentation of the pituitaries sensitivity to LRH was observed (Campbell and Schwartz, 1977).

Thus the action of AVT might involve a decrease in the estrogen induced incorporation of glucosamine into LH. This would necessitate LH synthesis involving the incorporation of carbohydrate moieties on the polypeptide chain to occur during the incubation. However, LH synthesis from amino acids precursors would not have to be occurring. Thus, AVT could be interfering with the final steps of LH biosynthesis. Another possible explanation concerning the antigonadtropic activity of AVT may involve interference with the potentiation that exists between estrogen and LRH concerning the release of LH. Closely related is the suggestion by Hoff et al. (1977) that "two pools" of gonadotropins exist in women. Pool 1 consists of releasable LH while pool 2 is involved with the synthesis and storage of LH. They suggest that estrogen exerts a positive effect on pool 2 while exerting a negative effect on pool 1. They further hypothesize that estrogen potentiates the LRH stimulated activation of pool 2 to pool 1. De Koning et al. (1976) also reported the existence of a readily releasable pool of LH and a second pool that requires the LRH induced synthesis of a protein before the pool could be released. They determined this new protein not to be newly synthesized LH. It is possible that AVT may interfere with this conversion of pool 2 to pool 1.

Perhaps closely related to the synergism or potentiation that exists between estrogen and LRH is yet another possible explanation for the antigonadotropic activity of AVT. It is possible that AVT could be interfering with LRH binding to its receptors. This would necessitate the existence of at least two different receptor types. One of these receptor types would need to be found predominantly in the estrogen-progesterone primed castrate male rat and not found in any great

quantity in either the intact or castrate rat. Spona (1974b) has described the existence of high affinity-low capacity and low affinity-high capacity receptors. He further reported that a pituitary binding site for LRH is lost upon ovariectomy and may be restored upon treatment with steroids. These receptors were apparently lost by 30 days after ovariectomy and are classified as high affinity-low capacity receptors. It was also reported that pituitaries from intact male rats also have two distinct receptor types (Spona, 1975). However, he concluded that the receptors found in the male were different from those found in the female. It is interesting to note that when a castrate male rat was primed with estradiol benzoate, the high affinity receptors disappeared leaving only the low affinity receptors. These low affinity receptors had the same affinity constant as the high affinity receptors of the female which were lost upon ovariectomy and reappeared with estrogen-progesterone replacement.

If these purported high affinity receptors are involved in the observed effects of AVT on LH release then the release of LH from the anterior pituitary of the intact female rat should be affected similar to that of the estrogen-progesterone primed castrate male rat. Indeed, when anterior pituitaries from intact female rats were challenged with LRH, 10 and 100 ng/ml AVT significantly ($P < .02$ and $P < .05$, respectively) inhibited the LRH stimulated LH release from these glands (Figure 10). AVT was found to have no effect on prolactin secretion from HAP obtained from these rats (Figure 11).

These data would add further support to the hypothesis that estrogen or progesterone or a combination of these steroids are necessary for AVT to exert an antigonadotropic action. As the rats utilized in

this experiment were females at various stages of estrous cycle, no statement can be made concerning the circulating levels of steroids other than the assumption that all rats should have experienced a surge of steroids within several days of sacrifice. The HAP incubated with AVT and LRH in all the incubations discussed contained less immunoassayable LH than the LRH treated control halves (Tables VIII, IX, X, XI and XII). Although usually not statistically significant, this reduction in LH nevertheless occurred in every treatment group regardless of the steroid environment. This observation suggests that AVT could be affecting the synthesis of LH in vitro. This reduction in synthesis need not involve the synthesis of LH from amino acid precursors but may involve the association of the a- and B-subunits or the addition of the carbohydrate moieties to the protein molecule which may not depend on the steroid environment of the rat. This further supports the hypothesis that the inhibition of LRH stimulated LH release observed in those rats exposed to estrogen and progesterone was due to a reduction of the facilitory action of estrogen on the LRH stimulated LH release. The facilitory action may occur at the LRH receptors or at some other step in the LH releasing mechanism.

The prolactin concentrations of these HAP were elevated in most groups receiving AVT (Tables VIII, X, and XI) except those intact male rats in pituitary incubation III (Table IX). In pituitary incubation II, significant ($P < .02$) elevation in glandular prolactin was observed after treatment with 0.01, 0.1 and 1.0 ng/ml AVT (Table VIII). In pituitary incubation IV, significant ($P < .05$ and $P < .01$, respectively) elevation was observed with 0.1 and 100 ng/ml AVT (Table X). Significant ($P < .01$) elevation in prolactin was observed in

those HAP incubated with 100 ng/ml AVT in pituitary incubation V (Table XI). The prolactin concentration in those HAP in pituitary incubation VI incubated with AVT was not significantly affected (Table XII).

The increase in immunoassayable prolactin in those HAP incubated with AVT along with no significant effect on release from these HAP implies an increase in synthesis of immunoassayable prolactin. This is of interest as a prehormone for prolactin has been identified (Evans et al., 1977; Maurer et al., 1977; Royal and Chrambach, 1975). It has been determined that preprolactin contains 29 extra amino acids attached to the N-terminal portion of the hormone. It is possible that AVT may stimulate the conversion of preprolactin to prolactin. Also of interest are the reports of Vaughan et al. (1975b) and Blask et al. (1976) that AVT was observed to release prolactin in vivo and in vitro using larger doses of AVT than utilized in the present study. It would be of interest to determine if these larger doses of AVT also stimulated the synthesis of prolactin and also if the results reported in this work would release prolactin with larger doses of AVT.

The one exception to the apparent AVT stimulation of gland concentrations of prolactin was in pituitary incubation III in which the HAP received 10 ng/ml LRH as well as AVT. In this experiment, 10 ng/ml AVT was found to significantly ($P < .02$) reduce prolactin glandular concentrations (Table IX). These results are difficult to reconcile when compared with the rest of the experiments; however, the effect of 10 ng/ml LRH on prolactin synthesis and release was not examined and may be a possible explanation.

The results of pituitary incubation VII indicate that 1 ng/ml AVT does not affect basal LH release from the non-LRH stimulated HAP obtained from intact female rats (Figure 12) but does affect the LRH stimulated LH release as observed in pituitary incubation VI (Figure 10). However, 1 ng/ml AVT did not significantly inhibit LRH stimulated LH release in either estrogen-progesterone primed castrate or intact female rats (Figures 8 and 10) although less release was observed in the AVT treated halves. In agreement with the previously discussed pituitary incubations, slightly less LH was found in the AVT treated HAP (Table XIII). This may indicate the inhibitory effect of AVT on LH synthesis is independent of either LRH or steroid effects on the pituitary. This adds more evidence to the suggestion previously discussed that the effect of AVT on LH synthesis is separate from the effect on LH release.

Again AVT was observed to have neither a significant effect on prolactin release nor synthesis in HAP obtained from intact female rats (Figure 13). This is in agreement with pituitary incubation VI where no affect of AVT was observed on prolactin gland concentration in the intact female rat.

Pituitary incubation VIII was performed as an attempt to determine whether the effects of AVT on LH and prolactin were specific to AVT or were shared with the neurohormone AVP. Like AVT, AVP was found to have no effect on either non-LRH stimulated LH release or on prolactin release (Figures 14 and 15); however, these glands were obtained from intact male rats. In contrast to the results observed in all experiments involving AVT, AVP was observed to have no effect on pituitary LH concentration (Table XIV). This may be interpreted as indicating

that AVT exhibits a specific effect on pituitary LH synthesis. AVP was also found to significantly ($P < .01$) reduce the glandular concentration of prolactin (Table XIV). This is in contrast with all incubations but pituitary incubation III.

The effects of AVP on LRH stimulated LH release from intact male rat HAP were found to be identical with those of AVT (Figure 16). However, AVP was found to significantly ($P < .02$) inhibit prolactin release (Figure 17). This is difficult to reconcile with the rest of the experiments, especially since LRH was found to have no effect on prolactin release from intact male HAP (Table II). Again, consistent with the hypothesis that the effects of AVT on glandular LH concentrations is specific, AVP was found to have no significant effect on glandular LH concentrations. AVP was also found to slightly, but non-significantly decrease glandular prolactin concentrations in these glands. This is in agreement with the previously discussed experiment where AVP was also observed to decrease prolactin concentration in HAP.

One of the possible modes of action of AVT is the interference of this peptide with LRH binding to LRH binding sites. The technique of Pedroza et al. (1977) was used to investigate this possibility. Using this technique, LRH may bind to such structures as secretory granules as well as membrane receptor sites (Sternberger and Petrali, 1975). However, such binding may be decreased due to the presence in the pituitary of enzymes involved with the degradation of LRH (Schally et al., 1976). In order to avoid the degradation of LRH, the peptide Bactracin was added to the buffers. This antibiotic is known to inhibit the <u>in vitro</u> degradation of LRH in brain tissue (McKelvey et al., 1976) and pituitary tissue (Pedroza et al., 1977) incubated

for 30 minutes. However, recent evidence has suggested that the peptidase inhibitory activity of Bacitracin may be short lived and that total protection may exist for only 5 minutes at 0°C (Gruber, 1978). If this is the case then enzymatic degradation was taking place during the 30 minute incubation used in this work.

Figure 18 depicts the ability of unlabeled LRH to displace ^{125}I-LRH from a pituitary homogenate obtained from intact male rat pituitaries. The iodinated LRH (4.5 nM) was almost entirely displaced from the pituitary homogenate with 40 nM unlabeled LRH. The addition of either 100 or 1000 nM unlabeled LRH resulted in very little additional displacement. In contrast, AVT concentrations up to 100 nM AVT was unable to displace ^{125}I-LRH (Figure 19). However, 1000 nM AVT was found to displace the iodinated LRH. Marshall et al. (1976) found that micromolar concentrations of AVP were capable of displacing ^{125}I-LRH from its membrane bound receptor sites. Therefore, the ability of AVT in micromolar concentrations to displace the binding of ^{125}I-LRH from pituitary homogenate binding sites is not unexpected. These results are also in agreement with those observations from the intact male pituitary incubation experiments. Log concentrations of AVT up to 100 ng/ml (83.5 nM) were unable to affect the LRH stimulated LH release (Figures 2 and 4).

In contrast, AVT was found to biphasically potentiate the binding of iodinated LRH to pituitary homogenates obtained from estrogen-progesterone primed castrate male rats (Figure 21). In pituitary homogenates from these same animals, unlabeled LRH was found to displace the ^{125}I-LRH as expected (Figure 20). These results are

consistent with the pituitary incubation experiments in which AVT was found to affect the LRH stimulated LH release in HAP obtained from the estrogen-progesterone primed castrate male rat. Thus, an effect of AVT on LRH binding in these animals is predictable. However, it is difficult to explain a potentiation of ^{125}I-LRH uptake in pituitary tissue by AVT. One possible explanation may involve AVT serving as a substrate for the peptidases that degrade LRH. Credence would be added to this hypothesis if Gruber (1978) is correct in his suggestion that Bacitracin only inhibits the peptidase for 5 minutes at 0^{o}C. This could be of physiological importance as degrading enzymes have been suggested as playing a role in the termination or regulation of local hormone action and concentration (Kuhl et al., 1977, 1978). However, it still remains difficult to explain the biphasic effect of AVT. This hypothesis also does not agree with the observations that AVT decreased the LRH stimulated LH release in pituitary incubations.

Another possible explanation may involve the binding of AVT to the unique type of receptor that Spona (1974) suggested exists in the estrogen-progesterone treated rats. This type of action would constitute heterotropic cooperativity. Heterotropic cooperativity is known to exist with regard to the effect of guanyl nucleotides on glucagon and angiotensin receptors (Kahn, 1976). It is believed that guanyl nucleotides may act in concert with the hormone receptor as an allosteric regulator of biological response. The mechanism(s) by which such an effect might occur include conformational changes in the structure of oligomeric receptors, or polymerization or depolymerization of receptors in the fluid membrane (Kahn, 1976). Therefore, it is possible that AVT could bind to an allosteric site on the receptor,

enhance the receptors' ability to bind LRH by changing the conformation of the receptors, but render the receptor incapable of eliciting a biological response. However, this does not explain the biphasic response of ^{125}I-LRH binding which was observed in this experiment.

Thus, pituitary incubation experiments have demonstrated that AVT will affect the LRH stimulated LH release in only those animal models exposed to estrogen and progesterone. These observations were confirmed by the ability of AVT to only affect the binding of ^{125}I-LRH in the estrogen-progesterone primed castrate male rat.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Much of the literature concerning the antireproductive properties of arginine vastocin is confusing and contradictory. Previous investigators have hypothesized that AVT can affect either the gonads, pituitary or hypothalamus. However, several investigators disagree as to the effect observed at these target organs. For the present study it was hypothesized that AVT influences the pituitary's sensitivity to LRH as measured by changes in LH release. The influence of AVT on prolactin was also examined. This modulation of tropic hormone secretion was examined both in vivo and in vitro in animal models of varying steroid environments.

The results of the in vivo experiment indicate that AVT is capable of reducing the pituitary's sensitivity to LRH in the intact male rat. However, this influence could be the result of either a direct effect of AVT on the pituitary or an indirect effect due to AVT modulation of gonadal steroids. Prolactin secretion was not affected by AVT in this experiment.

The results of the in vitro experiments indicate that AVT demonstrates an inhibitory effect on the LRH stimulated LH release only in those animals previously exposed to estrogen and progesterone. AVT was observed to have no effect on pituitaries obtained from intact or castrate male rats. The LH concentrations of those HAP incubated

with AVT in all pituitary incubation experiments contained less immunoassayable LH than their corresponding control halves. This might indicate that AVT may affect LH synthesis in all animal models investigated while it is capable of reducing only the estrogen-progesterone potentiation of the LRH stimulated LH release. These observations on the effect of AVT on LRH stimulated LH release in various animal models is strengthened by the observations of the influence of AVT on the uptake of ^{125}I-LRH in a pituitary homogenate. AVT was found to be without significant effect on the ^{125}I-LRH uptake in pituitary homogenate obtained from intact male rats. Predictably, AVT was observed to influence the uptake of ^{125}I-LRH in pituitary homogenate obtained from estrogen-progesterone primed castrate male rats. However, this effect was facilitory in nature. This may be explained on the basis of either an action of LRH peptidases on AVT or heterotropic cooperativity between AVT and the LRH receptor.

AVT was observed to have no effect on prolactin secretion in any of the animal models utilized for pituitary incubations. However, in most cases AVT was observed to increase the gland concentrations of immunoassayable prolactin.

In conclusion, these studies demonstrate that:

1. AVT can influence the responsiveness of the anterior pituitary to LRH both in vivo and in vitro;

2. At least the in vitro response is dependent upon animals being exposed to estrogen and progesterone;

3. AVT can also reduce the in vitro glandular concentration of LH and increase that of prolactin; and

4. AVT does not influence prolactin release *in vivo* or *in vitro* under the experimental conditions examined in the experiments described in this dissertation.

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APPENDIX A

KREBS-RINGERS BICARBONATE WITH GLUCOSE (KRBG)

TABLE XVI

KREBS-RINGERS BICARBONATE WITH GLUCOSE (KRBG)

	Stock Solution (g/l)	Working Solution[1] (ml)
NaCl	9.0	100
KCL	11.5	4
$CaCl_2$	12.2	3
KH_2PO_4	21.1	1
$MgSO_4 \cdot 7H_2O$	38.2	1
$NaHCO_3$	13.0	21

[1] 200 mg of glucose per 100 ml working solution.

Method of preparation as described in Mackie and McCartney's Handbook of Bacteriology. Robert Cruikshank (Ed.). London. E&S Livingstone Limited, 10th Edition. p. 291. 1960.

APPENDIX B

PEPTIDES UTILIZED IN EXPERIMENTS

TABLE XVII

PEPTIDES UTILIZED IN EXPERIMENTS

Peptide	Supplier	Specific Activity
LRH (Porcine) Synthetic	Beckman	---
AVT Synthetic	Calbiochem	225 U/mg[1]

[1]Expressed in terms of rat pressor bioassay.

APPENDIX C

PARALLEL INHIBITION CURVES FOR LH RIA

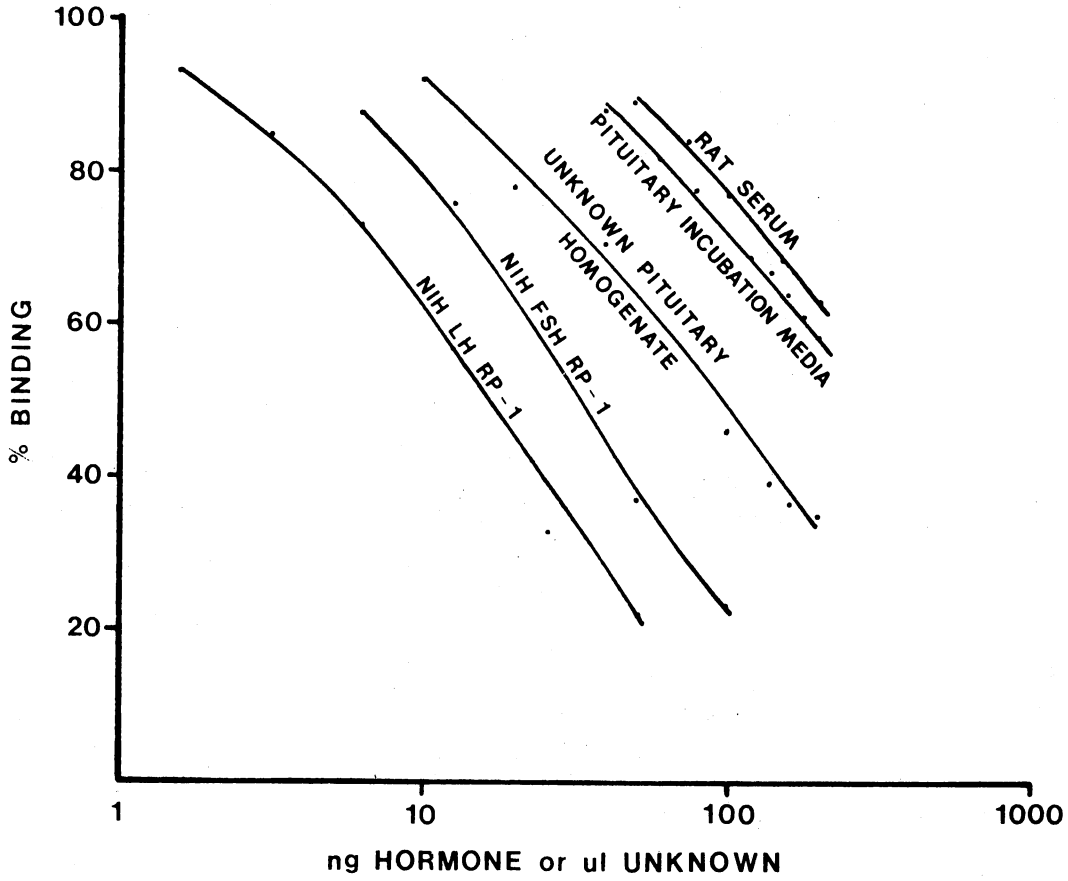

Figure 22. Parallel Inhibition Curves for LH RIA

APPENDIX D

PARALLEL INHIBITION CURVES FOR PROLACTIN RIA

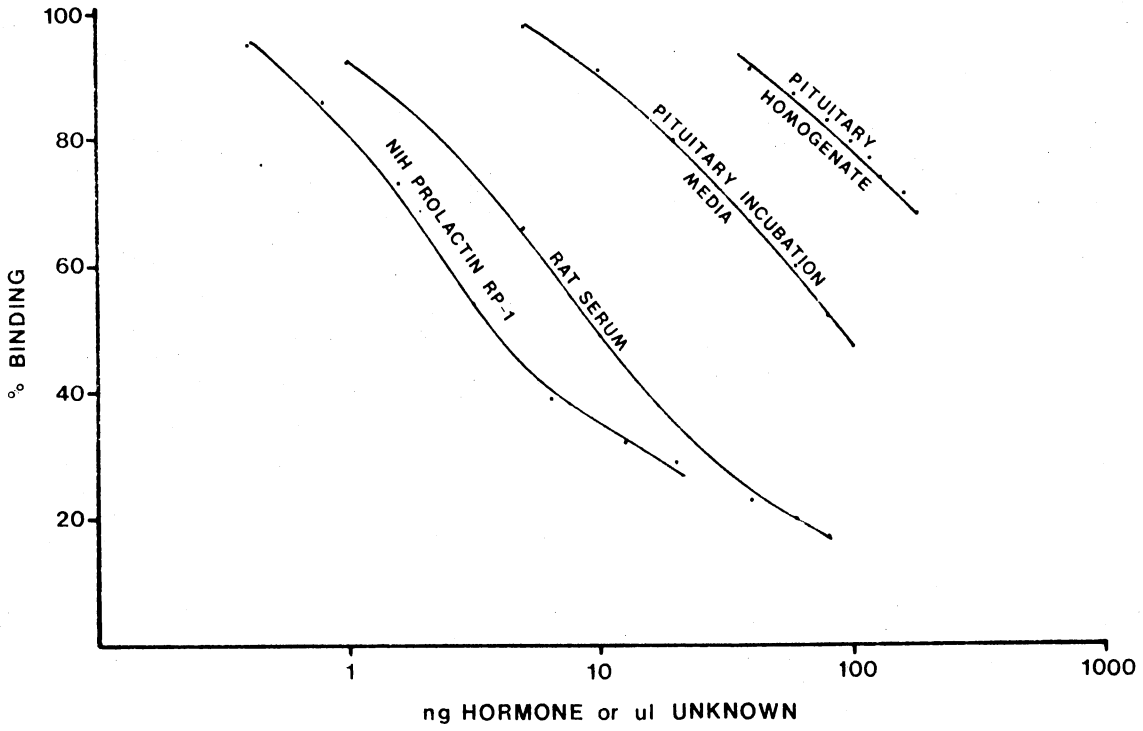

Figure 23. Parallel Inhibition Curves for Prolactin RIA

VITA 2

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