AN ABSTRACT OF THE THESIS OF

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Title: ROLE OF HYPOTHALAMIC BIOGENIC AMINES IN THE
RELEASE OF LUTEINIZING HORMONE IN THE EWE

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Fredrick Stormshak

The role of hypothalamic biogenic amines (norepinephrine, dopamine and serotonin) in regulating the release of luteinizing hormone (LH) in the ewe was investigated. Eleven experiments utilizing mature crossbred or Hampshire ewes were conducted to determine the ability of exogenous 17β-estradiol and various pharmacological agents to alter the levels of hypothalamic biogenic amines and affect the release of LH in the ewe. The stalk median eminence (SME) and hypothalamus proper (HP) were chemically analyzed for norepinephrine (NE), dopamine (DA) and serotonin (5-HT). Serum LH concentrations were determined using radioimmunoassay.

A single intramuscular injection of 750 μg of 17β-estradiol into each of four ewes on day 3 of the estrous cycle (first day of estrus = day 0 of the cycle) caused an increase in serum LH concentration in each ewe (range, 20 to 30 ng/ml) within 16 hours following treatment.
A similar injection of 17β-estradiol into five ewes 8 hours prior to necropsy on day 3 of the cycle was without affect on the levels of biogenic amines in the SME or HP. In both treated and control ewes, NE levels were higher (P < .01) in the HP than concentrations of NE in the SME. Conversely, DA and 5-HT concentrations were found to be higher (P < .01) in the SME than in the HP.

Treatment of six ovariectomized ewes with 750 μg of 17β-estradiol 3 hours prior to necropsy elevated NE levels in the SME (P ≤ .07) but failed to alter NE concentrations in the HP. Serotonin concentrations in both the SME and HP tended to increase after treatment with estradiol. One hour after intravenous injection of six ovariectomized ewes with L-dihydroxyphenylalanine (L-dopa, 12 mg/kg) biogenic amine levels qualitatively resembled those detected following injection of estradiol. Norepinephrine levels in the SME tended to increase while only a slight change in the concentration of NE in the HP was detected. Serotonin concentrations in both the SME and HP were higher after treatment with L-dopa than those observed in control ewes.

In an attempt to inhibit the ovulatory surge of LH 12 ewes were injected intravenously with α-methyltyrosine (α-MT), α-methyldopa (α-MD), 5-hydroxytryptophan (5-HTP) or vehicle as soon as they were detected in estrus and again 4 hours later. Three ewes each were injected with α-MT (12 mg/kg), α-MD (6 mg/kg), 5-HTP (6 mg/kg) or
vehicle. Elevated serum LH levels indicative of the ovulatory release of LH were detected in at least one serum sample from each ewe. Similarly, pretreatment of four anestrous ewes with α-MT (15 mg/kg) did not block or modify the ability of exogenous estradiol (20 μg) to induce a release of LH. Pretreatment of five ovariectomized ewes with p-chlorophenylalanine (p-cpa) did not inhibit the release of LH elicited by injection of estradiol (50 μg) but did increase the interval from injection of estradiol until the onset of LH release by 4 hours.

Experiments were conducted to investigate the effects of L-dopa and p-cpa on the release of LH. L-Dihydroxyphenylalanine was intravenously injected into four ewes on day 3 of the cycle. Forty seven ewes in various stages of anestrus (early, deep or late) were intravenously injected with graded doses of L-dopa (3 to 18 mg/kg), p-cpa (11 to 60 mg/kg) or vehicle. Treatment of ewes with L-dopa during the estrous cycle resulted in a release of LH from only one animal at 24 hours (20 ng/ml) and again at 32 hours (91 ng/ml) post-injection. Elevated serum LH concentrations were detected in three of the 24 ewes treated with L-dopa and in one of nine ewes injected with p-cpa. Each of the ewes that responded to treatment with a release of LH was in late anestrus.
Role of Hypothalamic Biogenic Amines in the Release of Luteinizing Hormone in the Ewe

by

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Serum LH concentrations of 4 ewes intramuscularly injected with 750 µg of 17β-estradiol (group 1) or 4 ewes intravenously injected with 7 mg/kg L-dopa (group 2) at 0800 hr (0 hr) on day 3 of the estrous cycle.

THE EFFECT OF VARIOUS PHARMACOLOGICAL AGENTS ON THE RELEASE OF LUTEINIZING HORMONE IN THE EWE

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Three ewes each were intravenously injected with α-MD (6 mg/kg), α-MT (12 mg/kg), 5-HTP (6 mg/kg) or vehicle as soon as they were detected in estrus (0 hr) and again 4 hr later.

Four anestrous ewes were intravenously injected with α-MT (15 mg/kg) at 0800 hr (0 hr) and again 4 hr later. Four control ewes were similarly injected with vehicle. All ewes were intramuscularly injected with 20 µg of 17β-estradiol at 1200 (4 hr).

Five ovariectomized ewes were intraperitoneally injected with p-cpa (75 mg/kg) at 1200 hr (-48 hr) and intravenously injected with p-cpa (25 mg/kg) at 0 hr. Five control ewes were similarly injected with vehicle. All ewes were intramuscularly injected with 50 µg of 17β-estradiol at 0 hr.
RELEASE OF LUTEINIZING HORMONE IN THE EWE DURING VARIOUS STAGES OF ANESTRUS FOLLOWING INJECTION OF L-DIHYDROXYPHENYLALANINE AND p-CHLOROPHENYLALANINE

1 Serum LH concentrations of 4 late anestrous ewes treated with L-dopa (group 1). Two ewes received 6 and 2 received 3 mg/kg L-dopa. Each dose level was injected intra-arterially into 1 ewe and intravenously into the other. Four ewes were injected with p-cpa (group 2); 2 ewes received 22 and 2 received 11 mg/kg p-cpa. All ewes were injected at 1200 hr (0 hr).

2 Serum LH concentrations of 3 late anestrous ewes intravenously injected with L-dopa (6 mg/kg) at 0800 hr (0 hr).
ROLE OF HYPOTHALAMIC BIOGENIC AMINES
IN THE RELEASE OF LUTEINIZING HORMONE IN THE EWE

REVIEW OF LITERATURE

The neurovascular hypothesis for the control of adenohypophysial function was first formulated by Green and Harris (1947). They postulated that neural impulses, monitored and integrated in the hypothalamus, affect the secretion of hypophysiotropic factors from the median eminence. Accordingly, these factors were presumed to enter the fenestrated capillaries comprising the proximal segment of the hypothalamo-hypophysial portal vascular system and thereby reach the anterior pituitary. The neurovascular hypothesis presented a plausible solution to the perplexing problem of how the central nervous system influenced adenohypophysial function in light of the absence of histological evidence demonstrating nervous innervation of this lobe of the pituitary. It also marked the beginning of intense neuroendocrine investigation.

Since then the many efforts throughout the world to isolate, characterize and synthesize the hypophysiotropic factors (recently termed hypothalamic hormones) in anticipation of their important clinical value culminated in the synthetic production of two of these neurohormones, one acting to stimulate the release of thyroid stimulating hormone and the other the release of luteinizing hormone.
and/or follicle stimulating hormone (Gay, 1972; Guillemin and Burgus, 1972; Schally, Kastin and Arimura, 1972, 1973). Although it is still a matter of some conjecture, it appears that a hypothalamic hormone promoting either release or inhibition of release exists for each of the anterior pituitary hormones (McCann and Porter, 1969; Meites, 1970).

Not long ago investigators interested in the integrative mechanisms of the nervous and endocrine systems were confronted with the challenge of elucidating the relationship between the hypothalamus and the anterior pituitary. Today, realization of the subserviency of the adenohypophysis to neurohormones has redirected the emphasis of neuroendocrine integrative research to the factors regulating the synthesis and release of these small polypeptides.

The median eminence can be divided into an external and internal layer. The outer surface of the external layer is surrounded by the pars tuberalis. Between these zones there are arterioles supplying the median eminence and also portal vessels which extend along the surface of the pars tuberalis passing into the adenohypophysis. Numerous nerve terminals are present in the external layer of the median eminence and frequently end in perivascular spaces or in contact with endothelial cells of the vascular system (Duffy and Menefee, 1965; Rinne, 1966; Monroe, 1967). These nerve endings or boutons contain electron dense vesicles of various dimensions. Experiments utilizing ultracentrifugation and bioassay techniques have
demonstrated that neurohormone activity resides in the vesicles localized in the neurosecretory cell boutons of the median eminence (Kobayashi and Matsui, 1969; Ishii, 1970). The results of these investigations coupled with the known anatomical proximity of the neurohormone containing terminals to the primary capillary plexus of the portal system has led to the generalized concept that neurosecretion is the mode by which hypothalamic hormones exit the median eminence. In this manner neurosecretory cells function as transducers of neuroendocrine integration, as clearly displayed by their function in the adrenal medulla and posterior pituitary. The location of these specialized neurons in the hypothalamus define that region known as the hypophysiotropic area (Halász, Pupp and Uhlarik, 1962; Halász, 1969).

Histochemical fluorescence studies designed to localize primary amines in the brain yield a diffuse green fluorescence in the infundibular region of the pituitary stalk (Carlsson, Falck and Hillarp, 1962). Studies by Fuxe and Hökfelt (1964, 1966) suggest that the fluorescence is attributable to catecholamines localized in nerve terminals present in the external layer of the median eminence. In addition to the hypothalamic hormones and the catecholamines, dopamine (DA) and norepinephrine (NE), the indolamine, serotonin (5-hydroxytryptamine) has been isolated from the bovine median eminence (Piezzi, Larin and Wurtman, 1970). Clementi et al. (1970)
using sucrose fractionation, chemical analysis, bioassay and electron microscopy reached the conclusion that in the rat DA, NE and serotonin (5-HT) are each stored in a different nerve ending in the median eminence. Similarly, combined neuropharmacological and electron microscopy studies of the median eminence indicate that DA and NE are associated with different axonal terminals and that dopaminergic endings are the most abundant (Hökfelt, 1967; Jonsson, Fuxe and Hökfelt, 1971). Weiner et al. (1972) surgically isolated the medial basal hypothalamic region of the rat which results in degeneration of neurons whose cell bodies lie outside this deafferentated island and observed little change in DA concentrations but NE levels were found to be totally depleted. These results support the supposition of Fuxe and Hökfelt (1966) that the majority of the dopaminergic neurons belong to the tubero-infundibular system with their cell bodies in the arcuate nucleus and their axons terminating in the external layer of the median eminence. In contrast, most NE fibers appear to originate outside the medial basal hypothalamic region. Björklund et al. (1970) described a modification of the histochemical fluorescence method that allows differentiation of DA- and NE-containing nerve fibers. They also detected a large group of DA-containing axons originating in the arcuate nucleus. A considerable number of NE axons originating beyond the mediobasal hypothalamus were observed to intermingle with the DA fibers in the median eminence. The origin of the
serotonergic fibers innervating the median eminence is not known; however, limited evidence suggests an extra-hypothalamic origin (Aghajanian, Graham and Sheard, 1970; Carrer and Taleisnik, 1970).

Even though DA, NE and 5-HT are present within only a fraction of all the neurons in the brain the close proximity of these amine containing neurons to the neurosecretory cells and to the hypothalamic portal capillaries suggests that these amines may be of special significance in the control of hypothalamic hormones (Anton-Tay and Wurtman, 1971). These monoamines are included in the classification of biosynthetic amines, the biogenic amines, that produce physiological effects in the proximity of their discharge. Other biogenic amines include histamine, bradykinin and epinephrine.

Participation of biogenic amines in regulating the function of the anterior pituitary was suspected some years ago (Friedgood and Bevin, 1938; Markee, Sawyer and Hollinshead, 1948; Markee, Everett and Sawyer, 1952). Only in the last few years, however, have advances in methodology permitted a more precise examination of the function they perform. The interest and intensity of research concerning the intervention of monoaminergic neurons in regulating gonadotropic hormone secretion is reflected in the frequency with which articles concerning this subject appear in the current literature. Considerable speculation as to the function of the various aminergic systems has been promoted, but to date, the data have not been
assimilated into a compelling hypothesis. Nevertheless, current investigations have lent unequivocal support for the critical intervention of biogenic monoamines in the functional interrelationships of the hypothalamo-hypophysial-gonadal axis. Some of the pertinent experimental results relating hypophysiotropic monoamines to the control of ovulation are reviewed below. Evidence for a monoaminergic mediation of gonadal-steroid feedback is also presented.

Evidence derived from neuropharmacological investigations has demonstrated that at least one of the catecholamines promotes or facilitates luteinizing hormone-releasing hormone (LRH) secretion. Appropriate administration of drugs to laboratory animals that interfere with catecholamine biosynthesis such as α-methyltyrosine or α-methyldopa inhibit ovulation (Brown, 1967; Lippmann, 1968; Kordon and Glowinski, 1969; Kordon, 1971a). Similar results have been obtained after injection of rats with reserpine or tetrabenazine which deplete intracellular monoamine stores (Coppola, Leonardi and Lippmann, 1966; Meyerson and Sawyer, 1968).

Selective restoration of normal brain concentrations of DA or NE following inhibition of catecholamine synthesis suggests that the anti-ovulatory action of diminished catecholamine levels in rats can be primarily attributed to a decrease in the concentration of DA (Kordon and Glowinski, 1970). Administration of the catecholamine precursor L-dihydroxyphenylalanine (L-dopa) to rats pretreated with
α-methyltyrosine reverses the anti-ovulatory effect of this synthesis inhibitor. Simultaneous injection of disulfiram, which blocks the transformation of DA into NE, has no affect on L-dopa’s ability to restore ovulation. Moreover, restoration of NE levels only by injection of dihydroxyphenylserine, which is decarboxylated to NE without giving rise to DA, does not reverse the inhibitory effect of α-methyltyrosine on ovulation.

Results of experimental investigations utilizing exogenous DA support the contention that DA may be the neurotransmitter that promotes discharge of LRH. Addition of DA to incubation flasks containing rat anterior pituitary and hypothalamic fragments results in a dose response release of pituitary luteinizing hormone (Schneider and McCann, 1969), whereas no change in luteinizing hormone (LH) release was noted when DA was added to flasks containing only pituitary fragments. Dopamine injected into the third ventricle of intact rats was also shown by Schneider and McCann (1970a) to bring about LH release. Furthermore, it was demonstrated that injection of DA into the third ventricle of hypophysectomized rats elicits hypothalamic LRH secretion as evidenced by increased levels of LRH present in the systemic circulation (Schneider and McCann, 1970b). Direct perfusion of the adenohypophysis with DA through a microcannula penetrating a portal vessel failed to alter plasma LH levels (Kamberi, Mical and Porter, 1970).
In contrast to the apparent positive action of DA on LRH secretion, other investigators have presented evidence to suggest the opposite. Dopamine microimplanted into the median eminence of the rat was found to inhibit the secretion of LRH (Kobayashi and Matsui, 1969; Uemura and Kobayashi, 1971). On the basis of DA turnover studies as determined by injecting α-methyltyrosine and histochemically estimating the rate of disappearance of the amine following synthesis inhibition, Fuxe (1969) has concluded that the tubero-infundibular DA neurons function to suppress LRH secretion in the rat. The rate of DA turnover within nerve terminals in the median eminence was found to undergo cyclic activity changes with the lowest rate of turnover recorded during proestrus and early estrus (Fuxe, Hökfelt and Nilsson, 1967). Subsequently, a similar but more defined experiment revealed that the activity of DA neurons is low at the time of LRH secretion (Ahren et al., 1971). Conversely, during anovulatory conditions, such as pregnancy, lactation, pseudopregnancy, androgen sterilization and exposure to constant light, the activity of DA neurons in the rat is markedly increased (Fuxe, Hökfelt and Nilsson, 1972). At the present time, the neuroendocrine function of DA remains unclear, perhaps obscured in the assumptions underlying the diversity of techniques used in the investigations of this catecholamine.

Limited evidence suggests that elevated hypothalamic NE levels
in the rat may be positively related to the secretion of LRH. Hypothalamic NE levels attain their highest concentration during proestrus prior to the release of LH and drop significantly after ovulation (Stefano and Donoso, 1967). In a pharmacological study using intact and castrated male rats, Donoso et al. (1971) found that selective blockade of NE synthesis resulted in decreased plasma LH levels. Weiner et al. (1972) associated degeneration of NE nerve terminals in the median eminence with a concomitant decrease in plasma LH levels.

More general agreement exists for the role of serotonergic neurons terminating in the median eminence than for either DA or NE nerve fibers. Serotonin appears to exert a negative influence on the processes regulating LRH secretion. Wheaton et al. (1972) detected a decrease in median eminence 5-HT concentration just prior to the ovulatory surge of LH in the ewe. Exogenous 5-HT blocks ovulation in mature rats (Labhsetwar, 1971) and chorionic gonadotropin pretreated immature rats (O'Steen, 1965). Injection of the immediate precursor of 5-HT, 5-hydroxytryptophan, which elevates 5-HT concentrations in the brain, was reported to have anti-ovulatory effects in immature rats (Kordon et al., 1968). Serotonin suppresses the enhanced LH release induced when rat hypothalamic fragments are added to anterior pituitaries incubated in vitro (Moszkowska, 1965). When hypothalamic fragments are not included in the incubation system, the presence of
5-HT does not directly modify LH release from the pituitaries. Contrary to the promoting action of intraventricularly injected DA on the release of LRH, similar administration of 5-HT decreases plasma LH levels in ovariectomized rats (Schneider and McCann, 1970a) and intact male rats (Kamberi, Mical and Porter, 1970). Direct infusion of the anterior pituitary with 5-HT via a portal vessel was without affect on plasma LH levels (Kamberi, Mical and Porter, 1970).

Systemic administration of monoamine oxidase inhibitors, such as tranylcypromine, iproniazid, pargyline or nialamine which elevate monoamine levels, interferes with ovulation in the hamster (Alleva, Overpeck and Umberger, 1966; Alleva and Umberger, 1966; Lippmann, 1968). This blockade of ovulation brought about by increasing monoamine levels has been singularly related to the increase in the concentration of 5-HT (Kordon et al., 1968; Lippmann, 1968). Microinjection of monoamine oxidase inhibitors has demonstrated that the median eminence is the only hypothalamic area where an increase in 5-HT levels inhibits the ovulatory response of immature rats pre-treated with pregnant mare serum gonadotropin and chorionic gonadotropin (Kordon, 1969).

Realization of the importance of hypothalamic monoaminergic systems and hypothalamic gonadal steroid feedback on the regulation of pituitary gonadotropin release suggests hypophysiotropic biogenic amines may be the common receptor for neural and endocrine
integration. Experimental evidence indicates that estrogen and progesterone affect monoamine levels and activity.

Ovariectomy of the rat markedly alters hypothalamic NE levels (Donoso and Stefano, 1967) as well as affecting the rate of NE turnover in the hypothalamus (Anton-Tay and Wurtman, 1968). Furthermore, injection of estrogen, progesterone or a combination of both steroids into ovariectomized rats modifies mid-brain monoamine levels (Tonge and Greengrass, 1971) and alters their rate of turnover (Bapna, Neft and Costa, 1971).

Enzymes which participate in the degradation of monoamines in the rat are influenced by ovarian hormones. Catechol-o-methyltransferase (Salseduc, Jofre and Izquierdo, 1966) and monoamine oxidase (Zolovic et al., 1968) activity changes during the estrous cycle. Following ovariectomy, hypothalamic monoamine oxidase levels markedly increase; however, normal levels of this enzyme are restored by treatment with ovarian steroids (Kobayashi, Kato and Minaguchi, 1964).

In addition to affecting the metabolism of monoamines, ovarian hormones may exert part of their influence directly on the neuro-secretory cell. Estrogen inhibits in vitro DA induced release of rat pituitary LH in the presence of hypothalamic fragments (Schneider and McCann, 1970c). Similarly, injection of estrogen into the third ventricle of rats prior to intraventricular injection of DA prevents
DA induced LRH secretion (Schneider and McCann, 1970b). Estrogen antagonism of DA induced LRH secretion is in accord with the negative influence of this steroid on gonadotropin secretion. On the other hand, a facilitory action of estrogen on DA induced LRH secretion is indicated from the experiment of Raziano et al. (1971) the results of which demonstrate that injection of antiserum to estrogen prevents intraventricular DA induced ovulation in the rat.

A working hypothesis has not emerged from the expanding body of literature concerning the effects of ovarian hormones on the monoamines. Nevertheless, data from current investigations strongly indicate that a relationship exists between the monoaminergic systems and circulating ovarian hormones.
STATEMENT OF THE PROBLEM

It is increasingly apparent that the worldwide production of food is being heavily taxed by population growth. In lieu of technical developments providing vast new areas of food production, fulfilling future nutrient requirements of this expanding population will depend upon further development and application of more efficient agricultural methods.

Sheep serve as an important source of protein for human consumption in many parts of the world. This is particularly true in those geographical areas where land is not suitable for the production of crops other than forages. In order to keep abreast of the world needs for mutton and lamb it will be necessary to improve the efficiency of sheep production. Both immediate and future gains in sheep production could be realized through increasing the reproductive efficiency of the ewe. The latter might be accomplished through increasing the number of lamb crops produced annually.

In most breeds of sheep the ewe is seasonally polyestrus exhibiting behavioral estrus only during the months of diminishing photoperiod. This natural phenomenon limits the ewe to one lamb crop annually. The interval between breeding seasons of the ewe is characterized by an absence of behavioral estrus and ovulation and is referred to as anestrus. Induction of behavioral estrus with concomitant ovulation during the anestrous interval would permit the
producer to obtain a second lamb crop. Attempts to induce ovulation in the anestrous ewe by use of exogenous ovarian or gonadotrophic hormones have met with varied success and have not been widely accepted by the producer. Consequently, further research on the control of ovulation in the anestrous ewe is warranted.

Recent experimental evidence derived from investigations utilizing laboratory animals has demonstrated that certain biogenic amines found in the central nervous system are of critical function in the periodic release of an intermediate neurohormone, luteinizing hormone-releasing hormone, which elicits the ovulatory release of LH from the anterior pituitary. The present experiments were conducted to gain insight into the function of hypothalamic biogenic amines in the regulation of luteinizing hormone-releasing hormone in the ewe.

The role of the central nervous system in reproduction provides a potentially critical site with which to modify reproductive processes. Research developments in this area may find wide application not only in the induction of ovulation, but also in controlling the number of ova ovulated and in preventing ovulation. Further knowledge of the regulatory mechanisms controlling ovulation in domestic species may contribute significantly to development of effective methods of human population control.
ESTROGEN INDUCED CHANGES IN HYPOTHALAMIC BIOGENIC AMINE LEVELS IN THE EWE

**Introduction**

In the rat and ewe the ovulatory surge of luteinizing hormone (LH) is preceded by an increase in the level of circulating estradiol. Moreover, elevation of plasma estradiol levels through injection of this steroid into the ovariectomized rat (Callantine, Humphrey and Nesset, 1966) or ewe (Scaramuzzi et al., 1971) or into the intact ewe during the early stages of the estrous cycle (Bolt, Kelley and Hawk, 1971) induces a release of LH characteristic of the ovulatory surge of this gonadotropin. It is generally accepted that estradiol acts on the hypothalamo-hypophysial axis to elicit the release of LH but the mechanism of action of this steroid at these sites is unknown.

It has been demonstrated that hypothalamic biogenic amines play a prominent role in regulating the release of neurohumors which affect the secretion of adenohypophysial gonadotropins (Fuxe, Hökfelt and Jonsson, 1970; Coppola, 1971; Kordon and Glowinski, 1972). The concentrations of hypothalamic biogenic amines are markedly altered in the rat (Stefano and Donoso, 1967) and ewe (Wheaton et al., 1972) immediately prior to the ovulatory surge of LH when plasma estradiol levels are elevated. It is possible that the ability of endogenous or exogenous estradiol to cause the release of LH in these species is
mediated via an effect of this steroid on the biogenic amines. Further support for the involvement of hypothalamic biogenic amines in regulating the release of LH has been presented by Dickey (1970) and Gay (1972). These investigators found that administration of L-α-3, 4-dihydroxyphenylalanine (L-dopa) to the rat causes a release of LH. Presumably the L-dopa acts to alter the levels of hypothalamic biogenic amines.

In the present experiments, the ability of exogenous estradiol to induce a release of LH in the ewe was used as a model to investigate the effects of this steroid on hypothalamic biogenic amines. In addition, the effect of L-dopa on hypothalamic biogenic amines and LH release in the ewe was studied.

**Materials and Methods**

**Animals:** Three experiments were conducted in which two-year-old crossbred ewes ranging in weight from 53 to 64 kg were used. The length of the estrous cycle of these ewes averaged 16 days with the first day of detected estrus designated as day 0 of the cycle. Ewes were penned with vasectomized marker rams and checked for estrus in the morning and evening of each day.

**Experiment 1:** Eleven ewes were assigned randomly to one of three groups. The number of ewes in each group and the treatments the ewes received are described below. In group 1, four ewes were
injected intramuscularly (im) with 750 μg of 17β-estradiol dissolved in 1 ml of corn oil. This group was included in the first experiment to compare the response of the ewes treated with estradiol to those receiving L-dopa. In group 2, four ewes received a single dose of L-dopa (7 mg/kg) injected via the jugular vein. L-Dopa (Sigma) was dissolved in warm 0.01 N HCl (8 mg/ml) immediately prior to use. Only a small fraction of systemically administered L-dopa enters the brain (Wurtman, Chou and Rose, 1970) and this rapidly elevates catecholamine levels (Everett and Borcharding, 1970). In order to obtain a maximum circulating L-dopa concentration in the ewe the drug was administered intravenously (iv). In the third group, three ewes were injected with vehicle; one ewe was injected im with corn oil and two animals received an iv injection of 0.01 N HCl. All treatments were initiated at 0800 hr on day 3 of the estrous cycle. A 10 ml venous blood sample was taken immediately before injection and at each 4 hr interval for 32 hr thereafter. The serum was subsequently analyzed for LH using radioimmunoassay (RIA). Three days after injection the ovaries of ewes treated with estradiol or L-dopa were exposed through a mid-ventral incision and the number, size and appearance of corpora lutea were recorded.

Experiment 2: Ten ewes were assigned randomly in equal numbers to a treatment or control group. Treatment consisted of a single im injection of 750 μg of 17β-estradiol at 2400 hr on day 2 of
the estrous cycle. Control ewes received a similar injection of corn oil only. All ewes were sacrificed by exsanguination at 0800 hr the next morning. At the abbatoir the brain was exposed and the stalk median eminence (SME) and hypothalamus proper (HP) were excised. The SME was severed from the pituitary and HP, exposing the opening to the third ventricle. The HP was defined rostrally by the posterior limit of the optic chiasm, 5 mm laterally on either side of the opening to the third ventricle, posteriorly by the anterior border of the mammillary body and dorsally by a depth of 10 mm. Mean wet weight of the SME and HP were 23 and 291 mg, respectively. Approximately 5 minutes elapsed from the time of exsanguination until the brain tissues were excised and packed in ice, another 15 minutes passed before the tissues were subjected to biogenic amine analysis which was completed the same day. Data were analyzed statistically using Student's unpaired and paired t tests for treatment and brain area comparisons, respectively.

Experiment 3: Eighteen ewes were bilaterally ovariectomized and allowed a 7 week recovery period before being assigned randomly in equal numbers to three groups. The following treatments were imposed: 750 µg of 17β-estradiol in corn oil injected intramuscularly; L-dopa, injected iv at a dose of 12 mg/kg dissolved in 0.01 N HCl; or vehicle. Estradiol was injected into ewes 3 hr prior to necropsy since a similar study with ovariectomized rats demonstrated that significant
changes in mid-brain biogenic amine levels occurred within 3 hr following estradiol injection (Tonge and Greengrass, 1971). L-Dopa was injected 1 hr before sacrifice. All ewes were killed by exsanguination at 0800 hr and the same experimental procedures for excising and assaying the SME and HP for biogenic amines were followed as described in experiment 2. The only notable exception being that two ewes were sacrificed each day. Treatments were assigned to pairs of animals to permit day of assay to be blocked in a balanced incomplete block design with three groups. Data were analyzed statistically using least squares analysis of variance.

Biogenic Amine Assay: The protocol for the simultaneous assay of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) reported by Shellenberger and Gordon (1971) was followed with slight modification. Briefly, the SME and HP were homogenized in perchloric acid solution, centrifuged and the supernatant adjusted to pH 7.8 with tricine-EDTA buffer solution which facilitates catecholamine retention on alumina. Instead of accomplishing the pH adjustment as suggested by these investigators, each sample was corrected using a pH meter equipped with a micro-electrode. This procedure was adopted because in our hands the tricine-EDTA buffer solution did not possess the same pH characteristics ascribed to it by Shellenberger and Gordon. Catecholamines were eluted from alumina with acid and oxidized with iodine to form fluorescent derivatives which were identified by their
characteristic activation and fluorescent wavelengths. Indolamines were separated by solvent extraction with heptanol and phosphate buffer and oxidized with ninhydrin to form fluorescent derivatives. Due to the limited tissue size, samples were not divided for duplicate oxidation. Fluorescent derivatives of NE, DA, and 5-HT were measured using an Aminco-Bowman spectrophotofluorometer. In order to quantify the assay, the HP was homogenized in 5 ml and divided into five aliquots, each adjusted to 3 ml with homogenization solution and utilized as follows: HP sample, HP tissue blank and HP plus three internal standards containing 25, 50 or 100 ng (free base) each of norepinephrine, dopamine hydrochloride and serotonin creatinine sulfate. Since it was not possible to develop a tissue blank for the SME, the fluorescence of the HP tissue blank was subtracted from both the HP and SME sample fluorescence. It was previously established that this procedure did not introduce significant error in the estimate of biogenic amine concentrations in the SME.

In experiment 3, the largest HP of the pair of ewes necropsied on each day of assay was used for the tissue blank as well as the internal standards. Dopamine levels in the SME and HP of ewes in the third experiment are not presented due to interference by exogenous L-dopa in the assay of DA. Normally, endogenous levels of dopa are negligible alleviating interference by this catechol. By chance the HP from the L-dopa treated ewes was frequently used as the source.
of tissue for the blank and standard tubes. Consequently a large part of the DA data obtained from the control and estradiol treated ewes, like that from the ewes injected with L-dopa, were exceedingly high and variable.

**LH Radioimmunoassay:** Blood samples were allowed to clot at room temperature and then refrigerated. Serum was separated by centrifugation and frozen for subsequent LH assay by the RIA protocol established and validated by Niswender *et al.* (1969). Antisera for ovine LH was generously supplied by Dr. G. D. Niswender. Dr. L. E. Reichert kindly supplied the highly purified ovine LH (LER-1056-C2) which was iodinated with $^{125}$I. Plasma LH was expressed in terms of an ovine LH standard (NIH-LH-S17) which was a gift provided by the National Institutes of Health. Ovine anti-rabbit gamma globulin prepared in this laboratory was used to precipitate the antisera to LH.

**Results**

**Experiment 1:** Treatment of ewes in group 1 with 750 µg of estradiol on day 3 of the estrous cycle was followed by a release of LH not later than 16 hr after injection in each of the four animals (Figure 1). Characteristics of the induced LH release such as duration, magnitude and the interval of time between injection and release, resembled those of the LH release in ewes injected with
Figure 1. Serum LH concentrations of 4 ewes intramuscularly injected with 750 µg of 17β-estradiol (group 1) or 4 ewes intravenously injected with 7 mg/kg L-dopa (group 2) at 0800 hr (0 hr) on day 3 of the estrous cycle.
estradiol at this stage of the cycle as reported by Bolt, Kelley and Hawk (1971) and in estradiol-treated overiectomized ewes (Goding et al., 1969). Three of the four ewes (0290, 0314, 0362) treated with estradiol were found to have newly ovulated follicles when examined.

A single dose of L-dopa, 7 mg/kg, injected into ewes on day 3 of the estrous cycle failed to elicit an LH release during the 32 hr sampling period in three of the four animals (Figure 1). Luteinizing hormone levels in these three ewes were not different from those receiving vehicle which had an average serum LH concentration of 3.2 ng/ml. Ewe 0575 was an exception; elevated serum LH levels of 19.6 and 91.3 ng/ml were detected at 24 and 32 hr post-injection, respectively (Figure 1). These LH values were verified using several serum dilutions in duplicate assays. The time of response relative to the injection of L-dopa, the double release pattern and the magnitude of the release were not indicative of an estrogen induced release. Upon ovarian examination 3 days after treatment a 4- to 5-day-old corpus luteum was observed in ewe 0575 which eliminated the possibility of a delayed endogenous ovulatory LH surge. None of the ewes treated with L-dopa were found to have newly ovulated follicles.

**Experiment 2:** Table 1 presents the concentrations of biogenic amines detected in the SME and HP of estradiol treated or control ewes on day 3 of the estrous cycle. No statistically significant changes in biogenic amine levels were found in the SME or HP of ewes 8 hr
Table 1. Concentrations of Biogenic Amines in the Stalk Median Eminence (SME) and Hypothalamus Proper (HP) of Intact Ewes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biogenic Amine (µg/g wet tissue weight)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Norepinephrine</td>
</tr>
<tr>
<td></td>
<td>SME</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>0.79±0.45</td>
</tr>
<tr>
<td>Controls</td>
<td>1.12±0.50</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SE of five ewes per group. Ewes were intramuscularly injected with 750 µg 17β-estradiol or corn oil 8 hr prior to necropsy at 0800 hr on day 3 of the estrous cycle.
after injection of estradiol. Norepinephrine levels in the SME of estradiol-treated ewes tended to be lower than those found in corn oil treated ewes, but in the HP, NE concentrations slightly increased in response to estradiol. In both the SME and HP, DA and 5-HT levels were inappreciably changed by estradiol. In all cases, biogenic amine levels in the SME were significantly different (P < .01) from those detected in the HP. The SME concentration of NE was lower than the concentration of NE in the HP but concentrations of DA and 5-HT in the SME were greater than the concentrations of these amines in the HP.

**Experiment 3:** The effects of exogenous estradiol and L-dopa on hypothalamic biogenic amine levels in ovariectomized ewes are presented in Table 2. Concentrations of biogenic amines in the SME and HP of ovariectomized ewes 3 hr following estradiol administration were significantly altered. Similarly, the changes in biogenic amine levels in the SME and HP measured 1 hr after L-dopa injection, although not statistically significant, reflect those changes in amines induced by estradiol. Exogenous estradiol elevated NE levels in the SME by over 100% (P ≤ .07) and L-dopa increased them by 80% over the levels detected in control ewes. Norepinephrine in the HP did not appear to be sensitive to either injected estradiol or L-dopa; both treatments had negligible effects on NE levels. Substantial quantities of 5-HT were detected in the SME of ovariectomized ewes and the
Table 2. Concentrations of Biogenic Amines in the Stalk Median Eminence (SME) and Hypothalamus Proper (HP) of Ovariectomized Ewes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biogenic Amine (µg/g wet tissue weight)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>Serotonin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SME</td>
<td>HP</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td></td>
<td>0.85±0.12</td>
<td>1.81±0.14</td>
</tr>
<tr>
<td>L-Dihydroxyphenylalanine (L-dopa)</td>
<td></td>
<td>0.67±0.12</td>
<td>1.79±0.14</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0.37±0.12</td>
<td>1.99±0.14</td>
</tr>
</tbody>
</table>

Mean ± SE of six ewes per group. Ovariectomized ewes were either intramuscularly injected with 750 µg of 17β-estradiol 3 hr prior to necropsy; intravenously injected with L-dopa (12 mg/kg) 1 hr before necropsy or injected with vehicle only. All ewes were necropsied at 0800 hr.

* p ≤ .07.
metabolism of this monoamine appears to be sensitive to both treatment with estradiol or L-dopa. Each treatment increased 5-HT levels in the SME and HP but to a different extent. Treatment of ewes with estradiol was followed by a 50% increase in 5-HT levels in the SME and HP while L-dopa increased the SME and HP 5-HT concentrations approximately 25% over those in control ewes. This same trend in hypothalamic 5-HT levels has been noted in the rat following injection of estradiol (Tonge and Greengrass, 1971) or L-dopa (Hyyppä, Lehtinen and Rinne, 1971).

Discussion

Biogenic amines not only influence gonadotropin release but appear to be in turn modified by ovarian steroids. Sites within the hypothalamus which concentrate estrogen correspond to the sites where microinjection of estrogen or drugs that alter monoamine metabolism affect pituitary release of gonadotropins (Kordon, 1971b). Castration produces marked effects on biogenic amine levels (Donoso and Stefano, 1967), turnover rates (Anton-Tay and Wurtman, 1968), enzymes of amine synthesis (Beattie, Rodgers and Soyka, 1972) and enzymes of amine degradation (Kobayashi, Kato and Minaguchi, 1964). All of the above effects of castration can be reversed by the injection of estrogen and progesterone.
Exogenous estradiol was capable of inducing a release of LH on day 3 of the estrous cycle in each of the ewes treated. The magnitude and duration of each LH release was similar but considerable variation did exist in the interval of time from estradiol injection until a release was detected. Injection of L-dopa into ewes on day 3 of the estrous cycle was followed by a release of LH in only one of the animals. It is possible this release of LH was facilitated by a favorable hormonal background prevalent in this ewe.

Biogenic amine levels in the SME and HP were not detected to be significantly different when measured 8 hours after injection of estradiol into ewes on day 3 of the cycle. It is conceivable that the temporal aspects of the experiment may not have been optimal for detection of estradiol induced changes in biogenic amine levels in the SME or HP. Eight hours after injection of estradiol was selected as the time of sacrifice because existing data indicated that LH levels generally rise in ewes shortly after this time interval (Bolt, Kelley and Hawk, 1971). Nevertheless, as pointed out earlier this response time was found to be subject to individual variation. If estradiol induced changes in the SME or HP biogenic amine levels responsible for triggering LH release are short lived, then this variation in time from injection of steroid to the onset of LH release could account for the inability to detect significant changes in amine levels in these hypophysiotropic areas at a constant time of necropsy.
Another possibility is that even the low levels of plasma progesterone reported to be present early in the estrous cycle of the ewe (Stabenfeldt, Holt and Ewing, 1969) may have a stabilizing effect on the response of the SME or HP biogenic amines to estradiol treatment.

Consequently, in another experiment designed to resolve the effect of exogenous estradiol on the SME and HP biogenic amine levels, ovariectomized ewes were used in anticipation of magnifying the response to estradiol by eliminating the primary source of endogenous estrogen and progesterone. Ovariectomized animals were necropsied 3 hr after injection of estradiol, an experimental design that Tonge and Greengrass (1971) used successfully to detect changes in mid-brain biogenic amine levels in rats after treatment with steroids. Marked changes in SME and HP biogenic amine levels were detected in ewes following injection of estradiol under these experimental conditions. Both concentrations of NE and 5-HT were elevated in the SME. In the HP, NE levels were not noticeably changed but 5-HT levels were increased. The ability of estradiol to alter biogenic amine levels in the central nervous system of the ewe, particularly in the SME, suggests that this steroid may act at least in part via monoaminergic systems to alter gonadotropin secretion in these animals. It is difficult to evaluate the relationship between the estradiol induced changes in hypophysiotropic biogenic amines and LH secretion in the ovariectomized ewe. Changes in monoamine
levels detected 3 hours after injection of estradiol may not only reflect the events triggering the ensuing induced LH release but may also be related to the circumstances responsible for the decrease in tonic serum LH levels observed within 3 hours after injection of this steroid into ovariectomized ewes (Scaramuzzi et al., 1971).

Injection of L-dopa into ovariectomized ewes 1 hour prior to necropsy did not produce significant changes in the concentration of biogenic amines in the SME and HP. It did, however, exert qualitative effects on the levels of NE and 5-HT in the SME resembling those detected in ewes 3 hours after injection of estradiol. Both NE and 5-HT concentrations in the SME tended to increase as a result of L-dopa treatment of ewes. The manner by which L-dopa affects 5-HT metabolism is not understood. Other investigations have demonstrated an interrelationship of this catecholamine precursor on indolamine metabolism (Everett and Borcherding, 1970; Ng et al., 1970; Goldstein and Frenkel, 1971). The common effect of estradiol and L-dopa on NE and 5-HT levels in the ovariectomized ewe indicate that one action of estradiol may be to increase the synthesis of L-dopa.

Highly significant differences were detected between the concentrations of biogenic amines present in the SME and HP of ewes. Dopamine is present in high concentrations in the SME yet almost undetectable in the HP while NE levels are low in the SME and high in the HP. These relative differences in concentrations of
catecholamines between the hypothalamus and median eminence of the ewe are similar to those reported to exist between the hypothalamus and median eminence of the rat as determined histochemically (Jonsson, Fuxe and Hökfelt, 1971). Greater concentrations of 5-HT were detected in the SME as compared to the HP in ewes. Recently, 5-HT has been chemically isolated from the infundibular area of the bovine (Piezzi, Larin and Wurtman, 1970) and rat (Clementi et al., 1970).

The results of the present studies demonstrate the contrast in monoamine concentrations between the SME and HP in the ewe as well as the ability of monoamines in each hypophysiotropic area to differentially respond to treatment of the animal with estradiol or L-dopa. These data also illustrate the advantage of independent chemical analysis of the median eminence and hypothalamus in investigating the role of biogenic amines in regulating the release of pituitary gonadotropins.
THE EFFECT OF VARIOUS PHARMACOLOGICAL AGENTS ON THE RELEASE OF LUTEINIZING HORMONE IN THE EWE

**Introduction**

In the ewe, as plasma progesterone concentrations decline during the estrous cycle, plasma estrogen levels rise initiating the onset of behavioral estrus. Evidence indicates this proestrus increase in estrogen elicits the ensuing ovulatory surge of luteinizing hormone (LH). A low dose of 17β-estradiol injected into anestrous or ovariectomized ewes brings about a release of LH characteristic of the endogenous ovulatory surge of LH (Goding et al., 1969; Scaramuzzi et al., 1971). Injection of 17β-estradiol into ewes early in the estrous cycle when plasma progesterone levels are low induces a release of LH and ovulation but a similar injection is without affect on the release of this gonadotropin in the presence of mid-cycle progesterone levels (Bolt, Kelley and Hawk, 1971). The mode by which estrogen stimulates LH release is not understood. In laboratory animals, investigations concerning the sites of estrogen feedback suggest that cells in the hypothalamus and anterior pituitary concentrate this steroid (Davidson, 1969). In addition it has been demonstrated that microimplantation of estradiol into the hypothalamus or the anterior pituitary influence the secretion of LH (Ramirez, Abrams and McCann, 1964; Chowers and McCann, 1967).
Microinjection of drugs that modify biogenic amine metabolism into the same hypothalamic sites that are sensitive to estrogen also influence the release of gonadotropins (Kordon, 1971). Hypothalamic biogenic amine levels (Tonge and Greengrass, 1971) and metabolism (Bapna, Neff and Costa, 1971) can be altered by injection of estrogen. The similar localization of action of estrogen and drugs and the metabolic link between estrogen and biogenic amines suggest that the effect of this steroid on LH release may be mediated in part via hypothalamic biogenic amines.

In the present experiments, attempts were made to inhibit the endogenous ovulatory surge of LH during the estrous cycle or an estrogen induced release of LH in anestrous and ovariectomized ewes by utilizing pharmacological agents which interfere or alter biogenic amine metabolism.

Materials and Methods

Animals: Mature, crossbred ewes averaging 60 kg were used in each of three experiments. Experiments were conducted during the breeding season and during anestrus. Under the regional environmental conditions, anestrus prevails from about February through August. Vasectomized marker rams were penned with the flock year-round and a ewe was considered to be in estrus as a consequence of being marked and when she stood for mounting by a ram. Ewes were
normally checked for estrus in the morning and evening of each day. Six daily checks for estrus were conducted at 4 hr intervals for the duration of the first experiment.

**Experiment 1:** Twelve ewes were assigned in replicate to four groups as they were detected in estrus. In group 1 ewes were injected with DL-\(\alpha\)-methyltyrosine methylester HCl (\(\alpha\)-MT), 12 mg/kg (free base) in saline (10 mg/ml). Administration of \(\alpha\)-MT decreases dopamine (DA) and norepinephrine (NE) concentrations by inhibiting tyrosine hydroxylase (Spector, Sjoerdsma and Udenfriend, 1965; Corrodi and Hanson, 1966). Ewes in group 2 were injected with DL-\(\alpha\)-methyl-3(3,4-dihydroxyphenyl)alanine (\(\alpha\)-MD), 6 mg/kg in saline (10 mg/ml). Injection of \(\alpha\)-MD gives rise to the false transmitter \(\alpha\)-methyl-DA in dopaminergic neurons (Iversen and Glowinski, 1966). In group 3 ewes were treated with 5-hydroxy-DL-tryptophan (5-HTP), 6 mg/kg in saline. The amino acid 5-HTP readily crosses the blood-brain barrier and enters serotoninergic neurons where it is decarboxylated to form and elevate serotonin (5-HT) levels (Udenfriend, Weissbach and Bogdanski, 1956). The ewes in group 4 served as controls and were injected with saline. Dosages of drugs injected into ewes in these studies were calculated on a metabolic body size \((\text{body weight}^{\frac{75}{75}})\) ratio from those used in rats. Based on these calculations a dose of 6 mg/kg administered to the ewe is equivalent to 25 mg/kg given to rats. Drugs were dissolved
immediately before injection at concentrations permitting equivalent injection volumes. Each ewe was injected with the appropriate drug twice, once immediately after being detected in standing estrus and again 4 hr later. A 10 ml blood sample was obtained via jugular puncture at the time of the first drug injection (0 hr) and at each 4 hr interval thereafter for 24 hr. Sera from these samples were stored frozen until assayed for LH using radioimmunoassay.

**Experiment 2:** Twelve anestrous ewes (July) were assigned randomly in equal numbers to three groups and treated with the following: group 1, α-MT and 17β-estradiol; group 2, saline and estradiol; and group 3, saline and corn oil. Ewes in group 1 received two equal intravenous injections of α-MT, 15 mg/kg in saline (32 mg/ml); the first injection was given at 0800 hr (0 hr) and the second injection 4 hr later. Along with the second injection of α-MT, ewes were given a single intramuscular injection of 20 μg of estradiol dissolved in corn oil. Group 2 ewes received saline at 0 hr and saline plus estradiol 4 hr later. Ewes in group 3 were treated with saline and corn oil following the appropriate injection schedule. Venous blood samples were taken for subsequent LH radioimmunoassay beginning at 0 hr and at 4 hr intervals for 32 hr. The ovaries of each ewe were examined through a mid-ventral incision 9 or 10 days after injection to check for the presence of new corpora lutea.

**Experiment 3:** Ten ewes were ovariectomized, allowed a
6 week recovery period, and assigned randomly to two groups of five animals each. Ewes in each group were treated as follows: group 1, DL-p-chlorophenylalanine (p-cpa) and 17β-estradiol; group 2, vehicle and estradiol. p-Chlorophenylalanine is an inhibitor of 5-HT synthesis and consequently lowers the levels of this amine (Koe and Weissman, 1966). Ewes in the first group received two injections of p-cpa. The first was administered intraperitoneally (ip) at 1200 hr, 75 mg/kg p-cpa (Sigma), in a 1% Tween-80 suspension (25 mg/ml). The second was given intravenously 48 hr later, 25 mg/kg (free base) p-cpa methylester HCl (Cal Biochem) in saline (20 mg/ml). A single intramuscular injection of 50 μg estradiol was given at the time of the second injection of p-cpa. Following the same injection schedule, group 2 ewes received 1% Tween-80, saline and estradiol. Venous blood samples were obtained for subsequent LH radioimmunoassay beginning 18 hr prior to the first p-cpa injection. Samples were taken at 6 hr intervals to the time of estradiol injection and then at 4 hr intervals for the next 32 hr.

**Results**

**Experiment 1:** Serum LH levels in each of the 12 ewes injected with α-MT, α-MD, 5-HTP or saline upon being detected in standing estrus are presented in Figure 1. It is evident from the high serum LH concentration (range, 80 to 300 ng/ml) detected in at least one
Figure 1. Three ewes each were intravenously injected with α-MD (6 mg/kg), α-MT (12 mg/kg), 5-HTP (6 mg/kg) or vehicle as soon as they were detected in estrus (0 hr) and again 4 hr later.
sample from each ewe that the endogenous ovulatory surge of LH was not blocked by any of the treatments. A third of the ewes were found to have ovulatory quantities of LH at the time of the first treatment injection, making it impossible to block the ovulatory LH release in these ewes. Data in the literature concerning the elapsed time from the onset of estrus until the commencement of the ovulatory surge range from 0 to 24 hr (Geschwind and Dewey, 1968; Niswender et al., 1968). A large part of this variation can be attributed to the various methods of heat detection and frequency of blood sampling used in each of these studies. In the present experiment, where ewes were checked for estrus every 4 hr with an ample number of rams and blood samples were obtained at 4 hr intervals, the onset of behavioral estrus and the ovulatory surge of LH were found to be less than 8 hr apart and in some cases, coincident.

**Experiment 2:** Subject to the experimental procedures of the second experiment, $\alpha$-MT did not inhibit the estradiol induced release of LH in anestrous ewes (Figure 2). No discernible difference in the character of the estradiol induced LH release was apparent between the ewes treated with $\alpha$-MT or those receiving saline. Ewes injected with saline and corn oil exhibited baseline serum LH levels of approximately 2 ng/ml throughout the sampling period. The uterine horns from all ewes in this experiment were observed to be pale and flaccid and no corpora lutea were evident at the time of laparotomy.
Figure 2. Four anestrous ewes were intravenously injected with α-MT (15 mg/kg) at 0800 hr (0 hr) and again 4 hr later. Four control ewes were similarly injected with vehicle. All ewes were intramuscularly injected with 20 μg of 17β-estradiol at 1200 (4 hr).
Experiment 3: It is evident from the data depicted in Figure 3 that treatment of ovariectomized ewes with p-cpa did not prevent the estradiol induced LH release. Nevertheless, a difference did exist between the ewes in the treated and control groups in the timing of the induced release. The onset of the estradiol induced LH release appeared to occur 4 hr later in time in the ewes injected with p-cpa. The magnitude and duration of the LH release in these ewes was not different from that of ewes injected with estradiol and vehicle. Tonic serum LH levels were elevated and variable (range, 1 to 22 ng/ml) in both the p-cpa treated and control ovariectomized ewes preceding the injection of estradiol. The typical decline in tonic serum levels (mean, 2.5 ng/ml) was evident 4 hr after the administration of estradiol.

Discussion

It has been demonstrated that the ovulatory surge of LH in the rat and ewe is dependent on the prior increase in serum estrogen levels. It has also been demonstrated in rats that administration of drugs capable of interfering with catecholamine synthesis and/or release, such as α-MT or α-MD (Kordon and Glowinski, 1969), or elevation of 5-HT concentrations by injection of large quantities of this indolamine (Labhsetwar, 1971), can block the ovulatory surge of LH. Together, these data suggest that the action of estrogen may be mediated via a biogenic amine mechanism.
Figure 3. Five ovariectomized ewes were intraperitoneally injected with p-Cpa (75 mg/kg) at 1200 hr (-48 hr) and intravenously injected with p-Cpa (25 mg/kg) at 0 hr. Five control ewes were similarly injected with vehicle. All ewes were intramuscularly injected with 50 μg of 17β-estradiol at 0 hr.
In the first experiment it was anticipated that injection of ewes at the time of detected estrus with either α-MT, α-MD or 5-HTP might inhibit the release of LH. The results of this experiment, however, failed to support biogenic aminergic intervention in the ovulatory release of LH. This lack of treatment effect may be attributed in part to the variability between animals in the time during which the ovulatory surge of LH occurs relative to detected estrus. In some ewes release of LH was maximal when the animals were detected in estrus making it impossible to inhibit the release. The longest time interval from drug injection to LH release was somewhere between 4 and 8 hours, perhaps too short a time for drugs to significantly modify biogenic amine metabolism. Alternatively, the drugs may have had sufficient time to act at least in those ewes releasing LH later into estrus. Assuming these circumstances, the lack of drug effect on inhibiting LH release brings to mind several possibilities. First, if there exists a functional neural center in the ewe containing estrogen sensitive biogenic amines responsible for triggering the cyclic secretion of luteinizing hormone-releasing hormone (LRH) as demonstrated in the rat, then it would appear this biogenic aminergic center in the ewe was triggered prior to the injection of drugs in this experiment. This is in agreement with results from studies with rats which demonstrate that the biogenic amine sensitive component to LRH secretion is activated during proestrus. Second, there may not
exist a monoaminergic neural intermediate involved in the action by which endogenous estradiol affects LH release in the ewe. Estradiol may by-pass monoaminergic neurons by directly stimulating the neurosecretory cells producing LRH. Another possibility that has not been excluded is the ability of estradiol to provoke LH release by acting directly on the anterior pituitary.

In all but one instance in these studies, drugs were injected intravenously in anticipation of expediting their time of action. Following intravenous injection, plasma drug concentrations are only temporarily increased, thus multiple iv injections were given to ewes in an attempt to maintain elevated drug levels. Nevertheless, it is possible that the dosages of drugs administered to ewes in the first experiment utilizing this injection regimen were not sufficient to alter the levels of hypothalamic biogenic amines.

It is evident that uncertainty pertaining to temporal relationships and drug dosage in the first experiment preclude any conclusions concerning the mechanism of estrogen action in stimulating LH release. Two additional studies were designed to minimize time as a variable and achieve a greater probability of drug effectiveness in inhibiting the release of LH. Advantage was taken of the predictable manner in which LH release follows the injection of estradiol in the anestrous and ovariectomized ewe. A drug antagonism to the ability
of estradiol to induce LH release in the ewe would suggest that the drug may be acting on hypothalamic biogenic amines.

In the second experiment, α-MT was administered 4 hours in advance of estradiol injection. Intraperitoneal administration of a metabolic body size equivalent dose of α-MT given to rats is effective within 8 hours after injection in lowering brain DA and NE levels. Consideration of the iv injection route of α-MT and the time needed for exogenous estradiol to reach stimulatory levels in the plasma of the ewe enhances the likelihood that the time of drug effectiveness and the time needed for estradiol to induce LH release would overlap. Absence of differences in the nature of the LH release between the α-MT treated and control ewes suggests that a catecholaminergic involvement may not be essential in the estradiol induced release of this gonadotropin.

In the third experiment, p-cpa was administered to ovariectomized ewes ip 48 hours prior to injection of estradiol. An equivalent dose of p-cpa given ip to dogs minimized mid-brain 5-HT concentrations within two days (Koe and Weissman, 1966). p-Chlorophenylalanine was used as a treatment to block estradiol induced LH release in experiment 3 because results from a previous study (p. 25) demonstrated that 5-HT concentrations increase in response to estradiol in the ovariectomized ewe. A similar increase in 5-HT levels was detected in ovariectomized rats following injection of estrogen
(Tonge and Greengrass, 1971). Although p-cpa did not block or reduce the estradiol induced release of LH, it appeared to shift the onset of the release 4 hours later in time. These data suggest that changes in the function of serotonergic neurons may be involved in facilitating the release of LH in the ewe. This is supported in part by experimental evidence demonstrating that a reduction in hypothalamic 5-HT levels at estrus precedes the ovulatory release of LH in the ewe (Wheaton et al., 1972).

Admittedly highly qualified, the absence of pharmacological blockade of the endogenous ovulatory surge of LH and the failure of α-MT and p-cpa to inhibit the ability of exogenous estrogen to induce LH release, collectively suggest that LH release in the ewe may occur via mechanism(s) which are not entirely dependent on the biogenic amines.
RELEASE OF LUTEINIZING HORMONE IN THE EWE DURING VARIOUS STAGES OF ANESTRUS FOLLOWING INJECTION OF L-DIHYDROXYPHENYLALANINE AND p-CHLOROPHENYLALANINE

Introduction

Seasonal reproductive quiescence of ewes provides a fortuitous neuroendocrine state for the investigation of neural mechanisms influencing gonadotropin release in this species. Absence of neurologic stimuli promoting gonadotropin releasing hormone(s) secretion presumably underlies anestrus since components of the hypothalamo-hypophysial axis appear to be functionally intact. Substantial luteinizing hormone-releasing hormone (LRH) has been demonstrated to be present in the hypophysiotropic area of the ewe during anestrus (Jackson et al., 1971) and LRH secretion has been provoked upon electrostimulation of the hypothalamus (Przekop and Domanski, 1970). In response to injection of synthetic gonadotropin releasing hormone, the adenohypophysis of the anestrous ewe is capable of releasing ovulatory quantities of luteinizing hormone (Reeves et al., 1972).

Whatever the origin of the neurologic stimuli triggering gonadotropin releasing hormone(s) secretion, be it environmental or internal, chemical mediation is required and most likely is monoaminergic in nature. Biogenic monoamines clearly play an integral role in the nervous regulation of gonadotropin releasing hormone(s) secretion.
(Kordon and Glowinski, 1972). It appears both a stimulatory and inhibitory influence are supplied by monoaminergic neurons to the neurosecretory neurons of the ventromedial hypothalamus. It has been proposed that catecholaminergic neurons function to stimulate whereas serotoninergic neurons function to inhibit the secretion of LRH (Kamberi, Mical and Porter, 1970).

In the present experiments, attempts were made to pharmaco-logically induce LRH secretion in anestrous ewes by elevating catecholamine levels through the administration of the catecholamine precursor L-dihydroxyphenylalanine (L-dopa) or by lowering serotonin levels using the serotonin synthesis inhibitor, p-chlorophenylalanine (p-cpa).

**Materials and Methods**

**Animals:** Crossbred ewes ranging in age from two to six years and averaging 60 kg were used in each of the studies except experiment number 5, in which two-year-old Hampshire ewes were utilized. Experiments were conducted during anestrus, which occurs under the regional environmental conditions from February through August. June is considered the time of deepest anestrus. Vasectomized marker rams were penned with the flock year-round and a ewe was considered to be in estrus as a consequence of being marked and when she stood for mounting by a ram at the morning or evening check. A
ram was kept with the experimental animals during each study. Animals were used on more than one experiment but never on consecutive studies, thus insuring the ewes ample recovery time before being used again.

Drugs: L-α-3, 4-Dihydroxyphenylalanine (Sigma) was dissolved in warm 0.01 N HCl on the day of use. A concentration of 10 mg/ml was used for the highest dose and appropriate dilutions were made for lower doses in order to maintain an equal injection volume. Control animals received an equivalent volume of 0.01 N HCl. p-Chlorophenylalanine (Sigma) was suspended in 1% Tween-80 shortly before use, the greatest concentration used being 15 mg/ml. p-Chlorophenylalanine methylester HCl (Cal-Biochem) was dissolved in saline at a concentration of 150 mg/ml calculated on the basis of the non-esterified compound. Equivalent volumes of 1% Tween-80 or saline were administered to corresponding control ewes. Dosages of L-dopa and p-cpa were derived from those used in rats calculated on a metabolic body size (body weight $^{0.75}$) ratio. Based on these calculations a dose of 6 mg/kg administered to ewes is equivalent to 25 mg/kg given to rats. 17β-Estradiol (Sigma) was dissolved in corn oil at concentrations permitting 1 ml injections.

Experiment 1: Prior to experimentation, the carotid artery in three ewes was exteriorized in a skin loop (Bone, Metcalfe and Parer, 1962) to facilitate intra-arterial (i-a) administration of drugs.
During late anestrus (August), these three and eight other ewes were assigned to the first experiment. Four ewes were treated with L-dopa; two were given a dose of 6 mg/kg body weight and two received 3 mg/kg. Each dose level was injected into the jugular vein (iv) into one ewe and i-a into the other. Two control ewes were injected with vehicle, one iv, the other i-a. p-Chlorophenylalanine was administered to four ewes, two at a dose of 22 and two at 11 mg/kg. A control animal was also treated with an equivalent volume of saline. All p-cpa injections were iv. Ten ml blood samples were obtained by jugular puncture immediately prior to injection, 1200 hr (0 hr), and at subsequent 4 hr intervals for 32 hr. Serum from each blood sample was analyzed for luteinizing hormone (LH) using radioimmunoassay.

**Experiment 2:** The second study was conducted in the spring succeeding experiment 1. Sixteen early anestrous (May) ewes were assigned randomly in equal numbers to four groups. Ewes in each group were intravenously injected with one of the following doses of L-dopa: 0, 6, 12 or 18 mg/kg. In the first experiment (p. 51) the two ewes injected with a 6 mg/kg dose of L-dopa, one i-a and the other iv, responded similarly to treatment with a release of LH. From these limited data on i-a vs. iv injection routes, the only conclusion reached was that a dose of 6 mg/kg was sufficient to be effective when injected iv. Therefore, in subsequent experiments L-dopa was administered iv at a minimum dose of 6 mg/kg. Ewes were injected
with L-dopa at 0800 hr (0 hr). Venous blood samples were obtained at 0 hr and at each 4 hr period thereafter for 40 hr. Shortly after three of the ewes were injected with the high dose of L-dopa, the first ewe injected exhibited tachypnea, collapsed and expired within 30 minutes. Instead of injecting the fourth ewe with 18 mg/kg L-dopa, two additional ewes were added to the experiment redefining the groups as follows: group 1, four ewes received 0 mg/kg L-dopa; group 2, six ewes were injected with 6 mg/kg; group 3, five ewes were given 12 mg/kg; and group 4, two ewes were injected with 18 mg/kg. Eight or nine days following injection the ovaries of each ewe were examined through a mid-ventral incision for presence of new corpora lutea.

**Experiment 3:** In early anestrus (May), 12 ewes were assigned randomly in equal numbers to three groups. Ewes were iv injected with one of the following doses of p-cpa-methylester HCl: 0, 30 or 60 mg/kg. p-Chlorophenylalanine methylester HCl was injected at 1200 hr (0 hr) and venous blood samples were taken at 0 hr and at every 6 hr interval thereafter for 48 hr. After injecting the high dose to two ewes, the second ewe injected died within minutes after treatment. The remaining two animals in this group were not injected, leaving one ewe in the high dose group. Nine or 10 days following injection ewes were laparotomized and the ovaries of each ewe were examined to determine whether ovulation had occurred.
**Experiment 4:** Eight deep anestrous (June) ewes were pre-treated with 17β-estradiol followed by treatment with L-dopa or vehicle. A single intramuscular (im) injection of 5 µg estradiol was given at 0800 hr followed by another 3 µg injection at 2000 hr. At 0800 hr (0 hr) the next morning, four ewes were injected iv with L-dopa (12 mg/kg) and four animals received an injection of vehicle. Blood was obtained at 0 hr and at 4 hr intervals for 36 hr. The ovaries from each ewe were examined 3 to 4 days after L-dopa or vehicle injection for the presence of corpora lutea.

**Experiment 5:** Ten Hampshire ewes were laparotomized during late anestrus (August). Corpora lutea were present in seven of the ewes. The three ewes which were found to be anestrus were injected iv with 6 mg/kg L-dopa at 0800 hr (0 hr) the morning after ovarian examination. Venous blood samples were obtained at 0 hr and at 4 hr intervals for the next 32 hr. The ovaries of these ewes were re-examined for signs of induced ovulation two days after injection.

**Results**

**Experiment 1:** Serum LH levels in the four late anestrous ewes treated with L-dopa are presented in Figure 1. Both ewes injected with 6 mg/kg were found to have elevated LH levels. Peak LH levels of 42 and 63 ng/ml serum were detected at 16 and 24 hr post-injection for ewes injected iv and i-a, respectively. The two ewes treated with
Group 1 - L-α-3,4-Dihydroxyphenylalanine (L-dopa)

Figure 1. Serum LH concentrations of 4 late anestrous ewes treated with L-dopa (group 1). Two ewes received 6 (---) and 2 received 3 mg/kg (----) L-dopa. Each dose level was injected intra-arterially (i-a) into 1 ewe and intravenously (iv) into the other. Four ewes were iv injected with p-Cpa (group 2); 2 ewes received 22 and 2 received 11 mg/kg p-Cpa. All ewes were injected at 1200 hr (0 hr).
3 mg/kg and the control ewes had baseline LH levels of approximately 2 ng/ml throughout the 32 hr sampling period. It can be seen from Figure 1 that one of the two ewes injected with the high dose of p-cpa (22 mg/kg) released LH. A peak LH serum concentration of 81 ng/ml was detected 28 hr after treatment in this ewe. Serum LH levels from the remaining treated and control ewes were baseline.

**Experiment 2:** Serum LH levels detected in the venous blood samples obtained from each early anestrous ewe during the 42 hr sampling period following treatment with L-dopa or vehicle were averaged and are presented in Table 1. Six, 12 or 18 mg/kg L-dopa administered to ewes at this stage of anestrus failed to significantly alter LH levels. In contrast to the quiescent ovaries and pale, flaccid uterine horns observed in 16 of the ewes, one control ewe possessed a corpus luteum and flush, tonus uterine horns. Data from this animal were not included in the analysis or presented.

**Experiment 3:** The effect of p-cpa-methylester HCl on serum LH levels of ewes in early anestrus are presented in Table 2. Data are expressed as in experiment 2. Administration of p-cpa-methylester HCl was without affect on serum LH levels throughout the 48 hr experiment. Nevertheless, nine days after injection two bright red corpora lutea weighing 100 and 122 mg, judged to be about two to three days old, were observed in the single ewe treated with 60 mg/kg. Since no elevated serum LH levels were detected in this ewe, it appears
Table 1. Serum LH Concentrations (ng/ml) in Anestrous Ewes Treated with L-Dihydroxyphenylalanine (L-dopa).  

<table>
<thead>
<tr>
<th>Dose of L-dopa (mg/kg)</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1.4 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.8</td>
<td>2.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6 ± 0.4</td>
<td>2.3 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 ± 0.5</td>
<td>1.7 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SE of 11 serum samples obtained from each anestrous ewe beginning at the time of intravenous injection of L-dopa, 0800 hr, and at each 4 hr interval thereafter for 40 hr.*
Table 2. Serum LH Concentrations (ng/ml) in Anestrous Ewes Treated with p-Chlorophenylalanine (p-cpa).\(^a\)

<table>
<thead>
<tr>
<th>Dose of p-cpa (mg/kg)</th>
<th>0</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.3±0.1</td>
<td>2.1±0.4</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td></td>
<td>2.1±0.2</td>
<td>2.8±0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2±0.2</td>
<td>1.6±0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.9±0.1</td>
<td>1.9±0.1</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SE of nine serum samples obtained from each anestrous ewe beginning at the time of intravenous injection of p-cpa, 1200 hr, and at each 6 hr interval thereafter for 48 hr.
that ovulatory quantities of LH were released after the 48 hr sampling period.

**Experiment 4:** Estrogen pretreatment during deep anestrus did not influence the response to injected L-dopa. No significant differences were noted between the serum LH levels of L-dopa treated and control ewes (Table 3). Some of the ewes released substantial quantities of LH during the pretreatment period as evidenced by the high LH levels detected in the control sample (0 hr). In this connection, at the time of ovarian examination, one ewe had a small corpus luteum and two others had luteinized follicles.

**Experiment 5:** As can be seen from Figure 2, one of three late anestrous ewes treated with L-dopa (6 mg/kg) released large amounts of LH. A peak serum LH value of 76 ng/ml was detected 24 hr post-injection. Ovulation was induced in this ewe as indicated by the presence of corpora lutea two days after L-dopa injection.

**Discussion**

Although the ewe remains overtly reproductively quiescent during late anestrus, there is some evidence indicating neuroendocrine adjustments of a transitional nature are taking place. Information on circulating levels of gonadal and pituitary hormones during anestrus is limited. It has been shown that LH levels, both in the blood and anterior pituitary, increase during late anestrus (Roche et al., 1970).
Table 3. The Effect of Exogenous L-dopa on Serum LH Concentrations (ng/ml) in Anestrous Ewes Pretreated with 17β-Estradiol. 

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after Injection of L-dopa (hr)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>8-36</td>
<td></td>
</tr>
<tr>
<td>L-Dihydroxyphenylalanine</td>
<td>19.3</td>
<td>1.7</td>
<td>1.2±0.6</td>
<td></td>
</tr>
<tr>
<td>(L-dopa)</td>
<td>6.5</td>
<td>2.4</td>
<td>0.5±0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>2.3</td>
<td>2.5±0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>6.3</td>
<td>NDc</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.0</td>
<td>2.4</td>
<td>1.5±1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>4.4</td>
<td>1.4±0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>3.5</td>
<td>0.7±0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>2.4</td>
<td>3.1±0.3</td>
<td></td>
</tr>
</tbody>
</table>

a A single intramuscular (im) injection of 5 µg 17β-estradiol into ewes at 0800 hr was followed by another 3 µg im injection at 2000 hr. At 0800 hr (0 hr) the next morning, ewes were intravenously (iv) injected with L-dopa (12 mg/kg) or vehicle.

b Mean ± SE of eight serum samples obtained from each ewe beginning at 8 hr after iv injection (0 hr) of L-dopa or vehicle and at each 4 hr interval thereafter for 28 hr.

c Non detectable.
Figure 2. Serum LH concentrations of 3 late anestrous ewes intravenously injected with L-α-3,4-dihydroxyphenylalanine (L-dopa, 6 mg/kg) at 0800 hr (0 hr).
Bioassayable LH can be released in rats injected with extracts of SME from late anestrous ewes but not when injected with extracts of SME from early anestrous ewes (Dománski and Kochman, 1968). In another experiment, more LRH activity was detected in late compared to early anestrous ewes (Jackson et al., 1971). Conceivably, a change in nervous activity induced in part by environmental stimuli underlies the neuroendocrine modulations occurring during this transitional stage of anestrus. Changes in the activity of afferent neurons impinging upon the hypophysiotropic area may be reflected in alterations of biogenic amine metabolism since it has been demonstrated that hypothalamic biogenic amines influence neurosecretory cells elaborating gonadotropin releasing factor(s) (Coppola, 1971).

Collectively, the experimental results demonstrate that systemically administered L-dopa and p-cpa can stimulate LH release in the anestrous ewe but this capability appears to be dependent on the stage of anestrus. It is interesting that ewes responding to treatment with L-dopa or p-cpa did so in late anestrus, suggesting that the likelihood of encountering favorable circumstances for a drug response increases as the anestrous season draws to an end. Nevertheless, even during late anestrus, only three of the seven ewes treated with L-dopa released LH. p-Chlorophenylalanine induced a release of LH from one of two ewes treated during this period. Undoubtedly contributing to these superficial hit-or-miss results is individual progress being made towards the onset of estrus.
Exogenous L-dopa is rapidly decarboxylated to DA by the ubiquitous enzyme L-amino-acid decarboxylase. The small fraction of L-dopa entering hypothalamic catecholaminergic neurons is primarily reflected in elevated DA levels (Everett and Borcherding, 1970), although a slight increase in NE levels is observed following L-dopa treatment in the rat (Romero et al., 1972). There is some evidence that L-dopa affects 5-HT metabolism as well (Ng et al., 1970). Thus, it is assumed L-dopa treatment effects are brought about by elevated catecholamine levels with perhaps complimentary changes in 5-HT metabolism.

Serotonin levels fall and remain low for several weeks following p-cpa administration to rats, rabbits and dogs (Koe and Weissman, 1966). Presumably, p-cpa inhibits the synthesis of 5-HT by blocking the enzyme tryptophan hydroxylase. Accordingly, p-cpa treatment effects are attributed to alterations in 5-HT metabolism.

Both L-dopa and p-cpa act via the hypothalamo-hypophysial axis since a peripheral drug effect has been eliminated by using serum LH concentrations as the criteria for drug response rather than the more commonly used endpoint, ovulation.

Ovulation without manifestations of behavioral estrus normally precedes the first behavioral estrus of the breeding season (Scaramuzzi et al., 1971). In experiment 5, corpora lutea (CL) were present in seven of 10 Hampshire ewes presumed to be in late
anestrus. In addition to checking for new corpora lutea resulting from treatment induced ovulation, laparotomy of ewes was normally performed after each experiment, except number 1, to detect the presence of existing CL. In the first experiment, CL may have been present at the time of treatment. This seems unlikely however, at least for the ewes that did release LH, in view of the limited evidence indicating that progesterone hinders the ability of L-dopa to induce LH release in the rat (Dickey, 1970). Silent ovulation during late anestrus implies an ovulatory LH release. The possibility of mistaking such an LH release for a treatment induced LH release cannot be completely ruled out; however, the probability leading to this circumstance is remote (calculated to be < 1%).

As mentioned earlier, little is known about plasma gonadotropin and ovarian steroid hormone levels in the anestrous ewe. In the rat, estrogen appears to facilitate the action of L-dopa. L-Dopa has been found to be successful in eliciting LH release during proestrus but not during other stages of the estrous cycle (Gay, 1972). A priming dose of estrogen facilitated a release of LH following L-dopa injection into ovariectomized rats (Dickey, 1970). In this connection, deep anestrous ewes were pretreated with low doses of estradiol before receiving L-dopa (experiment 4). Estradiol priming did not appear to facilitate the ability of exogenous L-dopa to cause a release of LH in these ewes.
These experimental observations support the existence of a fundamental difference in the responsiveness of the central nervous system to pharmacological agents affecting monoamine metabolism in deep anestrous ewes compared to ewes approaching the breeding season.


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