ROLE OF SEROTONIN AND DOPAMINE IN GONADOTROPIN RELEASE

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A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Physiology

1979

ABSTRACT

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Sustained administration of the dopamine (DA) agonist, piribedil, prevented the post-castration rise of both LH and FSH, whereas multiple injections of the serotonin (5-HT) precursor, 5-hydroxytryptophan (5-HTP), blocked only the increase in serum LH. The DA and 5-HT receptor blockers, pimozide and methysergide, had no effect on the increase in serum gonadotropin after orchidectomy, but reversed the inhibitory effects of piribedil and 5-HTP. These results indicate that both DA and 5-HT can inhibit the release of gonadotropin.

Orchidectomy had no effect on either the concentration or the turnover of norepinephrine (NE) in the median eminence (ME) of male rats. A significant increase in DA concentration in the ME occurred by 16 and 24 hrs post-castration. DA turnover was significantly elevated at 16 hrs after orchidectomy. Serum LH and FSH increased significantly by 8 hrs and 16 hrs post-castration, respectively. These observations suggest that tuberoinfundibular DA may not have an important role in steroid-mediated negative

feedback control of gonadotropin, but may have a role in prolactin secretion.

An afternoon surge of gonadotropin was induced in ovariectomized rats by either two injections of estradiol benzoate (EB) 72 hrs apart (EB-EB), or by progesterone (PRG) 72 hrs after EB priming (EB-PRG). Blockade of 5-HT synthesis with p-chlorophenylalanine (PCPA), administered 3 days earlier, significantly suppressed serum FSH in EB-EB treated rats, whereas the LH surge was not consistently affected. Subsequent injection of 5-HTP at 1000 hrs potentiated and also advanced the surge of gonadotropin. The LH surge could not be induced before 1200 hrs by earlier injection of 5-HTP. PCPA had no effect on the gonadotropin surge in EB-PRG treated rats. However, subsequent injection of 5-HTP potentiated both the LH and FSH surges. These findings demonstrate a time dependent facilitative action of 5-HT on the phasic release of gonadotropin.

The gonadotropin surges in EB-PRG, but not in EB-EB treated rats were significantly augmented by p-chloroamphetamine (PCA), a long-lasting 5-HT antagonist, and 5,7-dihydroxytryptamine (5,7-DHT), a 5-HT neurotoxic agent. Administration of PCA and 5,7-DHT resulted in a greater depletion of 5-HT in the medial basal hypothalamus (MBH) than in the anterior hypothalamic area (AHA). These results suggest an inhibitory serotonergic pathway in the MBH regulating the phasic release of gonadotropins.

The LH surge in EB-EB treated ovariectomized rats was abolished 24 hrs after PCPA injection, and this could be restored by subsequent injection of 5-HTP. The LH surge in rats pre-treated

with PCPA for 48 hrs was significantly augmented by the first but not the second dose of 5-HTP. Administration of GnRH to induce a huge surge of LH failed to prevent 5-HTP from potentiating the LH surge on the next day. These results suggest that the serotonergic system may develop supersensitivity to 5-HT agonists 48 hrs after PCPA treatment, and can be desensitized within 24 hrs by 5-HTP treatment.

The 5-HT agonists, 5-HT, quipazine, and 5-HTP, at low doses augmented the gonadotropin surges in EB-EB treated ovariectomized rats, whereas at higher doses, they tended to suppress the surges. It is concluded that 5-HT may exert a biphasic effect on the phasic release of gonadotropin, with a facilitative effect at low doses and an inhibitory effect at higher doses.

Administration of 5-HTP in EB-EB treated ovariectomized rats resulted in a 4-fold increase in serum progesterone within 30 mins, and significantly augmented the LH surge in PCPA pre-treated rats. Adenalectomy did not block the facilitative action of 5-HTP. These results suggest that adrenal progesterone is not required for 5-HTP to exert its facilitative action on the phasic release of LH.

In EB primed rats, the steady state concentration of 5-HT in the AHA and MBH increased, whereas the turnover decreased significantly by 4 hrs after PRG administration. These results indicate that the decrease in 5-HT turnover in the MBH after PRG treatment may be associated with its potentiation of the gonadotropin surge.

DEDICATION

This dissertation is dedicated to my parents

Mr. and Mrs. Shih-Hsi Chen.

ACKNOWLEDGMENTS

I wish to acknowledge thanks to Dr. Joseph Meites for providing me with the support and the opportunity to undertake these studies.

I also wish to thank the fellow members of our laboratory for their help and friendship during my graduate studies.

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INTRODUCTION

The stability of the internal environment which is vital to the survival of life depends on the coordination of both nervous and endocrine systems. The linkage between the two systems is provided by the neurosecretory cells located in the hypothalamus to act as neuroendocrine transducers (Wurtman, 1973). Therefore, a neural input from the central nervous system (CNS) triggers the release of hypophysiotropic hormones which travel through the hypothalamo-hypophyseal portal circulation and act on the anterior pituitary to regulate hormone secretion. Both physiological and pharmacological evidence indicate that hypothalamic monoamines are involved in regulation of gonadotropin secretion. There is general agreement that the secretion of luteinizing hormone (LH) is under the stimulatory influence of the central noradrenergic system, whereas evidence for possible roles by dopamine (DA) and serotonin (5-HT) in the secretion of gonadotropin is still confusing and conflicting, despite extensive investigation in the last decade. This thesis therefore was devoted to further investigating the role of central dopaminergic and serotonergic systems in regulating gonadotropin secretion in the rat.

It appears that DA may exert both inhibitory and stimulatory effects on LH secretion, depending on the steroid environment. The mechanisms involving the regulation of gonadotropin secretion in the

female rat are complicated by the fact that gonadal steroids can exert both positive and negative feedback regulation of gonadotropin secretion. A tonic center in the medial basal hypothalamus (MBH) has been suggested to maintain basal secretion, whereas the cyclic center in the pre-optic-anterior hypothalamic area (AHA) controls the phasic release of gonadotropin. Since multiple monoaminergic pathways may be involved in gonadotropin secretion, and both DA and 5-HT may exert different effects at the two centers, the early conflicting reports may simply be due to the different models used in these studies. Therefore, both positive and negative feedback control systems were examined in this thesis to assess the possible roles of DA and 5-HT on the secretion of gonadotropin.

Castration results in a rapid rise in gonadotropin in the male rat, but not in the female (Gay and Midgley, 1969). Thus, short-term orchidectomy was applied in the first part of this thesis as a model for studying the negative feedback control systems. The effects of DA and 5-HT on the post-castration rise of both LH and follicle-stimulating hormone (FSH) after acute orchidectomy were first investigated by using pharmacological approaches. In addition, changes in catecholamine turnover in the median eminence were also evaluated in order to correlate with the hormone changes following orchidectomy.

Early studies indicated that the serotonergic effects on control of gonadotropin secretion and ovulation were inhibitory in nature. However, new evidence also suggested a facilitative role for 5-HT in the cyclic release of LH. Therefore, the second part of this

thesis was devoted to further examining this stimulatory role of 5-HT by using ovarian steroid primed ovariectomized rats as a model (Caligaris et al., 1971a). Pharmacological agents, such as 5-HT synthesis inhibitors, neurotoxins, precursors, and agonists, were used to manipulate central serotonergic activity. It has been proposed that both a facilitative 5-HT center located in the AHA and an inhibitory center in the MBH are involved in regulating the phasic release of LH (Kordon and Glowinski, 1972). Therefore, 5-HT turnover in the AHA and MBH were measured in estradiol benzoate (EB) primed ovariectomized rats after progesterone (PRG) administration.

LITERATURE REVIEW

I. Hypothalamic Control of Anterior Pituitary Secretion

A. Classical Observations of Functional Relationship Between Hypothalamus and Adenohypophysis

It is well known that the secretion of anterior pituitary hormones is under the regulation and control of the central nervous system (CNS). Environmental changes, such as light, temperature, odor and touch, often perceived through the special sensory organs, are known to affect pituitary hormone secretion (Marshall, 1942; Harris, 1955). The pituitary gland lies directly underneath the hypothalamus with a pituitary stalk connecting the two structures together. In view of its close anatomic relationship with the pituitary gland, and its numerous afferent connections with the other parts of the brain, the hypothalamus is likely to be the center integrating nerve signals which regulate the secretion of adenohypophyseal hormones.

Early indications for an important role of the hypothalamus in control of pituitary hormone secretion has been derived from many kinds of experiments.

Electrical Lesions and Stimulation of the Hypothalamus.-- As early as 1921, Aschner demonstrated that gonadal deficiency could

be induced in dogs by hypothalamic lesions which spared the pituitary. Similar observations were confirmed later by Camus and Roussy (1920) in rats and by Dey (1943) in guinea pigs. In addition, hypothalamic lesions were shown to induce atrophy of the thyroid (Cahane and Cahane, 1938; Greer, 1952; Bogdanove and Hamli, 1953) and the adrenal cortex (deGroot and Harris, 1950), and to block stress induced hypertrophy of the adrenal glands (Ganong and Hume, 1954). On the other hand, electrical stimulation of certain areas of the hypothalamus was found to induce ovulation in rabbits (Harris, 1937; Haterius and Derbyshire, 1937), whereas direct stimulation of the adenohypophysis was ineffective (Markee et al., 1946; Harris, 1948a). Electrical stimulation of the hypothalamus also increased the activity of thyroid gland (Harris, 1948b) and adrenal cortex (deGroot and Harris, 1950).

Transection of Pituitary Stalk.--Sectioning of the pituitary stalk generally produces only transient effects on pituitary functions because of the regeneration of portal vessels (Harris, 1949). If regeneration of these portal vessels is prevented by placing a mechanical barrier between the pituitary and hypothalamus, the secretory function of the pituitary is seriously impaired. In 1923, Dott first demonstrated that transection of the pituitary stalk resulted in atrophy of both the gonads and the thyroids in dogs. Thereafter, in a series of classic experiments, Harris (1950) showed that stalk section in the rat caused loss of sexual function. It was also shown that stalk section impaired normal adrenal (Fortier

et al., 1957; Lazalo and DeWied, 1966) and thyroid functions (Brown-Grant et al., 1957).

Transplantation of the Pituitary Gland.--It was reported that when the pituitary gland was removed from its original position in the sella turcica and transplanted to either the anterior chamber of the eye or underneath the kidney capsule, a variety of physiological changes occurred, including atrophy of the gonads, adrenals and thyroid glands, with the exception of functional corpora lutea, which persisted for a prolonged period (Harris, 1948b; Harris and Jacobsohn, 1952; Everett, 1954). The pituitary failure resulting from ectopic transplantation of the gland could be corrected by re-transplanting the same pituitary back to its normal position beneath the median eminence, where regeneration of blood vessels occurred (Nikitovitch-Winer and Everett, 1958). These observations demonstrated that the pituitary fossa is a privileged site for the growth and function of the pituitary.

B. Anatomy of the Hypothalamus

The hypothalamus, which is located in the most ventral portion of the diencephalon (Netter, 1968; Jenkins, 1972), comprises the lateral walls of the third ventricle below the hypothalamic sulcus and those structures of the ventricular floor. The anterior and posterior boundaries of the hypothalamus are demarcated by the optic chiasma and the mammillary bodies, respectively. Laterally, the hypothalamus is indistinctly separated from the subthalamus.

There are three regions of gray matter arrayed in a rostro-caudal sequence in the hypothalamus; namely supraoptic, tuberal and mammillary area. In general, hypothalamic nuclei are located bilaterally on each side of the third ventricle with the exception of the median eminence. The supraoptic area lies above the optic chiasma and fuses rostrally with the preoptic area (POA), which is generally not considered to be part of the hypothalamus. However, the integrity of the POA and anterior hypothalamus is crucial for the cyclic release of luteinizing hormone (LH) (Halász and Pupp, 1965; Gorski, 1966; Tejasen and Everett, 1967). Lying directly upon the optic chiasma and immediately ventral and caudal to the medial pre-optic area is the well-defined supra-chiasmatic nucleus (SCN), which receives ascending, serotonin containing afferent fibers from the raphé nuclei (Dahlström and Fuxe, 1964; Fuxe 1965a,b; Aghajanian et al., 1969). It is believed that SCN may play an important role in maintenance of some circadian rhythms in rodents (Menaker et al., 1978). Lying dorsolateral to the SCN is the anterior hypothalamic nucleus (AHN). Two functionally well-defined nuclei, namely the supraoptic and the paraventricular nuclei, can also be localized in this area. The former is primarily concerned with the secretion of oxytocin and the latter with that of antidiuretic hormone (ADH) (Bargmann and Scharrer, 1951).

The tuberal region of the hypothalamus includes the area dorsal to the tuber cinereum, located on the ventral surface of the brain, between the optic chiasma and mammillary bodies. The median

eminence, a small but highly vascularized protrusion at the apex of the dome shaped base of the hypothalamus, can be further divided anatomically into three zones: (a) the inner ependymal zone, which consists of the ependymal cells lining the inferior portion of the third ventricle; (b) the inner palisade layer, which contains the hypothalamo-neurohypophyseal neurons; and (c) the outer palisade layer, wherein lies the neurovascular junction between the axons of the tuberohypophyseal tract and the capillary loops of the portal vessel (Knigge and Scott, 1970). The tuberoinfundibular dopaminergic axons, which end on the portal capillaries, appear to have their cell bodies located in the arcuate nucleus and the anterior periventricular nuclei (Fuxe and Hökfelt, 1966; Hökfelt and Fuxe, 1972). In addition to the median eminence, the arcuate nucleus and anterior periventricular nuclei, the tuberal region contains the lateral hypothalamic nucleus, the ventromedial nucleus, and the dorsomedial nucleus.

The mammillary region contains mammillary bodies which are not believed to be essential for central control of anterior pituitary hormone secretion. The hypothalamus receives afferent fibers mainly from two regions of the brain, including the brain stem reticular formation, from which afferents reach the hypothalamus via the mammillary peduncle, the dorsal longitudinal fasiculus and the medial forebrain bundle, and the limbic system from which afferents innervate the hypothalamus via the fornix, the medial forebrain bundle, the thalamo-hypothalamic fibers and the stria terminalis

(Nauta and Haymaker, 1969). In addition, evidence for the existence of retinohypothalamic tract has been reported (Riss et al., 1963). The major efferent pahtways leave the hypothalamus via the hypothalamo-hypophyseal, periventricular and mammillary tracts.

C. Hypothalamo-Hypophyseal Portal Vessels

A set of portal vessles connecting the median eminence region of the basal hypothalamus with the sinusoids in the anterior pituitary was first described by Popa and Fielding (1930, 1933). They concluded on the basis of morphological evidence that blood flow was directed from the pituitary to the hypothalamus. Working with the toads, Houssay et al. (1935) realized that the portal blood flowed from the hypothalamus toward the anterior pituitary. This observation was later confirmed by Wislocki and his co-worker (Wislocki and King, 1936; Wislocki, 1937, 1938) who injected dyes systemically and found that the dyes penetrated tissue surrounding the capillaries of the hypothalamus before reaching tissue surrounding the capillaries of the anterior pituitary. The first direct observation on the direction of blood flow of the portal vessles from the hypothalamus to the pituitary in the living rats was made by Green and Harris (1949). Thereafter, similar observations have been reported in the rat (Barrnett and Greep, 1951), mouse (Worthington, 1955), dog and cat (Török, 1954).

The anterior pituitary in mammals receives no direct arterial supply, its entire afferent vascular supply is provided by

portal vessels (Harris, 1947; Goldman and Sapirstein, 1962), with the exception of the rabbit, which is believed to have an additional arterial blood supply (Harris, 1947). There are two portal systems (long and short portal vessels) delivering blood to the anterior pituitary (Adams et al., 1965; Daniel, 1966). Long portal vessels, which travel along the surface of the pituitary stalk, consist of the primary capillaries in the median eminence and in the pituitary stalk, while the short portal vessels, which lie on the dorsal and anterior surfaces of the pituitary, consist of the primary capillaries in the infundibular process of the posterior pituitary (Porter et al., 1974). It has been calculated that 70-90% of the blood supplied to the anterior pituitary in mammals arrives via the long portal vessels and the rest comes from the short portal vessels (Adams et al., 1963; Porter et al., 1967).

In 1954, Török discovered that blood in some of the vessels along the pituitary stalk of the dog flowed toward the hypothalamus. Similar observation also has been reported in sheep (Jazdowska and Dobrowolski, 1965). It was believed that the blood flow within the neurohypophysis went toward the infundibulum (Török, 1954, 1964) and that part of the venous outflow of the anterior pituitary was by way of the vasculature of the posterior pituitary (Szentágothai et al., 1968; Bergland and Page, 1978). The discovery of high concentrations of pituitary hormones in the long portal vessels (Oliver et al., 1977) strongly suggests that pituitary hormones can be transported upward in certain vascular channels of the pituitary stalk up to the

hypothalamus, and provides physiological evidence for the significance of shortloop feedback in controlling brain and pituitary functions (Motta et al., 1969).

D. Portal Vessel-Chemotransmitter Hypothesis and Hypophysiotropic Hormones

The innervation of the anterior pituitary is remarkably sparse; virtually no nerve fibers pass directly from the hypothalamus to the anterior pituitary (Szentágothai et al., 1968). The neurons which are present in the anterior pituitary are probably exclusively of postganglionic sympathetic origin, and innervate predominantly blood vessels (Harris, 1955; Szentágothai et al., 1968). Since no direct neural connections between the hypothalamus and adenohypophysis were found, alternative pathways which might link the two structures were sought. Attention had been turned to the hypophyseal portal vessels soon after the direction of blood flow in those long portal vessels was established to drain from the median eminence to the anterior pituitary, and the concept was developed that the link between the hypothalamus and the anterior pituitary is vascular, rather than neural.

The concept of a neuron serving as a specialized glandular secretory cell dates back to 1919, when Speidel first discovered giant neurons with the appearance of secretory cells in the spinal cord of the fish. Morphologically similar neurons were described in a variety of vertebrate and invertebrate species by Scharrer and Scharrer (1940). Subsequent investigations in several vertebrate

species further demonstrated the neurosecretory phenomenon and the pathways for the synthesis, transport, and secretion of oxytocin and vasopressin (Bargmann and Scharrer, 1951; Scharrer, 1952; Scharrer and Scharrer, 1954).

Based on the previously proposed concept of neurosecretion and the realization of the crucial role of the hypophyseal portal system in the control of anterior pituitary hormone secretion, Harris (1948b) proposed that the hypothalamus secretes specific substances into the portal capillaries of the median eminence, which are transported to the anterior pituitary by the portal vessels to regulate the anterior pituitary hormone secretions. This portal vessel-chemotransmitter hypothesis has continued to serve as a basic model for the study of neuroendocrinology. During the past thirty years, an intense search has been carried out to identify the hypophysiotropic hormones of the hypothalamus that influence pituitary function (Guillemin et al., 1971; Blackwell and Guillemin, 1973; Schally et al., 1973).

Corticotropin-releasing factor (CRF) was the first hypothalamic hormone to be discovered by Saffran and Schally (1955) and Guillemin and Rosenberg (1955). By using a hypothalamic-pituitary co-incubation system, they demonstrated that hypothalamic extracts from the rat, bovine, and ovine stimulate adrenocorticotropic hormone (ACTH) release. Since then, several hypothalamic factors capable of altering the release of anterior pituitary hormones <u>in vitro</u> have been demonstrated. Those include hypothalamic factors which stimulate

the release of thyrotropin (TSH) (Shibusawa et al., 1956; Guillemin et al., 1963), luteinizing hormone (LH) (McCann et al., 1960), prolactin (Meites, et al., 1960), follicle-stimulating hormone (FSH) (Igarshi and McCann, 1964; Mittler and Meites, 1964), and growth hormone (GH) (Deuben and Meites, 1964). They also include hypothalamic factors which inhibit the release of prolactin (Pasteel, 1961; Talwalker et al., 1963), and GH (Krulich et al., 1968). Because dopamine (DA) has a direct action on the pituitary to inhibit prolactin release in vitro at physiological concentrations (Shaar and Clemens, 1974; MacLeod, 1976), and is present in the portal blood (Ben-Jonathan et al., 1977), it has recently been suggested that DA may contribute at least partially to the inhibitory activity of hypothalamic extracts on prolactin release (MacLeod, 1976).

Three hypothalamic hormones have been isolated, structurally identified and synthesized: thyrotropin-releasing hormone (TRH), a tripeptide identified in 1969 by Schally (Schally et al., 1969; Böler et al., 1969) and Guillemin (Burger et al., 1969), and their collaborators, was the first hypothalamic releasing hormone to be isolated. The second hormone was luteinizing hormone releasing hormone (LHRH), a decapeptide, which was initially isolated, characterized, and synthesized in 1971 (Matsuo et al., 1971a,b). Two years later, the third hypothalamic hormone, somatostatin or somatotropin releasing inhibitory factor (SRIF), was chemically identified and shown to be a tetradecapeptide (Brazeau et al., 1973), and was first synthesized by Rivier et al. (1973).

E. General Physiological and Anatomical Localization of Hypothalamic Releasing Hormones

It has been shown that synthetic TRH, LHRH, and somatostatin influence the release of their respective pituitary hormones in a dose-dependent manner, and are equipotent to purified native hormones (Schally et al., 1973; Vale et al., 1975). However, it was realized that certain hypothalamic hormones have an effect on more than one pituitary hormone soon after the synthetic hypophysiotropic hormones became available (Schally et al., 1973; Vale et al., 1975). Synthetic TRH has been shown to cause release of both TSH and prolactin *in vivo* and *in vitro* (Jacobs et al., 1971; Tashjian et al., 1971; Mueller et al., 1973; Convey et al., 1973), whereas the antiserum against TRH can suppress both TSH and prolactin secretion in the rat (Koch et al., 1977). LHRH is effective in stimulating both LH and FSH releases (Schally et al., 1971). The different secretion patterns of the two gonadotropins under certain physiological conditions could be explained by their different biological half-lives (Gay et al., 1970), and by the modulation of gonadal steroids on the response of the two gonadotropins to LHRH (Schally et al., 1973; Yen et al., 1975). Recently, Wise et al. (1979) were able to demonstrate a dissociated secretion pattern of LH and FSH by manipulating the method of LHRH administration. Thus, a brief pulse injection of a high concentration of LHRH to nembutal-blocked proestrous rats elicited a selective release of LH, whereas a low concentration of LHRH delivered over prolonged periods released primarily FSH. On the other hand, both

LH and FSH were released if a high dose of LHRH was administered for prolonged periods. Somatostatin has been shown to suppress the release of GH from dispersed human and rat pituitary cells in vitro (Brazeau et al., 1973), and pentobarbital (Brazeau et al., 1974), morphine (Martin et al., 1975), or suckling (Chen et al., 1974) stimulated GH release in the rat in vivo. In addition to its inhibitory action on GH secretion, somatostatin has also been shown to inhibit TRH induced TSH secretion (Hall et al., 1973; Vale et al., 1974). Recently, somatostatin antiserum was reported to be able to increase the basal level of both GH and TSH in rats (Ferland et al., 1976; Arimura and Schally, 1976). In addition, somatostatin has also been reported to inhibit both insulin and glucagon secretion in the pancreas (Alberti et al., 1973; Fujimato et al., 1974; Koerker et al., 1974) and gastrin secretion in the gut (Bloom et al., 1974).

Based on the studies of pituitary microimplantation in the early 1960's, Halász and co-workers (1962) and Knigge (1962) described a region of the basal hypothalamus which contains trophic substances capable of maintaining the cellular structure and secretory function of the anterior pituitary, i.e., the "hypophysiotrophic area." Studies on the localization of the three known hypothalamic hormones, by using microdissection and radioimmunoassays, have shown a widespread distribution in the hypothalamus with the highest concentration in the median eminence (Brownstein et al., 1976a).

The recent development of immunohistochemical techniques has provided insight into the distribution and the nature of peptidergic

neuronal systems. It has been shown that TRH, LHRH, and somatostatin all are located in the external layer of the median eminence with their cell bodies originating from the pre-optic area and the periventricular region dorsal to the optic chiasma (Hökfelt et al., 1975a; Barry, 1976; Setalo et al., 1976; Alpert et al., 1976). These projections have been established by showing the disappearance of the specific peptidergic terminals in the external layer of the median eminence after anterior deafferentation of the medial basal hypothalamus (Weiner et al., 1975; Brownstein et al., 1977; Elde and Hökfelt, 1978). Both TRH and somatostatin have been shown to be widely distributed throughout the central nervous system outside of the hypothalamus (Hökfelt et al., 1975b; Vale et al., 1975; Brownstein et al., 1975). In addition, immunoactive somatostatin has been located in the gut and pancreas (Hökfelt et al., 1975c; Polak et al., 1975), and in the substantia gelatinosa of the dorsal horn of the spinal cord (Hökfelt et al., 1975d; 1976).

II. Hypothalamic Biogenic Amines

A. General

The presence of norepinephrine (NE) in the brain was first demonstrated by Holtz (1939) and its distribution pattern was studied by Vogt (1954). The uneven distribution and characteristic regional localization within the brain suggest that NE might serve as a central neurotransmitter. This view was further supported by the finding that the relative distribution of NE is quite similar in most mammalian species (Holzbauer and Sharman, 1972).

Since NE was shown to be present in the mammalian brain, the existence of dopamine (DA) (an immediate precursor of NE) in the brain was to be expected. The first evidence showing the presence of DA in mammalian brain was provided by Montagu (1957) and Weil-Malherbe and Bone (1957). In 1959, Bertler and Rosengren, as well as Carlsson, reported that there is a marked difference in the regional distribution between NE and DA in the brain. The concentration of DA in the caudate nucleus, which is almost devoid of NE, is on the average of 10 μg/g of fresh tissue. Approximately 80% of the total DA in the brain is present in the caudate nucleus and the putamen regions, whereas DA concentration in the hypothalamus rarely exceeds 10% of the concentration of NE which has concentration in the range between 1 and 2 μg/g. The different distribution pattern of DA and NE indicates strongly that DA might also serve as a central neurotransmitter instead of just being an immediate precursor of NE. Both epinephrine (E) and the enzyme, phenylethanolamine-N-methyltransferase (PNMT), which converts NE to E recently have been found in several regions of the brain (Hökfelt et al., 1974; Saavedra et al., 1974a). However, E concentration in the mammalian brain is relatively low as compared to NE (Cooper et al., 1974).

The first evidence of the presence of serotonin (5-HT) in the CNS was provided by Amin et al. (1954), who found that the highest concentration of 5-HT is present in the hypothalamus and the lowest in the cerebellum of the dog. The development of The Falck-Hillarp histofluorescence technique with high sensitivity and sufficient

specificity has permitted the cellular localization of monoamines in the CNS (Falck et al., 1962), and the recent development of sensitive radioenzymatic assays for catecholamines (Cuello et al., 1973; Coyle and Henry, 1973; Ben-Jonathan and Porter, 1976) and 5-HT (Saavedra et al., 1973), and the micropunch techniques (Palkovits, 1973) have made it possible to determine the manoamine content in individual nuclei of the hypothalamus.

B. Monoaminergic Pathways Innervating the Hypothalamus

Noradrenergic Pathways.--The classic work of Fuxe (1965a,b) indicated that 7 out of 10 NE cell groups are located in the pons and medulla oblongata. The A_1, A_2, A_5, and A_7 cell groups give rise to the ventral NE pathway with axons ascending to the mid-reticular formation, and entering the medial forebrain bundle (MFB). This pathway provides NE nerve terminals to the lower brainstem, hypothalamus, median eminence, and limbic system. Descending fibers arise in most caudal cells (A_1) and project to both the ventral horn and dorsal horn of the spinal cord (Fuxe and Hökfelt, 1969; Ungerstedt, 1971). The NE cell bodies in the locus ceruleus (A_6) give rise to the dorsal bundle with the ascending fibers innervating the cerebral cortex, the hippocampus, and the anterior hypothalamus.

Hypothalamic NE is not evenly distributed. Highly concentrated NE was found in the retrochiasmatic area of the anterior hypothalamus, dorsomedial nucleus, supraoptic and paraventricular nuclei, periventricular nucleus and median eminence (Fuxe, 1965a,b;

Palkovits et al., 1974). Median eminence NE terminals are mainly located within the internal layer (Jonsson et al., 1972; Björklund and Nobin, 1973), with few terminals projecting to the external layer as implied by localization of dopamine-β-hydroxylase (Goldstein et al., 1974) or by studies with 6-hydroxydopamine (6-OHDA) (Cuello et al., 1974).

The importance of this noradrenergic input into the hypothalamus has been demonstrated by the fact that lesions in the midbrain tegmentum (Andén et al., 1966a), locus ceruleus (Loizou, 1969) and medial forebrain bundle (Kobayashi et al., 1974) result in a decrease in NE content in the hypothalamus. Besides, hypothalamic deafferentation with a Halász knife resulted in a total loss of dopamine-β-hydroxylase activity and NE content in the hypothalamus (Brownstein et al., 1976b).

Dopaminergic Pathways.--Dopaminergic pathways in the CNS have a more localized distribution. The cell bodies of A_9 DA cell group in the zona compacta of the substantia nigra and A_8 cell group in the adjacent ventral tegmental area give rise to the nigrostriatal pathway which extends rostrally to provide terminals to the structures of striatum (putamen and caudate nuclei) (Andén et al., 1965; 1966b; Hökfelt and Ungerstedt, 1969; Hökfelt et al., 1976). The mesolimbic system which has A_{10} cell group clustered around the mesencephalic interpeduncular nucleus, ascends along with the axons of the nigrostriatal DA system, and innervates the nucleus accumbens and the olfactory tubercles (Andén et al., 1966c; Ungerstedt, 1971). The

tuberoinfundibular DA system arises and ends entirely within the hypothalamus. The neuronal cell bodies of this system are located in the arcuate (A_{12}) and anterior periventricular nuclei with their axons projecting to the external layer of the median eminence (Fuxé, 1964; Fuxé and Hökfelt, 1966; Björklund et al., 1970; Jonsson et al., 1972). As observed, ultrastructurally, the catecholaminergic fibers in the zona externa do not seem to form any true synaptic connections to other tissue elements, but show sites of close contact with non-monoaminergic axons, ependymal cells and the pericapillary space of the hypophyseal portal vessels (Ajika and Hökfelt, 1963; Hökfelt, 1973).

In addition to the arcuate nucleus (A_{12}), DA containing cell bodies have been reported to be located in the posterior hypothalamus, the medial zona incerta (A_{11} and A_{13} according to Fuxé et al., 1969a; Björklund and Nobin, 1973), and the rostral periventricular nucleus (A_{14} according to Björklund and Nobin, 1973). The axons with cell bodies originating from A_{11} and A_{13} have terminals projecting to anterior and dorsal hypothalamic areas and to the dorsal part of the dorsomedial nucleus, and those with cell bodies originating from A_{14} have terminals extending to the medial pre-optic area and supra-chiasmatic nucleus (Björklund et al., 1975a).

Serotonergic Pathways.--In spite of the relative difficulty of detecting 5-HT by histofluorometric techniques as compared to measuring catecholamines, several methods have been applied to improve the visualization of 5-HT neurons and make it possible to map central serotonergic pathways. Two main ascending bundles of 5-HT axons have been described, namely medial and lateral ascending 5-HT

pathways. Both pathways originate from cell bodies located in the mesencephalic raphé (B_7, B_8, and B_9) and pontine raphé (B_5 and B_6) nuclei, and run near the midline in the medial forebrain bundle to innervate the hypothalamus (particularly the suprachiasmatic region) and other regions of the forebrain (Fuxe, 1965a,b, Ungerstedt, 1971; Fuxe and Jonsson, 1974). It is believed that both the dorsal and median midbrain raphé nuclei innervate the hypothalamus (Geyer et al., 1976; Kellar et al., 1977; Palkovits et al., 1977). Recent evidence provided by Van DeKar and Lorens (1979) indicated that the median raphé nucleus seems to be the primary source of 5-HT fibers innervating the suprachiasmatic nucleus, anterior hypothalamic area and medial preoptic area, whereas the arcuate nucleus seems to receive an almost equal innervation from both the dorsal and median raphé nuclei.

Recently, the concentration of 5-HT in the individual nuclei of the rat hypothalamus has been measured by using the microenzymatic assay (Saavedra et al., 1974b). High concentration of 5-HT are found in the suprachiasmatic nucleus, the median eminence, the arcuate nucleus, and the medial forebrain bundle. The 5-HT terminals in the median eminence, like DA terminals, are localized mainly in the external layer in contrast to the NE terminals which are distributed mainly in the internal and subependymal layer.

C. Metabolism of Catecholamines and Serotonin

Biosynthesis.--

Catecholamines: The biosynthesis of catecholamines takes place in the catecholaminergic nerve endings. Those enzymes

responsible for amine synthesis are produced in the neuron bodies and transported to presynaptic nerve terminals by axonal transport (McClure, 1972; Jarrott and Geffen, 1972). L-tyrosine, the precursor of catecholamines, is actively transported into the axon through a stereospecific 'carrier' mechanism common to other neutral amino acids (Wurtman and Fernström, 1972), and hydroxylated to L-dihydroxyphenylalanine (L-dopa) by the soluble enzyme tyrosine hydroxylase, a rate limiting enzyme in catecholamine synthesis (Spector et al., 1963; Levitt et al., 1965). Tyrosine hydroxylase, which requires molecular O_2 and Fe^{++} and has tetrahydropteridine as a cofactor (Nagatsu et al., 1964; Levitt, 1967), is stereospecific, and is saturated by its substrate L-tyrosine under most conditions (Nagatsu et al., 1964; Cooper et al., 1974). The activity of this enzyme is regulated by feedback inhibition of DA and NE, which competes with the cofactor for binding to it (Costa and Neff, 1966; Spector et al., 1967). It is believed that an increase in neuron activity can stimulate the activity of tyrosine hydroxylase acutely by removing the end product inhibition after the release of releasible catecholamines (Sedvall et al., 1968). It also increases the synthesis of new enzymes. However, the latter mechanism operates more slowly (Axelrod, 1974).

The conversion of DA from L-dopa is catalyzed by L-aromatic amino acid decarboxylase (AAAD), an enzyme which displays little substrate specificity and catalyzes decarboxylation in the synthesis of both catecholamines and serotonin (Carlsson et al., 1972; Christenson et al., 1972). This enzyme requires pyridoxal phosphate

as a cofactor, and is tightly bound to the apoenzyme as a schiff base (Christenson et al., 1970).

In noradrenergic neurons, DA is converted to NE by dopamine-β-hydroxylase which lacks substrate specificity and can hydroxylate a variety of phenylethylamines (Kaufman, 1966; Cooper et al., 1974). This enzyme is a tetrameric glycoprotein containing 4 moles of Cu^{++} (Goldstein et al., 1965; Wallace et al., 1973), and is localized on the membrane of NE storage granules (Potter and Axelrod, 1963; Thomas et al., 1973).

NE can be further methylated to form epinephrine by phenylethanolamine-N-methyltransferase (PNMT) with S-adenosylmethionine as a methyl donor. PNMT is highly localized in the cytoplasm of the adrenal medulla (Axelrod, 1962) and is present in certain areas of the mammalian CNS with the highest levels in some nuclei in the brain stem (A_1 and A_2) and hypothalamus (Hökfelt et al., 1974; Saavedra et al., 1974).

Serotonin (5-HT): Evidence shows that brain 5-HT is synthesized within the neurons where it is found (Graham-Smith, 1967). The synthesis of 5-HT involves uptake of L-tryptophan by the nerve endings, hydroxylation of tryptophan to 5-hydroxytryptophan (5-HTP), and finally decarboxylation of 5-HTP to 5-HT. Tryptophan hydroxylase, the rate limiting enzyme in the synthesis of 5-HT, appears to exist in two forms, a soluble and a bound form, each exhibiting different characteristics (Ichiyama et al., 1970; Knapp and Mandell, 1972). Like tyrosine hydroxylase, tryptophan hydroxylase requires molecular

O_2, Fe^{++}, and a reduced pteridine cofactor for optimal activity (Ichiyama et al., 1970), and its distribution correlates with that of 5-HT very well (Kizer et al., 1975). Since tryptophan hydroxylase is not saturated by the concentration of tryptophan in the brain at physiological conditions (Lovenberg et al., 1968; Friedman et al., 1972), changes in the availability of brain tryptophan affect the formation of 5-HT (Fernstrom and Wurtman, 1971; Graham-Smith, 1971).

Because tryptophan is the only essential amino acid partially bound to plasma albumin (McMenamy and Oncley, 1958), the amount of tryptophan which gets into the brain seems to be closely dependent on the free form of plasma tryptophan (10-20% instead of the total (Knott and Curzon, 1972; Tagliamonte et al., 1973). A variety of lipid-soluble compounds such as hormones, drugs and nonesterified fatty acids (NEFA), which bind to albumin in the serum may release tryptophan from its binding sites to plasma proteins, and therefore increase plasma concentration of free form tryptophan (Curzon, 1974). However, no correlation could be found between plasma-free tryptophan levels and brain concentrations of tryptophan and 5-HT in some studies. It has been suggested that the factor limiting the availability of plasma tryptophan to the brain might be the ratio of plasma tryptophan to other neutral amino acids which compete with tryptophan for the same uptake system (Fernström and Wurtman, 1972; Fernström, 1976).

The natural rhythm in serotonin metabolism, however, probably does not reflect the variations in plasma tryptophan. It is more likely that these changes result from fluctuations in the rate of

uptake of tryptophan by brain neurons themselves (Héry et al., 1972). Recent evidence shows that there are two or more 5-HT storage compartments in the brain (Shields and Eccleston, 1973), implying that brain 5-HT concentration and functional brain 5-HT might be unrelated (Green and Graham-Smith, 1976).

It is generally believed that tryptophan hydroxylase activity is not subject to feedback inhibition by 5-HT under normal conditions (Lin et al., 1969; Jequier et al., 1969; Millard et al., 1972). However, end-product inhibition of 5-HT synthesis may occur following the administration of monoamine oxidase (MAO) inhibitor when 5-HT levels are very high (Macon et al., 1971; Hamon et al., 1972). Whether this feedback inhibition of 5-HT has any physiological significance is yet to be elucidated.

Storage.--At nerve endings, monoamines are stored in the vesicles in synaptosomes, and thus protected from enzymatic degradation (Hökfelt, 1968; Fillenz, 1971; Tranzer and Thoenen, 1968). Vesicles are synthesized in the neuron cell body and transported to the terminals by axonal flow (Dählström et al., 1973). Within the granules NE has been shown to be in part free and in part bound to ATP at a ratio of 4 to 1 (Iversen, 1967, 1971), whereas, 5-HT is stored together with nucleotides and bivalent cations (Ca^{++} and Mg^{++}) (DaPrada et al., 1971). The existence of two pools of catecholamines has been suggested, based on a functional point of view. The two pools, with one being more readily releasible, are present in vesicles closest to the presynaptic membrane; the other larger pool

is located far from the neuronal membrane. They seem to be linked in such a dynamic way that the larger pool might serve as a reservoir for the more readily releasible pool (Axelrod, 1974).

Release.--Neurotransmitters can be released from their nerve endings by nerve impulses, electrical stimulation, and drugs (Costa et al., 1971; Glowinski, 1972; Carlsson et al., 1972). Evidence shows that newly synthesized or stored transmitters are more readily released by nerve stimulation in comparison to older stored ones (Kopin et al., 1968; Besson et al., 1969; Glowinski, 1972). It is believed that the release of neurotransmitters from the vesicles into the extracellular space is conducted by a process of exocytosis. This was proposed in 1957 as a mechanism for the release of catecholamines on the basis of an electron-microscopic study of the adrenal medulla (DeRobertis and Vaz Ferreira, 1957). This process is Ca^{++} dependent (Rubin, 1970; Smith and Winkler, 1972) and consists of the fusion of vesicles and neuronal membrane. The discovery of two vesicle proteins, chromogranin and dopamine-β-hydroxylase, together with NE at the noradrenergic terminals after electrical stimulation supports exocytosis as the mechanism of amine release (Douglas and Poisner, 1966; Malamed et al., 1968; Geffen et al., 1969).

Uptake.--One of the most important mechanisms to terminate the action of released neurotransmitters on post-synaptic receptors is the active re-uptake system located on the membrane of the presynaptic nerve terminal (also called $uptake_1$ system) (Iversen,

1967; 1971). This re-uptake process appears to be stereochemically specific, saturable with a high affinity constant, and Na^+ dependent (Iversen, 1974).

In various peripheral tissues innervated by the sympathetic nervous system, such as cardiac muscle of the heart and smooth muscle in blood vessels, NE can be taken up by a second uptake system located in extra-neuronal sites ($uptake_2$) (Iversen, 1971). Drugs, such as imipramine and cocaine, which are potent inhibitors of the $uptake_1$ system have no effect on the $uptake_2$ system. Biochemical studies demonstrated that if the $uptake_2$ system was inhibited, the enzymatic catabolism of catecholamines was markedly reduced, but if the $uptake_1$ system were inhibited, enzymatic catabolism of catecholamines increased (Eisenfeld et al., 1967). Therefore, NE accumulated by $uptake_2$ is rapidly metabolized by exposing it to MAO and/or catechol-O-methyltransferase (COMT) instead of re-incorporating it into the vesicle (Lightman and Iversen, 1969).

Since the uptake system is not entirely specific to each particular neurotransmitter, structurally related compounds by displace and replace the normal transmitter, and serve as a false transmitter (Kopin, 1968; Muscholl, 1972). The uptake and retention of 5-HT or 5-HTP by catecholaminergic neurons in the brain has been demonstrated by Lichtensteiger et al. (1967). Similarly, DA can be formed in serotonergic neurons after administration of large doses of L-dopa (Bartholini et al., 1968).

Metabolic Degradation.--Enzymatic degradation of catecholamines is accomplished by the action of MAO and/or COMT. A MAO which deaminates DA and NE to 3,4-dihydroxyphenylacetaldehyde, and 3,4-dihydroxyphenylglycolaldehyde, respectively, is an intraneuronal enzyme widely distributed throughout the brain and is associated with the mitochondria (Nukada et al., 1963; Tipton, 1967; Wurtman, 1972). COMT, which catalyzes the O-methylation of DA and NE to 3-methoxytyramine and normetanephrine, respectively, is an extraneuronal enzyme present in the synaptic cleft (Alberici et al., 1965; Broch and Fonnum, 1972). It has been shown that MAO is not a single enzyme, but rather a family of enzymes with different substrate specificity (see Costa and Sandler, 1972).

MAO is mainly responsible for the oxidation of 5-HT to 5-hydroxyindoleacetylaldehyde, which is further oxidized by aldehyde-dehydrogenase to 5-hydroxyindoleacetic acid (5-HIAA) (Sjoerdsma et al., 1955; Udenfriend et al., 1956). The other metabolic pathway for inactivating 5-HT is O-sulfation. 5-HT-O-sulfate is formed from 5-HT and 3'-phosphoadenosine, 5'-phosphosulfate (PAPS) through the action of a sulfotransferase system (Hidaka et al., 1966). The discovery of 5-HT sulfotransferase in the brain (Hidaka et al., 1969) raises the possibility that deamination by MAO may not be the only pathway to inactivate 5-HT in the brain. Under normal conditions, it appears that conversion of 5-HT to 5-HIAA is the major route for the inactivation of brain 5-HT. However, when MAO is inhibited, the sulfation of 5-HT may be an important mechanism for continuing the removal of 5-HT.

The third pathway for 5-HT metabolsim involves N-methylation. Indoleamine N-methyltransferase, which uses S-adenosylmethionine as the methyl donor, has been shown to be present in small amounts in the brain (Morgan and Mandell, 1969; Saavedra and Axelrod, 1972).

Turnover.--Neurotransmitters are constantly being synthesized, released and metabolized, and the rate at which the processes occur, is called turnover. To measure the turnover of brain amines provides an index of the physiological functions of those aminergic neurons since the steady state concentrations per se do not indicate whether a rise in amines reflects as increase in their synthesis or decrease in their degradation.

A close relationship between nerve impulse flows and the turnover of neurotransmitter has been shown in both catecholaminergic and serotonergic neurons. It has been demonstrated that the NE turnover in the cerebral cortex and hippocampus is increased after electrical stimulation of the locus ceruleus or its projections (Arbuthnott et al., 1970; Korf et al., 1973), whereas decrease in cerebral cortical NE turnover occurs after the lesions of locus ceruleus (Korf et al., 1973a). Electrical stimulation of the substantia nigra which contains DA cell bodies results in an increase in DA turnover in the striatum (VonVoightlander and Moore, 1971). Similarly, 5-HT turnover in the forebrain is decreased after 5-HT neurons from the midbrain are transected (Andén et al., 1966) or after raphé nucleus lesions (Kuhar et al., 1972) and is increased

after electrical stimulation of the raphé nucleus (Sheard and Aghajanian, 1968; Shields and Eccleston, 1973).

The relationship between total turnover of brain 5-HT and functional activity of 5-HT has been intensively discussed by Grahame-Smith (1974). There is only a small rise in the concentration of brain 5-HT, a large increase in the production of 5-HIAA, and no behavioral changes after the administration of tryptophan alone. On the other hand, after pre-treating the animals with a MAO inhibitor, even small doses of tryptophan produce hyperactivity related to the accumulation of 5-HT in the brain. It is possible that under normal conditions the synthesis rate of 5-HT is in excess of that required to fulfill the functional needs of the brain, and the excess 5-HT is either stored in the vesicles or metabolized by MAO. Therefore, measurements of total turnover rate may not accurately reflect serotonergic neuronal function.

Several steady-state and non-steady-state methods for measuring the turnover rate of brain monoamines have been described (Anton-Tay and Wurtman, 1971; Morot-Gaudry et al., 1974), and each method has certain limitations as indicated by the review of Costa (1970). In the steady-state methods, trace doses of radio-labeled tyrosine or catecholamines which do not disturb the steady-state are administered. The acute accumulation of labeled catecholamines following systemic injection of labeled catecholamine precursors is taken as an index of catecholamine synthesis (Zigmond and Wurtman, 1970; Zschaeck and Wurtman, 1973), whereas the disappearance of

labeled catecholamines from the brain over the first few hours after intraventricular infusion is taken as an index of catecholamine turnover (Glowinski et al., 1965). In the non-steady-state approaches, brain catecholamines are estimated by the decline in brain catecholamine levels after treatment with α-methyl-para-tyrosine (α-mpt) (Brodie et al., 1966; Costa and Neff, 1966; Fuxe and Hökfelt, 1969), the increase in brain catecholamine after treatment with a MAO inhibitor (Anton-Tay and Wurtman, 1971), or accumulation of acid DA metabolites after inhibition with probenecid (Sharman, 1966).

The turnover of 5-HT can be estimated by either (a) measuring the accumulation of labeled 5-HT and 5-HIAA after systemic injection of labeled 5-HT precursor and taking them as an index of 5-HT synthesis and degradation, respectively (Neff and Tozer, 1968; Neff et al., 1971; Morot-Guadry et al., 1974); or by (b) measuring the rate of increase in 5-HT or decrease in 5-HIAA after MAO inhibition (Neff and Tozer, 1968; Morot-Gaudry et al., 1974); or by (c) measuring the accumulation of 5-HIAA in CSF or in the brain after blocking the acid transport system with probenecid (Neff et al., 1967; Neff and Tozer, 1968). In addition, the synthesis rate of 5-HT was also estimated from the initial rate of 5-hydroxytryptophan (5-HTP) accumulation, following 5-hydroxytryptophan decarboxylase inhibition (Carlsson and Lindqvist, 1970).

The recent advent of high sensitive radioenzymatic assays for measuring catecholamines (Coyle and Henry, 1973; Ben-Jonathan and Porter, 1976) and 5-HT (Saavedra et al., 1973) has made the

non-steady-state methods the most popular to measure biogenic amine turnover in small pieces of brain tissue. The two methods of measuring the turnover of catecholamines and 5-HT after administrations of α-mpt and pargyline, respectively, were used in some experiments in this thesis.

III. Hypothalamic Control of Gonadotropin Secretion

A. Feedback of Gonadal Steroids on Gonadotropin Secretion

Negative Feedback.--The discovery of increased plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels after castration in both male and female rats (Ramirez and McCann, 1963; Gay and Midgley, 1969), indicates that the secretion of gonadotropin is under tonic inhibition by gonadal steroids. This negative feedback mechanism is further evidenced by the decline in plasma levels of gonadotropin after administration of steroids to castrated rats (Ramirez et al., 1964; Chowers and McCann, 1967). There is a sex difference in the secretion of gonadotropin after castration. In male rats, increased serum LH can be detected within 8 hrs after gonadectomy (Gay and Midgley, 1969), whereas the increase in serum LH in female rats does not occur until several days later. It is generally agreed that estrogen is the most potent ovarian steroid to inhibit gonadotropin secretion (Schwartz and McCormack, 1972), and decreases in serum LH have been shown to occur within 2 hrs after estrogen administration (Blake, 1977a). On the other hand, progesterone alone has little, if any, effect on the high levels of

serum LH in ovariectomized rats (McCann, 1962; McPherson, J. C., III, 1975; Chen et al., 1977). It appears that progesterone is able to synergize with estrogen to inhibit gonadotropin secretion.

The action sites where gonadal steroids exert their negative feedback inhibition seem to involve both the hypothalamus (Flerkó and Bárdos, 1961; Sawyer, 1964; Ramirez et al., 1964), and anterior pituitary (Rose and Nelson, 1957; Bogdanove, 1963; Ramirez et al., 1964). A widely used experimental approach to distinguish between the two action sites has been the implantation of small amounts of steroids into either the hypothalamus or the pituitary (Rose and Nelson, 1957; Flerkó and Bárdos, 1961; Bogdanove, 1963; Ramirez et al., 1964). The initial acute decrease in serum LH following estrogen administration appears to involve a negative feedback at the level of both pituitary (Negro-Vilar et al., 1973; Blake et al., 1974; Ferland et al., 1976) and hypothalamus (Blake et al., 1974; Orias et al., 1974), whereas the long-term suppression in serum LH is due to the action of estrogen at the hypothalamic level (Blake et al., 1974, 1977a). The hypothalamic action site seems to be restricted to the medial basal hypothalamus since surgical disconnection of all the neural inputs to this area does not impede the negative feedback action of gonadal steroids (Blake et al., 1977a).

The effect of androgens on serum levels of gonadotropin is exclusively inhibitory (Bogdanove, 1967; Schally et al., 1967; Ferland et al., 1976). They exert their inhibitory action at the levels of both the hypothalamus (Smith and Davidson, 1967; Ferland

et al., 1976; Cheung and Davidson, 1977), and the pituitary (Kingsley and Bogdanove, 1973; Ferland et al., 1976; Cheung and Davidson, 1977), while their inhibitory effect on FSH release appears to be restricted to the hypothalamus (Ferland et al., 1976).

Since LH-RH stimulates both LH and FSH release (Schally et al., 1971), the divergence between LH and FSH release occurring under different physiological conditions can be explained by the differential effect of steroids at the pituitary on the release of these two gonadotropins in response to GnRH stimulation.

Positive Feedback.--The gonadotropin secretion pattern in the male is different from that in the female showing cyclic activity. In normal female rats, the basal serum LH level is interrupted by a dramatic surge every 4 or 5 days on the afternoon of proestrus (Monroe et al., 1969; Gay et al., 1970). The rise in serum estrogen which starts from the afternoon of diestrus 2 and reaches a peak on the morning of proestrus (Hori et al., 1968; Brown-Grant et al., 1970), appears to be crucial in triggering the LH surge because administration of antibodies against estrogen at a critical time can abolish the ovulatory surge of LH (Ferin et al., 1969, 1974). In addition to the LH surge, serum FSH and PRL also increase and reach a peak in the afternoon of proestrus (Taya and Igarashi, 1973; Butcher et al., 1974; Smith et al., 1975).

The stimulatory effect of gonadal steroids on gonadotropin secretion was first demonstrated by Hohlweg (1934) who showed that the formation of corpora lutea can be induced by estrogen administration

in pre-pubertal rats. Administration of estrogen to cycling rats during diestrus was shown to advance the time of ovulation (Everett, 1948; Brown-Grant, 1969; Weick et al., 1971). This positive feedback action of estrogen on LH secretion could be only demonstrated in the female (Taleisnik et al., 1971). The development of the positive feedback mechanism in response to estrogen is determined by the steroid environment of the hypothalamus during the neonatal 'critical period' in the rat (Gorski, 1968). The effects of progesterone on pre-ovulatory LH surge appear to depend upon the time when it is administered. Thus, progesterone is able to advance the LH surge if it is administered on diestrus 3 in 5-day cycling rats or on proestrus in 4-day cycling rats (Everett, 1948; Zeilmaker, 1966; Brown-Grant and Naftolin, 1972). On the other hand, progesterone treatment in early diestrus delays ovulation by 24 hrs (Schwartz, 1969). Progesterone has also been shown to facilitate estrogen-induced ovulation (Döcke and Dörner, 1966) and PMS induced superovulation (McCormack and Meyer, 1963) in immature rats. In normal cycling female rats, it has been shown that a diurnal rhythm exists in adrenal progesterone secretion with a peak during the early morning hours (0100-0500 hrs) and a nadir between 1000 and 1400 hrs (Mann and Barraclough, 1973). It is possible that this early morning rise in progesterone may synergize with estrogen to regulate the pre-ovulatory surge of gonadotropin.

The positive feedback action of estrogen was first investigated in long-term ovariectomized rats by Caligaris et al. (1971a).

Administration of large doses of estrogen to ovariectomized rats causes a daily LH surge for 3 to 4 days (Caligaris et al., 1971a; Legan et al., 1975; Blake, 1977b), which strongly supports the idea that the neural signal triggering the LH surge occurs daily in the rat, as originally proposed by Everett and Sawyer (1949). Progesterone appears to act synergistically with estrogen to facilitate the LH surge since progesterone alone is ineffective in stimulating the LH surge in long-term ovariectomized rats (Caligaris et al,, 1971b).

In normal cycling female rats, the LH surge on the afternoon of proestrus initiates a simultaneous surge of progesterone (Barraclough et al., 1971; Feder et al., 1971; Piacsek et al., 1971). It is this progesterone secretion which may be responsible for preventing the release of LH on subsequent days (Freeman et al., 1976; Blake, 1977b) since progesterone has been shown to be able to block the LH surge induced by estrogen in ovariectomized rats when it is administered prior to estrogen treatment (Caligaris et al., 1971b). Since the LH surge induced by gonadal steroids in long-term ovariectomized rats is similar in timing and duration to that in normal proestrous rats (Caligaris et al., 1971a,b; Neill, 1972; Jackson, 1972), this model was used in this thesis to study the role of hypothalamic 5-HT in regulating the LH surge.

The sites of the positive feedback action of gonadal steroids appear to involve both the hypothalamus and anterior pituitary. By using techniques, such as electrolytic lesion, hormone implantation, electrical stimulation, and hypothalamic deafferentation,

investigators have provided evidence to show that the arcuate region of the medial basal hypothalamus regulates tonic release of gonadotropin, whereas phasic release of gonadotropin involves the pre-optic area (Flerkó, 1966; Halász, 1969; Gorski, 1971). Anterior deafferentation, which disconnects the medial basal hypothalamus from pre-optic area, blocks ovulation (Halász and Gorski, 1967; Halász, 1969) and gonadotropin secretion (Blake et al., 1972; Weiner et al., 1972b). Deafferentation and anterior hypothalamic lesions also block the steroid induced LH surge in ovariectomized rats (Neill, 1972; Blake, 1977b). The two center control mechanisms of gonadotropin secretion has recently been questioned in primates (Krey et al., 1975), because normal cyclicity and normal response of LH to the positive feedback action of estrogen continue in monkeys after anterior deafferentation of the medial basal hypothalamus. Tritiated estradiol has been demonstrated to be taken up in significant amounts in the anterior hypothalamus, especially in the pre-optic and median eminence areas as determined by autoradiography (Anderson and Greenwald, 1969; Stumpf, 1970), and estrogen receptors in this area were found later by Kato (1973, 1977).

The direct action of estrogen on the anterior pituitary to facilitate gonadotropin release has been repeatedly demonstrated. The cyclic variations of pituitary sensitivity in response to LHRH stimulation have been reported with the most sensitive stage on the afternoon of proestrus when circulating estrogen titer is high (Gordon and Reinchlin, 1974; Aiyer et al., 1974; Zeballos and McCann, 1975). Later studies <u>in vitro</u> further demonstrated that estrogen can sensitize the pituitary to potentiate LHRH induced LH

release (Drouin et al., 1976). Since the specific binding of I^{125} labeled LHRH to the anterior pituitary membrane has been shown to be highest on proestrus (Park et al., 1975), estrogen may exert its action by increasing LHRH receptors on the pituitary.

B. Effects of Monoamines on Gonadotropin Secretion

Norepinephrine.--The existence of central neurotransmitters involved in control of gonadotropin secretion was first proposed by Sawyer et al. (1947) on the basis of pharmacological studies. It was shown that α-adrenergic blockers are able to prevent the reflex release of LH in rabbits (Sawyer et al., 1947; Markee et al., 1948), and the spontaneous release of LH in rats (Markee et al., 1952; Everett, 1961). The effects of these drugs appear to be fairly specific because they were only effective when administered to rabbits before coitus, and in rats prior to the expected time of the LH surge. Brain monoamine depletors, like reserpine, were reported to inhibit LH secretion induced by pregnant mare serum (PMS) in immature rats (Barraclough and Sawyer, 1957; Hopkins and Pincus, 1963; Coppola et al., 1966). The effect of these drugs was mediated by a central rather than peripheral action because they were only effective when given prior to the expected time of LH release, and their inhibitory effect was prevented by simultaneous administration of human chorionic gonadotropin (HCG) (Coppola, 1968). In addition to receptor blockers and depleting agents, catecholamine synthesis inhibitors elicit comparable effects on gonadotropin. α-Methyl-p-tyrosine, which depletes central catecholamines by

competitively inhibiting the activity of tyrosine hydroxylase (Spector et al., 1965; Corrodi and Hansen, 1966) has been shown to block PMS-induced ovulation in immature rats (Lippmann et al., 1967; Coppola, 1968; Kordon and Glowinski, 1969), the proestrus LH surge (Kalra and McCann, 1974) and gonadal steroid-induced LH surge (Kalra et al., 1972).

The above evidence suggests that NE may be the central catecholamine responsible for the stimulation of gonadotropin release and was amplified by Kalra and his colleagues in a series of experiments. Administration of α-methyl-para-tyrosine, which lowers both DA and NE levels in the hypothalamus, or dopamine-β-hydroxylase inhibitors, such as diethyldithiocarbarmate (DDC) and 1-phelyl-3-(2-thiazolyl)-thiourea (U-14,624), which are believed to only lower NE, blocked the LH surge in proestrous rats (Kalra and McCann, 1974), in proestrus rats after electrochemical stimulation of the pre-optic area (Kalra and McCann, 1973) and in estrogen-primed progesterone treated ovariectomized rats (Kalra et al., 1972). These blockades could be partially reversed by the treatment with dihydroxyphenylserine (DOPS) which selectively increases NE, but not by L-dopa which mainly increases DA.

6-Hydroxydopamine (6-OHDA) has been shown to be selective in destroying catecholaminergic neurons when it is injected into the brain or ventricles (Bloom et al., 1969; Breese and Traylor, 1970; Ungerstedt, 1968, 1971a). At low doses, 6-OHDA selectively depletes NE only and leaves DA stores unchanged (Breese and Traylor, 1971). Administration of 6-OHDA has been shown to block the LH surge on the

afternoon of proestrus, and LH surge induced by steroids (Martinovic and McCann, 1977; Simpkins et al., 1979). However, these blocking effects seem to be transient because normal estrous cycles (Nicholsson et al., 1978) and normal LH and FSH surges (Martinovic and McCann, 1977) continue in rats injected with 6-OHDA for more than 10 days, when increased activity of the surviving neurons and development of supersensitivity of adrenergic receptors become evident (Uretsky et al., 1971; Kostrzewa and Jacobowitz, 1974). Additional evidence for the involvement of positive NE inputs on gonadotropin secretion came from the deafferentation studies. Complete hypothalamic deafferentation which causes over 50% depletion of hypothalamic NE, while DA levels remain normal (Weiner et al., 1972b) blocked the gonadotropin surge on proestrus (Blake et al., 1972).

Even though results obtained from intraventricular amine injections are conflicting, recent works suggest the stimulatory role of NE in controlling gonadotropin secretion. Intraventricular infusion of NE has been shown to induce ovulation in pentobarbital-blocked proestrus rats (Rubinstein and Sawyer, 1970), and in constant estrous rats induced by electrolytic lesion in the anterior hypothalamus, or by exposure to continuous illumination (Tima and Flerkó, 1975). Recently Sawyer and his co-workers (Sawyer et al., 1974; Sawyer, 1975) reported that intraventricular injections of NE, but not DA stimulated LH release in estrogen-primed rabbits, and similar results have also been confirmed in rats (Krieg and Sawyer, 1976).

NE may also be concerned with the tonic basal secretion of gonadotropin. Ojeda and McCann (1973) have shown that both α-methyl-tyrosine and DDC are able to lower serum LH levels in short term castrated male rats, and administration of DOPS was effective in restoring to normal levels. α-Methyl-tyrosine also has been demonstrated to prevent the compensatory hypertrophy of the remaining ovary after unilateral castration, and this effect could be overcome by both DOPA and DOPS (Müller et al., 1972). The pulsatile secretion of LH also appears to be under the stimulatory control of NE. In long-term ovariectomized rats, disappearance of pulsations resulted after selective inhibition of NE synthesis by U-14,624 or FLA-63, whereas α-methyl-tyrosine had no effect (Doura and Gallo, 1976; Gnodde and Schuiling, 1976). Phenoxybenzamine, an α-adrenergic receptor blocker, was reported to prevent the post-castration rise of gonadotropin in rats (Ojeda and McCann, 1973) and to block the pulsatile release of LH in ovariectomized monkeys (Bhattacharya et al., 1972).

Additional evidence which suggests a stimulatory role of NE on gonadotropin secretions came from amine turnover studies. It has been shown that castration selectively increases NE content and synthesis in the anterior hypothalamus in both male and female rats (Stefano et al., 1965; Donoso et al., 1967, 1969). Increases in NE turnover in adult male and female rats 20 days after gonadectomy were reported by Anton-Tay and Wurtman (1968) based on the increased depletion rate of ^{3}H-NE from whole brain after intraventricular injection of ^{3}H-NE. Consistent with these data, the synthesis of

^{3}H-NE from ^{3}H-tyrosine was also shown to be increased after ovariectomy, and replacement of gonadal steroids to ovariectomized rats decreased the formation of ^{3}H-NE from ^{3}H-tyrosine (Anton-Tay et al., 1970; Bapna et al., 1971). Also, a two- to three-fold increase in tyrosine hydroxylase activity has been detected on day 4 after ovariectomy, which lasted for at least 60 days (Beattie et al., 1972). Estradiol replacement further stimulated the enzyme activity, whereas progesterone decreased it in both ovariectomized and ovariectomized, estrogen-treated rats.

Changes in NE concentration and turnover in the hypothalamus have also been shown at various stages during the estrous cycle. Donoso and deGutierrez Moyano (1970) reported that the content and turnover of hypothalamic NE increased on the afternoon of proestrus. In agreement with this, catecholamine content increases in the anterior hypothalamus and catecholamine synthesis is significantly enhanced during proestrus as compared with that on diestrus (Zschaeck and Wurtman, 1973). Recently, Advis et al. (1978) provided evidence to show that NE turnover in the hypothalamus increased at early proestrus in prepubertal rats during the first estrus cycle. In general, all of these studies suggest a central stimulatory mechanism of NE in regulating gonadotropin secretion under physiological conditions.

Dopamine.--The role of DA in controlling gonadotropin secretion is still not clear. The early work which suggested a stimulatory role for DA was reported by McCann, Kamberi and their co-workers,

using in vitro co-incubation techniques and intraventricular injections of relatively high doses of DA. Increased serum LH and FSH have been reported after intraventricular injections of DA to intact male and female rats and to ovariectomized rats pre-treated with ovarian steroids (Kamberi et al., 1969, 1970, 1971a; Schneider, and McCann, 1970a,b), while direct perfusion of the amine into the anterior pituitary was ineffective (Kamberi et al., 1970, 1971a). These authors also claimed that intraventricular injections of DA increased LRF activity in portal vessel plasma (Kamberi et al., 1969) and in peripheral plasma of hypophysectomized rats (Schneider and McCann, 1970b).

In vitro studies conducted by these same authors also reached the same conclusion. DA had no direct effect on pituitary gonadotropin release in vitro. However, dopamine stimulated the hypothalamus to release LRF as judged by the increased release of gonadotropin in pituitary-hypothalamus co-incubations (Schneider and McCann, 1969a, b) and this response to DA could be completely blocked by α-adrenergic blocking agents but not by β-adrenergic blockers (Schneider and McCann, 1969a). Recently, the release of radioimmunoassayable LHRH was reported to be stimulated in vitro by DA from synaptosome preparations isolated from sheep hypothalamus or median eminence (Bennett et al., 1975), and from rat medial basal hypothalamus (Rotsztejn et al., 1976, 1977). This stimulatory effect of DA seemed to be steroid-dependent and could be blocked by pimozide, a specific DA receptor blocker.

Further evidence for its stimulatory role in control of gonadotropin secretion was provided by Kordon and Glowinski (1969). They reported that α-methyl-tyrosine blocked superovulation in immature rats induced by PMS and HCG, and restoration of NE and DA levels with DOPA restored ovulation, whereas restoration of NE alone had no effect. Pimozide, a DA receptor blocker, has been shown to suppress the pre-ovulatory surge in rats (Beattie et al., 1976), and to reduce mid-cycle surges of LH without significantly affecting the FSH surge in humans (Leppäluoto et al., 1976).

In contrast, Fuxe and co-workers first suggested an inhibitory role for the tuberoinfundibular DA system in regulating gonadotropin secretion. They have shown a negative correlation between the turnover of median eminance DA and the secretion of gonadotropin. A selective decrease in DA turnover has been shown to occur on proestrus and early estrus as compared with other stages of the cycle in rats (Fuxe et al., 1967; Ahrén et al., 1971; Löfström, 1977). On the other hand, activation of the tuberoinfundibular DA system was associated with several physiological conditions such as pregnancy, pseudopregnancy and lactation, which share in common, low circulating gonadotropin and high PRL levels (Fuxe and Hökfelt, 1969). Intraventricular infusion of DA in some studies has been reported to have no effect on ovulation in pentobarbital-blocked proestrous rats (Rubinstein and Sawyer, 1970), and on serum LH in intact or estrogen-treated rats (Cramer and Porter, 1973; Ojeda et al., 1974). In addition, implantation of DA into the median eminence (Uemura and Kobayashi, 1971), and infusion of DA into the

arcuate nucleus (Craven and McDonald, 1973), actually prolonged diestrus and suppressed both phasic and tonic release of LH. More recently, Sawyer and his co-workers (1974, 1975, 1976) showed that intraventricular infusion of DA was not effective in stimulating LH secretion, but was actually able to block LH secretion induced by NE. Some in vitro studies also indicated that DA had no effect (Quijada et al., 1974) or even inhibited the release of LHRH from the rat hypothalamus (Miyachi et al., 1973). One possible explanation which has been suggested to interpret the apparent disagreement between these reports and the earlier work reported by McCann and his collaborators, is that DA may be taken up by noradrenergic neurons and converted to NE before it affects gonadotropin secretion (Fuxe and Hökfelt, 1970). This idea is actually supported by the data showing that α-adrenergic receptor blockers prevented the stimulatory action of DA on gonadotropin secretion (Schneider and McCann, 1969, 1970a).

Studies with systemic administration of DA agonists have also shown that DA is able to inhibit gonadotropin release. Fuxe et al. (1975) and Löfström et al. (1977) found that DA-receptor stimulators, such as ergocornine, 2-Br-α-ergocryptine, apomorphine, piribedil and lergotrile could block PMS-induced ovulation in immature rats, and this blockage could be overcome by pimozide. Apomorphine and piribedil have also been shown to block the premature, preovulatory type LH surges in 15-day old female rats (Beck et al., 1978). However, apomorphine or piribedil were unable to block the LH surge in proestrus rats (Beck et al., 1978) or in steroid treated

ovariectomized rats (Simpkins, 1979). In castrated rats, stimulation of DA receptor with apomorphine or CB-154 reduced serum LH levels (Beck and Wuttke, 1977; Beck et al., 1978), and blocked the pulsatile secretion of LH (Drouva and Gallo, 1976). On the other hand, pimozide had no effect on the acute rise of LH in male rats following castration (Ojeda and McCann, 1973) or on pulsatile release of LH in long-term ovariectomized rats (Drouva and Gallo, 1976). These results might indicate that dopaminergic systems do not exert tonic inhibition of LH secretion under normal conditions. Recently, Vijayan and McCann (1978) demonstrated different actions of DA on the LH secretion depending upon the dose and the steroid background of the rat. Therefore, systemic injection of low doses of DA stimulated LH release in steroid-primed ovariectomized rats, where high doses of DA suppressed serum LH in ovariectomized rats. It appears that the dose and steroid dependency for DA action could account for most contradictory results in the literature.

Serotonin.--Most of the early studies which demonstrated an inhibitory role of 5-HT on gonadotropin secretion have been conducted by administration of 5-HT peripherally. It has been shown that systemic injection of 5-HT caused atrophy of the reproductive organs and delay of puberty in immature mice (Robson and Botros, 1961), and prevented the compensatory hypertrophy of the ovary in unilaterally ovariectomized rats (Vaughn et al., 1970). Besides, peripheral administrations of 5-HT were also reported to block ovulation in cycling rats (Endersby et al., 1970; Labhsetwar, 1971)

and in immature rats treated with PMS (O'Steen, 1965). Since 5-HT does not easily penetrate the blood-brain barrier, these anti-ovulatory effects of 5-HT have been attributed to its direct actions on the ovary (Wilson and McDonald, 1974).

Intraventricular injection of 5-HT was reported to suppress both LH and FSH release in intact and castrated rats (Kamberi et al., 1970, 1971b; Schneider and McCann, 1970a; Kamberi, 1973). The action of 5-HT appears to be at hypothalamic level because it had no effect on gonadotropin release when infused directly into hypophyseal portal vessel (Kamberi et al., 1970, 1971b). Recently, Arendash and Gallo (1978) provided evidence to show that 5-HT is involved in inhibition of episodic release of LH during electrical stimulation of the mid-brain dorsal raphé nucleus in ovariectomized rats. Results on the effect of intraventricular administration of 5-HT on ovulation were contradictory. It has been shown that 5-HT did not block ovulation when it was infused intraventricularly to proestrus rats (Rubinstein and Sawyer, 1970; Schneider and McCann, 1970a; Wilson and McDonald, 1974). However, supperssion of the proestrus surge of LH and FSH, and blockade of ovulation after intraventricular injection of 5-HT were also reported in rats (Kamberi, 1973) and in sheep (Domanski et al., 1975). 5-HTP, the precursor of 5-HT was also effective in blocking ovulation (Kordon et al., 1968; Kamberi, 1973) and suppressing gonadotropin surge (Kamberi, 1973), whereas a decrease in 5-HT levels after administration of P-chlorophenylalanine (PCPA) around the 'critical time' facilitated ovulation in PMS-treated immature rats (Kordon et al., 1968). In addition, electrochemical

stimulation of the raphé nuclei, which has been shown to increase hypothalamic 5-HT turnover, inhibited ovulation and reduced serum LH levels (Carrer and Taleisnik, 1970, 1972). The inhibitory action of 5-HT on gonadotropin secretion has been suggested to occur at the level of the medial basal hypothalamus, based on the observation that microinjection of drugs which increase the concentration of 5-HT blocks ovulation only when it is given within the arcuate-median eminence region (Kordon, 1969). Similar conclusions were reached by Domanski et al. (1975).

Changes in brain concentration and turnover of 5-HT during different endocrine states have been reported. However the work was not done as extensively as that on catecholamines. Wheaton et al. (1972) found that 5-HT levels in the median eminence fell significantly just before the LH surge in sheep. Administration of estrogen was shown to increase midbrain 5-HT concentration in castrated rats (Tonge and Greengrass, 1971) and brain 5-HT levels in immature rats (Giulian et al., 1973). Recent work by Fuxe et al. (1974) showed that estrogen increased 5-HT turnover in castrated female rats, and progesterone restored this increased turnover to control level. In agreement with this, Kordon and Glowinski (1972) reported that accumulation of ^{3}H-5HT was reduced in castrated rats pretreated with progesterone.

Even though most studies indicate an inhibitory role for 5-HT on gonadotropin release, there are some reports suggesting that 5-HT may be responsible for phasic release of gonadotropin. It has been reported that PCPA prevented the onset of puberty (Fajer et al., 1970)

and the sudden release of FSH normally seen around puberty (Brown, 1971). Intra-ocular or subcutaneous injection of 5-HT was able to restore ovulation in persistent estrous rats induced by constant light exposure (Takahashi et al., 1973). In 1972 Kordon et al. found that ovulation could be blocked by PCPA in PMS treated immature rats if the drug was given 20 hr before the 'critical period.' In later experiments Héry et al. (1976) further demonstrated that blockage of 5-HT synthesis by PCPA or 5-HT receptor by methiothepin inhibited the daily afternoon surge of LH in estrogen implanted, ovariectomized rats, whereas replacement of 5-HT by administration of 5-HTP resulted in the reappearance of LH surge. Similar studies were also reported by Coen and MacKinnon (1976) who were able to prevent the afternoon surge of LH in estrogen primed ovariectomized rats with PCPA, P-chloroamphetamine (PCA) and 5,6-dihydroxytryptamine (5,6-DHT), all of which deplete central 5-HT levels. In a recent study, Héry et al. (1978) showed that maximal depression of hypothalamic 5-HT and 5-HIAA concentrations after complete basal mediopontine transections or after lesion of the medial and the dorsal raphé nuclei, reduced the daily LH surge by more than 70%. In contrast to those earlier pharmacological studies, none of these surgical procedures abolished the surge completely. These authors therefore concluded that the dorsal raphé is involved in the regulation of rhythmic LH secretion by modulating the amplitude of this circadian rhythm rather than by generating the rhythmic pattern itself.

The stimulatory 5-HT system seems to be located in the suprachiasmatic nucleus (SCN) which receives a large serotonergic

input (Fuxe, 1965a,b; Ungerstedt, 1971). The integrity of this nucleus is necessary for phasic release of LH (Clemens et al., 1976; Coen and MacKinnon, 1976). Also, midbrain raphé lesions with 5,7-DHT or 5,6-DHT, which completely destroy 5-HT inputs into SCN (Björklund et al., 1973), blocked PMS induced ovulation in immature rats (Meyer, 1978) and abolished the afternoon surge of LH in ovariectomized rats primed with estrogen (Coen and MacKinnon, 1976).

MATERIALS AND METHODS

I. Animals, Treatments, and Blood Collection

Mature male and female rats used in these studies were obtained from two sources (Spartan Research Animals, Haslett, MI; and Harlan Industries, Cumberland, IN). Animals were housed in a temperature-controlled (25° ± 1°C) and artificially illuminated room (lights on from 0500-1900 hr) and were fed with Purina Rat Chow (Ralston Purina Co., St. Louis, MO), and tap water ad libitum. Animals were orchidectomized or ovariectomized under deep ether anesthesia. Lateral ventricle cannulation was conducted by the method of DeBalbian-Verster (1971), whereas third ventricle cannulae were implanted with the aid of a Kopf stereotaxic instrument, using coordinates described in the rat brain atlas of DeGroot (1959). Animals were anesthetized with 8% chloral hydrate (5 ml/kg Mallinckrodt Inc., Paris, Kentucky) during cannulation, and were kept in individual cages thereafter.

Piribedil mesylate (provided by Dr. M. Derome Tremblay, Les Laboratories Servier, Neuilly-sur-Seine, France), L-5-hydroxy-tryptophan ethyl ester HCL (Calbiochem, La Jolla, CA), DL-p-chlorophenylalanine methyl ester HCL (Sigma Chemical Co., St. Louis, MO), methysergide maleate (Sandoz Pharmaceuticals, Hanover, N.J.), alpha-methyl-para-tyrosine methyl ester HCl (Regis Chemical Co., Morton Grove, IL), P-chloroamphetamine HCl (Regis Chemical Co.,

Morton Grove, IL), quipazine (Miles Laboratories, Elkhardt, IN), desipramine HCl (Merrell National Laboratories, Cincinnati, OH), fluoxetine HCl (Eli Lilly and Co., Indianapolis, IN), pargyline HCl (Sigma Chemical Co., St. Louis, MO), and synthetic gonadotropin-releasing hormone (provided by Dr. K. Folkers, Institute for Biomedical Research, University of Texas, Austin, TX) were dissolved in 0.9% NaCl. 5,7-Dihydroxytryptamine creatinine sulfate (Sigma Chemical Co., St. Louis, MO) and 5-hydroxytryptamine creatinine sulfate (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% NaCl solution, containing 0.02% ascorbic acid. Pimozide (obtained from Dr. P. A. J. Janseen, Janssen Pharmaceutical Research Laboratories, Beerse, Belgium) was dissolved in 0.3% tartaric acid. Estradiol benzoate, progesterone, testosterone propionate, and hydrocortisone (Sigma Chemical Co., St. Louis, MO) were dissolved in corn oil. The glossary of drugs used in this thesis is shown in Appendix C.

Blood samples were collected by decapitation or by cardiac puncture under light ether anesthesia. Blood samples were stored at 4°C for overnight to allow clot formation and serum was separated and stored at -20°C until assayed for hormone concentration.

II. Radioimmunoassay of Serum Hormones

Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined using standard double antibody radioimmunoassay procedures. Serum LH was assayed by the method of Niswender et al. (1968), and serum FSH by the method described in the NIAMDD kit. Hormone concentrations are expressed as ng/ml in

terms of the standard reference preparations, NIAMDD-rat-LH-RP-1 and NIAMDD-rat-FSH-RP-1. All serum samples were assayed in duplicate or triplicate. Samples from individual experiments were all tested in the same assay to avoid interassay variability.

Methods used for ether extraction of plasma progesterone, separation of bound from free steroids by charcoal dextran and scintillation counting were previously described by Campbell et al. (1977). Antiprogesterone-11-BSA, GDN #337, 1:2500 was provided through the courtesy of Dr. G. D. Niswender of Colorado State University. Specificity of the progesterone antiserum had been determined by Gibori et al. (1977).

III. Assay of Dopamine, Norepinephrine, and Serotonin in Brain Tissues

A. Isolation and Preparation of Brain Tissue

Immediately after decapitation, the brains were removed from the cranium and laid on the dorsal side of a 30° incline, with the anterior portion facing the bottom. The anterior hypothalamic area (AHA) was dissected by cutting rostrally and caudally to the optic chiasm and laterally at the hypothalamic sulci, at a depth to the level of the anterior commissure. The medial basal hypothalamus (MBH) included an area caudal to the optic chiasm and rostral to the mammillary bodies, with its lateral boundary at the hypothalamic sulci. The block of tissue was produced by cutting at a depth of 1-2 mm (Figure 1). The hypothalamic fragments were immediately frozen on dry ice until assayed for biogenic amines. The median

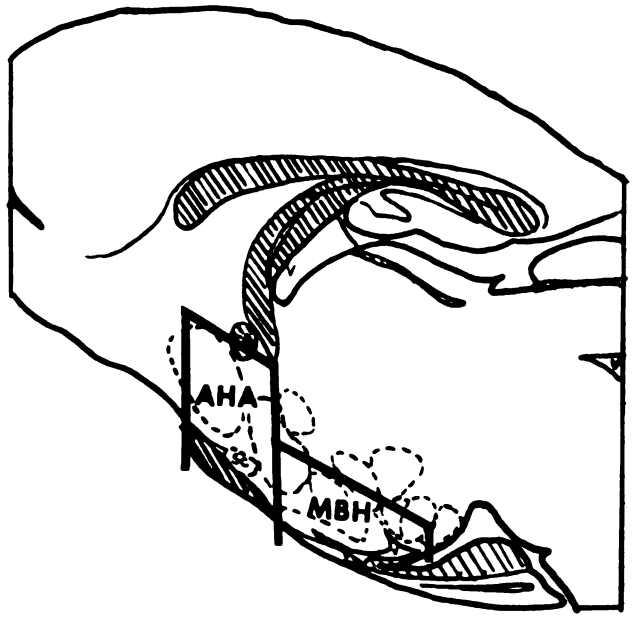

Figure 1. Sagittal Section of the Rat Brain Showing Pre-Optic-Anterior Hypothalamic Area (AHA) and Medial Basal Hypothalamus (MBH).

eminence (ME) was dissected by using a fine iris scissors. Cuts were made at the posterior border of the infundibular stalk and along the lateral aspects of the tuber-cinereum at an angle of about 20° from the ventral hypothalamic surface.

The AHA and MBH were then weighed and homogenized in 100 µl of either 0.4 N perchloric acid (containing 10 mg % EDTA) when both catecholamines and serotonin were measured, or 0.1 N HCl (plus 10 mg % EDTA) when only serotonin was measured, whereas the ME was homogenized in 30 µl of 0.4 N perchloric acid (containing 10 mg % EDTA). Tissues were homogenized with a sonifier cell disruptor (Model W140D, Heat System-Ultrasonics, Inc.) and centrifuged to separate the particulate portion from the supernatant. Both catecholamines and serotonin were assayed in 10 µl of supernatant by the methods described below. ME protein content was determined by the micro-method of Lowry et al. (1951).

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

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

Normetanephrine and methoxytyramine were separated utilizing solvant extraction and thin layer chromatography. Amine content of

samples were determined after separation by counting chromatographic spots containing the ^{3}H-labeled metabolites in glass scintillation vials containing 10 ml of aqueous counting scintillant (Amersham Corp., Arlington Heights, IL). Samples were counted in a Beckman LS-100 liquid scintillation counter.

C. Radioenzymatic Assay of Serotonin (5-HT)

Tissue 5-HT was assayed by a modification of the method of Saavedra et al. (1973) (see Appendix B). Rat liver N-acetyl transferase was prepared by the method of Weissbach et al. (1961). Hydroxyindole-o-methyl transferase extracted from bovine pineals (Pel-Freez Biologicals, Inc., Rogers, AK), was prepared by the method of Axelrod and Weissbach (1961).

IV. Methods of Statistical Analysis

Data were analyzed statistically by either one way analysis of variance, followed by the Student-Neuman-Keuls multiple range test, or Student's 't' test when it was appropriate. The level of significance chosen was $p < 0.05$.

EXPERIMENTAL

I. Effects of Dopaminergic and Serotonergic Drugs on Post-Castration Rise of Serum Gonadotropin in Male Rats

A. Objective

Involvement of biogenic amines in the regulation of gonadotropin secretion has been well established (Sawyer, 1975; Meites et al., 1977). It is generally believed that NE has a stimulatory role in controlling the secretion of gonadotropin. However, controversy still remains as to whether DA has an inhibitory or stimulatory role on the release of LH and FSH. The early work reported by McCann, Kamberi and their co-workers suggested a stimulatory role for DA (Kamberi et al., 1969, 1970; Schneider and McCann, 1970). On the other hand, studies with systemic administration of DA agonists have shown that DA can inhibit gonadotropin release. Stimulation of DA receptors with apomorphine, CB-154, and piribedil suppressed the pulsatile LH secretion (Gnoddle and Schuiling, 1976; Drouva and Gallo, 1976, 1977), and decreased serum levels of LH in long-term ovariectomized rats (Fuxe et al., 1976; Beck and Wuttke, 1977; Beck et al., 1978). However, no work was reported on acute castrated rats.

Intraventricular injection of 5-HT has been shown to inhibit gonadotropin secretion in both castrated male and female rats (Kamberi, 1970, 1971b; Schneider and McCann, 1970). Recently Arendash

and Gallo (1978) provided evidence that the inhibition of episodic LH release following electrical stimulation of the dorsal raphé nucleus (DRN) was mediated through 5-HT.

Castration results in a rapid increase in serum levels of gonadotropin in male rats. The increase in serum LH can be detected within 8 hrs after orchidectomy (Gay and Midgley, 1969). It was of interest to see whether or not administration of dopaminergic and serotonergic drugs could influence this rapid rise of gonadotropin in short-term orchidectomized rats.

B. Materials and Methods

Male Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-400 g each were used in this study. Rats were maintained in an air conditioned (25° ± 1°C) and light-controlled (lights on from 0500-1900 hrs) room and were supplied with Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. Orchidectomy was always conducted under ether anesthesia at about 0900 hrs. Piribedil mesylate (PIR), L-5-hydroxytryptophan ethyl ester HCl (5-HTP), methysergide maleate (MES), DL-P-chlorophenylalanine methyl ester HCl (PCPA) were dissolved in 0.9% NaCl solution, whereas pimozide (PIM) was dissolved in 0.3% tartaric acid. Testosterone propionate (TP) was injected s.c. in oil.

Experiment I.--Rats were orchidectomized at 0900 hrs and separated into three groups. The control group was further divided into two subgroups. Thus blood samples could be collected every 4 hr from each group alternately. Rats in the two experimental

groups were injected i.p. with either piribedil (10 mg/kg) or 5-HTP (50 mg/kg) every 4 hrs for five consecutive injections, starting at 1300 hrs. Controls were treated in the same way, except that only 0.9% saline was given. Blood samples were taken by cardiac puncture under light ether anesthesia from each rat in control group (A) and experimental groups, 1 hr before, and 8, 16 and 24 hrs after orchidectomy, and from control group (B) 4, 12 and 20 hrs after orchidectomy.

Experiment II.--In the first trial, rats were castrated and divided into three groups. They were injected i.p. with multiple doses of either 1 or 10 mg/kg of piribedil starting 4 hrs after orchidectomy, and continued every 4 hrs for three consecutive injections. Rats given saline only were used as controls. Half of the rats in each group were further treated (i.p.) with either 1 mg/kg of pimozide, or 0.3% tartaric acid at 4 and 10 hrs after orchidectomy.

In the second trial, rats were orchidectomized and divided into four groups. They were treated with either piribedil (10 mg/kg, i.p.) or pimozide (2 mg/kg, s.c.), or a combination of the two drugs. The controls were given saline and 0.3% tartaric acid. Piribedil was administered as described in the first trial, whereas pimozide was injected 1 hr after orchidectomy. Blood samples were collected by decapitation 16 hrs after orchidectomy.

Experiment III.--Rats were castrated and divided into four groups. They were treated i.p. with either 5-HTP (50 mg/kg) or methysergide (5 mg/kg), or a combination of the two drugs. The

controls were given saline only. Both drugs were injected at 4, 8 and 12 hrs after orchidectomy. Blood samples were collected by decapitation 4 hrs after the last drug injection.

Experiment IV.--Long-term orchidectomized rats (for more than 1 mo) were separated into two groups. PCPA (300 mg/kg) was injected i.p. into one group of rats 3 days prior to the experiment, whereas the other group received saline only. On the day of the experiment, half of the rats in each group were injected s.c. with TP (0.5 mg/ 300 g B.W.), at 0900 hrs, whereas the other half received corn oil only. Blood samples were collected from the trunk after decapitation, 8 hrs after injection of TP.

C. Results

Effects of Multiple Doses of Piribedil and 5-HTP on Post-Castration Increases in Serum LH and FSH.--Serum LH levels in the controls remained at basal levels within the first 4 hrs following orchidectomy. By 8 hrs after orchidectomy, serum LH increased significantly from the basal levels of 44 $\pm$ 7 ng/ml to 84 $\pm$ 10 ng/ml ($p < 0.05$), and reached a plateau by 16 hrs after orchidectomy (Figure 2). Both piribedil and 5-HTP effectively suppressed the increase in serum LH through the entire sampling period. Serum FSH increased slightly, but not significantly 4 hrs after orchidectomy (361 $\pm$ 24 vs. 434 $\pm$ 43 ng/ml). The increase in serum FSH reached significant levels by 8 hrs after orchidectomy (361 $\pm$ 24 vs 542 $\pm$ 57 ng/ml, $p < 0.05$) (Table 1). Administration of piribedil suppressed the post-castration rise in serum FSH. However, it was not

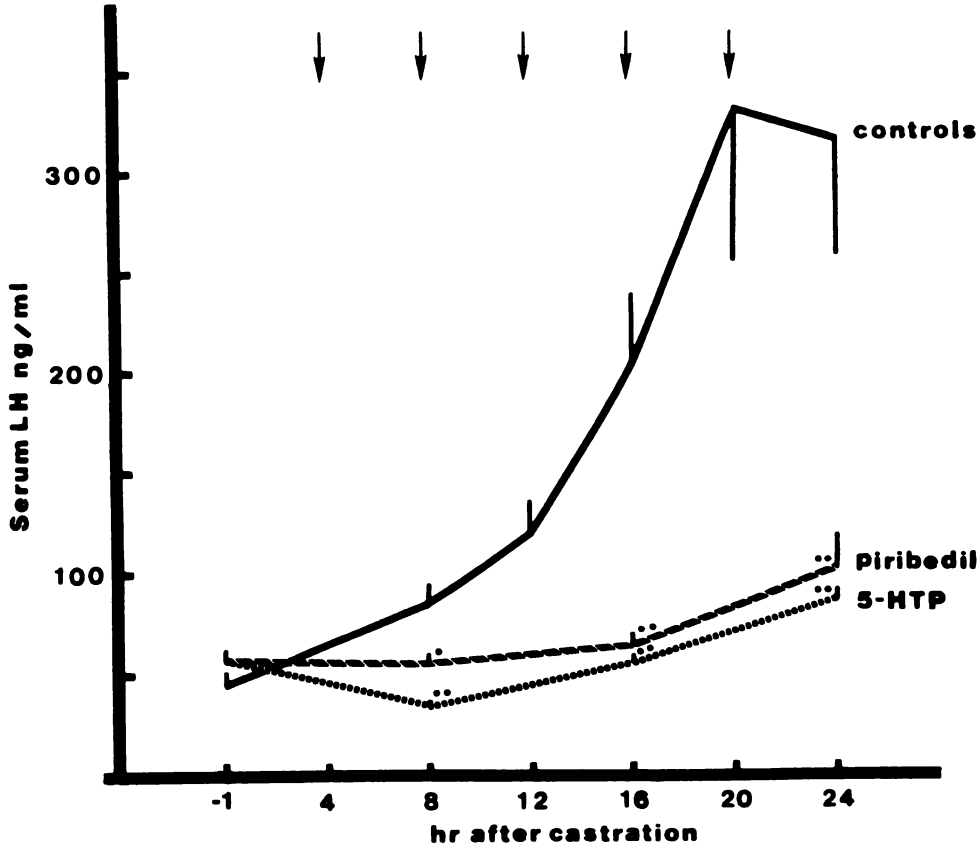

Figure 2. Effects of Sustained Administration of Piribedil and 5-Hydroxytryptophan (5-HTP) on Post-Castration Rise of Serum Luteinizing Hormone (LH) in Male Rats.

Rats were injected i.p. with either piribedil (10 mg/kg) or 5-HTP (50 mg/kg), or 0.9% NaCl solution at 4, 8, 12, 16, and 20 hrs after orchidectomy. Each point represents the mean of 5-6 determinations, and the vertical lines indicate 1 SEM.

Table 1. Effects of Sustained Administration of Piribedil and 5-Hydroxytryptophan (5-HTP) on Post-Castration Rise of Serum FSH in Male Rats

Group	Time (hrs) After Orchidectomy						
	-1	4	8	12	16	20	24
Controls (A)	361±24[a]	---	542±57[b]	---	624±54[b]	---	733±45[b]
Controls (B)	---	434±43	---	623±72	---	669±110	---
5-HTP (50 mg/kg)	396±19	---	554±52	---	593±71	---	685±100
Piribedil (10 mg/kg)	436±23	---	489±18	---	574±18	---	471±32*

a Mean ± SEM (Standard Error of Mean) of 5-6 determinations.
* $P < 0.05$ vs. controls.
b $P < 0.05$ vs. -1 hr sample.

significant, except at 24 hrs post-castration (471 ± 32 vs 733 ± 45 ng/ml in controls, $p < 0.05$). On the other hand, 5 HTP had no effect on the increase in serum FSH at any time throughout the experiment.

Effects of Multiple Doses of Piribedil, Pimozide and Their Combination on the Post-Castration Rise of Serum LH and FSH.--Figure 3 shows that administration of piribedil resulted in a dose dependent suppression of serum LH ($p < 0.01$). Pimozide (1 mg/kg) alone had no effect on the post-castration rise of serum LH, and failed to overcome the action exerted by the low dose of piribedil (1 mg/kg). However, it partially reversed the suppression of serum LH after administration of the higher dose of piribedil (10 mg/kg) (198 ± 30 vs. 65 ± 11 ng/ml, $p < 0.01$). The effects of piribedil and pimozide on serum FSH are shown in Figure 4. Piribedil was effective in suppressing the post-castration rise of serum FSH only at the higher dose (460 ± 31 vs. 864 ± 53 ng/ml in controls, $p < 0.01$). Pimozide alone had no significant effect on serum FSH and failed to reverse the suppression of serum FSH by 10 mg/kg of piribedil. Serum FSH in piribedil (1 mg/kg)-pimozide treated group was significantly lower than in the controls (642 ± 73 vs. 864 ± 53 ng/ml, $p < 0.05$).

The effects of piribedil and pimozide on the post-castration increase in serum LH and FSH in the second trial are shown in Table 2. Piribedil at a dose of 10 mg/kg significantly suppressed both LH and FSH ($p < 0.05$). Pimozide alone had no significant effect on either LH or FSH, but either partially reversed the piribedil

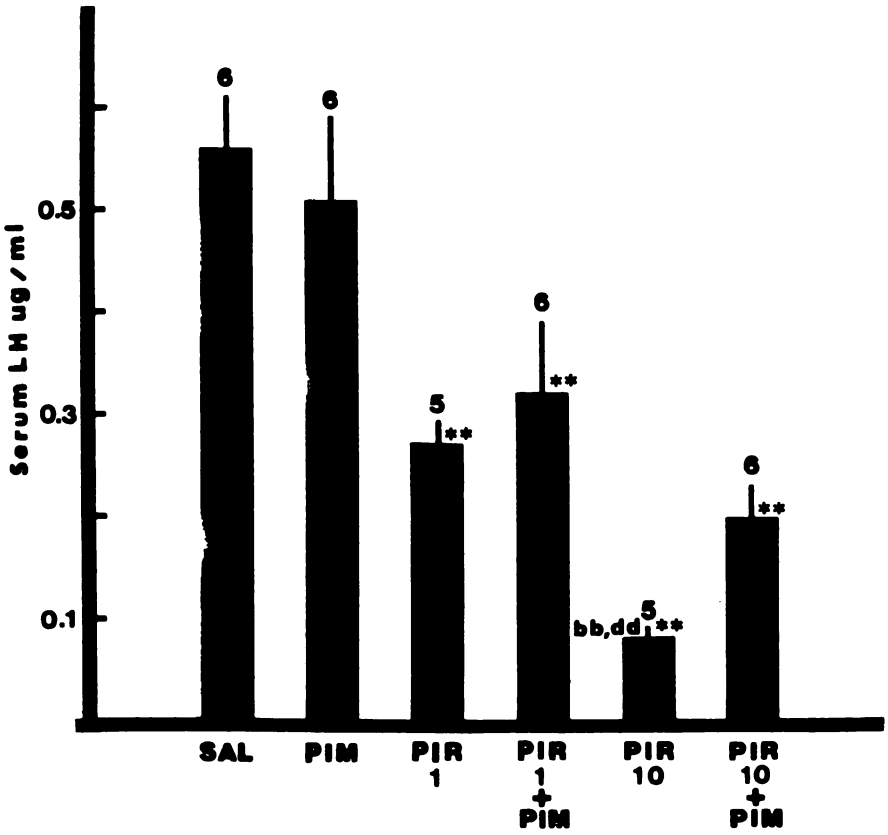

Figure 3. Effects of Sustained Administration of Piribedil (PIR) and Pimozide (PIM) on Post-Castration Rise of Serum LH in Male Rats.

Rats were injected i.p. with either 1 or 10 mg/kg of piribedil, or 0.9% saline at 4, 8, 12 hrs after orchidectomy. Half of the rats of each treatment were further injected i.p. with either pimozide (1 mg/kg) or 0.3% tartaric acid at 4 and 10 hrs post-castration. Blood samples were collected by decapitation at 16 hrs after orchidectomy. Each bar represents the mean of 5-6 determinations. Vertical lines indicate 1 SEM.

** = $p < 0.01$ vs. saline treated controls.
bb = $p < 0.01$ vs. piribedil (1 mg/kg) treated group.
dd = $p < 0.01$ vs. piribedil (10 mg/kg) plus pimozide treated group.

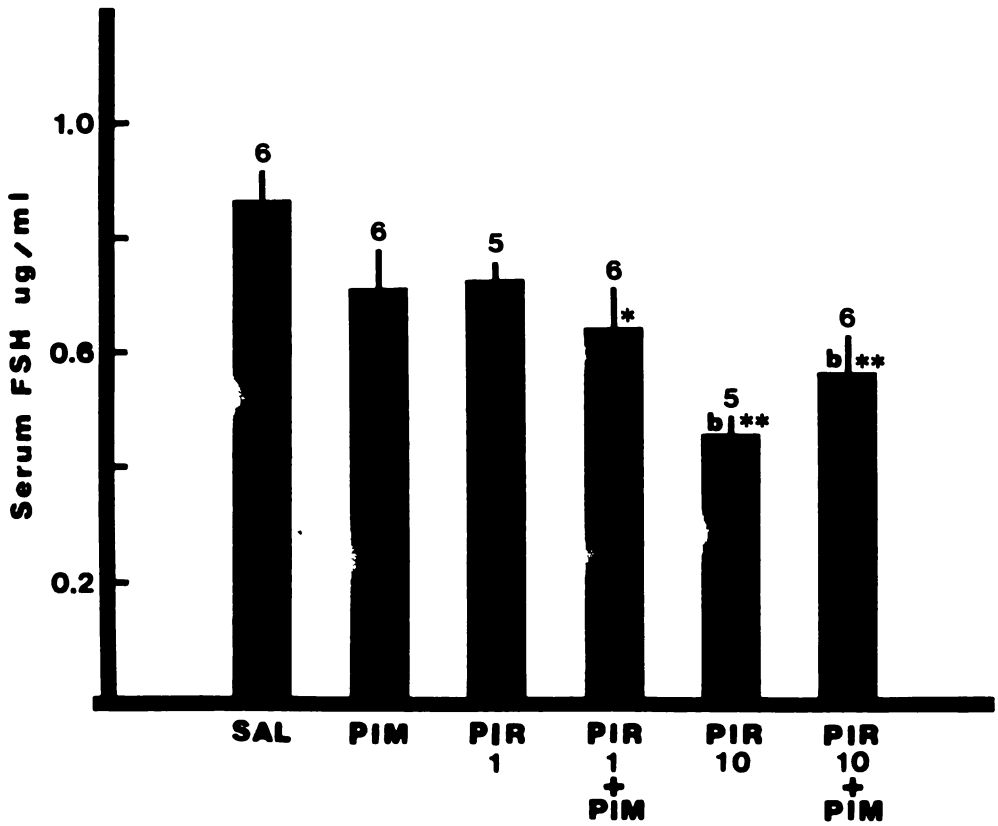

Figure 4. Effects of Sustained Administration of Piribedil (PIR) and Pimozide (PIM) on Post-Castration Rise of Serum Follicle-Stimulating Hormone (FSH) in Male Rats.

See Figure 3 for explanation. *, **; $p < 0.05$ and 0.01, respectively vs. saline treated controls. b, $p < 0.05$ vs. piribedil (1 mg/kg) treated group.

Table 2. Effects of Piribedil and Pimozide on Post-Castration Rise of Serum Gonadotropin in Male Rats

Treatment	No. of Rats	Serum Levels of Hormone (ng/ml) LH	FSH
Vehicles	8	589 ± 62[a]	1300 ± 57
Pimozide	8	409 ± 57	1188 ± 51
Piribedil	8	198 ± 36*	853 ± 55*
Pimozide + Piribedil	8	323 ± 99	1088 ± 89[b]

Piribedil (10 mg/kg) was dissolved in saline and injected i.p. in rats every 4 hrs for 3 consecutive injections, starting at 4 hrs after orchidectomy, whereas pimozide (2 mg/kg) was dissolved in 0.3% tartaric acid and injected s.c. at 1 hr after orchidectomy.

a Mean ± SEM.

* $P < 0.05$ vs. vehicle treated controls.

b $P < 0.05$ vs. piribedil treated group.

induced suppression of serum LH or completely prevented the decrease in serum FSH in orchidectomized rats after piribedil administration.

Effects of Multiple Doses of 5-HTP, Methysergide, and Their Combination on the Post-Castration Rise of Serum LH and FSH.--Figure 5 shows the effects of 5-HTP and methysergide on serum LH and FSH in rats shortly after orchidectomy. 5-HTP at a dose of 50 mg/kg significantly suppressed the post-castration increase in serum LH (182 $\pm$ 37 vs. 462 $\pm$ 86 ng/ml in controls, $p < 0.01$). Methysergide (5 mg/kg) alone had no effect on the post-castration rise of serum LH, but completely blocked the inhibitory effect of 5-HTP on serum LH. Neither 5-HTP nor methysergide was able to affect the post-castration rise of serum FSH.

Effect of PCPA on Testosterone-Induced Negative Feedback Inhibition of LH in Long-Term Orchidectomized Rats.--The serum level of LH in long-term orchidectomized rats was 1169 $\pm$ 125 ng/ml (Table 3). Administration of TP resulted in a significant decrease in serum LH within 8 hrs ($p < 0.05$). PCPA not only had no effect on the increased serum LH in response to chronic orchidectomy, but also did not impair the testosterone induced negative feedback inhibition of LH.

D. Discussion

The present study demonstrates that piribedil, a dopamine agonist, can inhibit the rise in serum LH and FSH, and 5-HTP is able to suppress the increase in serum LH but not FSH in rats shortly

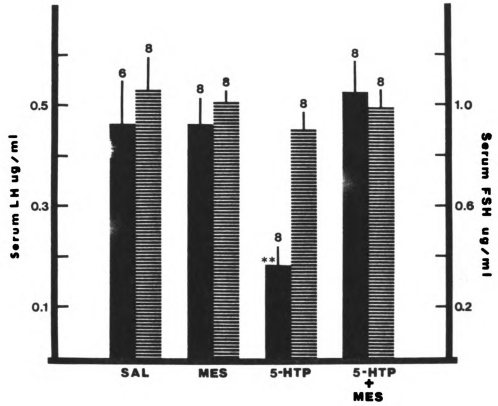

Figure 5. Effects of Sustained Administration of 5-HTP and Methysergide (MES) on Post-Castration Rise of Serum LH and FSH in Male Rats.

Rats were injected i.p. with either 5-HTP (50 mg/kg) or methysergide (5 mg/kg), or a combination of the two drugs at 4, 8, and 12 hrs after orchidectomy. Blood samples were collected by decapitation at 16 hrs post-castration. Solid bars indicate serum levels of LH, whereas striped bars indicate serum FSH. Vertical lines indicate 1 SEM. Number above each vertical line indicates the number of determinations. **, $p < 0.01$ vs. saline treated controls.

Table 3. Effect of P-Chlorophenylalanine (PCPA) on Testosterone Propionate (TP)-Induced Negative Feedback Inhibition of Serum LH in Long Term Orchidectomized Rats

Treatment	No. of Rats	Serum Levels of LH (ng/ml)
Saline		
Oil	6	1169 ± 125[a]
TP	6	592 ± 96*
PCPA		
Oil	6	975 ± 119
TP	6	467 ± 40*

PCPA (300 mg/kg) was injected i.p. to the rats 3 days prior to the experiment, whereas TP (0.5 mg/300 g) was injected s.c. at 0900 hrs.

a Mean ± SEM.

* $P < 0.05$ vs. saline-oil treated controls.

after orchidectomy. This inhibition appears to be quite specific since pimozide, a dopamine receptor blocker, and methysergide, a 5-HT receptor blocker, could either partially or completely prevent the inhibitory action of piribedil and 5-HTP on gonadotropin release.

Fuxe and co-workers were the first to suggest an inhibitory role of the tuberoinfundibular dopaminergic system in controlling gonadotropin secretion (Fuxe and Hökfelt, 1969; Ahren et al., 1971). Studies using systemic administration of DA agonists provided evidence for this inhibitory effect of DA. Stimulation of the DA receptor with apomorphine or CB-154 reduced serum LH levels in castrated rats (Beck and Wuttke, 1977; Beck et al., 1978), and both apomorphine and piribedil have been shown to block the pulsatile secretion of LH in long-term ovariectomized rats (Drouva and Gallo, 1976, 1977). Recently, Vijayan and McCann (1978) reported that relatively low doses of DA and apomorphine stimulated LH release in steroid-primed ovariectomized rats, whereas high doses of DA agonists suppressed serum LH in ovariectomized rats. These authors suggested a dual action of DA on LH secretion depending on the dose and the endocrine state of the animal.

In a number of physiological and experimental conditions, an inverse relationship between serum PRL and gonadotropin exists. During post-partum lactation in rats, serum PRL is high, whereas serum LH and FSH are low (Meites, 1966; Lu et al., 1976). Induction of hyperprolactinemia by either exogenous administration of ovine PRL (Gudelsky et al., 1976) or implantation of anterior pituitary glands underneath the kidney capsule, or s.c. implantation of

pituitary tumor tissue (Grandison et al., 1977) decreases LH secretion in castrated male and female rats. The hyperprolactinemia induced inhibition of gonadotropin secretion appears to be mediated through the dopaminergic system, since systemic injection of PRL and s.c. pituitary tumor grafts have been shown to increase DA turnover in the median eminence (Hökfelt and Fuxe, 1972; Gudelsky et al., 1977; Hodson et al., 1978).

Administration of pimozide had no effect on the rapid rise of LH following orchidectomy, which agrees with the result reported by Ojeda and McCann (1973). Pimozide also was shown to have no effect on the pulsatile LH release in long-term ovariectomized rats (Drouva and Gallo, 1976). These data may indicate that the central dopaminergic system does not exert a tonic inhibition of LH secretion under most physiological conditions.

The possible role of DA on the secretion of FSH has not yet been well studied. Multiple injections of piribedil suppressed the post-castration rise of FSH suggesting an inhibitory role of DA. The failure to detect any acute effects on serum levels of FSH after apomorphine injection in ovariectomized rats (Vijayan and McCann, 1978; Beck et al., 1978) could be due to the short period between drug administration and blood collection since the half life of serum FSH is very long (Gay et al., 1970). Administration of pimozide had no effect on the post-castration rise of serum FSH, which was not in agreement with the result reported by Ojeda and McCann (1973). We found that frequent injection of pimozide, or even tartaric acid, suppressed the increase in serum LH and FSH after

orchidectomy (unpublished observation), suggesting that interpretations based on single drug injections, without checking the specificity of the drugs have to be interpreted with caution.

The effects of 5-HT on the tonic release of gonadotropin generally are believed to be inhibitory. It has been shown that intraventricular injections of 5-HT suppressed LH and FSH release in both intact and castrated rats (Schneider and McCann, 1970; Kamberi et al., 1970; 1971b). However, in our study, the post-castration rise of serum FSH was not affected by 5-HTP. This discrepancy could be due to an insufficient dose of 5-HTP employed in the present study. Recently, Arendash and Gallo (1978) reported that inhibition of episodic LH release in ovariectomized rats by following electrical stimulation of the dorsal raphé nucleus, appeared to be mediated through the serotonergic system.

Despite the fact that 5-HT is able to inhibit gonadotropin secretion, the central serotonergic system may not play an important role in controlling gonadotropin secretion in castrated rats, since the data showed that a 5-HT receptor blocker, methysergide, failed to influence the post-castration increase in serum levels of gonadotropin. In addition, PCPA and metergoline, another 5-HT receptor blocker, were shown to have no effect on episodic LH secretion (Arendash and Gallo, 1978). Our data also indicated that the negative feedback inhibition of testosterone on LH secretion is not mediated through 5-HT since blockade of 5-HT synthesis by PCPA did not impair the negative feedback inhibition of LH exerted by testosterone.

Although the present results show that dopaminergic and serotonergic drugs can block the rise in serum LH that occurs soon after orchidectomy, they do not necessarily indicate that these amines are involved in the physiological regulation of gonadotropin secretion except in special states, i.e. hyperprolactinemia, lactation and perhaps other conditions.

II. Effects of Orchidectomy on Median Eminence Catecholamine Turnover and Serum Levels of Gonadotropin in Male Rats

A. Objective

The pharmacological study in Experiment I showed that activation of DA receptors by piribedil could effectively prevent the rapid post-castration rise of serum LH in male rats. However, the dopaminergic system does not appear to exert a tonic inhibition of LH secretion since pimozide, a dopamine receptor blocker, did not alter the acute rise of LH following orchidectomy. Also pimozide has no effect on the pulsatile LH release in ovariectomized rats (Drouva and Gallo, 1976).

The tonic release of LH appears to be under the stimulatory control of NE. Ojeda and McCann (1973) have shown that both diethyldithiocarbamate (DDC), a DA-β-hydroxylase inhibitor, and phenoxybenzamine, an α-adrenergic receptor blocker, were able to prevent the post-castration rise of gonadotropin in male rats. In addition, decreased serum LH and disappearance of pulsations in long-term ovariectomized rats resulted after selective inhibition of NE synthesis by U-14,624 or FLA-63 (Doura and Gallo, 1976).

Orchidectomy results in a rapid increase in serum LH in male rats, whereas serum prolactin levels decrease following castration (Meites et al., 1972). The purpose of this study was to attempt to correlate changes in median eminence catecholamine metabolism with the release of pituitary gonadotropin in male rats soon after orchidectomy.

B. Materials and Methods

Male Sprague-Dawley rats, weighing 200-300 g each were used in this study. The rats used for the α-methyl-p-tyrosine (α-mpt) time course study were purchased from Spartan Research Animals (Haslett, MI), whereas the rats used in catecholamine turnover study were purchased from Harlan Ind. (Cumberland, IN).

To study the time course effects of α-mpt on the depletion of catecholamines in the medial basal hypothalamus, α-mpt (250 mg/kg) was administered i.p. to the normal intact rats at 0.5, 1, 2, 4, or 6 hrs prior to decapitation. The control rats were given 0.9% NaCl only. All of the rats, including the controls, were killed between 1150 and 1320 hrs. The acute effect of orchidectomy on the median eminence (ME) catecholamine turnover was investigated in a second experiment. Three groups of 12 rats each were orchidectomized at 8, 16, or 24 hrs prior to decapitation. One group of 12 rats was sham operated 16 hrs before decapitation by making an incision on the scrotum. DA and NE turnover were estimated by a modification of the non-steady state method described by Brodie et al, (1966). Each group of rats received an i.p. injection of either α-mpt

(250 mg/kg) or 0.9% NaCl 1 hr before decapitation. (The depletions of both NE and DA by 1 hr following α-mpt administration are linear as shown in Results.) All of the rats were killed within 3 hrs (0900 to 1200 hrs).

After decapitation, the trunk blood was collected for hormone assays, and the brain was immediately removed from the cranium. The MBH and ME were dissected, and catecholamines were assayed as described in the General Materials and Methods section. The average weight of the MBH was 14.2 ± 0.3 mg, and the average protein content of the ME was 46.6 ± 1.5 μg (due to its small size, tissue weight could not be recorded). NE and DA in the MBH are expressed as ng/g wet weight, whereas NE and DA in the ME are expressed as ng/mg protein.

The depletion of catecholamines with time after α-mpt injection was analyzed by a least squares regression analysis after logarithmical transformation (Sokal and Rohlf, 1969). The slope of the regression line was used to determine the turnover rates of NE and DA in the MBH in μg/g/hr.

C. Results

Time Course Effects of α-mpt on Concentrations of Medial Basal Hypothalamic Norepinephrine and Dopamine in Intact Male Rats.--The time course effects of a single injection of α-mpt on the depletion of NE and DA in the MBH are shown in Figure 6. The concentrations declined in an exponential manner having a calculated overall rate constant of 0.0889 hr^{-1} for NE and 0.4267 hr^{-1} for DA.

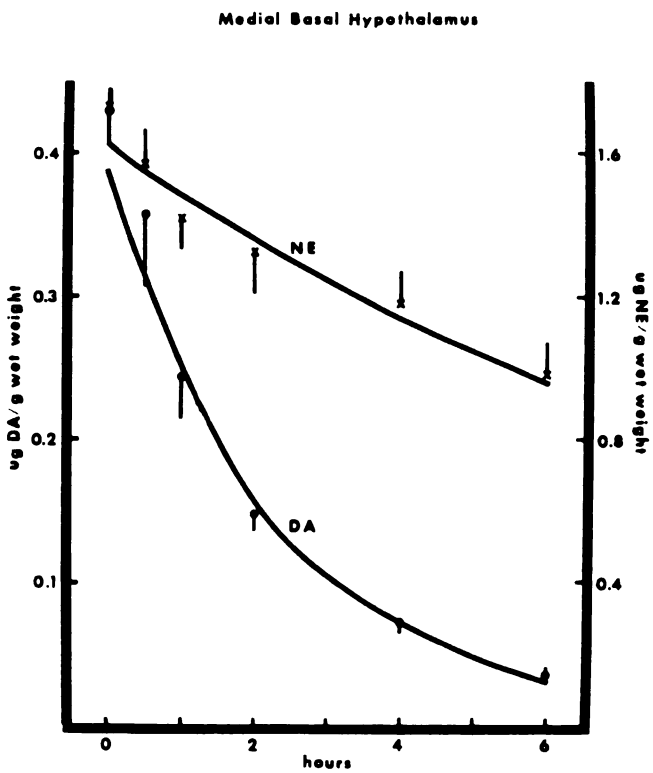

Figure 6. Time Course of the Effect of Alpha-Methyl-Para-Tyrosine (α-mpt) on Medial Basal Hypothalamic Concentration of Norepinephrine (NE) and Dopamine (DA) in Male Rats.

Rats were injected i.p. with α-mpt (250 mg/kg) and killed at various times thereafter. Each symbol represents the mean of 5-7 determinations. Vertical lines indicate 1 SEM.

The turnover rates of NE and DA in the MBH were calculated to be 0.15 μg/g/hr and 0.18 μg/g/hr, respectively (Table 4). α-mpt at a dose of 250 mg/kg caused a linear depletion of MBH NE and DA for 2 hrs after injection.

Effects of Acute Orchidectomy on Median Eminence Catecholamine Turnover and Serum Levels of LH and FSH.--Neither the concentration nor the turnover of median eminence NE changed by 24 hrs after orchidectomy (Figure 7). On the other hand, a significant increase in median eminence DA concentration occurred by 16 and 24 hrs post-castration ($p < 0.05$). DA turnover as measured by the percent depletion of the monoamine after α-mpt injection was significantly elevated at 16 hrs after orchidectomy ($p < 0.05$).

Serum LH increased ($p < 0.05$) from the basal levels of 29 ± 9 ng/ml in sham orchidectomized rats to 151 ± 28 ng/ml by 8 hrs post-castration (Table 5). By 16 and 24 hrs post-castration, serum LH levels were greater than 300 ng/ml. Basal levels of serum LH were not affected by α-mpt 1 hr after its injection. On the other hand, the rapid rise in LH by 8 hrs after orchidectomy was significantly suppressed by α-mpt ($p < 0.05$). By 16 hrs post-castration, elevated serum LH was no longer suppressed by α-mpt.

Serum FSH in sham orchidectomized rats was 468 ± 23 ng/ml (Table 5). A significant increase in serum FSH after castration was first detected at 16 hrs post-castration (801 ± 68 ng/ml vs. 468 ± 23 ng/ml in sham castrates, $p < 0.01$). Administration of α-mpt for 1 hr had no effect on either the basal or the post-castration level of FSH.

Table 4. Turnover Rates and Turnover Times of Medial Basal Hypothalamic Norepinephrine (NE) and Dopamine (DA) in Male Rats

	Initial Level		Rate Constant of Amine Loss		Turnover	
Amine	µg/g ± SEM	N	K (hr^{-1}) ± SEM	N	Time hr	Rate µg/ g /h
NE	1.732 ± 0.052	7	0.0889 ± 0.009	41	11.2	0.15
DA	0.429 ± 0.025	7	0.4267 ± 0.0216	40	2.3	0.18

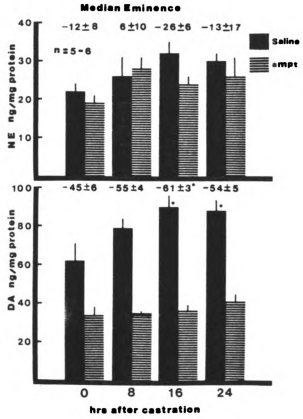

Figure 7. Effect of Orchidectomy on Steady State Concentration and Alpha-Methyl-Para-Tyrosine Induced Depletion of NE and DA in the Median Eminence.

The solid bars indicate steady state concentration and striped bars indicate amine concentration 1 hr after i.p. injection of α-mpt. The number above each set of bars indicates percentage depletion of amines induced by α-mpt (Mean $\pm$ SEM). *, $p < 0.05$ vs. zero hr value.

Table 5. Serum Levels of Gonadotropin in Male Rats after Orchidectomy

Hormone	Group	No. of Rats	Time (hrs) after Orchidectomy 0	8	16	24
LH						
	Saline	6	29 ± 9[a]	151 ± 28*	314 ± 55*[b]	691 ± 37*[bc]
	α- mpt	6	16 ± 2	53 ± 13†	207 ± 44*[b]	577 ± 93*[bc]
FSH						
	Saline	6	468 ± 23	582 ± 46	801 ± 68*[b]	1127 ± 52*[bc]
	α- mpt	6	506 ± 34	514 ± 41	825 ± 72*[b]	1159 ± 61*[bc]

a Mean ± SEM.
* $P < 0.05$ vs. 0 hr.
b $P < 0.05$ vs. 8 hrs.
c $P < 0.05$ vs. 16 hrs.
† $P < 0.05$ vs. saline treated group.

D. Discussion

The decline in the concentrations of both NE and DA in the medial basal hypothalamus after 250 mg/kg of α-mpt is not a simple exponential curve, but appears to have two major components. Our results are in agreement with those reported by Doteuchi et al. (1974) and Kizer et al. (1974). The rapid initial decline in catecholamines after treatment with α-mpt has been related to the generation of amphetamine-like metabolites of α-mpt, p-hydroxyamphetamine and p-hydroxynorephedrine, which increase the release of catecholamines (Doteuchi et al., 1974). In addition, the onset of tyrosine hydroxylase inhibition following α-mpt injection is slow. The conversion index of radioactive tyrosine into striatal DA has been shown to be inhibited about 53% between 5 and 15 min after α-mpt and about 76% by 40 min after α-mpt (Doteuchi et al., 1974). It is important, therefore, to measure the depletion of catecholamine by α-mpt over an extended period in order to get an appropriate estimation of turnover rate.

Neither the concentration nor the turnover of NE in the ME changed within 24 hrs after orchidectomy in male rats. Our data do not agree with those reported by Chiocchio et al. (1976), who showed a transient increase in NE content in the ME at 4 and 8 hrs after orchidectomy. The reason for this discrepancy is not clear. It is possible that the divergent results may be due to the quantity of ME tissue assayed. Our ME samples, in general, only contained very small amounts of NE.

On the other hand, our data demonstrated that both the steady state concentration and turnover of DA in the ME were significantly increased by 16 hrs after orchidectomy. The increased steady state concentration and turnover of DA suggested that DA synthesis and release were accelerated. The increased DA turnover in response to castration appears to be a long lasting phenomenon since Kizer et al. (1978) reported a similar increase in DA turnover following 10 days of castration.

The tuberoinfundibular dopaminergic system does not appear to play an important role in the tonic, negative feedback regulation of gonadotropin secretion, since Greeley et al. (1978) have shown that the post-castration rises of LH and FSH in both male and female rats were not impaired by neonatal treatment of monosodium glutamate (MSG) which selectively destroys the DA cell bodies located within the arcuate nucleus (Nemeroff et al., 1977). On the other hand, the tuberoinfundibular dopaminergic system has been demonstrated to be involved in the regulation of prolactin (PRL) secretion (Neill, 1974; Macleod, 1976). Therefore, increases in the activity of the tuberoinfundibular DA system consistently decrease the secretion of PRL. It is possible that the decreased pituitary and serum PRL observed after castration in both male and female rats (Meites et al., 1972) may be due to the increased DA turnover following castration.

Based on turnover studies, Fuxe and co-workers proposed an inhibitory tuberoinfundibular DA system in the control of gonadotropin secretion. These authors found that pre-treatment of male

rats with estradiol increased tuberoinfundibular DA turnover (Fuxe et al., 1969). However, recent studies by Eikenburg et al. (1977) indicated that the increase in DA turnover in the ME after estrogen administration is mediated through PRL which has been shown to increase tuberoinfundibular DA turnover (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976).

Serum levels of LH at 8 hrs after orchidectomy were significantly suppressed by α-mpt, which is in agreement with the result reported by Ojeda and McCann (1973). The failure of α-mpt to suppress the post-castration rise of LH and FSH at 16 hrs after orchidectomy could be because the injection of α-mpt for only 1 hr is too short for the drug to exert its action. In supporting this idea, our data show that the depletion of MBH NE is much slower than that of DA following α-mpt injection. In general, it is believed that the central NE system has a stimulatory role in regulating gonadotropin secretion (Meites et al., 1977; Weiner and Ganong, 1978).

III. Effects of Suppression of Serotonin Synthesis by P-Chlorophenylalanine and Subsequent Replacement of Serotonin by 5-Hydroxytryptophan on Gonadotropin Secretion in Estrogen Treated Ovariectomized Rats

A. Objective

The role of serotonin (5-HT) in controlling cyclic release of gonadotropin is still not clear. Most early studies indicated an inhibitory role for 5-HT. Therefore, intraventricular injection of 5-HT resulted in the suppression of proestrous surges of LH and FSH, and blocked ovulation in rats (Kamberi, 1973) and sheep (Domanski

et al., 1975). 5-hydroxytryptophan (5-HTP), the precursor of 5-HT, was also effective in blocking ovulation (Kordon et al., 1968; Kamberi, 1973) and suppressing the gonadotropin surge (Kamberi, 1973). Also, electrochemical stimulation of the raphé nuclei, which has been shown to increase hypothalamic 5-HT turnover, inhibited ovulation and reduced serum LH levels (Carrer and Taleisnik, 1970, 1972).

In addition to this well documented inhibitory action of 5-HT, accumulated evidence also suggests a facilitative role for 5-HT in phasic release of LH and ovulation. Based on pharmacological studies, Héry et al. (1976) and Coen and MacKinnon (1976) demonstrated a permissive role of serotonergic system in estrogen-induced cyclic release of LH. Coen and MacKinnon (1976) showed that the blocked LH surge in estrogen-primed ovariectomized rats pre-treated with PCPA, could be reinstated only when 5-HTP was given at 1000 hrs, but not at 1800 hrs or at 1000 and 1800 hrs. This suggested that the facilitative action of 5-HT is time dependent. The purpose of the present study was to determine whether or not administration of 5-HTP either in the morning or in the afternoon could have different effects on gonadotropin surges in long-term ovariectomized rats treated with estrogen.

B. Materials and Methods

Mature Sprague-Dawley female rats ovariectomized for 1 wk were used in this experiment. Each rat was primed s.c. with 20 µg estradiol benzoate (EB) in 0.1 ml corn oil at 1200 hrs. Seventy-two

hrs after EB-priming, rats received a second injection of 20 μg EB. Blood samples were taken by cardiac puncture under ether anesthesia at 1000 and 1800 hrs for 3 consecutive days, started on the day of the second EB injection. In order to suppress brain 5-HT, DL-P-chlorophenylalanine-methyl-ester, hydrochloride (PCPA, 300 mg/kg) was dissolved in saline and injected i.p. at 1200 hrs 2 days prior to the first day of bleeding. L-5-hydroxytryptophan-ethyl-ester, hydrochloride (5-HTP, 50 mg/kg) was also dissolved in saline and injected i.p. once a day at either 1000 or 1400 hrs for 3 days to restore brain 5-HT. Control animals were given physiological saline solution.

C. Results

In control rats, EB-treatment induced a daily afternoon rise of serum LH through the 3 day sampling period (Figure 8). Forty-eight hrs after PCPA administration, neither basal levels of LH nor the afternoon LH surge was affected, even though the basal levels of LH had a tendency to be lowered after PCPA, but they were not significantly different from the controls. Administration of 5-HTP at either 1000 or 1400 hrs for 3 consecutive days potentiated the LH surge significantly (1411 $\pm$ 345 and 2530 $\pm$ 617 ng/ml, respectively, vs. 268 $\pm$ 45 ng/ml in controls, $p < 0.01$) on the first day, but not on the second or third day of bleeding.

In contrast to the effects observed in LH, basal levels of serum FSH on the first day of bleeding were decreased significantly in two out of three groups pre-treated with PCPA 48 hrs in advance

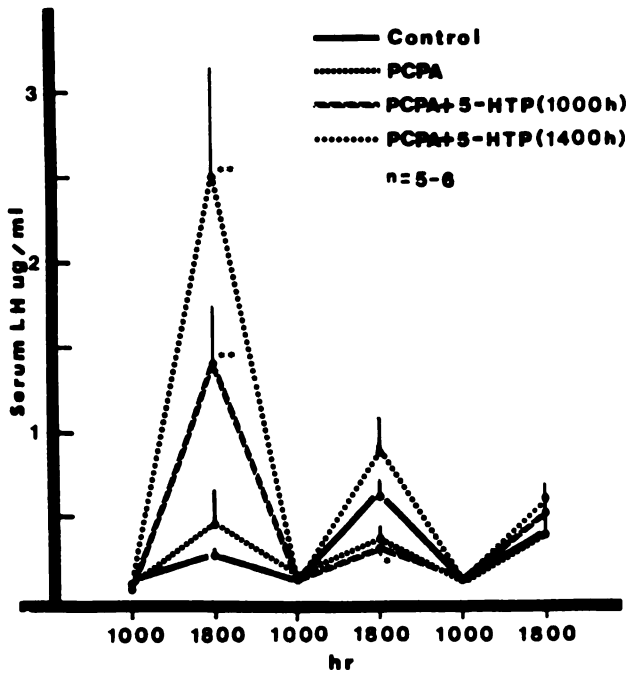

Figure 8. Effects of P-Chlorophenylalanine (PCPA) and 5-HTP on Serum LH in Estrogen Treated Ovariectomized Rats.

PCPA (300 mg/kg) was injected i.p. to rats 2 days earlier, whereas 5-HTP (50 mg/kg) was injected i.p. once a day at either 1000 or 1400 hrs for 3 days. Blood samples were collected starting on the day of the second estradiol benzoate (EB) injection. Each point represents mean and the vertical lines indicate 1 SEM. *, **; $p < 0.05$ and 0.01, respectively vs. saline treated controls.

($p < 0.05$) (Figure 9). In addition, PCPA also decreased the afternoon rise in serum FSH (987 $\pm$ 128 ng/ml vs. 1500 $\pm$ 109 ng/ml in controls, $p < 0.05$). Similar to serum LH, the afternoon surge of FSH on the first day, but not on the second day of bleeding was significantly increased by administration of 5-HTP to rats pretreated with PCPA (2007 $\pm$ 118 and 2393 $\pm$ 375 ng/ml vs. 1500 $\pm$ 109 ng/ml in controls, $p < 0.05$).

D. Discussion

These results suggest that 5-HT may have a facilitative action on the phasic releases of both LH and FSH. Administration of PCPA did not affect the afternoon surge of LH, which is not in agreement with the results reported by Héry et al. (1976). These authors showed that the LH surge could be completely abolished by PCPA if the drug was given to rats 24-48 hrs, but no more than 72 hrs earlier. The possible reason for this difference may be due to the animal model which we used in this experiment. The afternoon rise in serum LH in controls on the first day of bleeding was very small, which is in agreement with the results reported by Mennin and Gorski (1975). Kawakami et al. (1978) recently showed that the effect of a second EB treatment on the LH surge in EB-primed ovariectomized rats could not be seen until the day after injection. Therefore, the blocking effect of PCPA on the LH surge under this condition cannot be detected. The effect of PCPA on the phasic release of FSH has not been demonstrated thus far. Our data clearly

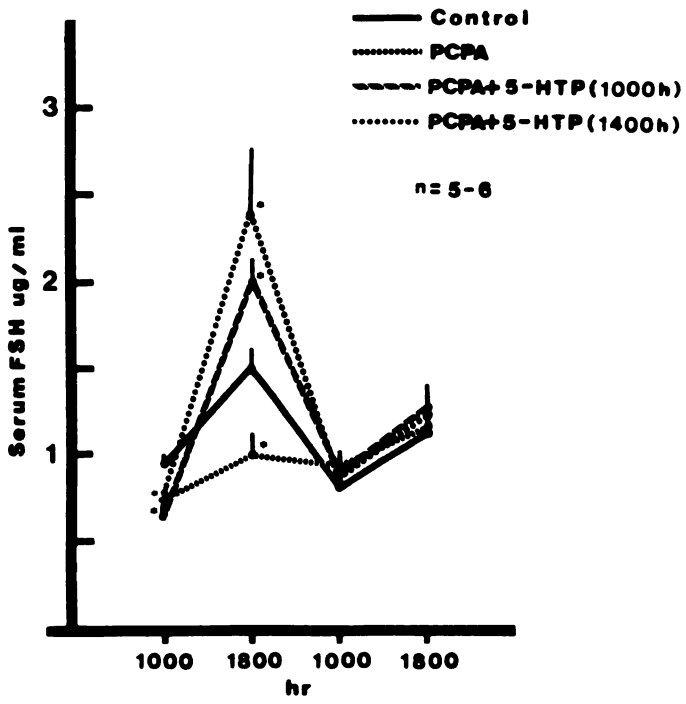

Figure 9. Effects of PCPA and 5-HTP on Serum FSH in Estrogen Treated Ovariectomized Rats.

See Figure 8 for explanation.

show that both the morning and afternoon serum levels of FSH could be suppressed by PCPA when it was given 48 hrs earlier.

Restoration of brain 5-HT in PCPA pre-treated rats by giving 5-HTP at either 1000 or 1400 hrs significantly potentiated the afternoon surges of both LH and FSH on the first day of bleeding. The increase in both LH and FSH was consistently higher in rats receiving 5-HTP at 1400 hrs than at 1000 hrs, even though the difference was not significant. It is not clear whether or not these data indicate a different onset time for the stimulatory action of 5-HTP on gonadotropin secretion.

Interestingly enough, the potentiating effect of 5-HTP on gonadotropin release could not be seen on the second and third days of bleeding, despite the continued injections of 5-HTP. However, administration of 5-HTP at 1400 hrs on the second day did significantly increase the afternoon LH surge above the levels in rats pre-treated with PCPA alone. This finding suggests that desensitization could occur after increased agonism of 5-HT receptors. A similar phenomenon has been reported by Steward et al. (1978) who showed that the strength of the myoclonic response to 5-HTP in rats previously lesioned by 5,7-dihydroxytryptamine (5,7-DHT) could be attenuated by early repeated injections of 5-HTP. An alternative explanation for the failure of 5-HTP on the second and third days to potentiate gonadotropin release in PCPA pre-treated rats could be due to the augmented release of gonadotropin after the first injection of 5-HTP resulting in depletion of the releasable pool

of gonadotropin in the pituitary, which therefore could no longer respond to the subsequent challenge of 5-HTP.

The development of supersensitivity in the 5-HT system might be the mechanism for the potentiation of gonadotropin secretion with 5-HTP in rats pre-treated with PCPA. However, based on behavioral studies, Trulson et al. (1976) reported that supersensitivity which occurred after 5,7-DHT treatment could not be developed by chronic treatment with PCPA. The failure to induce supersensitivity after depletion of 5-HT by PCPA is not paralleled in the catecholamine (CA) system, since depletion of CA by synthesis inhibition has been shown to potentiate the behavioral response to CA precursors and agonists (Dominic and Moore, 1969; Thornburg and Moore, 1973). There is no good explanation for this difference. On the other hand, it is possible that different 5-HT systems may have different characteristics. Therefore, the 5-HT system which is responsible for endocrine effects may be able to develop supersensitivity following PCPA administration.

IV. Temporal Effect of 5-Hydroxytryptophan on Gonadotropin Secretion in Gonadal Steroid Treated Ovariectomized Rats

A. Objective

In the previous experiment, we found that 5-HTP injected on the first day of bleeding at either 1000 or 1400 hrs potentiated the afternoon surges of both LH and FSH. However, serum levels of LH at 1800 hrs showed a tendency to be higher in rats given 5-HTP at 1400 hrs than in rats receiving 5-HTP at 1000 hrs, even though this

difference was not statistically significant. Two possibilities may account for this observation. First, the stimulatory effect of 5-HTP on LH surge may be time dependent, and its action may be more effective at 1400 hrs than at 1000 hrs. The other possibility is that the LH surges stimulated by 5-HTP may have a different onset time, depending on the time of day when 5-HTP is given. The purpose of these experiments was to distinguish between these two possibilities.

B. Materials and Methods

Two different models were used to induce gonadotropin surges in these experiments. The protocol of steroid treatment is shown in Figure 10. Rats ovariectomized at least 2 wks were primed by s.c. injection of 20 μg EB at 1200 hrs. Seventy-two hours after EB priming, rats received either a second injection of 20 μg of EB or 2.5 mg of progesterone (PRG). Rats treated with EB-EB were bled on the day after the second EB injection since the rise in serum gonadotropin on the day of the second EB injection is very small as the previous experiment showed, whereas rats treated with EB-PRG were bled on the day of PRG injection. Sequential blood samples were collected by cardiac puncture under light ether anesthesia at the time indicated in Results. PCPA (300 mg/kg) was always administered to rats 72 hrs prior to further treatment, whereas 5-HTP (50 mg/kg) was injected on the day of bleeding at different times as indicated in Results.

In order to measure hypothalamic biogenic amines, rats in one study were killed by decapitation after the last blood sample

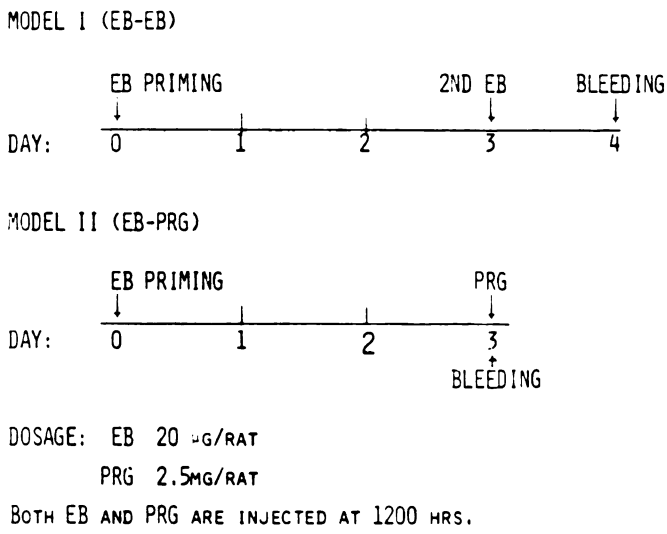

Figure 10. Protocol for Induction of Gonadotropin Surges by Gonadal Steroids in Ovariectomized Rats.

was taken. The anterior hypothalamic area (AHA) and medial basal hypothalamus (MBH) were dissected out and frozen on dry ice. Tissue samples were then homogenized in 100 µl of 0.4 N perchloric acid containing 10 mg EDTA/100 ml. Dopamine (DA) and norepinephrine (NE) were assayed by the radioenzymatic method of Ben-Jonathan and Porter (1976), and 5-HT was assayed according to the radioenzymatic method of Saavedra et al. (1973) as described in the section on General Materials and Methods.

C. Results

Effects of Administration of 5-HTP at Various Times of the Day on Serum Levels of Gonadotropin in Estradiol Benzoate Treated Ovariectomized Rats.--Serum LH in controls increased continuously from a basal level of 160 ± 13 ng/ml and reached a peak value of 1027 ± 309 ng/ml at 1800 hrs (Figure 11). Administration of PCPA (300 mg/kg) for 72 hrs suppressed serum levels of LH, even though it was not statistically significant except at 2000 hrs (789 ± 297 vs. 239 ± 22 ng/ml, $p < 0.05$). Administration of 5-HTP (50 mg/kg) at 1000 hrs to rats pre-treated with PCPA not only potentiated, but advanced the afternoon surge of LH. Serum LH levels already were stimulated significantly above the controls at 1200 hrs (646 ± 96 vs. 160 ± 13 ng/ml, $p < 0.01$) and increased continuously with a peak at 1600 hrs. On the other hand, the peak of the LH surge was not reached until 1800 hrs and the elevated LH levels remained above the controls ($p < 0.01$) throughout the remainder of the period of bleeding when 5-HTP was administered at 1400 hrs.

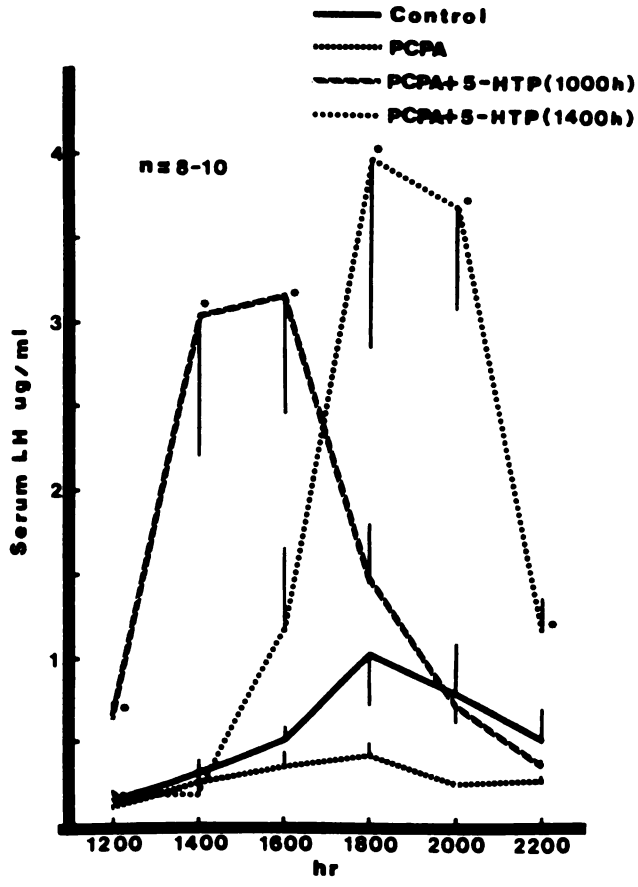

Figure 11. Temporal Effect of 5-HTP on Serum LH in Estrogen Treated Ovariectomized Rats Pretreated with PCPA.

PCPA (300 mg/kg) was injected i.p. to rats 3 days prior to the experiment. 5-HTP (50 mg/kg) administration and blood collection were conducted 1 day after the second EB injection. Each point represents mean and the vertical lines indicate 1 SEM *, $p < 0.05$ vs. saline treated controls.

In another trial, neither the basal levels nor the surge of LH was suppressed by PCPA (72 hrs) (Table 6). Replacement of 5-HTP at 1000 hrs significantly increased serum LH at 1300 hrs (1801 ± 866 vs. 350 ± 42 ng/ml in controls, $p < 0.05$), with a peak at 1600 hrs. However, due to the big standard error and small sample number, the increase in serum LH at 1600 hrs was not significant as compared with control values. Again, the LH surge was significantly potentiated with a peak value of 5949 ± 1743 ng/ml at 2000 hrs when 5-HTP was given at 1400 hrs to rats pre-treated with PCPA. A significant increase in serum LH at 2200 hrs (1335 ± 298 vs. 374 ± 8 ng/ml in controls, $p < 0.05$) could still be induced by a delayed injection of 5-HTP at 1700 hrs. However, the stimulatory effect of 5-HTP appeared to be attenuated. On the other hand, no LH surge could be induced before 1200 hrs by the injection of 5-HTP at either 0600 or 0000 hrs (Tables 8 and 9).

The temporal effects of PCPA and 5-HTP on serum FSH are shown in Figure 12. As can be seen, serum FSH levels were significantly suppressed by PCPA administration. Replacement of 5-HTP at 1000 hrs in PCPA pre-treated rats not only restored, but also potentiated the FSH surge with a significant increase at 1600 hrs (2111 ± 167 vs. 1522 ± 93 ng/ml in controls, $p < 0.05$). The FSH surge was also potentiated by 5-HTP injected at 1400 hrs, with a significant increase above the controls at 2000 hrs (2482 ± 211 vs. 1615 ± 170 ng/ml, $p < 0.01$). In the second trial, both the basal level and the surge of FSH were significantly suppressed by PCPA, and the suppressed FSH level could be restored by injection of 5-HTP at either 1000 or

Table 6. Temporal Effect of 5-HTP (50 mg/kg) on Serum LH in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

Group	No. of Rats	Time of Bleeding (hrs) 1300	1600	1800	2000	2200
Controls	7	350±42[a]	1118±134	763±72	687±46	374±8
PCPA	6	274±34	1608±654	1032±263	709±41	345±30
PCPA + 5-HTP (1000 hrs)	3	1801±866*	3242±903	2057±381	850±114	622±95
(1400 hrs)	6	265±41	1733±814	4643±1976*[d]	5949±1743*[bcd]	3027±1124*[b]
(1700 hrs)	6	249±23	944±126	686±98	1053±93	1335±298*[b]

[a] Mean ± SEM
* P<0.05 vs. controls.
[b] P<0.05 vs. PCPA.
[c] P<0.05 vs. PCPA + 5-HTP (1000 hrs).
[d] P<0.05 vs. PCPA + 5-HTP (1700 hrs).

Table 7. Temporal Effect of 5-HTP (50 mg/kg) on Serum FSH in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

Group	No. of Rats	Time of Bleeding (hrs) 1300	1600	1800	2000	2200
Controls	7	1314±141[a]	1864±153	1745±185	1780±117	1539±167
PCPA	6	711±47*	1369±91*	1150±131	1140±91*	882±71*
PCPA + 5-HTP (1000 hrs)	3	1090±103	1908±156	1765±144	1533±80[c]	1319±186[c]
(1400 hrs)	6	886±24*	1396±143*	1741±241	2238±238	2114±259*
(1700 hrs)	6	819±93*[b]	1251±83*	1337±181	1480±252[c]	1278±73[c]

a Mean ± SEM
* $P < 0.05$ vs. controls.
b $P < 0.05$ vs. PCPA + 5-HTP (1000 hrs).
c $P < 0.05$ vs. PCPA + 5-HTP (1400 hrs).

Table 8. Effect of 5-HTP (at 0600 hr) on Serum Gonadotropin in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

Hormone	Group	No. of Rats	Time (hrs) of Bleeding 0600	0800	1000	1200	1400
LH							
	Controls	7	226±13[a]	251±29	282±36	308±35	300±34
	PCPA + 5-HTP	6	152±6	259±35	191±35	441±209	893±419
FSH							
	Controls	7	1235±69	1144±57	1231±101	1355±88	1335±80
	PCPA + 5-HTP	6	970±40*	1070±122	1096±83	1199±74	1634±147†

PCPA (300 mg/kg) was injected i.p. 3 days before the day of bleeding.
a Mean ± SEM
* $P < 0.05$ vs. controls.
† $P < 0.05$ vs. 0600 hr value.

Table 9. Effect of 5-HTP (at 0000 hr) on Serum Gonadotropin in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

Hormone	Group	No. of Rats	Time (hrs) of Bleeding 0200	0400	0600	0800
LH						
	Controls	8	228±19[a]	201±11	233±35	208±10
	PCPA + 5-HTP	12	250±26	210±17	201±13	222±21
FSH						
	Controls	8	1236±89	1367±96	1486±102	1445±74
	PCPA + 5-HTP	12	1104±77	1485±116	1420±69	1497±110

PCPA (300 mg/kg) was injected i.p. 3 days before the day of bleeding.
a Mean ± SEM.

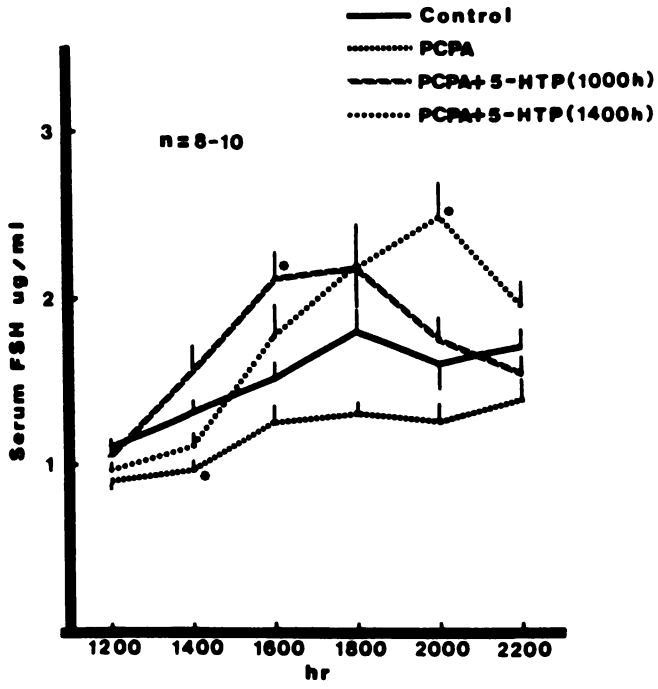

Figure 12. Temporal Effect of 5-HTP on Serum FSH in Estrogen Treated Ovariectomized Rats Pretreated with PCPA.

See Figure 11 for explanation.

1700 hrs (Table 7). Administration of 5-HTP at 1400 hrs to PCPA pre-treated rats not only restored, but also potentiated FSH surge with a significant increase at 2200 hrs, when compared with the control value (2114 ± 259 vs. 1539 ± 167, $p < 0.05$). On the other hand, administration of 5-HTP at either 0600 or 0000 hrs did not stimulate serum FSH in rats pre-treated with PCPA (Tables 8 and 9).

Effects of PCPA and Subsequent Injection of 5-HTP on Gonadotropin Surges in Estradiol Benzoate and Progesterone Treated Ovariectomized Rats.--Serum LH in controls showed a significant increase by 1600 hrs and reached a peak at 1800 hrs (Figure 13). Administration of PCPA for 72 hrs had no effect on the LH surge. Similar to EB-EB treated rats, restoration of brain 5-HT with 5-HTP administered at 1000 hrs not only advanced but also significantly potentiated the LH surge, with a peak value of 8968 ± 978 ng/ml at 1600 hrs ($p < 0.05$). The LH surge in PCPA pre-treated rats was also potentiated significantly by 1800 hrs (9899 ± 1967 vs. 3188 ± 676 ng/ml, $p < 0.01$), and remained above the control level at 2000 hrs (5939 ± 469 vs. 1110 ± 200 ng/ml, $p < 0.05$), when the injection of 5-HTP was postponed to 1400 hrs.

On the other hand, basal levels of FSH at 1300 hrs, but not the surge of FSH, was significantly suppressed by PCPA treatment in one group of rats (642 ± 45 vs. 844 ± 33 ng/ml in controls, $p < 0.05$, Figure 14). Administration of 5-HTP at 1000 hrs restored the basal level of FSH to control levels and significantly potentiated the FSH surge by 1600 hrs (2450 ± 263 vs. 1448 ± 30 ng/ml in controls, $p < 0.01$),

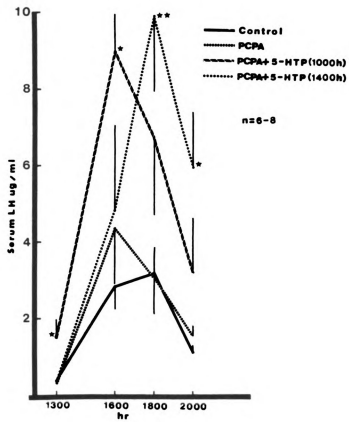

Figure 13. Temporal Effect of 5-HTP on Serum LH in Estrogen-Progesterone Treated Ovariectomized Rats Pretreated with PCPA.

PCPA (300 mg/kg) was injected i.p. to rats 3 days prior to the experiment. 5-HTP (50 mg/kg) administration and blood collection were conducted on the day of progesterone injection. Each point represents mean and the vertical lines indicate 1 SEM. *, **; $p < 0.05$ and 0.01, respectively vs. saline treated controls.

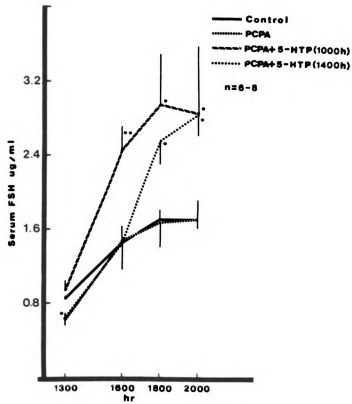

Figure 14. Temporal Effect of 5-HTP on Serum FSH in Estrogen-Progesterone Treated Ovariectomized Rats Pretreated with PCPA.

See Figure 13 for explanation.

whereas injection of 5-HTP at 1400 hrs did not significantly potentiate the FSH surge until 1800 hrs (2563 ± 266 vs. 1698 ± 106 ng/ml in controls, $p < 0.05$).

Effects of PCPA and Subsequent Injection of 5-HTP on Hypothalamic Concentration of Biogenic Amines.--Table 10 shows that 90% of 5-HT in both the AHA and MBH was depleted by 72 hrs after PCPA treatment. In addition to 5-HT, DA concentration in both areas was also decreased significantly by PCPA, whereas NE was not affected. Even though the normal level of 5-HT in AHA was not restored, 5-HT levels in both AHA and MBH were significantly increased 5 hrs after 5-HTP injection as compared to those in rats treated with PCPA alone ($p < 0.05$). Neither NE nor DA was affected by subsequent treatment of 5-HTP. The hormone changes in this experiment were similar to those in the previous two experiments and hence data are not shown here.

D. Discussion

This study clearly shows that the onset of the LH surge can be modulated by varying the time of 5-HTP injection. Thus, administration of 5-HTP at 1000 hrs advanced the LH surge for at least 2 hrs in rats pre-treated with PCPA. The peak of the LH surge, in general, occurred approximately 4-6 hrs after 5-HTP injection. It appears that the facilitative action of 5-HTP is time-dependent because the potentiation on LH surge was attenuated when the administration of 5-HTP was delayed to 1700 hrs. Also, 5-HTP was unable to induce

Table 10. Effects of PCPA and 5-HTP on Hypothalamic Biogenic Amine Concentration

	Biogenic Amine Concentration (ng/g Tissue)					
	AHA			MBH		
Group	5-HT	NE	DA	5-HT	NE	DA
Controls	903±44[a]	1676±126	605±86	799±53	1648±124	668±71
PCPA	101±24*[b]	1570±85	321±14*	82±23*[b]	1623±66	323±34*
PCPA + 5-HTP	469±121*	1377±74	445±53	571±104	1323±192	423±76*

PCPA (300 mg/kg) was injected 3 days earlier. Animal were killed 5 hrs after 5-HTP (50 mg/kg) injection.

a Mean ± SEM of 5-7 determinations.

* $P < 0.05$ vs. controls.

b $P < 0.05$ vs. PCPA + 5-HTP.

an LH surge before 1200 hrs by advancing the 5-HTP injection to 0600 hrs.

An intrinsic neural mechanism with a 24 hr rhythm has been proposed by Everett and Sawyer (1950) to be responsible for triggering gonadotropin surges. This intrinsic neural mechanism is in some way synchronized with the environmental light-dark cycle. Therefore, phase shifts in the onset of the light period cause a corresponding temporal shift in the activation of the central mechanism and the 'critical period' for the cyclic release of gonadotropin in both cycling (Everett et al., 1949; Critchlow, 1963) and estrogen primed ovariectomized rats (Colombo et al., 1974). Accumulated evidence suggests that the suprachiasmatic nucleus which receives a large serotonergic input (Fuxe, 1965a,b; Ungerstedt, 1971) is involved in the regulation of several circardian rhythms (Menaker et al., 1978), and is necessary for spontaneous ovulation (Barraclough et al., 1964; Clemens, et al., 1976). In addition, circadian variation in hypothalamic 5-HT has also been reported with a brief peak at the time coinciding with the beginning of the 'critical period' for LH surge (Quay, 1968). Therefore, it is possible that 5-HT might be one of the neurotransmitters responsible for transmitting the intrinsic neural signal. Our data suggest that the facilitative action of 5-HT on gonadotropin surges may be mediated through neural events which are not activated before 1000 hrs.

Administration of PCPA consistently suppressed the basal level of serum FSH, but not the surge of gonadotropin. The failure to abolish the gonadotropin surge 72 hrs after PCPA treatment, when

the concentrations of 5-HT in both the AHA and MBH were depleted by 90%, is in agreement with the findings of Héry et al. (1976). These authors found that hypothalamic 5-HIAA, but not 5-HT, had started to rise at 72 hrs after PCPA administration when the LH surge was restored. This increase in 5-HIAA suggested an increased 5-HT neuronal activity which could compensate for the impaired 5-HT function following PCPA treatment.

5-HTP has been shown to increase the secretion of ACTH (Fuller et al., 1976; Fuller and Wong, 1977) and prolactin (Chen and Meites, 1976; Clemens et al., 1977). Since both ACTH (Feder and Ruf, 1969; Feder et al., 1971) and prolactin (Piva et al., 1973) have been demonstrated to cause a release of adrenal progesterone, and progesterone, in turn, has been shown to be able to potentiate the gonadotropin surges in EB-primed ovariectomized rats (Caligaris et al., 1971; Mann et al., 1976), the possibility of involving adrenal progesterone in the facilitative action of 5-HTP on gonadotropin surges cannot be excluded. It has been shown that progesterone could overcome the antiovulatory effect of PCPA in immature rats given pregnant mare serum (PMS) (Wilson et al., 1977). Our results show that neither the LH nor the FSH surge in EB-PRG treated ovariectomized rats was suppressed by PCPA treatment.

The effect of PCPA is not specific to 5-HT alone. A slight but significant decrease in brain catecholamines also has been shown by Koe and Weissman (1966). Under our experimental conditions, a 50% reduction of DA in both AHA and MBH resulted from PCPA treatment. However, the subsequent injection of 5-HTP did not induce any

further change in DA levels. The role of dopamine in the regulation of the gonadotropin surge in the adult female rats appears not to be inhibitory. Neither apomorphine nor piribedil, two DA agonists, were able to inhibit the LH surge in proestrus rats (Beck et al., 1978). On the other hand, implantation of DA into the pre-optic area, a region containing incertohypothalamic dopaminergic neurons, stimulated LH release in ovariectomized EB-primed rats (Kawakami et al., 1975), whereas haloperidol (Dickerman et al., 1974) and pimozide (Beattie et al., 1976) inhibited the pre-ovulatory surge. The latter drug was also able to inhibit the LH surge in EB-PRG-treated ovariectomized rats when it was injected during the critical period (unpublished data). Therefore, the blockade of the LH surge 24 to 48 hrs after PCPA administration, as shown by Héry et al. (1978), cannot be totally excluded as a possible secondary effect of PCPA on DA.

V. Effect of Methysergide on Gonadotropin Secretion in Estrogen Treated Ovariectomized Rats

A. Objective

Previous experiments using PCPA and 5-HTP suggested a facilitative role for 5-HT in controlling the phasic release of gonadotropin. 5-HT antagonists such as LSD and methysergide have been shown to inhibit PMS induced ovulation in immature mice (Brown, 1967). It was of interest to see whether or not methysergide, a 5-HT receptor blocker, could inhibit the afternoon surges of gonadotropin in EB treated ovariectomized rats.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-300 g, were ovariectomized and given EB treatment as described in previous experiments. Methysergide maleate (MES, 10 mg/kg) was dissolved in saline and injected i.p. at either 1000 or 1600 hrs. In order to examine the effect of methysergide on pituitary release of gonadotropin in response to exogenous GnRH, nembutal (35 mg/kg, Abbott Labs., North Chicago, IL) was injected i.p. into rats at 1330 hrs to block the endogenous surge of gonadotropin. Synthetic GnRH (0.5 μg/kg) was dissolved in saline and injected s.c. every half hr for 8 consecutive injections, with the first at 1400 hrs. The controls were given saline only. Blood samples were collected under ether anesthesia by cardiac puncture at the time indicated in the Results.

C. Results

The effect of methysergide (MES) on the afternoon surge of LH is shown in Figure 15. Administration of MES (10 mg/kg) at 1000 hrs suppressed the LH surge with significant inhibition at 1800 ($p < 0.05$) and 2000 hrs ($p < 0.01$) as compared with controls. Significant inhibition of the LH surge could still be seen when the injection of MES was delayed to 1600 hrs ($p < 0.05$). On the other hand, MES administered at either time period had no effect on the FSH surge (Table 11).

Nembutal at a dose of 35 mg/kg completely blocked the LH but not the FSH surge in the control group (Tables 12 and 13).

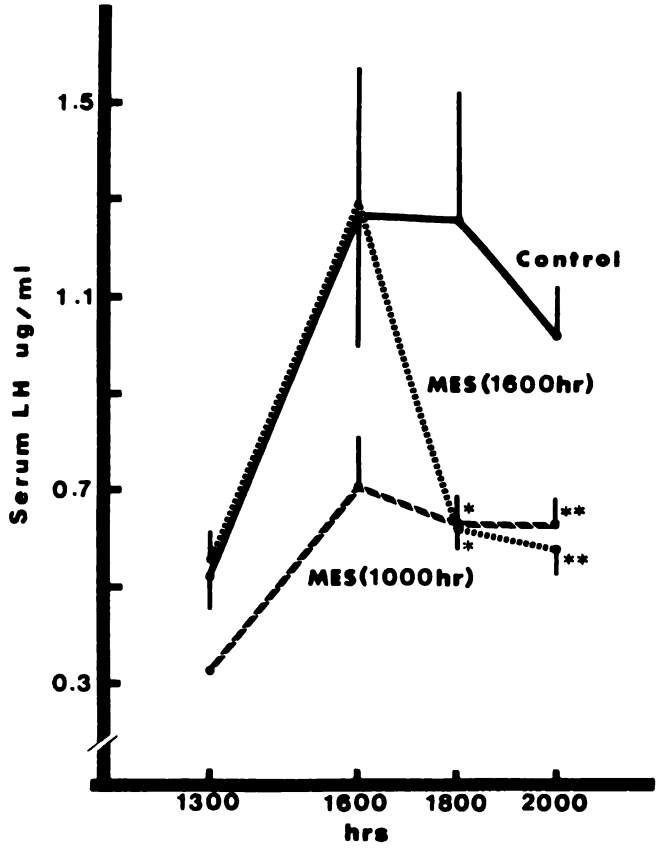

Figure 15. Effect of Methysergide (MES) on Serum LH in Estrogen Treated Ovariectomized Rats.

Rats were injected i.p. with either methysergide (10 mg/kg) or 0.9% NaCl solution. Each point represents the mean of 5-6 determinations and the vertical lines indicate 1 SEM. *, **; $p < 0.05$ and 0.01, respectively vs. saline treated controls.

Table 11. Effect of Methysergide (10 mg/kg) on Serum FSH in Estrogen Treated Ovariectomized Rats

Group	No. of Rats	Time (hrs) of Bleeding			
		1300	1600	1800	2000
Controls	5	1421±150[a]	1812±140	2201±204	1971±212
Methysergide (1000 hrs)	6	1363±106	1825±148	1960±157	2159±297
Methysergide (1600 hrs)	6	1683±112	2245±100	2228±188	2110±151

a Mean ± SEM.

Table 12. Effect of Methysergide on Pituitary Release of LH in Response to Synthetic GnRH in Estrogen Treated Ovariectomized Rats

	Time (hrs) of Bleeding				
Group	1400	1500	1600	1700	1800
Controls	276±15[a]	187±18	295±64	251±28	209±21
GnRH	282±16	2301±120*	5345±398*	6744±872*	7122±1460*
Methysergide + GnRH	160±11*[b]	2007±120*	4565±522*	5400±755*	5405±735*

Nembutal (35 mg/kg) was injected i.p. at 1330 hrs, whereas methysergide (10 mg/kg) was injected i.p. at 1000 hrs.
a Mean ± SEM of 7-8 determinations. * $P < 0.05$ vs. controls.
b $P < 0.05$ vs. GnRH alone.

Table 13. Effect of Methysergide on Pituitary Release of FSH in Response to Synthetic GnRH in Estrogen Treated Ovariectomized Rats

	Time (hrs) of Bleeding				
Group	1400	1500	1600	1700	1800
Controls	1188±36[a]	1471±54†	1552±85†	1190±92	1259±72
GnRH	1283±88	1680±131	1978±109*	2302±142*	2987±192*
Methysergide + GnRH	1172±62	1640±66	1962±77*	2408±146*	2371±155*[b]

a Mean ± SEM of 7-8 determinations.
* $P < 0.05$ vs. controls.
b $P < 0.05$ vs. GnRH alone.
† $P < 0.05$ vs. 1400 hr value.

Serum levels of LH at 1400 hrs (before GnRH injection) in the MES-treated group was significantly lower than in the controls (160 $\pm$ 11 vs. 276 $\pm$ 15 ng/ml, $p < 0.05$). The release of both LH and FSH was significantly stimulated and was increased continuously through the whole period of GnRH injection. Neither LH nor FSH release from the pituitary in response to multiple injections of GnRH was significantly impaired by MES administered at 1000 hrs, with the exception of FSH at 1800 hrs ($p < 0.05$).

D. Discussion

The present study demonstrates that MES, classified as a competitive 5-HT receptor blocker, inhibits the afternoon surge of LH but not FSH in EB treated ovariectomized rats. Consistent with our results, methiothepin, another 5-HT receptor blocker, also has been shown to inhibit the circadian variations of serum LH in EB implanted ovariectomized rats (Héry et al., 1976). Our data suggests a central nervous system site of action for MES because the release of gonadotropin from the anterior pituitary in response to exogenous GnRH was not affected by MES. Nembutal treatment completely prevented the afternoon surge of LH, but not FSH, which is consistent with the results reported by Brown-Grant and Greig (1975) and Wise et al. (1979).

MES has been suggested to be a peripheral 5-HT receptor blocker (Gyermeck, 1961). However, it has been shown to block some central effects of 5-HT (Boakes et al., 1970; Haigler and Aghajanian, 1974). MES is an ergot derivative of lysergic acid, and its primary

metabolite, methergine, has been shown to act on the pituitary directly as a DA agonist to inhibit prolactin secretion (Lamberts and MacLeod, 1978). Since the LH surges in proestrus adult female rats or in EB primed, progesterone treated ovariectomized rats were not inhibited by either apomorphine or piribedil, two DA agonists (Beck et al., 1978; Simpkins, 1979), it is unlikely that the secondary action of MES as a DA agonist can account for the inhibition of LH release.

The observation that delayed administration of MES at 1600 hrs was still able to suppress the already raised serum level of LH suggests that the continuous activation of the 5-HT system beyond the 2 hrs of the 'critical period' is required to maintain the normal LH surge.

The failure of MES to inhibit the FSH surge is difficult to understand, since 5-HTP, in the previous experiment, was shown to potentiate the FSH surge in EB treated ovariectomized rats pretreated with PCPA. The variation in serum FSH is much less than LH during the surge. In other words, serum levels of FSH are more stable than that of LH. Therefore, higher doses of MES might be required to inhibit FSH surge.

VI. Effect of P-Chloroamphetamine on Gonadotropin Secretion in Gonadal Steroid Treated Ovariectomized Rats

A. Objective

p-Chloroamphetamine (PCA), a long-lasting 5-HT antagonist, has been shown to be selective on 5-HT neurons at 2-3 days after

its injection (Sanders-Bush and Steranka, 1978). Unlike PCPA, PCA does not inhibit the synthesis of 5-HT in peripheral tissue (Sanders-Bush and Sulser, 1973). Coen and MacKinnon (1976) claimed that PCA, at a dose of 10 mg/kg, abolished EB induced LH surges in ovariectomized rats 2-3 days after PCA treatment. However, Clemens (1978) recently reported that PCA has no effect on the proestrus surge of LH. The purpose of these studies was to further determine the role of 5-HT in the control of gonadotropin surges, using PCA as an antagonist.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Industries, Cumberland, IN), weighing 250-300 g, were ovariectomized and received EB or EB-PRG treatment as described in Experiment IV. p-Chloroamphetamine hydrochloride (PCA, 5 mg/kg, i.p.) was dissolved in saline and injected into the rat 3 days before blood collection. Blood samples were collected by cardiac puncture under ether anesthesia at the times indicated in the Results. In one study, the blood samples were collected by decapitation at either 1200 or 1600 hrs. Hypothalamic tissue was quickly dissected into AHA and MBH for biogenic amine assays.

In order to examine the possible development of supersensitivity in 5-HT neurons to exogenous 5-HTP after PCA treatment, 5-HTP (50 mg/kg) was administered i.p. once a day at 1200 hrs for two consecutive days to EB-EB treated ovariectomized rats with or without PCA pre-treatment. PCA was injected 3 days prior to the

first day of bleeding. Rats given physiological saline served as controls. Blood samples were collected by cardiac puncture under ether anesthesia at either 1000 or 1800 hrs on each day. Statistical significance was determined by Student's 't' test or by analysis of variance and Student-Neuman Keuls' test (Sokal and Rohlf, 1969).

C. Results

Effect of p-Chloroamphetamine on Gonadotropin Surges in EB-EB and EB-Progesterone Treated Ovariectomized Rats.--Seventy-two hrs after PCA treatment, the LH surge in EB-EB treated ovariectomized rats was unaffected by the treatment. On the other hand, PCA significantly potentiated the LH surge at 1800 and 2000 hrs in EB-PRG treated ovariectomized rats ($p < 0.05$; Table 14). The effect of PCA on the FSH surge is shown in Table 15. In EB-EB treated ovariectomized rats, serum FSH was not altered by PCA during the whole sampling period. Like serum LH, PCA potentiated FSH surge throughout the entire sampling period in EB-PRG treated ovariectomized rats. However, the potentiation was significant only at 1300 and 2000 hrs ($p < 0.05$).

Effect of p-Chloroamphetamine on Gonadotropin Surges and Hypothalamic Biogenic Amines in EB-Progesterone Treated Ovariectomized Rats.--Neither the basal level of LH nor FSH at 1200 hrs in EB-PRG treated ovariectomized rats was affected by PCA (Figure 16). However, administration of PCA for 72 hrs significantly potentiated the afternoon rises of both LH (9839 $\pm$ 845 ng/ml vs. 4182 $\pm$ 998 ng/ml in controls, $p < 0.01$), and FSH (3419 $\pm$ 71 ng/ml vs.

Table 14. Effect of P-Chloroamphetamine (PCA, 5 mg/kg) on Serum LH in Estrogen or Estrogen-Progesterone Treated Ovariectomized Rats

Group	No. of Rats	Time (nrs) of Bleeding 1300	1600	1800	2000
EB-EB					
Saline	7	248±35[a]	704±150	584±97	443±54
PCA	7	255±34	587±120	612±76	340±47
EB-PRG					
Saline	7	284±91	1632±771	902±384	352±125
PCA	7	289±42	2055±358	2272±442*	954±215*

Blood samples from EB-EB treated rats were bled 1 day after the 2nd EB injection, whereas samples from EB-PRG treated rats were bled on the day of PRG injection.

a Mean ± SEM. * $P < 0.05$ vs. saline treated controls.

Table 15. Effect of P-Chloroamphetamine (PCA, 5 mg/kg) on Serum FSH in Estrogen or Estrogen-Progesterone Treated Ovariectomized Rats

Group	No. of Rats	Time (hrs) of Bleeding 1300	1600	1800	2000
EB-EB					
Saline	7	1174±81[a]	1577±92	1793±81	1465±91
PCA	7	1122±83	1509±81	1516±70*	1569±25
EB-PRG					
Saline	7	1128±82	1788±315	1851±290	1741±181
PCA	7	1418±79*	2328±62	2415±155	2326±119*

a Mean ± SEM. * $P < 0.05$ vs. saline.

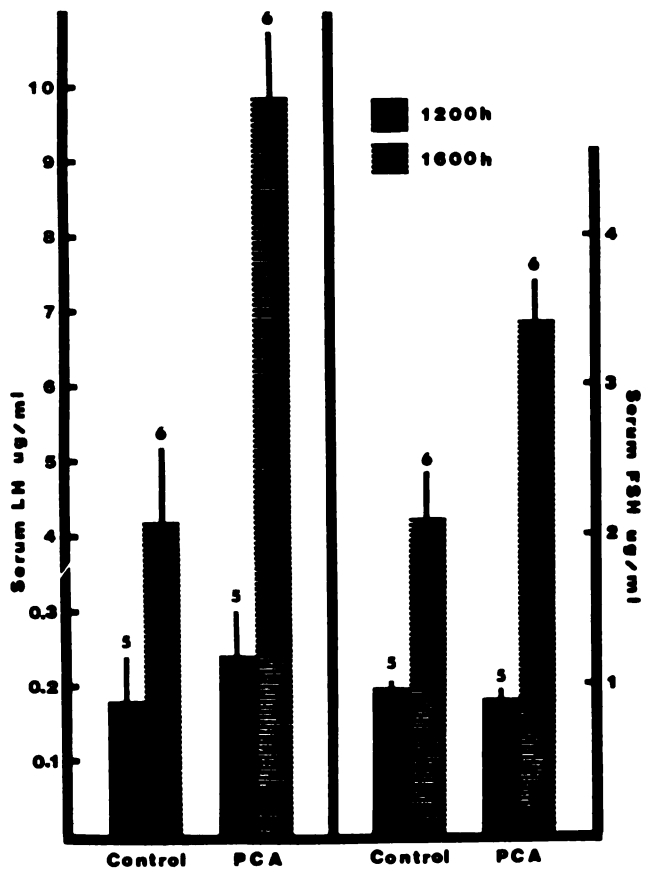

Figure 16. Effect of P-Chloroamphetamine (PCA) on Serum Gonadotropin in Estrogen-Progesterone Treated Ovariectomized Rats.

PCA (5 mg/kg) was injected i.p. to rats 3 days earlier. Serum LH is shown in the left panel, whereas serum FSH in the right panel. Each bar represents mean and the vertical lines indicate 1 SEM. Number above each vertical line indicates the number of determinations.

2100 ± 305 ng/ml in controls, $p < 0.05$) at 1600 hrs. The serum level of LH at 1600 hrs was almost doubled after PCA treatment as compared to controls.

Hypothalamic levels of 5-HT are shown in Figure 17. 5-HT content in both the AHA and MBH was significantly decreased by PCA ($p < 0.01$). The depletion of 5-HT after PCA treatment in the AHA (41 ± 1%) was significantly less than that in the MBH (64 ± 2%) ($p < 0.001$). NE contents in the AHA but not in the MBH at 1600 hrs was significantly decreased by PCA (Figure 18). On the other hand, DA in neither AHA nor MBH was significantly altered after PCA treatment (Figure 19).

Effect of 5-Hydroxytryptophan on Gonadotropin Surges in EB-EB Treated Ovariectomized Rats Pre-Treated with p-Chloroamphetamine.--Serum levels of LH in controls 24 hrs after the second EB injection displayed a typical daily variation through the 2 days of bleeding (Table 16). Administration of PCA 3 days earlier had no significant effect on LH surge on the first day of bleeding. On the other hand, the surge value of LH at 1800 hrs was stimulated after 5-HTP injection at 1200 hrs on the first day of bleeding, even though the increase in serum LH was not significant as compared to the controls. PCA pre-treatment significantly potentiated the stimulatory action of 5-HTP on the LH surge (2223 ± 655 ng/ml vs. 1038 ± 224 ng/ml in the group treated with 5-HTP alone, $p < 0.05$). However, the potentiated 5-HTP action on the LH surge in PCA pre-treated rats was no longer seen when the second dose of 5-HTP was given on the

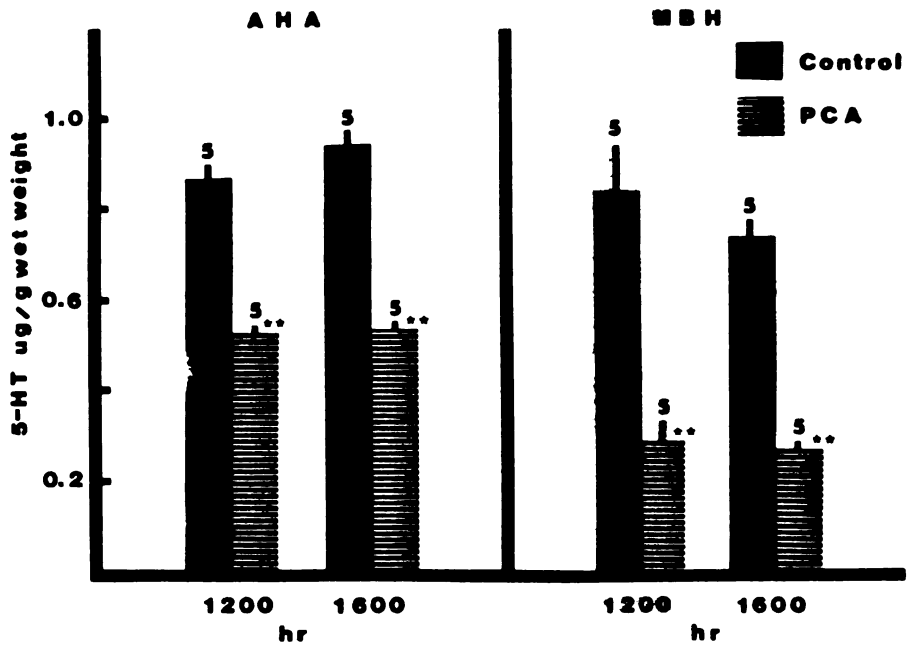

Figure 17. Effect of P-Chloroamphetamine (PCA) on 5-HT Concentration in the Anterior Hypothalamic Area (AHA) and Medial Basal Hypothalamus (MBH) in Estrogen-Progesterone Treated Ovariectomized Rats.

Left panel shows AHA and Right panel shows MBH amine concentration. Each bar represents mean and the vertical lines indicate 1 SEM. Number above each vertical line indicates the number of determinations. **, $p < 0.01$ vs. controls.

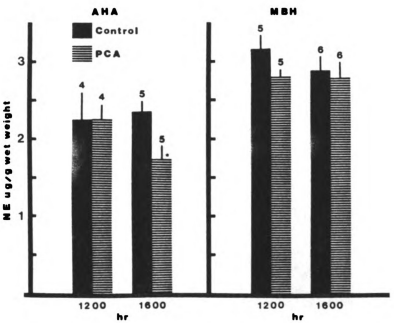

Figure 18. Effect of P-Chloroamphetamine on Norepinephrine Concentration in the Anterior Hypothalamic Area and Medial Basal Hypothalamus in Estrogen-Progesterone Treated Ovariectomized Rats.

See Figure 17 for explanation. *, $p < 0.05$ vs. controls.

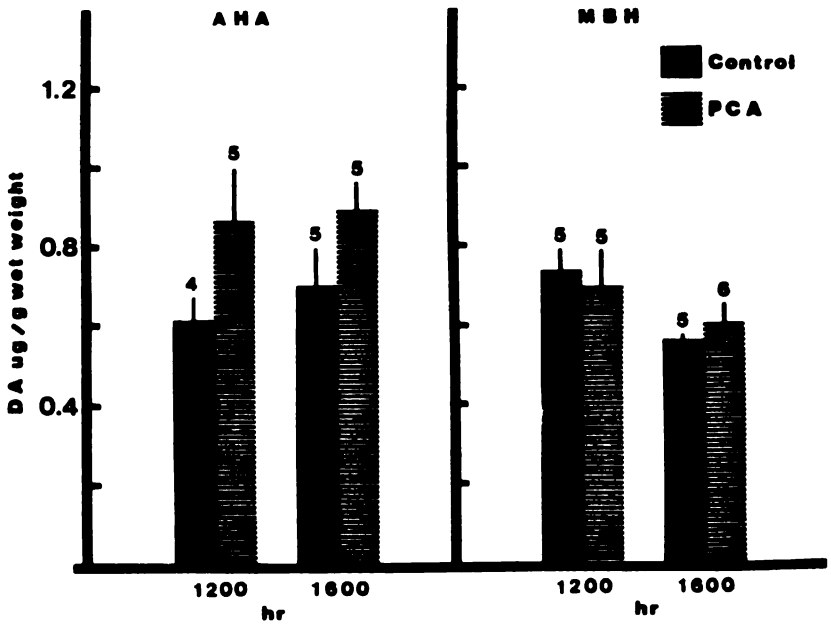

Figure 19. Effect of P-Chloroamphetamine on Dopamine Concentration in the Anterior Hypothalamic Area and Medial Basal Hypothalamus in Estrogen-Progesterone Treated Ovariectomized Rats.

See Figure 17 for explanation.

Table 16. Effect of 5-HTP (50 mg/kg) on Serum LH in Estrogen Treated Ovariectomized Rats Pretreated with PCA

	Time (hrs) of Bleeding			
	1st Day		2nd Day	
Group	1000	1800	1000	1800
Controls	234±20[a]	500±144	185±34	335±104
PCA	197±35	792±241[b]	173±34	408±87
5-HTP	164±19	1038±224[b]	207±38	441±103
PCA+5-HTP	162±13	2223±655*	231±23	413±61

a Mean ± SEM of 7-8 determinations.
b $P < 0.05$ vs. PCA+5-HTP.
* $P < 0.05$ vs. controls.

Table 17. Effect of 5-HTP (50 mg/kg) on Serum FSH in Estrogen Treated Ovariectomized Rats Pretreated with PCA

	Time (hrs) of Bleeding			
	1st Day		2nd Day	
Group	1000	1800	1000	1800
Controls	1288±87[a]	1462±92	1097±62	1378±101
PCA	1189±105	1651±126[b]	1150±61	1213±64
5-HTP	1189±70	2003±259	1473±304	1868±349
PCA+5-HTP	1301±79	2546±321*	1728±228*	1883±226

a Mean ± SEM of 7-8 determinations.
b $P < 0.05$ vs. PCA+5-HTP.
* $P < 0.05$ vs. controls.

next day. Similarly, serum levels of FSH at 1800 hrs on the first day, but not on the second day, of bleeding following 5-HTP treatment was significantly potentiated by PCA (2546 ± 321 ng/ml vs. 1462 ± 92 ng/ml in controls, $p < 0.05$) (Table 17).

D. Discussion

The results of this study demonstrate that 72 hrs after PCA administration neither the LH nor the FSH surge in EB treated ovariectomized rats is affected. Our data are consistent with those reported by Clemens (1978) who found that the proestrous surge of LH was not altered by PCA administration. There is no good explanation for the discrepancy between our results and those reported by Coen and MacKinnon (1972) who claimed that PCA at a dose of 10 mg/kg abolished the EB-induced LH surge in ovariectomized rats 2-3 days after its treatment. One possible reason for this difference could be the different doses of PCA used by Coen and MacKinnon in their experiment.

Based on the 5-HTP replacement study, it appears that a 5-HT denervation supersensitivity develops in rats pre-treated with PCA. After 5-HTP injection, the increase in the LH surge on the first day of bleeding in rats pre-treated with PCA was significantly greater than the increase seen in control rats after 5-HTP treatment. A similar effect of PCA on serum prolactin in response to 5-HTP also was reported by Clemens (1978).

The long-term biochemical effects of PCA has been shown to be selective on 5-HT neurons, and PCA acts as a 5-HT neurotoxin

(Sanders-Bush and Steranka, 1978). Direct morphologic evidence of degeneration of 5-HT cell bodies (Harvey et al., 1975) and axon terminals (Hattori et al., 1976) has recently been demonstrated. The enhanced efficacy of 5-HTP in PCA pre-treated rats may be attributed to pre-synaptic mechanisms. It has been shown that the high affinity uptake of 5-HT is impaired by PCA (Sanders-Bush et al., 1975). Therefore, the loss of re-uptake of 5-HT may partially account for the supersensitivity observed.

The potentiated LH surge in response to 5-HTP in rats pre-treated with PCA was evident on the first day of bleeding, but not on the next day after the second injection of 5-HTP. This finding suggests that the first dose of 5-HTP may diminish the supersensitivity of 5-HT neurons developed after PCA administration. A similar result was also seen in previous experiments when PCPA was administered to rats.

In contrast to ovariectomized rats treated only with EB-EB, PCA significantly potentiated the surges of both LH and FSH induced by PRG in EB-primed ovariectomized rats. This finding is of interest since administration of PCPA for 72 hrs has been shown in previous experiments to have no effect on gonadotropin surges in EB-PRG treated ovariectomized rats.

The decrease in hypothalamic 5-HT content after PCA administration was not the same in the AHA and MBH. It appears that PCA was more effective in depleting 5-HT in the MBH than in the AHA. Regional differences in the effects of PCA have been reported by Sanders-Bush et al. (1975), who attributed this differential effect

of PCA on 5-HT at least partially to the different responses of axonal and terminal 5-HT to PCA administration. PCA causes a significant and long-lasting decrease in terminal 5-HT, whereas 5-HT levels in axonal regions are simultaneously increased (see Sanders-Bush and Steranka, 1978). In our study, a relatively large piece of AHA tissue as compared to MBH was removed. Therefore, it is possible that more axonal regions were present in the AHA than in the MBH. However, the possibility that PCA may be more effective in depleting 5-HT in the MBH than in the AHA cannot be excluded. PCA has been shown to cause selective long-term biochemical (Bertilsson et al., 1975; Neckers et al., 1975) and histological (Harvey et al., 1975) changes only in neurons in the B_9 area, but not in B_7 or B_8 areas.

If the difference in the depletion of 5-HT between the AHA and the MBH after PCA treatment truly reflects the different regional effects of PCA on these two areas, this might explain the difference in gonadotropin surges between EB-EB and EB-PRG treated ovariectomized rats after PCA treatment. Kordon and Glowinski (1972) previously suggested that existence of an inhibitory 5-HT system in the MBH region and a facilitative center in the pre-optic-suprachiasmatic region. Therefore, the balance between the two 5-HT systems may be important in regulating the phasic release of gonadotropin. Since there is more destruction of 5-HT terminals in the MBH after PCA administration than that in the AHA, based on the depletion of 5-HT in these two areas, it is expected that the development of 5-HT neuron supersensitivity in the MBH will be greater than that in the AHA. These two events, denervation and development of

supersensitivity, could well compensate for each other to keep the surge of gonadotropin at the control level. On the other hand, decreased 5-HT turnover in the hypothalamus (Fuxe et al., 1974) and particularly in the MBH (Experiment XI) after PRG could diminish the compensatory effect exerted by the developed supersensitivity. Under this latter condition, the net result would be a larger surge of gonadotropin following PCA administration.

VII. Effect of 5,7-Dihydroxytryptamine on Gonadotropin Secretion in Gonadal Steroid Treated Ovariectomized Rats

A. Objective

The role of 5-HT in regulating the phasic release of gonadotropin was investigated in previous experiments by using PCPA, a 5-HT synthesis inhibitor; methysergide, a 5-HT receptor blocker; or PCA, a 5-HT neurotoxic agent. Recently, intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT), a hydroxylated derivative of tryptamine, was shown to produce a rather selective degeneration of central indoleaminergic axons in rats provided desmethylimipramine (DMI), an NE re-uptake inhibitor, was given first to protect NE neurons from the damage by 5,7-DHT (Gerson and Baldessarini, 1975; Björklund et al., 1975). The purposes of the present study were to use 5,7-DHT to further investigate the effect of reducing brain 5-HT on the phasic release of gonadotropin.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-350 g, were ovariectomized for at least 2 wks

before use. All rats were kept in individual cages after cannulation.

In the first experiment, 5,7-DHT at a dose of 50 µg (calculated as free base) in 4 µl of saline solution (plus 0.02% ascorbic acid, pH = 3.7) was injected into the third ventricle via a chronically implanted cannula made from a 20 gauge disposable hypodermic needle 1 wk before the first day of bleeding. Control rats were injected with vehicle only. Rats receiving 5,7-DHT injections were pre-treated with 25 mg/kg of desipramine HCl (DMI) in saline 1 hr before 5,7-DHT injection. All the rats were first primed s.c. with 20 µg of EB in 0.1 ml corn oil at 1200 hrs 4 days after 5,7-DHT injection. Seventy-two hrs later, half of the rats in each group were given either a second dose of 20 µg of EB (EB-EB) or 2.5 mg of progesterone (EB-PRG) administered s.c. in 0.2 ml corn oil at 1200 hrs. Blood samples were taken by cardiac puncture under ether anesthesia at 1000, 1600, and 1800 hrs on the day of the second EB or PRG injection. Blood samples from EB-EB treated groups were taken again at the same time on the next day.

The time course effect of 5,7-DHT on gonadotropin surges was studied in the second experiment. Fifty µg of 5,7-DHT (calculated as free base) in 10 µl of saline solution (plus 0.02% ascorbic acid) was injected into the lateral ventricle via a chronically implanted cannula as described by DeBalbian-Verster (1971) 3, 7, and 14 days prior to the day of bleeding.

Control rats were injected intraventricularly with vehicle alone 3 days prior to bleeding. All the rats including controls

were pre-treated with 25 mg/kg of DMI 1 hr before the injection of 5,7-DHT. Gonadotropin surges were induced by two injections of EB with an interval of 72 hrs. Blood samples were collected by cardiac puncture under ether anesthesia at 1000, 1600, 1800, and 2000 hrs on the day after the second EB injection.

Immediately following the last bleeding, rats were decapitated and brain was immediately removed from the cranium. AHA and MBH were removed, and catecholamines and 5-HT were assayed as described in General Materials and Methods. The average wt of the AHA and MBH were 17.4 $\pm$ 0.3 and 10.9 $\pm$ 0.2 mg, respectively, in the first experiment, and 16.3 $\pm$ 0.4 and 9.4 $\pm$ 0.3 mg, respectively, in the second experiment.

C. Results

Effect of 5,7-Dihydroxytryptamine on Gonadotropin Surges and on Hypothalamic Biogenic Amines in EB-EB or EB-PRG Treated Ovariectomized Rats.--The afternoon rise of LH on the day of the second EB injection was very small in EB-EB treated rat (Table 18). Administration of 5,7-DHT for 7 days had no effect on the basal level of LH, but significantly increased the LH surge at 1600 hrs (563 $\pm$ 101 vs. 335 $\pm$ 27 ng/ml in saline treated controls, $p < 0.05$). There was no difference in serum LH on the next day between 5,7-DHT and saline treated groups. 5,7-DHT had no effect on serum FSH. On the other hand, administration of 5,7-DHT significantly decreased the basal level of LH ($p < 0.05$), but significantly potentiated the afternoon surge of LH in EB-PRG treated rats as compared to saline treated

Table 18. Effect of 5,7-Dihydroxytryptamine (5,7-DHT) on Serum Gonadotropin in Estrogen Treated Ovariectomized Rats

		Time (hrs) of Bleeding					
		1st Day			2nd Day		
Hormone	Group	1000	1600	1800	1000	1600	1800
LH							
	Saline	237±21[a]	335±27	362±42	212±17	1515±621	802±156
	5,7-DHT	272±27	563±101*	576±111	218±20	1393±425	1469±425
FSH							
	Saline	1283±119	1530±103	1738±138	1352±71	2116±199	2518±466
	5,7-DHT	1220±67	1677±165	1884±131	1581±97	2330±209	2500±180

Fifty μg (free base) of 5,7-DHT was injected in 4 μl volume of saline into the 3rd ventricle of the rats.

a Mean ± SEM of 5-7 determinations.

* $P < 0.05$ vs. saline.

controls (11630 ± 940 vs. 4826 ± 1367 ng/ml at 1600 hrs, $p < 0.01$; 4809 ± 803 vs. 2436 ± 375 ng/ml at 1800 hrs, $p < 0.05$) (Figure 20). The FSH surge in EB-PRG treated rats also was significantly potentiated by 5,7-DHT ($p < 0.05$).

The effect of 5,7-DHT on hypothalamic biogenic amines are shown in Table 19. The 5-HT levels in both the AHA and the MBH decreased significantly 8 days after 5,7-DHT injection. The decrease in 5-HT in the MBH was significantly greater than that in the AHA (66-77% depletion in MBH vs. 41-43% depletion in AHA, $p < 0.05$). Neither the AHA nor the MBH DA was affected by 5,7-DHT treatment. On the other hand, 5,7-DHT increased MBH levels of NE in both EB-EB and EB-PRG treated rats ($p < 0.05$). In addition, NE concentration in the MBH was significantly higher in EB-PRG treated rats as compared to that in EB-EB treated rats (2169 ± 67 vs. 1759 ± 60 ng/g, $p < 0.05$).

Time Course Effect of 5,7-DHT on Gonadotropin Surges and on Hypothalamic Biogenic Amines in EB-EB Treated Ovariectomized Rats.--Tables 20 and 21 show the time course effect of 5,7-DHT on the EB induced LH and FSH surges, respectively. Neither the LH nor the FSH surge was affected by the intraventricular injections of 5,7-DHT. The changes in hypothalamic biogenic amines after 5,7-DHT injection in this experiment were similar to those in the first experiment. 5,7-DHT was much more effective in suppressing 5-HT in the MBH than that in the AHA (Table 22) (67-73% depletion in MBH vs. 31-37% depletion in AHA, $p < 0.05$). The DA levels in both the

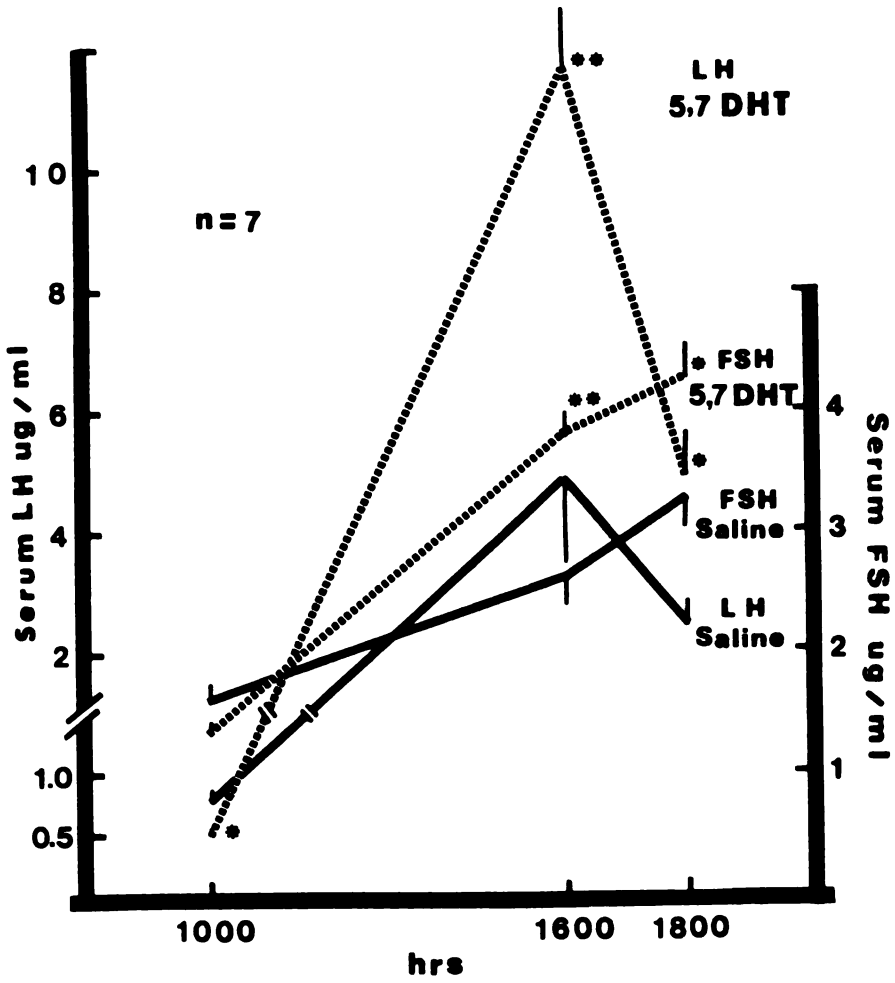

Figure 20. Effect of 5,7-Dihydroxytryptamine (5,7-DHT) on Serum Gonadotropin in Estrogen-Progesterone Treated Ovariectomized Rats.

Fifty μg (free base) of 5,7-DHT was injected in 4 μl volume into the third ventricle of the rats pretreated with desipramine (DMI, 25 mg/kg) for 1 hr. Experiment was conducted 1 wk after 5,7-DHT injection. Each point represents mean and the vertical lines indicate 1 SEM. *, **; $p < 0.05$ and 0.01, respectively vs. vehicle (saline solution containing 0.02% ascorbic acid) treated controls.

Table 19. Effect of 5,7-Dihydroxytryptamine (5,7-DHT) on Hypothalamic Biogenic Amine Concentration

Group	Amine Concentration (ng/g tissue) 5-HT	DA	NE	% Depletion of 5-HT
		AHA		
EB-EB				
Saline	758±62[a]	715±54	1892±100	---
5,7-DHT	445±31*	611±64	1951±115	41±4
EB-PRG				
Saline	745±41	579±49	2367±152	---
5,7-DHT	423±41*	442±36[b]	2577±115[bc]	43±5
		MBH		
EB-EB				
Saline	993±78	468±25	1759±60	---
5,7-DHT	225±38*	542±38	2285±142*	77±4†
EB-PRG				
Saline	1050±43	478±26	2169±67[b]	---
5,7-DHT	352±36*	428±25	2536±116*[b]	66±3†

a Mean ± SEM of 3-7 determinations.
b $P < 0.05$ vs. EB-EB treated saline controls.
c $P < 0.05$ vs. 5,7-DHT group in EB-EB treated rats.
* $P < 0.05$ vs. individal saline treated controls.
† $P < 0.05$ vs. AHA value in the same group.

Table 20. Time Course Effect of 5,7-DHT on Serum LH in Estrogen Treated Ovariectomized Rats

	Time (hrs) of Bleeding			
Group	1000	1600	1800	2000
Controls	327±28[a]	1142±269	774±153	525±109
5,7-DHT				
3 Day	309±20	1278±286	728±85	488±63
7 Day	288±16	1547±567	758±220	514±85
14 Day	263±22	1129±253	878±235	494±104

Fifty µg (free base) of 5,7-DHT was injected in 10 µl volume of saline into the lateral ventricle of the rats.

a Mean ± SEM of 7-8 determinations.

Table 21. Time Course Effect of 5,7-DHT on Serum FSH in Estrogen Treated Ovariectomized Rats

	Time (hrs) of Bleeding			
Group	1000	1600	1800	2000
Controls	1015±51[a]	1212±99	1329±130	1365±213
5,7-DHT				
3 Day	977±107	1178±105	1275±115	1091±120
7 Day	1149±65	1404±210	1324±143	1237±152
14 Day	1241±102	1453±179	1442±251	1264±169

a Mean ± SEM of 7-8 determinations.

Table 22. Time Course Effect of 5,7-DHT on Hypothalamic Biogenic Amine Concentration

Group	Amine Concentration (ng/g tissue) 5-HT	DA	NE	% Depletion of 5-HT
		AHA		
Controls	926±93[a]	519±37	1571±168	---
5,7-DHT				
3 Day	637±52*	561±100	1635±99[b]	31±6
7 Day	626±84*	636±110	1844±166	32±9
14 Day	583±84*	416±76	2153±130*	37±9
		MBH		
Controls	782±70	771±49	1961±136	---
5,7-DHT				
3 Day	208±58*	869±100	1924±94	73±7†
7 Day	236±87*	921±69	2062±193	70±11†
14 Day	258±72*	907±36	2188±87	67±9†

Fifty µg (free base) of 5,7-DHT was injected in 10 µl volume of saline into the lateral ventricle of the rats.

a Mean ± SEM of 6-8 determinations.

b $P < 0.05$ vs. 5,7-DHT (14 day) group.

* $P < 0.05$ vs. controls.

† $P < 0.05$ vs. AHA value in the same group.

AHA and the MBH were not affected at any time after 5,7-DHT injection. On the other hand, the NE level in the AHA increased significantly 14 days after 5,7-DHT injection (2153 $\pm$ 130 vs. 1571 $\pm$ 168 ng/g in controls, $p < 0.05$).

D. Discussion

In EB-EB treated rats, administration of 5,7-DHT did not impair the gonadotropin surges occurring on the day after the second EB injection. In fact, the LH surge on the day of the second EB injection was significantly enhanced in 5,7-DHT treated groups. The reason for the enhanced LH surge on the first day of bleeding, but not on the second day, is not clear. One possible explanation could be the small surge in controls. Consistent with our results are the findings of Clemens (1978) and Wuttke et al. (1978) who recently showed that the normal preovulatory surge of LH could still occur in 5,7-DHT treated rats. The failure of 5,7-DHT to suppress gonadotropin surges may actually be due to the development of supersensitivity in 5-HT neurons after the destruction of 5-HT nerve terminals, since it has been shown that the development of supersensitivity in 5-HT neurons occurs within 24-48 hrs after administration of 5,7-DHT (Trulson et al., 1976; Steward et al., 1976).

Like PCA, 5,7-DHT significantly potentiated the afternoon surges of both LH and FSH in EB-PRG treated ovariectomized rats. The steroid environment of the animal has been shown to be a critical factor in determining the LH response to brain stimulation (Gallo and Osland, 1976; Arendash and Gallo, 1979). Therefore, the dramatic

increase in gonadotropin surges in EB-PRG treated rats after 5,7-DHT administration could be due to the influence of PRG.

The depletion of 5-HT in the MBH was much greater than that in the AHA after 5,7-DHT injection. This regional difference in depletion of 5-HT is probably due to the non-homogenous distribution of 5,7-DHT after intraventricular injection. Actually, Baumgarten et al. (1975) found a rapid and long-lasting degeneration of indoleamine axons located near the ventricles after injection with 50 µg of 5,7-DHT, whereas terminals remote from the ventricles were less affected. Both 5,7-DHT and PCA appeared to be more effective in the depletion of 5-HT in the MBH as compared to that in the AHA, and both potentiated the afternoon surges of gonadotropin in EB-PRG treated rats. On the other hand, PCPA which was equally effective in decreasing the 5-HT levels in both the AHA and the MBH did not potentiate the surges in EB-PRG treated rats. In order to explain these results, one may assume that the gonadotropin surges are under the control of dual serotonergic systems. Kordon and Glowinski (1972) had previously postulated the existence of an inhibitory 5-HT center in the medial basal hypothalamic region and a stimulatory center in the pre-optic area. As discussed in the previous PCA study, the balance between the two 5-HT systems could be critical in regulating gonadotropin surges. The normal surges seen in EB-EB treated rats after 5,7-DHT administration could be due to the greater supersensitivity developed in the MBH as compared to that in the AHA, which compensate for the greater damage of 5-HT terminals in the MBH. Administration of PRG in EB-primed ovariectomized rats decreased

5-HT turnover in the hypothalamus (Fuxe et al., 1974), and therefore could diminish the compensatory effect exerted by the developing supersensitivity. Under this condition, the net result would be a potentiated gonadotropin surge following 5,7-DHT treatment. The hypothesis of dual 5-HT systems in controlling gonadotropin secretion also can easily explain the contradictory results found in the literature.

As reported by others, the specificity of 5,7-DHT in reducing brain 5-HT was improved after pre-treating rats with DMI to protect the NE system (Gerson and Baldessarini, 1975; Björklund et al., 1975). In fact, the NE levels in the AHA in one experiment and in the MBH in another increased significantly in 5,7-DHT injected rats as compared to that in controls. Similar increases in NE after 5,7-DHT administration in DMI pre-treated rats were also found by other investigators (Björklund et al., 1975; Clemens, 1978).

VIII. Effects of 5-HTP and Quipazine on Luteinizing Hormone Secretion in Estrogen Treated Ovariectomized Rats Pre-Treated with P-Chlorophenylalanine or P-Chloroamphetamine

A. Objective

It has been shown in several previous experiments that LH surges in EB-EB treated ovariectomized rats could be potentiated by 5-HTP if PCPA or PCA was administered 48 or 72 hrs earlier. These potentiated LH surges could only be seen on the first day of 5-HTP injection, but not on the second day, despite continued injection of 5-HTP. Based on behavioral studies, Trulson et al. (1976) concluded that denervation supersensitivity in 5-HT neurons could

be induced by 5,7-Dihydroxytryptamine (5,7-DHT), but not by PCPA.

There are two possible reasons for the failure of potentiation of the gonadotropin surges by the second injection of 5-HTP. First, 5-HTP injected on the first day could desensitize 5-HT neurons and prevent the second dose of 5-HTP from potentiating LH surges on the next day. Second, the potentiated LH surge following the first injection of 5-HTP may deplete the releasable pool of LH in the anterior pituitary, and thus the gland would not respond to the subsequent challenge of 5-HTP.

The purpose of the present study was to investigate the possibility of developing supersensitivity in 5-HT neurons on the LH surge after PCPA or PCA treatment and to distinguish among the two possible explanations mentioned above.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-275 g, received surgical and EB-treatments as described in Experiment IV. Previous results in Experiment IV indicated that the LH surge reaches its peak 4 to 6 hrs after 5-HTP. Thus 5-HTP (50 mg/kg) was given at 1200 hrs. In the first experiment, rats were separated into two groups and were treated with either PCPA (300 mg/kg i.p.) or saline at 1200 hrs on the day of the second EB injection (i.e., one day before the first day of bleeding). On the first day of bleeding, both groups were further divided into A and B subgroups and treated with either saline on the first day followed

by 5-HTP (50 mg/kg i.p.) on the next 2 days or with 5-HTP for 3 consecutive days. Blood samples were collected by cardiac puncture under light ether anesthesia at 1000 and 1800 hrs for 3 consecutive days.

In the second experiment, rats pre-treated with PCPA 48 hrs earlier were either injected with saline or 5-HTP at 1200 hrs on the first day of bleeding or given 6 consecutive injections of synthetic gonadotropin-releasing hormone (GnRH) at a dose of 50 ng/100 g B.W. every 30 mins beginning at 1500 hrs to induce an extra large surge of LH. On the next day, rats in all three groups were injected with 5-HTP. Blood samples were collected at 1000 and 1800 hrs for 2 consecutive days.

In the third experiment, either PCPA (300 mg/kg, i.p.) or PCA (5 mg/kg, i.p.) was injected into rats 72 hrs before the day of bleeding. On the day of bleeding, quipazine at a dose of 5 mg/kg was injected i.p. into each rat at 1400 hrs. Rats given saline alone were used as controls. Blood samples were collected at the times indicated in the Results.

C. Results

Effects of First, Second or Third Dose of 5-HTP on LH Surge in EB Treated Ovariectomized Rats Pre-Treated with PCPA.--On the first day of bleeding, the LH surge at 1800 hrs in saline treated controls was 1095 $\pm$ 198 ng/ml (Figure 21). Administration of 5-HTP alone at 1200 hrs tended to increase the LH surge. However, this increase was not significant. Administration of PCPA for 24 hrs

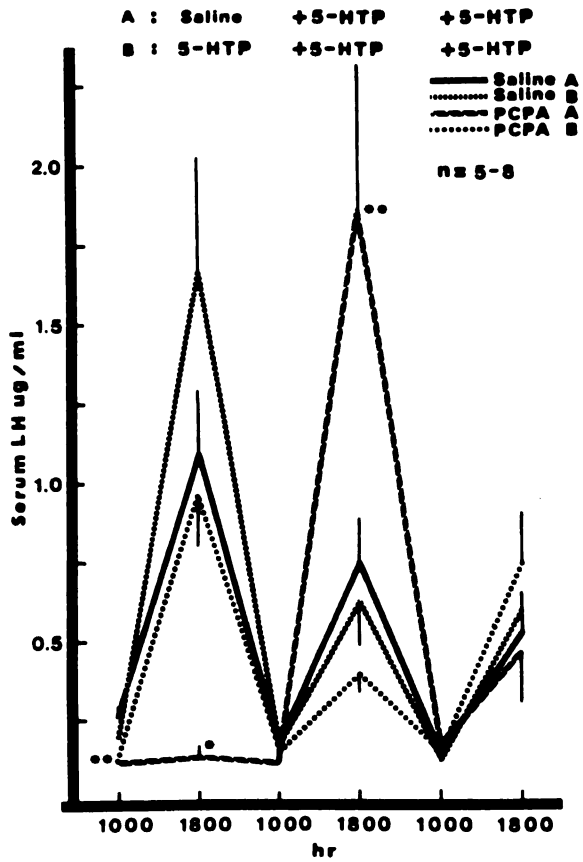

Figure 21. Effects of First, Second or Third Dose of 5-HTP on Serum LH in Estrogen Treated Ovariectomized Rats Pretreated with P-Chlorophenylalanine.

PCPA (300 mg/kg) was injected i.p. to rats 1 day before the first day of bleeding, whereas 5-HTP (50 mg/kg) was injected i.p. once a day at 1200 hrs. Protocol for 5-HTP injection is shown on the top of the figure. Each point represents mean and the vertical lines indicate 1 SEM. *, **; $p < 0.05$ and 0.01, respectively vs. saline A.

significantly decreased morning levels of LH and completely abolished the afternoon surge of LH. Replacement with 5-HTP returned the LH surge to control levels. On the second day of bleeding the LH surge following the first dose of 5-HTP in rats pre-treated with PCPA 48 hrs earlier (PCPA A) was significantly higher than that in rats pre-treated with saline alone (Saline A) (1858 $\pm$ 461 vs. 752 $\pm$ 145 ng/ml, $p < 0.01$). On the other hand, the second dose of 5-HTP failed to potentiate the LH surge. On the third day of bleeding there was no difference in the LH surge among these four groups.

Effects of 5-HTP and GnRH on the LH Surge in Response to the Second Injection of 5-HTP in EB Treated Ovariectomized Rats Pre-treated with PCPA.--On the first day of bleeding, the LH surge in the group (A) pre-treated with PCPA 48 hrs earlier was suppressed (Table 23). Only one rat in this group showed an LH surge which caused a large standard error. 5-HTP and GnRH injections resulted in huge LH surges. Actually, the increase in serum LH after GnRH injection was significantly greater than that after 5-HTP injection (5126 $\pm$ 626 vs. 3096 $\pm$ 636 ng/ml, $p < 0.05$). LH surges on the second day following 5-HTP injections were the same in both groups, A and C, even though there had been a very large surge in group C one day earlier. On the other hand, the second dose of 5-HTP in group B failed to potentiate the LH surge.

Effect of Quipazine on the LH Surge in EB Treated Ovariectomized Rats Pre-Treated with PCPA or PCA.--Quipazine alone administered at 1400 hrs had no effect on the LH surge (Figure 22). Rats

Table 23. Effects of 5-HTP and GnRH on Serum LH in Response to Second Injection of 5-HTP in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

		Time (hrs) of Bleeding					
			1st Day			2nd Day	
Group	No. of Rats	Treatment	1000	1800	Treatment	1000	1800
A	5	Saline	171±23[a]	391±194	5-HTP	161±25	4002±1094
B	6	5-HTP	143±10	3096±636*	5-HTP	216±38	448±55*
C	5	GnRH	177±24	5126±626*[b]	5-HTP	178±24	3818±1099[b]

PCPA (300 mg/kg) was injected 48 hrs before the 1st day of bleeding.
a Mean ± SEM.
* $P < 0.05$ vs. group A.
b $P < 0.05$ vs. group B.

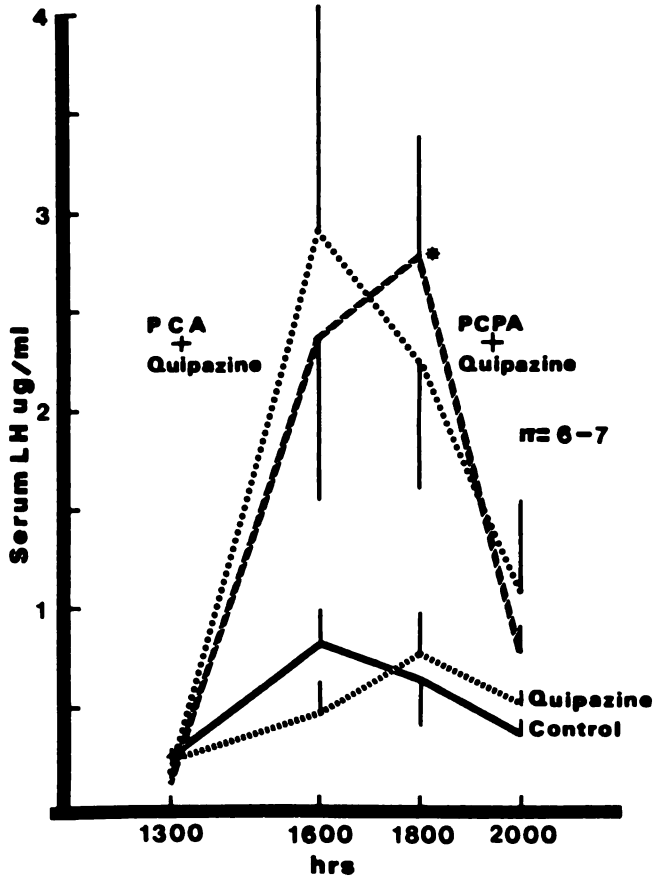

Figure 22. Effect of Quipazine on Serum LH in Estrogen Treated Ovariectomized Rats Pretreated with P-Chlorophenylalanine or P-Chloroamphetamine.

PCPA (300 mg/kg) and PCA (5 mg/kg) were injected i.p. to rats 3 days earlier, whereas quipazine (5 mg/kg) was injected i.p. at 1400 hrs. Each point represents mean and the vertical lines indicate 1 SEM. *, $P < 0.05$ vs. saline treated controls.

given PCPA 72 hrs earlier showed a slight, but not significant decrease in basal levels of LH at 1300 hrs. Administration of quipazine at 1400 hrs in rats pre-treated with either PCPA or PCA significantly increased the LH surges at 1800 hrs above that in rats without any pre-treatment ($p < 0.05$). Actually the LH surges at 1800 hrs in rats pre-treated with PCPA was significantly potentiated by quipazine as compared to that of controls (2786 ± 597 vs. 640 ± 236 ng/ml in controls, $p < 0.05$).

D. Discussion

These results suggest that the serotonergic neurons responsible for the phasic release of LH may develop supersensitivity to 5-HT agonists 48-72 hrs after PCPA or PCA treatment. Based on behavioral studies, Trulson et al. (1976) concluded that supersensitivity could not be developed in the serotonergic system after chronic treatment with PCPA. Similarly, Stewart et al. (1976) reported that the myoclonic syndrome that occurs after administration of 5-HTP to rats lesioned with 5,7-dihydroxytryptamine (5,7-DHT) could not be produced after long-term treatment with PCPA or PCA. On the other hand, pre-treatment with PCPA has been shown to enhance the hyperactivity induced by quipazine (Grabowska et al., 1974; Green et al., 1976). It has been shown that large doses of 5-HTP have a central effect to increase motor activity in mice, while decreasing motor activity by peripheral action (Modigh, 1972). It is possible that both central and peripheral 5-HT related mechanisms responsible for the motor activity become supersensitive to 5-HT agonists after

peripheral injection of either PCPA or PCA. If so, the two actions would tend to cancel each other leading to little change in motor activity. Accordingly, conclusions based on behavioral studies may not be valid.

The development of supersensitivity in the serotonergic system in rats after 5,7-DHT injection was evident within 24 to 48 hrs (Trulson et al., 1976; Stewart et al., 1976). In agreement with those, our data showed that the potentiation of LH surges by 5-HTP required PCPA pre-treatment for approximately 48 hrs. Quipazine has been shown to act as a 5-HT agonist both peripherally (Hong et al., 1969) and centrally (Rodriguez et al., 1973). The potentiation of the LH surge after quipazine administration in rats pre-treated with either PCPA or PCA suggests that the mechanism for the development of supersensitivity to 5-HT agonist is at the post-synaptic site.

The failure of the second dose of 5-HTP to potentiate the LH surge in rats pre-treated with PCPA does not appear to be due to the depletion of a releasable pool of pituitary LH following the augmented release in LH after the first injection of 5-HTP, since the potentiation of the LH surge after 5-HTP treatment was the same in both group A and C. This occurred even though there was a huge surge in group C induced by exogenous GnRH 1 day earlier. On the other hand, the second dose of 5-HTP on the following day failed to potentiate the LH surge in group B. It is possible that the first dose of 5-HTP administered 24 hrs earlier desensitized 5-HT neurons,

and therefore diminished the supersensitivity after administration of PCPA or PCA.

IX. Dose-Response Effects of 5-HT, Quipazine and 5-HTP on Gonadotropin Secretion in Estrogen Treated Ovariectomized Rats

A. Objective

In several previous experiments, a stimulatory role of 5-HT in the phasic release of gonadotropin was clearly demonstrated in rats pre-treated with 5-HT depletors. However, neither 5-HTP nor quipazine alone had a significant effect on the gonadotropin surges. Both 5-HT and its precursor, 5-HTP, have been shown to suppress the proestrous surges of LH and FSH and to block ovulation (Kamberi, 1973). On the other hand, intraventricular injection of 5-HT at various times on the day of proestrus were reported to have no effect on ovulation (Rubinstein and Sawyer, 1970; Schneider and McCann, 1970; Wilson and McDonald, 1974).

Since the dose of 5-HT used by Kamberi (1973) to inhibit the gonadotropin surge was relatively small (1-5 μg) as compared to that used in other studies, it is possible that the dosage may be a critical factor in determining the 5-HT action. The purpose of this study was to investigate the dose-response effects of 5-HT agonists on the afternoon surge of gonadotropin in EB-EB treated ovariectomized rats.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-350 g, were ovariectomized and subjected to EB

treatment as described in Experiment IV. Cannula for intraventricular injection of 5-HT were implanted as described in Experiment VII. All the rats were kept in individual cages after cannulation.

In the first experiment, 5-HT at doses of 1, 5, and 25 μg (calculated as free base) were injected at 1400 hrs in 4 μl of saline solution (with 0.02% ascorbic acid, pH = 3.7) into the third ventricle in one trial, and in 10 μl of saline solution into the lateral ventricle in another trial. Control rats were injected with vehicle only. Blood samples were collected by cardiac puncture under light ether anesthesia at 1300, 1600, 1800 and 2000 hrs.

In the second experiment, 5, 10 or 20 mg/kg of quipazine was injected i.p. either at 1200 hrs in one trial or at 1400 hrs in another. Rats given saline only were used as controls. Blood samples were taken at the times indicated in the Results.

In the third experiment, rats were injected i.p. with either 50, 100 or 200 mg/kg of 5-HTP at 1200 hrs, whereas controls were given saline only. Blood samples were collected from the trunk after decapitation at 1800 hrs.

In the fourth experiment, either 5-HTP (30 mg/kg), fluoxetine (10 mg/kg), a 5-HT re-uptake blocker, or the combination of the two drugs was injected. 5-HTP was administered at 1400 hrs, whereas fluoxetine was injected 1 hr earlier. The controls received saline only. Blood samples were taken at 1300, 1600, 1800 and 2000 hrs.

C. Results

Dose-Response Effect of 5-HT on Gonadotropin Surges in EB-EB Treated Ovariectomized Rats.--In the first trial, third ventricle injection of 1 μg of 5-HT augmented the LH surge. The increase in serum LH at 2000 hrs was statistically significant as compared to the controls (1511 ± 472 vs. 640 ± 178 ng/ml in controls, $p < 0.05$) (Table 24). On the other hand, 5-HT at doses of 5 and 25 μg not only failed to induce a dose dependent increase, but actually had a tendency to reduce serum LH. However, the decrease in serum LH was not significant as compared to the controls. The trend of the changes in serum LH in the second trial following lateral ventricle injection of increased doses of 5-HT was, in general, similar to that found in the first trial, except the increase in serum LH after 1 μg of 5-HT was not statistically significant. Serum levels of LH in pre-treatment samples (1300 hrs) in the group treated with 25 μg of 5-HT were significantly higher than in the controls ($p < 0.05$) because of the earlier rise in LH surges found in four out of eight rats in this group.

The dose-response effects of 5-HT on serum FSH are shown in Table 25. Serum levels of FSH at 1200 hrs was significantly stimulated by 1 μg of 5-HT, but not by either of the two higher doses of 5-HT in the first trial. On the other hand, none of the three doses of 5-HT had a significant effect on serum FSH in the second trial.

Dose-Response Effect of Quipazine on Gonadotropin Surges in EB-EB Treated Ovariectomized Rats.--In the first trial, quipazine at

Table 24. Dose Response Effect of 5-HT on Serum LH in Estrogen Treated Ovariectomized Rats

Treatment	Time (hrs) of Bleeding 1300	1600	1800	2000
		I		
Saline	321±79[a]	817±311	1034±381	640±178
5-HT				
1 µg	195±19	690±123	2072±676	1511±472*
5 µg	212±25	334±66	332±45[b]	273±46[b]
25 µg	176±17	315±45	742±172	467±80[b]
		II		
Saline	307±51	1223±306	693±116	379±31
5-HT				
1 µg	251±22	1948±595	883±189	456±58
5 µg	287±25	985±173	1067±416	813±231
25 µg	555±108*	643±151[b]	697±192	538±101

5-HT was injected in 4 µl volume into the 3rd ventricle of the rats in the 1st trial and in 10 µl volume into the lateral ventricle in the 2nd trial. 5-HT was injected at 1400 hrs.

a Mean ± SEM of 8 determinations.

b $P < 0.05$ vs. 5-HT (1 µg) treated group.

* $P < 0.05$ vs. saline treated controls.

Table 25. Dose Response Effect of 5-HT on Serum FSH in Estrogen Treated Ovariectomized Rats

Treatment	Time (hrs) of Bleeding 1300	1600	1800	2000
		I		
Saline	862±49[a]	1280±99	1533±147	1431±77
5-HT				
1 µg	817±35	1282±115	1554±167	1890±172*
5 µg	754±34	1098±58	1450±207	1339±98
25 µg	878±73	1159±79	1479±91	1518±107
		II		
Saline	979±33	1259±102	1463±121	1461±91
5-HT				
1 µg	1047±115	1499±188	1812±216	1463±144
5 µg	936±50	1312±145	1593±140	1545±134
25 µg	943±51	1371±63	1327±138	1533±147

5-HT was injected in 4 µl volume into the 3rd ventricle of the rats in the 1st trial and in 10 µl volume into the lateral ventricle in the 2nd trial. 5-HT was injected at 1400 hrs.

a Mean ± SEM of 8 determinations.

* $P < 0.05$ vs. saline treated controls.

a dose of 5 mg/kg resulted in a prompt increase in serum LH 2 hrs after its injection (803 ± 267 vs. 255 ± 24 ng/ml in saline treated controls, $p < 0.05$) (Table 26). Administration of 10 mg/kg of quipazine had no significant effect on serum LH. In the second trial, 5 mg/kg of quipazine injected at 1400 hrs increased the LH surge significantly at 1800 hrs (2589 ± 642 vs. 761 ± 201 ng/ml in controls, $p < 0.05$). Quipazine at a dose of 10 mg/kg also resulted in a significant increase in the LH surge at 1800 hrs ($p < 0.05$). On the other hand, administration of 20 mg/kg of quipazine resulted in a significant decrease in serum LH at 1600 hrs (340 ± 50 vs. 1110 ± 287 ng/ml in controls, $p < 0.05$). Following the inhibition there was a significant increase in serum LH later, at 2000 hrs (2277 ± 585 vs. 626 ± 246 ng/ml in controls, $p < 0.05$). In the first trial, serum FSH in quipazine treated rats at 1600 hrs was significantly higher than that in saline treated controls ($p < 0.05$). However, this stimulatory effect of quipazine was not seen in the second trial (Table 27).

Dose-Response Effect of 5-HTP on Gonadotropin Surges in EB-EB Treated Ovariectomized Rats.--5-HTP at a dose range from 50 to 200 mg/kg had no significant effect on serum LH (Table 28). However, a low dose of 5-HTP appeared to stimulate it. Serum FSH was significantly stimulated by 200 mg/kg of 5-HTP (1714 ± 170 vs. 1007 ± 159 in controls, $p < 0.05$).

Table 26. Dose Response Effect of Quipazine on Serum LH in Estrogen Treated Ovariectomized Rats

		Time (hrs) of Bleeding			
Trial	Treatment	1400	1600	1800	2000
1	Saline	255±24[a]	505±72	573±66	554±64
	Quipazine 5 mg/kg	803±267*	1077±233*	981±229	640±189
	10 mg/kg	326±61	1014±232	848±224	568±165
Trial	Treatment	1300	1600	1800	2000
2	Saline	362±43	1110±287	761±201	626±246
	Quipazine 5 mg/kg	415±132	1780±658	2589±642*	1323±420
	10 mg/kg	449±95	791±212	1957±482*	1041±221
	20 mg/kg	437±61	340±50*[b]	2140±1100	2277±585*

Quipazine was injected i.p. in saline at 1200 hrs in the 1st trial and at 1400 hrs in the 2nd trial.

a Mean ± SEM of 7-8 determinations.

* $P < 0.05$ vs. saline treated controls.

b $P < 0.05$ vs. quipazine (5 mg/kg) treated group.

Table 27. Dose Response Effect of Quipazine on Serum FSH in Estrogen Treated Ovariectomized Rats

		Time (hrs) of Bleeding			
Trial	Treatment	1400	1600	1800	2000
1	Saline	1095±38[a]	1093±74	1359±133	1238±107
	Quipazine 5 mg/kg	1258±84	1436±106*	1440±116	1399±122
	10 mg/kg	902±71	1446±136*	1426±83	1401±196
Trial	Treatment	1300	1600	1800	2000
2	Saline	1269±49	1538±62	1598±86	1678±123
	Quipazine 5 mg/kg	1147±85	1384±125	1751±240	1703±168
	10 mg/kg	1220±59	1402±74	1792±112	1673±94
	20 mg/kg	1369±116	1407±119	1870±209	1862±245

Quipazine was injected i.p. in saline at 1200 hrs in the 1st trial and at 1400 hrs in the 2nd trial.

a Mean ± SEM of 7-8 determinations.

* $P < 0.05$ vs. saline treated controls.

Table 28. Dose Response Effect of 5-HTP on Serum Gonadotropin in Estrogen Treated Ovariectomized Rats

Treatment	No. of Rats	Serum Levels of Hormone (ng/ml) LH	FSH
Saline	6	397±52[a]	1007±159
5-HTP			
50 mg/kg	7	966±395	1472±275
100 mg/kg	6	821±184	1481±158
200 mg/kg	6	476±106	1714±170*

5-HTP was injected i.p. in saline at 1200 hrs. Blood samples were collected by decapitation at 1800 hrs.

a Mean ± SEM.

* $P < 0.05$ vs. saline treated controls.

Effects of 5-HTP and Fluoxetine on Gonadotropin Surges in EB-EB Treated Ovariectomized Rats.--Administration of either 5-HTP, fluoxetine, or the combination of the two drugs had no significant effect on the LH surge, even though 5-HTP alone tended to increase serum LH (Table 29). On the other hand, serum levels of FSH at 2000 hrs was significantly increased by 5-HTP injection as compared to the controls (1408 $\pm$ 145 vs. 1022 $\pm$ 72 ng/ml, $p < 0.05$). Neither fluoxetine alone, nor the combination of 5-HTP and fluoxetine, had any effect on serum FSH.

D. Discussion

The results of this study indicate that 5-HT may exert a biphasic effect on the phasic release of gonadotropin with a facilitative effect at low doses and an inhibitory effect at higher doses. Even though the 5-HT agonists did not produce a significant effect on the gonadotropin surges in some experiments, the increase in serum LH following administration of low doses of 5-HT agonists was always reproducible.

It appeared that the LH surge was only delayed, but not blocked after a high dose of quipazine (20 mg/kg). The reason for this early transient decrease followed by a later increase in serum LH may be due to the quick decrease in serum concentration of the drug following a bolus i.p. injection. Therefore, facilitation of LH release resumed when the concentration of quipazine decreased to a lower level.

Table 29. Effects of 5-HTP and Fluoxetine on Serum Gonadotropin in Estrogen Treated Ovariectomized Rats

Hormone (ng/ml)	Treatment	Time (hrs) of Bleeding 1300	1600	1800	2000
LH	Saline	244±18[a]	498±85	501±48	510±30
	Fluoxetine	261±44	715±171	750±135	653±158
	5-HTP	227±19	785±150	1084±296	927±308
	Fluoxetine + 5-HTP	237±25	452±47	607±153	516±79
FSH	Saline	741±44	962±98	1082±79	1022±72
	Fluoxetine	645±36	837±40	1016±31	1055±79
	5-HTP	628±46	951±67	1082±114	1408±145*
	Fluoxetine + 5-HTP	608±56	761±54	887±44	1089±43

Fluoxetine (10 mg/kg) was injected i.p. in saline immediately after taking the pretreatment sample at 1300 hrs, whereas 5-HTP (30 mg/kg) was injected i.p. in saline at 1400 hrs.

a Mean ± SEM of 7 determinations.

* $P < 0.05$ vs. saline treated controls.

Fluoxetine has been shown to be a potent and specific inhibitor for 5-HT neuron re-uptake (Fuller et al., 1975a,b). Since the re-uptake mechanism is a major means to inactivate 5-HT at the synapse (Wurtman, 1972; Iversen, 1974), fluoxetine should potentiate 5-HT action at serotonergic synapses. In fact, fluoxetine has been shown to potentiate the stimulatory action of 5-HTP on prolactin release (Krulich, 1975; Clemens et al., 1977). In this study, however, we found that fluoxetine not only failed to potentiate but actually eliminated the increase in serum FSH following a low dose of 5-HTP. This also suggests a biphasic action of 5-HT on the phasic release of gonadotropin.

The facilitative, instead of inhibitory, action of a low dose of 5-HT is not in agreement with the results reported by Kamberi (1973). On the other hand, our data are consistent with the finding observed by most other investigators that intraventricular injection of 5-HT had no effect on ovulation (Rubinstein and Sawyer, 1970; Schneider and McCann, 1970; Wilson and McConald, 1974). It has been suggested that the phasic release of gonadotropin is under the control of a dual 5-HT system with an inhibitory center in the MBH and a facilitative center in the pre-optic-suprachiasmatic region (Kordon and Glowinski, 1972). Therefore, 5-HT at low doses may act at the facilitative center to stimulate gonadotropin release, whereas high doses of 5-HT may exert effects on both facilitative and inhibitory centers, with the net result either no effect or inhibition of secretion of gonadotropin.

In the second trial of the first experiment, serum LH in the pre-treatment samples showed significantly higher values in one group. Four out of eight rats in that group displayed a premature surge of LH. This premature rise in serum LH could have been due to stress since it stimulated secretion of adrenal progesterone, which has been shown to advance the gonadotropin surge in proestrous rats (Nequin et al., 1975; Campbell et al., 1977).

X. Possible Role of Adrenal Progesterone in Mediating Stimulation by 5-HTP of Luteinizing Hormone Release in Estrogen Treated Ovariectomized Rats

A. Objective

Evidence was presented in the previous experiments showing that relatively low doses of 5-HT agonists facilitated gonadotropin surges in estrogen treated ovariectomized rats. It has been shown that both 5-HT and its precursor, 5-HTP, stimulated secretion of ACTH (Fuller et al., 1976; Rose and Ganong, 1976; Fuller and Wong, 1977), and prolactin (Kamberi et al., 1971b; Chen and Meites, 1975; Clemens et al., 1977). Since both ACTH (Feder and Ruf, 1969; Feder et al., 1971) and prolactin (Piva et al., 1973) can release adrenal progesterone, and progesterone can potentiate the gonadotropin surge in EB primed ovariectomized rats (Caligaris et al., 1971b; Mann et al., 1976), it is possible that the facilitation of gonadotropin secretion by 5-HT agonists is mediated via adrenal progesterone. The purpose of this experiment was to examine this possibility.

B. Materials and Methods

Female Sprague-Dawley rats (Harlan Ind., Cumberland, IN), weighing 250-300 g, received surgical and EB treatments as described in Experiment IV. In the first experiment, rats were separated into three groups. One group was pre-treated with PCPA (300 mg/kg, i.p.) 3 days prior to the experiment. On the day of the experiment, 5-HTP (50 mg/kg) was injected i.p. at 1000 hrs. Rats given saline only served as controls. The first blood samples were collected by cardiac puncture under light ether anesthesia 30 mins after 5-HTP injection, whereas the second samples were collected by decapitation at 1600 hrs.

In the second experiment, rats were separated into two groups, and either adrenalectomized under deep ether anesthesia or sham operated to serve as controls, 6 days earlier. Hydrocortisone replacement (0.2 mg/rat/day) was started 1 day after the surgery. Two days prior to the experiment, part of the rats in each group were pre-treated with PCPA. On the day of the experiment, rats pre-treated with PCPA were given 5-HTP injections once a day at 1200 hrs for 2 consecutive days, whereas rats with no pre-treatment were given saline only. Blood samples were collected by cardiac puncture under light ether anesthesia at the times indicated in the Results.

C. Results

Effect of 5-HTP on Serum Levels of Progesterone and LH in EB Treated Ovariectomized Rats.--Administration of 5-HTP at 1000 hrs resulted in a four-fold increase in serum progesterone (23.9 $\pm$ 1.9

vs. 6.6 ± 2.2 ng/ml in saline treated controls, $p < 0.05$) (Table 30). PCPA pre-treatment did not alter the stimulatory effect of 5-HTP on serum progesterone. Administration of 5-HTP produced a significant increase in the LH surge at 1600 hrs (2080 ± 506 vs. 803 ± 146 ng/ml in controls, $p < 0.05$). This facilitative action of 5-HTP was further potentiated by the pre-treatment of PCPA (8665 ± 1711 vs. 2080 ± 506 ng/ml, $p < 0.05$).

Effects of 5-HTP and Adrenalectomy on the LH Surge in EB Treated Ovariectomized Rats Pre-Treated with PCPA.--On the first day of bleeding, PCPA pre-treatment significantly decreased the basal level of LH at 1000 hrs in sham operated rats (69 ± 5 vs. 195 ± 32 ng/ml in saline treated controls, $p < 0.05$) (Table 31). Administration of 5-HTP at 1200 hrs augmented the LH surge in sham operated rats pre-treated with PCPA. However, this potentiation was not statistically significant. Adrenalectomy had no effect on the LH surge, and did not impede the facilitative effect of 5-HTP either. Actually, the LH surge in adrenalectomized rats pre-treated with PCPA was significantly potentiated by 5-HTP ($p < 0.05$). On the second day of bleeding, the basal level of LH at 1000 hrs in the PCPA pre-treated adrenalectomized group was significantly higher than in the other group ($p < 0.05$). The second dose of 5-HTP failed to potentiate the LH surge in either sham operated or adrenalectomized rats.

Table 30. Effect of 5-HTP on Serum Levels of LH and Progesterone in Estrogen Treated Ovariectomized Rats

Treatment	No. of Rats	Serum Levels of Hormone (ng/ml)	
		Progesterone	LH
Saline	7	6.6 ± 2.2[a]	803 ± 146
5-HTP	7	23.9 ± 1.9*	2080 ± 506*
PCPA + 5-HTP	7	20.5 ± 1.6*	8665 ± 1711*[b]

PCPA (300 mg/kg) was injected i.p. to the rat 3 days prior to the experiment, and 5-HTP (50 mg/kg) was injected i.p. at 1000 hrs. Serum samples for progesterone assay was collected by cardiac puncture under ether anesthesia 30 min after 5-HTP injection, whereas the samples for LH assay was collected by decapitation at 1600 hrs.

a Mean ± SEM.

* $P < 0.05$ vs. saline treated controls.

b $P < 0.05$ vs. 5-HTP treated group.

Table 31. Effect of Adrenalectomy on Serum LH in Response to 5-HTP in Estrogen Treated Ovariectomized Rats Pretreated with PCPA

Treatment	Time (hrs) of Bleeding					
		1st Day			2nd Day	
	No. of Rats	1000	1800	No. of Rats	1000	1800
Sham						
Saline	6	195±32[a]	923±394	5	98±3	709±223
PCPA+5-HTP	8	69±5*	3440±1170	8	73±9	716±405
Adrenalectomy						
Saline	8	192±42	757±111	8	107±7	534±126
PCPA+5-HTP	11	189±35	5237±1313*	10	196±37*	429±81

PCPA (300 mg/kg) was injected i.p. to the rats 2 days prior to the experiment, and 5-HTP (50 mg/kg) was injected i.p. at 1200 hrs. Adrenalectomy was conducted 6 days earlier, and hydrocortisone replacement (0.2 mg/rat/day) was started 1 day after the surgery.

a Mean ± SEM.

* $P < 0.05$ vs. saline treated controls.

D. Discussion

This study indicates that the adrenal gland is not required for 5-HTP to exert its facilitative effect on the phasic release of LH, since adrenalectomized did not block the facilitative action of 5-HTP. However, this observation does not necessarily exclude the possibility of a secondary effect of 5-HTP by mediating through adrenal progesterone, since serum progesterone did increase after 5-HTP injection, and progesterone can potentiate the gonadotropin surge in EB primed ovariectomized rats (Caligaris et al., 1971b, Mann et al., 1976).

The increase in serum progesterone in estrogen treated ovariectomized rats after 5-HTP injection appears to be due to the increased secretion of ACTH (Fuller et al., 1976; Fuller and Wong, 1977) and prolactin (Chen and Meites, 1975; Clemens et al., 1977), since both ACTH (Feder and Ruf, 1969; Feder et al., 1971) and prolactin (Piva et al., 1973) have been shown to stimulate progesterone secretion from the adrenal gland.

Administration of progesterone has been shown to block the daily LH surge beginning on the second day in EB treated ovariectomized rats (Freeman et al., 1976). Since the second dose of 5-HTP failed to potentiate the LH surge in PCPA pre-treated-adrenalectomized rats, the possibility that the increased serum level of progesterone after the first dose of 5-HTP may be responsible for preventing the action of the second dose of 5-HTP on the following day, can be excluded.

XI. Effect of Progesterone on Steady State Concentration and Turnover of 5-HT in Anterior Hypothalamic Area (AHA) and Medial Basal Hypothalamus (MBH), and on Serum Gonadotropin in Estrogen Primed Ovariectomized Rats

A. Objective

Most evidence which suggested involvement of the central serotonergic system in the control of cyclic release of gonadotropin and ovulation was based on pharmacological studies (Kordon et al., 1968; Kordon and Glowinski, 1972; Kamberi, 1973; Weiner and Ganong, 1978). Changes in 5-HT concentrations and metabolism in the hypothalamus at different endocrine states have been studied. However, the work is not as extensive as that on the catecholamines. The ovarian steroids acting in the hypothalamus have both inhibitory and stimulatory effects on the secretion of the gonadotropin. The stimulatory influence is believed to be exerted on the anterior hypothalamic area. Thus, lesions placed in the pre-optic-supra-chiasmatic area or anterior deafferentation of the medial basal hypothalamus resulted in constant estrus and blocked ovulation (Halasz, 1972; Clemens et al., 1976), blocked the proestrus LH surge (Blake et al., 1972), and the LH surge normally seen after progesterone administration to ovariectomized, estrogen primed rats (Taleisnik, et al., 1970; Bishop et al., 1972; Kawakami et al., 1978).

The purpose of this study was to determine if any changes occurred in 5-HT metabolism in the anterior hypothalamus or medial

basal hypothalamus after progesterone administration in estrogen primed ovariectomized rats.

B. Materials and Methods

Adult female Sprague-Dawley rats (Spartan Research Farms, Haslett, MI) were bilaterally ovariectomized for at least 2 wks before being used in these experiments. In the first experiment, ovariectomized rats were given a single s.c. injection of estradiol benzoate (EB, 50 μg/rat) at 1200 hrs. Three days after the EB injection, animals were killed by decapitation at 0900, 1200, 1500, and 1800 hrs. In the second experiment, rats were primed with a single s.c. injection of 20 μg/rat of EB at 1200 hrs. Three days after EB priming, each rat received a single s.c. injection of progesterone (PRG; 2.5 mg/rat) at 1200 hrs. Animals were killed by decapitation 0, 2, 4, and 6 hrs after PRG administration. 5-HT turnover was estimated by a modification of the non-steady state method described by Neff and Tozer (1968).

Half of the rats in each group were injected i.p. with pargyline hydrochloride (75 mg/kg) either 30 (first experiment) or 60 mins (second experiment) before decapitation. The other half of the rats received vehicle (0.9% NaCl) only. After decapitation, trunk blood was collected for hormone assays and the brain was quickly removed from the cranium. The anterior hypothalamic area (AHA) and medial basal hypothalamus (MBH) were dissected as described in General Materials and Methods. AHA and MBH weighed 16.8 $\pm$ 0.4 and 13.8 $\pm$ 0.3 mg, respectively in the first experiment,

and 14.2 ± 0.3 and 11.2 ± 0.2 mg, respectively in the second experiment. Tissue samples were homogenized in 100 µl of 0.1 N HCl (containing 10 mg EDTA/100 ml). Hypothalamic 5-HT was assayed according to the radioenzymatic method of Saavedra et al. (1973). The turnover of 5-HT was calculated as the percent accumulation of 5-HT following pargyline injection.

C. Results

Steady State Concentration and Turnover of 5-HT in EB Primed Ovariectomized Rats.--The upper panel of Figure 23 shows that the steady state concentration of 5-HT in the MBH increased by 1200 hrs. However, due to the small N number, the increase was not significant. On the other hand, 5-HT turnover decreased significantly at 1200 hrs (36 ± 4 vs. 78 ± 7 at 0900 hrs, $p < 0.05$). Neither the steady state concentration nor the turnover of 5-HT in the AHA changed significantly between 0900 and 1800 hrs (lower panel of Figure 23). Serum levels of LH increased dramatically in rats killed at 1800 hrs (Table 32).

Steady State Concentration and Turnover of 5-HT in EB Primed Ovariectomized Rats Following PRG Administration.--The steady state concentration of MBH 5-HT increased significantly by 2 hrs after PRG administration (upper panel of Figure 24) ($p < 0.01$). 5-HT turnover, as estimated by the accumulation of 5-HT after 1 hr of pargyline injection, decreased to almost half of initial level by 2 hrs after PRG administration and remained at the low level throughout the 6 hr period ($p < 0.01$). Similar changes in 5-HT

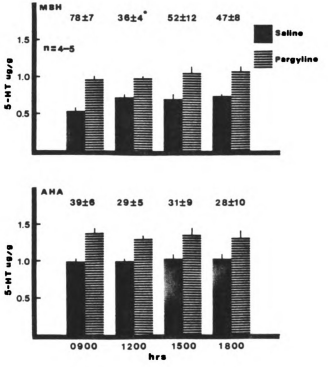

Figure 23. Anterior Hypothalamic and Medial Basal Hypothalamic Concentration of 5-HT and Levels 30 Mins After Pargyline Administration in Estrogen Primed Ovariectomized Rats.

Rats received a single i.p. injection of pargyline (75 mg/kg) or saline, 30 mins before decapitation. Upper panel shows 5-HT concentration in the MBH, whereas lower panel shows that in the AHA. Each bar represents mean and the vertical lines indicate 1 SEM. Number above each set of bars indicates percentage accumulation of 5-HT after pargyline treatment (Mean $\pm$ SEM). *, $p < 0.05$ vs. zero hr value.

Table 32. Serum LH in Estrogen Primed Ovariectomized Rats

Time (hrs) after PRG	No. of Rats	Serum Levels of LH (ng/ml)
0	10†	253±19[a]
2	10	342±16
4	10	399±21
6	10	789±198*

† Data from both saline and pargyline (30 mins) treated groups were combined for the calculation of Mean ± SEM since there was no difference in hormone levels between the two groups.
a Mean ± SEM.
* $P < 0.05$ vs. zero hr value.

Table 33. Serum Levels of Gonadotropin in Estrogen Primed Ovariectomized Rats Following Progesterone Administration

Hormone (ng/ml)	No. of Rats	Time (hrs) after Progesterone 0	2	4	6
LH	12†	334±43[a]	329±31	2291±462*	5114±716*[b]
FSH	12	1304±51	1341±111	2031±140*	3104±144*[b]

† Data from both saline and pargyline (1 hr) treated groups were combined since there was no difference in hormone levels between the two groups.
a Mean ±SEM.
* $P < 0.05$ vs. zero hr value.
b $P < 0.05$ vs. 4 hrs value.

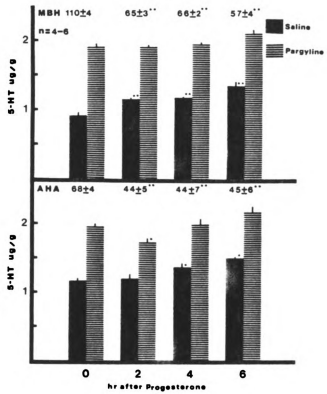

Figure 24. Effect of Progesterone on Steady State Concentration and Pargyline Induced Accumulation of Anterior Hypothalamic and Medial Basal Hypothalamic 5-HT in Estrogen Primed Ovariectomized Rats.

Rats received a single i.p. injection of pargyline or saline, 1 hr before decapitation. See Figure 23 for further explanation. *, **; $p < 0.05$ and 0.01, respectively vs. zero hr value.

also were seen in the AHA (lower panel of Figure 24). The steady state concentration of 5-HT in the AHA increased significantly by 4 hrs after PRG administration ($p < 0.05$). The accumulation of 5-HT 1 hr after pargyline injection was significantly lower at 2 hrs than at 0 hrs after PRG administration ($p < 0.05$). 5-HT turnover decreased significantly throughout the 6 hr post-progesterone period ($p < 0.01$). Serum levels of both LH and FSH increased significantly by 4 hrs after PRG administration (Table 33).

D. Discussion

The decrease in 5-HT turnover in the MBH after a stimulatory regimen of gonadal steroids is consistent with the hypothesis that the serotonergic system in the medial basal hypothalamic region has an inhibitory role in regulating gonadotropin surges (Kordon and Glowinski, 1972). Based on a microinjection study, Kordon (1969) showed that the inhibitory action of 5-HT was located in the arcuate-median eminence region.

It has been shown that the 5-HT levels in the hypothalamus display a circadian rhythm, and the most striking changes in 5-HT levels occur at the end of the light period and in the early hours of the dark period (Quay, 1968; Héry et al., 1972). The decreased endogenous 5-HT levels during the dark period appear to be due to the decreased synthesis and enhanced utilization of the newly synthesized 5-HT (Héry et al., 1972).

The significant increase in 5-HT levels and nearly 50% decrease in turnover in the MBH after PRG administration cannot

be attributed to the circadian change of 5-HT since no significant change in either the steady state concentration or turnover occurred between 1200 and 1800 hrs in EB primed rats. Consistent with our results, Tonge and Greengrass (1971) showed that progesterone increased 5-HT concentration in the mid- and hind-brain in ovariectomized rats. By using similar protocol for steroid treatment, Fuxe et al. (1974) reported that estrogen increased 5-HT turnover in ovariectomized rats, whereas progesterone in estrogen-primed rats suppressed the increased 5-HT turnover. These results showing either no change or a decrease in 5-HT turnover in the AHA after steroid treatment are not necessarily incompatible with the hypothesis that a stimulatory 5-HT center to regulate the phasic release of gonadotropin is located in the anterior hypothalamus (Kordon and Glowinski, 1972).

It has been shown that the timing of the stimulatory vs. inhibitory effects of 5-HT is quite different, and pharmacological data suggest an early facilitative effect of serotonergic systems on the afternoon surges of gonadotropin (Héry et al., 1976; Wilson et al., 1977). Therefore, it is possible that the 5-HT metabolism in the AHA increases sometime early in the morning. Consistent with this possibility, Everitt et al., (1975) recently suggested that the effect of ovarian steroids on the metabolism of brain 5-HT varies depending on the time when the hormones are administered. These authors showed that estrogen accelerated brain 5-HT turnover in ovariectomized rats in the evening and PRG prevented this increase. On the other hand, the opposite seemed to be the case when PRG was

injected and turnover was measured in the morning. A similar increase in anterior hypothalamic 5-HT turnover at 1100 hrs following PRG injection in EB primed ovariectomized rats also was observed by Munaro (1978). Furthermore, daily morning PRG peaks during the estrous cycle (Mann and Barraclough, 1973) has been demonstrated in normal cycling female rats.

In addition to its central effect on 5-HT metabolism, progesterone has been shown to increase NE turnover in the anterior hypothalamus (Simpkins et al., 1979), and to potentiate the sensitizing effect of estrogen on pituitary release of gonadotropin in vitro in response to GnRH during the first 4-8 hrs of incubation (Labrie et al., 1979). Therefore, progesterone may potentiate the gonadotropin surge by its multiple actions exerted at both the hypothalamus and pituitary.

GENERAL DISCUSSION

Several new findings have been made during the course of this thesis study. First, inhibitory effects were demonstrated for the first time for piribedil, a DA agonist, and 5-HTP, a 5-HT precursor, on the rapid rise in serum LH after short-term orchidectomy. Second, a facilitative effect of 5-HTP on the phasic release of LH was confirmed and extended. It was found that the facilitative action of 5-HT on the LH surge in gonadal steroid treated ovariectomized rats was time dependent, and could be potentiated in rats whose brain 5-HT had been depleted previously by pre-treatment for 2-3 days with either PCPA, a tryptophan hydroxylase inhibitor, or PCA, a long-lasting 5-HT antagonist. In addition, data presented in this thesis demonstrated for the first time that 5-HT also may have a facilitative role in the phasic release of FSH. Third, both systemic injection of PCA, and intraventricular injection of 5,7-DHT, a 5-HT neurotoxic agent, resulted in greater depletion of 5-HT in the MBH than in the AHA, and demonstrated for the first time that these treatments potentiated the surges of both LH and FSH in EB-PRG treated ovariectomized rats. These results suggest that presence of an inhibitory 5-HT center in the MBH to regulate the phasic release of gonadotropins, in addition to a facilitative center possibly located in the AHA. Fourth, evidence was provided for a

biphasic effect of 5-HT agonists on the phasic release of gonadotropins, with facilitation at low doses and inhibition at higher doses. Fifth, results from the adrenalectomy study excluded the possibility that facilitation of gonadotropic surges by 5-HTP is mediated entirely through adrenal progesterone.

The data presented in this thesis provide further evidence for the possible roles of central dopaminergic and serotonergic systems in the regulation of pituitary gonadotropin secretion. Administration of piribedil, a DA agonist, caused a dose-dependent inhibition of the post-castration increase in LH in male rats, suggesting an inhibitory role of DA in the tonic release of LH. Consistent with this view, stimulation of DA receptors with DA agonists has been shown to reduce serum LH (Beck et al., 1978) and to block the pulsatile release of LH (Drouva and Gallo, 1976). However, DA does not appear to exert its tonic inhibition on LH release under most physiological conditions since pimozide, a DA receptor blocker, had no effect on the acute rise of LH following orchidectomy (Experiment I; Ojeda and McCann, 1973), and on the pulsatile LH release in chronically ovariectomized rats (Drouva and Gallo, 1976).

Recent studies by Vijayan and McCann (1978) suggested that DA may have a differential action on LH secretion depending upon the dose and the endocrine state of the animal. Relatively low doses of DA effectively stimulated LH release in steroid-primed ovariectomized rats, whereas high doses of DA suppressed serum LH in ovariectomized rats. The importance of the steroid condition is

further strengthened by the observation that the MBH from normal male and estradiol-implanted ovariectomized rats were able to release LHRH in vitro in response to DA. On the other hand, DA failed to stimulate LHRH release from MBH of castrated rats (Rotsztejn et al., 1976, 1977).

DA appears to have both stimulatory and inhibitory effects on the phasic release of LH and ovulation. A selective decrease in median eminence DA turnover has been shown to occur during the afternoon of proestrus and early estrus as compared to other stages of the cycle in rats (Fuxe et al., 1967; Ahrén et al., 1971, Löfström, 1977), suggesting an inhibitory tuberoinfundibular DA system in controlling gonadotropin secretion. In agreement with this view, DA receptor stimulators have been shown to block PMS-induced ovulation (Fuxe et al., 1975) and the premature, pre-ovulatory-type LH surges (Beck et al., 1978) in immature rats. On the other hand, apomorphine or piribedil, both DA receptor stimulators, at doses effective in immature rats were unable to block the pre-ovulatory LH surge in proestrous rats (Beck et al., 1978) or in steroid treated ovariectomized adult rats (Simpkins et al., 1979). The former authors, therefore, suggested that DA receptors which are inhibitory to LH release became desensitized during development, and the inhibitory role of DA on the phasic release of LH in adult rats may not have physiological importance.

The incertohypothalamic dopaminergic neurons with their axons projecting to the medial pre-optic area and the suprachiasmatic nucleus (Björklund et al., 1975) may be a stimulatory

dopaminergic pathway regulating the LH surge since implantation of DA into the pre-optic area has been shown to stimulate LH release in estrogen-primed ovariectomized rats (Kawakami et al., 1975). In addition, administration of pimozide, a DA receptor blocker, inhibited the LH surge in proestrus rats (Beattie et al., 1976) and in steroid treated ovariectomized rats (unpublished data).

Very few studies have been done so far to investigate dopaminergic influence on FSH secretion. LHRH has been shown to release both LH and FSH (Schally et al., 1971) and it may well be a physiological releasor of FSH (Wise et al., 1978). Aminergic regulation of secretion of FSH would be expected to be similar to that of LH. The data presented in this thesis favor an inhibitory role for DA. The failure to detect any change in serum FSH after apomorphine injection in ovariectomized rats (Vijayan and McCann, 1978; Beck et al., 1978) may be due to the short period after drug administration to blood collection, since serum FSH has been shown to have a long half life (Gay et al., 1970).

The secretion of pituitary prolactin is believed to be under the tonic inhibition of the tuberoinfundibular DA system (Neill, 1974; MacLeod, 1976). In accord with this view, data presented in this thesis revealed an increased DA turnover in the median eminence in response to orchidectomy, a condition known to result in decreased serum prolactin (Meites, 1972). On the other hand, these data suggest that the tuberoinfundibular DA system does not play a major role in the negative feedback regulation of LH secretion. This latter idea is further supported by the failure of the DA receptor blocker,

haloperidol, to alter the basal levels of LH in rats (Meuller et al., 1976) and the persistent negative feedback action of testosterone on LH secretion in hamsters after a monosodium glutamate (MSG) induced lesion of the arcuate nucleus (Lamperti and Baldwin, 1979).

Most evidence indicates that 5-HT is an inhibitory neurotransmitter in regulating gonadotropin secretion (see III, B3 of Literature Review). The result presented in this thesis shows that systemic injection of 5-HTP, a 5-HT precursor, inhibited the post-castration rise in serum LH, and favors an inhibitory role for 5-HT in the tonic release of LH. In agreement with the present observations, Arendash and Gallo (1978) recently reported inhibition of episodic release of LH resulting from electrical stimulation of the dorsal raphé nucleus, again implicating a mediation through 5-HT. However, under most physiological conditions, 5-HT does not appear to play an important role in the negative feedback regulation of serum LH since interruption of the 5-HT system with either PCPA, a 5-HT synthesis inhibitor, or methysergide, a 5-HT receptor blocker, had no effect on the increase in serum LH in response to orchidectomy, or on the reduction of serum LH by testosterone in long-term orchidectomized rats (Experiment I). On the other hand, the inhibitory 5-HT mechanism under certain conditions may have some physiological importance. In post-partum lactating rats, the suckling stimulus has been shown to increase 5-HT turnover in the hypothalamus (Mena et al., 1976), whereas the post-castration increase in serum LH is absent (Ford and Melampy, 1973; Hodson et al., 1978), It

still remains to be definitely determined whether or not inhibition of LH secretion by the suckling stimulus is mediated through 5-HT.

With regard to the phasic release of LH, most early studies based on the ability of systemically injected 5-HT agonists to block ovulation, suggested an inhibitory role for 5-HT. However, evidence accumulated in recent years also suggests the existence of a facilitative serotonergic pathway. Therefore, the second part of this thesis was totally devoted to investigating the serotonergic mechanism involved in the phasic release of gonadotropin.

The data presented here indicate that the functional integrity of the central serotonergic system is required for estrogen to exert a positive feedback action on gonadotropin secretion, since both PCPA and methysergide were able to block the LH surge. These results are in agreement with those reported by Héry et al. (1976) and Marko and Flückiger (1975).

The facilitative effect of a 5-HT agonist on the gonadotropin surge was potentiated in rats whose brain 5-HT had been previously depleted by either PCPA or PCA suggesting an increased sensitivity of the 5-HT receptors. It was also shown that this increased receptor sensitivity could be desensitized within 24 hrs by increased 5-HT activity. A similar observation of increased sensitivity to 5-HTP in rats pre-treated with 5,7-DHT and PCA, demonstrated by an exaggerated release of prolactin, was also reported by Clemens (1978). It is of interest to speculate that the changes in receptor sensitivity may have some physiological importance.

Like progesterone, 5-HT was able to advance the LH surge only for several hrs (Experiment IV). The LH surge was always confined to the afternoon hrs no matter how early 5-HT was administered. This observation appears to rule out the possibility that the light-dark cycle maintains the 24 hr rhytimicity of the 'critical period' for the cyclic release of LH via the circadian rhythm of 5-HT in the brain. Instead, it suggests that the facilitative action of 5-HT may be mediated through some neural events which are not activated until 5 hrs after the lights go on.

Several lines of evidence presented in this thesis also suggest the existence of an inhibitory serotonergic pathway. First, the dose-response studies showed that the effect of 5-HT agonists on the gonadotropin surge was biphasic, with a facilitation at low doses and an inhibition at high doses. Therefore, dose dependency for 5-HT action could account for some contradictory results in the literature. Second, both PCA and 5,7-DHT, which caused a greater depletion of 5-HT in the MBH than in the AHA, potentiated the progesterone-induced gonadotropin surge in EB primed rats, whereas PCPA which equally depleted 5-HT in both areas failed to do so. This observation is consistent with the hypothesis proposed by Kordon and Glowinski (1972), suggesting the existence of an inhibitory 5-HT center in the MBH and a stimulatory center in the pre-optic AHA region. Third, progesterone, which potentiated the gonadotropin surge in response to EB, decreased 5-HT turnover in the hypothalamus, suggesting an inhibitory role for 5-HT. Recently, Everitt et al. (1975) pointed out that progesterone at different times of the day

may exert different action on 5-HT turnover in estrogen primed rats. These authors found that progesterone decreased 5-HT turnover at night, but increased it in the morning. Therefore, the ability of progesterone to potentiate the gonadotropin surge in response to estrogen could well be due to its dual action on 5-HT turnover, since the facilitative action of 5-HT has been shown to have an earlier time-course than the inhibitory action (Héry et al., 1976; Wilson et al., 1977). Of course, the possible effect of progesterone on other neurotransmitters cannot be ignored.

The data presented in this thesis, together with previous pharmacological observations, suggest that both stimulatory and inhibitory dopaminergic and serotonergic pathways may be involved in the regulation of gonadotropin secretion, via control of the release of LHRH. The inhibitory pathways appear to terminate in the MBH region, whereas the stimulatory pathways innervate the pre-optic AHA region. These two regions previously were proposed to control the tonic and cyclic release of gonadotropin, respectively (Halász, 1969; Gorski, 1971).

Further studies, such as local implantation of drugs or chemical lesions induced with neurotoxins on specific areas in the brain, are required in order to differentiate the function of DA and 5-HT pathways in regulation of the phasic and tonic release of gonadotropin.

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APPENDICES

APPENDIX A

BEN-JONATHEN AND PORTER CATECHOLAMINE ASSAY PROCEDURES

APPENDIX A

BEN-JONATHEN AND PORTER CATECHOLAMINE

ASSAY PROCEDURES

1. Homogenize pieces of brain tissue in desired volume of 0.4 N perchloric acid (plus 10 mg% EDTA).

2. Centrifuge at 3,000 RPM for 20 min at 4°C.

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

Reagent	Proportion
20 mM EGTA-Na salt (0.760 gm/100 ml H_2O and pH to 7.2)	1
Pargyline Solution (to 4 mg pargyline add 25 µl β-mercaptoethenol and 225 µl H_2O)	1
1 M Tris base (with 3 mM $MgCl_2$) (to 6.05 gm Tris add 50 ml H_2O plus 30.5 mg $MgCl_2$)	6.5
S-adenosyl-l-methionine (Methyl-^3H) (11.6 Ci/m mole diluted 1:3.5 with H_2O)	3.0
Catecholamine-o-methyl transferase (COMT, partially purified by the method of Nikodijevic et al., 1970)	5.1

4. Incubate for 60 min at 37°C.

5. Add 30 µl of 0.45 M borate buffer (pH 10.0) and 5 µl of carrier methoxyamine mix (50 mg 3-methoxytyramine, 50 mg DL-methanephrine and 50 mg DL-normetanephrine; 10 mg of each amine/ml of 0.1 N HCl). Add 500 µl of toluene: isoamyl alcohol (3:2, v:v), vortex for 30 sec, and centrifuge at 3,000 RPM for 20 min at 4°C.

6. Transfer 400 µl of organic phase to conical centrifuge tubes containing 40 µl of 0.1 N HCl. Vortex for 30 sec and centrifuge at 5/7 speed on IEC clinical centrifuge (International Equipment Co., Needham Hts., Mass.).

7. Apply 25 µl of acid phase to LQ-60 silica gel plates previously spotted with 5 µl of carrier methoxyamine mix. Allow plates to dry.

8. Place plates in thin-layer chromatography tanks containing chloroform, ethanol and methylamine (40:18:5 by volume). Allow plates to run 1 1/2 to 2 hrs and remove from tank to allow for drying.

9. Visualize and outline spots under ultraviolet light.

10. Scrape plates and place scrapings into scintillation vials containing 1.0 ml of ethyl acetate, acetic acid and H_2O (3:3:1 by volume) and shake for 30 min. Add 10 ml of Aqueous Counting Scintillant and count.

APPENDIX B

SAAVEDRA SEROTONIN ASSAY PROCEDURES

APPENDIX B

SAAVEDRA SEROTONIN ASSAY PROCEDURES

Reagent

PO_4 buffer (0.2 M, pH 7.9)
Titrate dibasic (2.84 g/100 ml) with Monobasic (2.76 g/100 ml) such that pH = 7.9.

Borate-Melatonin
Mix 9 parts of Borate (0.5 M, pH 10) (6.2 g/200 ml H_2O brought to pH 10 with 5 N NaOH solution) with 1 part of Melatonin (5 mg/ml) (50 mg Melatonin/2.5 ml Ethanol, brought up to 10 ml with H_2O).

0.1 N HCl (containing 10 mg% EDTA).

PO_4 buffer-NaOH
Mix 22.5 ml PO_4 buffer with 2.5 ml of 1 N NaOH.

Assay

1. Homogenize tissue in desired volume of 0.1 N HCl (containing 10 mg% EDTA).

2. Centrifuge at 3,000 RPM for 20 min at 4°C.

3. Transfer 10 µl of supernatant (or of working 5-HT standard solution) to glass culture tubes containing 10 µl of PO_4 buffer -NaOH.

4. Add 20 µl of a mixture of equal parts of Acetyl Co A (1 mg/ml in PO_4 buffer) and N-acetyl transferase (NAT).

5. Incubate at 37°C for 30 min.

6. Add 25 µl of a mixture containing 14.5 parts of PO_4 buffer, 10 parts of hydroxyindole-o-methyl transferase (HIOMT), and 0.5 parts of S-adenosyl-l-methionine (Methyl-^3H).

7. Incubate at 37°C for another 10 min.

8. Stop reaction with 100 µl of Borate-Melatonin.

9. Extract in 2 ml Toluene (vortex for 30 sec and centrifuge at 3,000 RPM for 10 min at 4°C.

10. Aspirate 1.5 ml supernatant (organic phase) and transfer to a scintillation vial containing 2 ml fresh Toluene.

11. Dry overnight at 80°C in hood.

12. Add 1 ml Ethanol and 10 ml Aqueous Counting Scintillant and count.

APPENDIX C

GLOSSARY OF DRUGS USED

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GLOSSARY OF DRUGS USED

Alpha-methyl-paratyrosine (α-mpt): A tyrosine hydroxylase inhibitor which inhibits the synthesis of catecholamines.

Desipramine (DMI): A norepinephrine re-uptake blocker.

5,7-Dihydroxytryptamine (5,7-DHT): A neurotoxin for serotonergic neurons, which destroys 5-HT nerve terminals.

Fluoxetine: A serotonin re-uptake blocker, which prolongs 5-HT action.

5-Hydroxytryptamine (5-HT): A neurotransmitter (serotonin).

5-Hydroxytryptophan (5-HTP): A 5-HT precursor.

Methysergide: A 5-HT receptor blocker, which inhibits the action of 5-HT.

Para-chloroamphetamine (PCA): A long lasting 5-HT antagonist; recently it was classified as a neurotoxin for serotonergic neurons.

Para-chlorophenylalanine (PCPA): A tryptophan hydroxylase inhibitor which inhibits the synthesis of 5-HT.

Pargyline: A monoamine oxidase inhibitor, which prevents degradation of catecholamines and 5-HT.

Pimozide: A specific DA receptor blocker, which inhibits DA action.

Piribedil: A long-lasting DA agonist, which stimulates DA receptors.

Quipazine: A 5-HT agonist, which stimulates 5-HT receptors.

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FUTURE ADDRESS: Department of Obstetrics, Gynecology
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MAJOR ACADEMIC ADVISOR: Dr. Joseph Meites
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EDUCATION:

National Taiwan Normal University, 1969, B.S., Biology
National Taiwan University, 1973, M.S., Zoology
Michigan State University, 1979, Ph.D., Physiology

POSITIONS HELD:

Teaching Assistant in Physiology, M.S.U., 1974-1977.
Research Assistant in Physiology, M.S.U., 1977-1979.

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ACCOMPLISHMENTS:

a. Established gonadotropin-releasing hormone (GnRH) radioimmunoassay and conducted research on the control of GnRH secretion, 1976-1977.

b. Established radioenzymatic assay of serotonin and conducted research on the control of hypothalamic serotonin on anterior pituitary hormone secretion, 1977-1978.

c. Maintained and supplied reagents for the radioenzymatic assay of catecholamines for general lab. usage, and conducted research on the control of hypothalamic catecholamines on anterior pituitary hormone secretion, 1977-1978.

AWARDS:

Fellowship Award from the graduate school at National Taiwan University, 1972-1973.

Sigma Xi Graduate Student Award for Meritorious Research in Physiology, May 1979.

TALKS PRESENTED AT SCIENTIFIC MEETINGS:

Meeting	Date	Topic
63rd Annual FASEB Meeting Dallas, Texas	April, 1979	Role of serotonin (5-HT) in phasic release of gonadotropins

RESEARCH PUBLICATIONS:

1. W. C. M. Wan and H. T. Chen. Adenohypophyseal interstitial-cell stimulating hormone (ICSH) in rats after thyroidectomy. J. of Formosan Medical Assoc. 73(7):387-392, 1974.

2. G. P. Mueller, H. T. Chen, J. A. Dibbet, H. J. Chen, and J. Meites. Effects of warm and cold temperatures on release of TSH, GH, and Prolactin in rats. Proc. Soc. Exp. Biol. Med. 147:698-700 (1974).

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3. K. H. Lu, H. T. Chen, L. Grandison, H. H. Huang, and J. Meites. Reduced luteinizing hormone release by synthetic luteinizing hormone-releasing hormone (LHRH) in postpartum lactating rats. Endocrinology 98:1235-1240, 1976.

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

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

6. H. T. Chen, K. H. Lu, and J. Meites. Effects of hypo- and hyperthyroidism on 5-hydroxytryptophan and chlorpromazine-induced prolactin release in rat. Proc. Soc. Exp. Biol. Med. 151: 739-741, 1976.

7. K. H. Lu, H. H. Huang, H. T. Chen, M. Kurcz, R. Mioduszewski, and J. Meites. Positive feedback by estrogen and progesterone on LH release in old and young rats. Proc. Soc. Exp. Biol. Med. 154:82-85, 1977.

8. H. T. Chen, J. Geneau, and J. Meites. Effects of castration, steroid replacement, and hypophysectomy on hypothalamic LHRH and serum LH. Proc. Soc. Exp. Biol. Med. 156:127-131, 1977.

9. H. T. Chen, J. W. Simpkins, G. P. Mueller, and J. Meites. Effects of pargyline on hypothalamic biogenic amines and serum prolactin, LH and TSH in male rats. Life Sciences 21:533-542, 1977.

10. L. Grandison, C. Hodson, H. T. Chen, J. Advis, J. Simpkins, and J. Meites. Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology 23:312-322, 1977.

11. J. P. Advis, J. W. Simpkins, H. T. Chen, and J. Meites. Relation of biogenic amines to onset of puberty in the female rat. Endocrinology 103:11-16, 1978.

12. P. W. Sylvester, H. T. Chen, and J. Meites. Interactions of morphine with dopaminergic and cholinergic drugs on release of prolactin in the rat. IRCS J. of Med. Sci. 6:510, 1978.

13. D. A. Van Vugt, J. F. Bruni, P. W. Sylvester, H. T. Chen, T. Ieiri, and J. Meites. Interaction between opiates and hypothalamic dopamine on prolactin release. Life Sciences 24:2361-2368, 1979.

14. T. Ieiri, H. T. Chen, and J. Meites. Effects of morphine and naloxone on serum levels of luteinizing hormone and prolactin in prepubertal male and female rats. Neuroendocrinology (in press), 1979.

15. T. Ieiri, H. T. Chen, G. A. Campbell, and J. Meites. Effects of naloxone and morphine on proestrous surge of prolactin and gonadotropins in the rat. (Submitted to Endocrinology), 1979.