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Serotonin, a Neurotransmitter Involved in the Regulation of Luteinizing Hormone Release*

MARIA L. VITALE AND SARA R. CHIOCCHIO

Instituto de Neurobiología, Serrano 665, (1414) Buenos Aires, Argentina

I. Introduction

FOLLOWING the discovery of various neurotransmitters in the brain, neuroendocrinologists have been challenged over the years with identifying those involved in the control of hormone secretion by the pituitary gland. A decade ago, Weiner and Ganong (1) and Kalra and Kalra (2) reviewed the role of serotonin (5-HT), among other neurotransmitters, in the release of luteinizing hormone (LH). This early literature was filled with conflict and controversy. As technology became more sophisticated and especially as immunohistochemistry and neurochemistry developed, it was possible to correlate physiological and experimental changes in LH secretion with changes in 5-HT metabolism in specific, discrete neuroanatomical areas. Nevertheless, at present, the physiological role of 5-HT in the control of LH secretion is still a matter of debate.

The present review has been written in an effort to clarify the confusion resulting from the comparison of data from incompatible experimental designs. According to this, the information available on the participation of 5-HT in LH secretion was classified and discussed on the basis of the different experimental and physiological models.

Address requests for reprints to: Sara R. Chiocchio, M.D., Instituto de Neurobiología, Serrano 665, 1414 Buenos Aires, Argentina.

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II. Male Rats

LH secretion in male rats can be described as a series of low amplitude pulses of irregular frequency. These erratic LH pulses are always followed by an episode of testosterone (T) secretion which, in turn, is followed by a period without LH pulses (3). This sequence describes the reciprocal control between the pituitary and the testes: the stimulatory effect of LH on T secretion and the negative feedback action of the steroid on LH release. The most direct basis for the pulsatile discharge of LH seems to be the pulsatile secretion of LH-releasing hormone (LHRH) from the median eminence (4).

LH pulse frequency and amplitude as well as mean LH concentration increase dramatically after orchidectomy (5). Administration of T reduces blood LH concentration. This negative-feedback effect is exerted at the level of the pituitary gland and the hypothalamus (4).

The neurotransmitters involved in the negative feedback effect of T and the neural control of the LHRH pulse generator are not completely defined. For instance, the participation of 5-HT in the regulation of LH secretion in male rats is still a matter of debate. This could be due, in part, to the fact that early investigations took into account the pulsatile pattern of LH release in male rats, perhaps because at that time the pulsatile secretion was not well defined. Therefore, the possibility of a serotonergic influence on the pulse frequency or amplitude of LH secretion was not studied.

A. 5-HT metabolism in intact and castrated male rats

5-HT content and metabolism of areas related to the control of hormone secretion and sexual behavior, including the hypothalamus, the lateral brain stem, and the frontal

cortex, display a circadian rhythm in the intact male rat (6, 7). The most dramatic changes occur at the end of the light period and at the beginning of the dark phase. For instance, there is an increase in 5-HT metabolism in the suprachiasmatic nucleus (SCN) in the early hours of darkness whereas in the median eminence (ME), the increase takes place at the end of the light period (8). In addition, there are three peaks of hypothalamic [^{3}H]5-HT uptake while there is only one in the SCN (9).

Removal of the testes has little effect on brain 5-HT content and metabolism. There is no change in 5-HT content of the dorsal and median raphe nuclei (DRN and MRN), ME, SCN, and anterior hypothalamic nucleus, when evaluated 14 days after orchidectomy (10). An increase of 5-HT content in the ventromedial nucleus of the hypothalamus (VMN) and in the medial forebrain bundle has been reported 1 week after castration (11). T therapy returns the 5-HT content of the latter areas to control levels, while it decreases the 5-HT content in the arcuate nucleus (AN) and SCN (11) below normal levels. However, since the high doses of T utilized to suppress the effect of castration on hypothalamic 5-HT content only partially depress the elevated serum LH levels, it has been suggested that hypothalamic 5-HT might be involved in T-regulated brain functions other than LH secretion, such as male sexual behavior (12).

Orchidectomy does not affect the activity of the rate limiting enzyme of 5-HT synthesis, tryptophan hydroxylase, in the hippocampus, amygdala, and hypothalamic nuclei (13). In the DRN and MRN, Long *et al.* (10) observed a decrease in the activity of the enzyme 2 weeks after orchidectomy but the effect was not reversed by T treatment, suggesting that tryptophan hydroxylase activity in these areas is not involved in the negative feedback effect of T.

No changes in 5-HT metabolism occur in the preoptic area (POA), SCN, anterior hypothalamic area (AH), and VMN after castration (14). In agreement with these results, castration does not alter 5-HT metabolism in the frontal cortex and hypothalamus (15). Nevertheless, the lack of effect of the ablation of the testes on 5-HT metabolism does not rule out the possibility of its participation in the regulation of LH secretion in male rats since, to our knowledge, no studies were performed on the effect of castration on the circadian rhythm of hypothalamic 5-HT metabolism. For example, in female rats, an oscillatory pattern in hypothalamic 5-HT metabolism has been described to correlate with phasic LH secretion (see below).

Removal of the testes in males lowers the number of 5-HT_1 binding sites in the septum, hypothalamus, and midbrain. The effect in the midbrain is abolished by estrogen therapy. Estrogen (E_2) administration to castrated male rats decreases B_{max} and increases the dissociation constant (K_d) in the POA (16). These experimental data show that gonadal hormones modulate the levels and the affinity of 5-HT_1 binding sites within the brain; however, the physiological significance of such changes is not clear.

B. Lesion of 5-HT neurons

The majority of 5-HT containing neurons innervating the hypothalamus are located within the raphe nuclei in the brain stem (17–19). Damage of these "serotonergic" areas was also used as an approach to study the participation of 5-HT in the regulation of LH secretion in male rats. However, the results obtained in these experiments have to be regarded with caution. When neurotoxins are infused into a lateral or into the third ventricle, different populations of serotonergic neurons, expressing opposite influences on LH secretion, could be affected. On the other hand, in most studies, rats were killed at different postlesion intervals and, therefore, different compensatory mechanisms: denervation-induced hypersensitivity and/or sprouting of injured axons could have developed, making the comparison and discussion of results very difficult.

Initially, Ladosky and Noronha (20) reported that the infusion of the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) into the third ventricle induces a massive increase in LH secretion in intact and castrated rats. However, since in these rats the uptake of 5,6-DHT by catecholaminergic neurons was not blocked with desmethylimipramine (DMI), the effect of the neurotoxin on LH secretion could be due to the destruction of catecholaminergic neurons. In fact, the injection of this neurotoxin into the third ventricle of DMI-pretreated castrated male rats has no significant effect on either mean plasma LH concentrations or mean LH pulse amplitude (21). The results suggest that the serotonergic fibers damaged by the infusion of 5,6-DHT into the third ventricle may not be critical for LH secretion in males. Instead, local injection of 5,7-dihydroxytryptamine (5,7-DHT), a more specific serotonergic neurotoxin (22), into the medial preoptic area (MPOA) but not into the medial basal hypothalamus (MBH) or the amygdala causes a dramatic increase in serum LH and T content in intact males, suggesting an inhibitory role of serotonergic terminals within the MPOA (23).

A stimulatory influence of 5-HT on LH secretion was reported by other authors. For instance, Wuttke *et al.* (24) observed that after the infusion of 5,7-DHT into the left lateral ventricle, a significant decrease in serum LH concentration occurs. LH levels remain low up to 26 days postlesion but 55 days after the treatment, control serum LH levels have recuperated. The recovery of LH concentration parallels the recovery of 5-HT metabolism and [^{3}H]5-HT uptake in the hypothalamus and tegmentum mesencephali, suggesting that the sprouting of serotonergic fibers may have overcome the effect of the axotomy caused by the neurotoxin (24). Local injection of 5,7-DHT into the DRN and MBH reduces serum LH levels in intact male rats (25). Infusion of the neurotoxin into the MRN, MPOA, or AH does not affect LH secretion. Neither treatment affects serum LH concentration in castrated rats (25).

C. Pharmacological and in vitro studies

Systemic injections of 5-HT into either intact, chronically castrated, or T-treated chronically castrated male rats do not alter LH secretion (26, 27). No changes in serum LH levels occur after administration of 5-hydroxytryptophan (5-HTP), an immediate precursor of 5-HT (28).

Blockade of 5-HT synthesis with *p*-chlorophenylalanine

(pCPA) in chronically castrated males does not modify mean blood LH concentration (29) or the pulsatile pattern of LH secretion (21). These results suggest a lack of effect of 5-HT on LH secretion in male rats. However, administration of 5-HT to acutely orchidectomized rats decreases LH secretion (26). Therefore, it is possible that the subtle influence of 5-HT in LH secretion in male rats only appears when the mechanism responsible for LH secretion is forced to lose the steady state condition (acute orchidectomy).

Incubation of the MBH with 5-HT reduces the *in vitro* LHRH secretion (30) and infusion of 5-HT into the third ventricle inhibits the release of LH (31), suggesting a central inhibitory role of the indolamine on LH secretion. Soon after, however, the same group reported that the infusion of larger amounts of 5-HT into the third ventricle actually increases LH secretion (32). This experimental datum is in accordance with the findings of Ruzsas *et al.* (33), who observed that when two factors increasing extraneuronal 5-HT levels act together, a permissive effect on LH secretion becomes apparent.

This possibility is sustained by recent *in vitro* studies. In these experiments, ME and anterior pituitaries (AP) of male rats were incubated in a sequential-double chamber perifusion system (34). Addition of 5-HT into the chamber containing the ME stimulates the release of LH into the incubation medium (34). The discrepancy between this result and the inhibitory effect reported by Charli *et al.* (30) could be related to the fact that they employed a tissue preparation that included, together with the ME, other hypothalamic nuclei that could mediate opposite influences on LHRH secretion.

On the other hand, the 5-HT-induced increase on LH secretion reported by Vitale *et al.* (34) only represents 33% of the stimulatory effect of 5-HT on LH secretion from tissues taken from proestrous female rats (34). Since the 5-HT metabolism in the hypothalamus, POA, and limbic areas is higher in female than in male rats (35), and that 5-HT was shown to stimulate LH secretion in male rats orchidectomized at birth and treated with E_2 in adulthood (27), it is evident that the effect of 5-HT on LH secretion is very sensitive to the estrogen milieu.

III. Pregnant Mare Serum-Treated Immature Rats

Ovulation in immature (27-day-old) rats can be evoked by injecting pregnant mare serum gonadotropin (PMS). The hormone, which has an FSH-like activity, induces the maturation of ovarian follicles and stimulates the secretion of estrogens by Graafian follicles (36). This increased blood concentration of E_2 has a positive feedback effect at the hypothalamus-pituitary level, inducing an LH surge on the second day (52–56 h) after PMS administration. This surge is followed by ovulation 12 h later (37). The secretion pattern of ovarian hormones and LH after PMS administration to immature rats is similar to that found in adult rats during the estrous cycle (37, 38). PMS-induced LH secretion has a "critical period," defined as the time of activation of the neural mechanisms associated with the initiation of the surge (39), just like the "normal" preovulatory discharge of LH (40). Therefore, the PMS-induced LH surge and ovulation have been judged as a suitable model for the proestrous discharge of LH and ovulation in the adult rat.

Pioneer studies on the participation of 5-HT in LH secretion were performed in PMS-induced ovulation. Systemic administration of 5-HT (12.5–25 mg/kg) was reported to inhibit PMS-evoked ovulation and luteinization and to cause a delay in the time of vaginal opening (41, 42). Subcutaneous administration of larger amounts of 5-HT (100 mg/kg) was also found to inhibit induced ovulation when given at least 3 h after the critical period (43). However, it is likely that 5-HT, administered systemically, inhibits induced ovulation acting at a peripheral site since the inhibitory effect can be overcome by injection of progesterone (P). 5-HT reduces the incidence of ovulation in hypophysectomized, PMS-human CG-treated immature rats and 5-HT still inhibits the induced LH surge when injected after the critical period for the release of the hormone, that is, when the neurogenic mechanisms had already occurred (43). Nevertheless, experiments involving 5-HT infusion into the third ventricle (44) or substances that block 5-HT and catecholamine catabolism, such as monoamine oxidase inhibitors (45–46), suggest a central inhibitory effect of 5-HT on induced ovulation.

At that time, another series of experiments demonstrated that 5-HT may have a stimulatory influence on PMS-provoked ovulation. Brown (47, 48) observed that the ovulatory effect of low doses of PMS was actually potentiated by injections of 5-HT. Wilson *et al.* (43) later reported that intraventricular infusion of 5-HT stimulates induced ovulation, provided it is injected at the beginning of the critical period for LH secretion. Depletion of brain 5-HT levels with pCPA or impairment of 5-HT transmission with serotonergic antagonists also block PMS-induced ovulation when given at the appropriate time (43, 46, 48, 49). Furthermore, Wilson *et al.* (50) reported that pCPA administration prevents the PMS-induced LH surge.

The facilitatory role of 5-HT in PMS-induced ovulation in immature rats was confirmed by lesion experiments. In this experimental approach, serotonergic neurons were damaged via the injection of the neurotoxin 5,7-DHT into either the fourth ventricle, a lateral ventricle, or locally into the DRN (51). As a result of the lesions, rats showed a reduced [^{3}H]5-HT uptake in the SCN and a blockade of PMS-evoked ovulation (51). It was concluded that the integrity of a serotonergic projection arising from the DRN and terminating in the SCN may be important for the mechanism involved in ovulation in this experimental model (51).

A circadian rhythm in the concentration of 5-HT and of its major catabolite 5-hydroxyindolacetic acid (5-HIAA) in the hypothalamus of immature (27- to 30- day-old) rats has been described (50). This circadian rhythm is not altered by PMS administration, although it is necessary but not sufficient for a normal PMA-induced ovulation to occur (52). It has been shown that in immature rats with body weights below 60 g, PMS cannot induce ovulation (52). Interestingly, in these animals, the amplitude of daily hypothalamic 5-HT oscillations is smaller than in rats weighing more than 60 g (53, 54).

Recently, a role for 5-HT in the paraventricular nucleus of the hypothalamus on induced ovulation has been suggested (55). However, in this case, 5-HT is related to GH release rather than to LH secretion (55).

IV. Ovariectomized Rats

Removal of the ovaries releases the hypothalamus and the AP from the inhibitory feedback of estrogens. As a consequence of this, increased amounts of gonadotropins are secreted into the blood (56). The rise of blood gonadotropins in the castrated male rat is very fast. On the contrary, in the castrated female, the rise is delayed and occurs only after several days. Apparently, the delay depends on the circulating levels of E_2 at the time of ovariectomy (57). LH secretion in ovariectomized rats is characterized by periodic discharges that occur with a relatively constant frequency and amplitude (58, 59). Such pulsatile secretion depends on the pulsatile pattern of LHRH release, which in turn is modulated by a rhythmic neurogenic signal (60). Several studies were undertaken to investigate the involvement of the serotonergic system in the neural mechanism controlling LH secretion in ovariectomized rats.

A. 5-HT metabolism

Initially, the effects of the ablation of the gonads on the steady state concentrations and metabolism of the amine were studied. The determinations were carried out in whole brain samples or in grossly dissected brain areas. More recently, measurements were performed in discrete brain nuclei facilitating the detection of subtle changes that are unique to specific small areas. In most hypothalamic nuclei 5-HT concentration is not affected by castration; only the SCN shows an increase with respect to diestrous I values (61). A circadian rhythm of 5-HT metabolism occurs in the POA, the medial preoptic nucleus (MPON) (62, 63), AH (64), SCN (8, 63), MBH (62), and AN (63) of ovariectomized rats. The metabolism is higher in the morning than in the afternoon. No changes in 5-HT turnover are observed in the ME (63).

Few investigations have been undertaken to examine the effect of castration on brain 5-HT binding sites. Kendall *et al.* (65) reported that ovariectomy has no effect on 5-HT_2 receptors in the cerebral cortex and hippocampus.

B. Stimulation and lesion of 5-HT neurons

Stimulation of the raphe nuclei in the brain stem or damage of 5-HT nerve terminals were used as strategies to alter serotonergic neurotransmission and to evaluate their effects on LH secretion. Electrical stimulation of the DRN decreases blood LH levels (66, 67). The effect is mediated by serotonergic neurons since blockade of 5-HT synthesis with pCPA or blockade of 5-HT receptors with metergoline abolish the inhibition (66). These findings suggest that 5-HT has an inhibitory influence on LH secretion in ovariectomized rats.

Accordingly, the destruction of serotonergic nerve terminals should increase circulating LH concentrations. In fact, that is the effect observed 10 days after the infusion of the serotonergic neurotoxin 5,6-DHT into the third ventricle (20). Moreover, localized depletion of 5-HT produced by intracerebral injection of another neurotoxin, 5,7-DHT, also alters LH release (68). Four days after the microinjection of 5,7-DHT into the MBH there is an increased LH secretion. The microinjection of the same neurotoxin into the MPOA decreases plasma LH levels. The results suggest that the MBH represents an area where 5-HT exerts its inhibitory influence on LH secretion whereas the MPOA represents the region where the stimulatory effect would take place (68). However, it has also been reported that 5 days after intraventricular injection of 5,7-DHT there are no changes in LH peak height but there is a decrease in the frequency of the peaks, hence a decrease in average serum LH levels (69). A return to the oscillatory pattern present in control animals occurs 55 days after lesioning, even though hypothalamic 5-HT levels are not recovered. These results indicate a facilitatory influence of 5-HT on LH secretion. They also suggest that after lesioning, other neurotransmitter systems may overcome the lack of hypothalamic 5-HT and/or hypersensitivity of postsynaptic serotonergic receptors develops in order to reestablish LH secretion (69). The discrepancy in the results obtained in these works on the effect of neurochemical damage of 5-HT fibers on LH secretion is difficult to explain although some differences in the protocols may account for the opposite effects. The results of the first paper (20) agree with the inhibitory role of 5-HT suggested by the "stimulation" experiments. However, they used 5,6-DHT, a neurotoxin that is less specific than 5,7-DHT, and the animals were not previously treated with DMI to prevent the uptake of the neurotoxin by noradrenergic neurons. Moreover, plasma LH concentrations were evaluated only once 10 days after lesioning. Therefore, changes, if any, in the circadian pattern of LH secretion could not be detected. The third paper (69) does not explain whether the neurotoxin was injected into a lateral ventricle or into the third ventricle. This is important since the extent of the lesion produced in each case is very different and probably damages different serotonergic pathways having opposite effects on LH release (70).

C. Pharmacological studies

Experimental evidence strongly indicates an inhibitory influence of 5-HT neurons on LH secretion in ovariectomized animals. Infusion of 5-HT into the third ventricle decreases plasma LH levels (71). Systemic administration of 5-HT fails to modify LH secretion (27), indicating that the inhibitory action of 5-HT must be exerted within the blood brain barrier, since 5-HT does not practically penetrate the barrier (72). Systemic administration of 5-HTP (28) or of fluoxetine (73), a 5-HT uptake blocker, increases extraneuronal 5-HT concentrations and concomitantly decreases the amplitude of LH peaks without affecting the interpeak interval. However, the destruction of 5-HT-containing fibers with *p*-chloroamphetamine or blockade of 5-HT metabolism with pCPA does not affect any characteristic of the pulsatile release of LH (73). The authors interpreted the results as an indication that normal physiological levels of 5-HT are not necessary to regulate LH secretion in the ovariectomized rat. However,

when pharmacological manipulations lead to an increase of central 5-HT concentrations, an inhibitory effect can be observed.

On the contrary, an elevation of brain 5-HT levels, via the intraventricular infusion of fluoxetine in combination with the systemic administration of 5-HTP, causes a significant rise in serum LH, indicating that 5-HT could be a facilitatory influence on LH release although not a major one (33). The discrepancies between these results on the effect of fluoxetine could be due to the route of administration of the drug (intraventricular *vs.* systemic). In accordance with the inhibitory action of 5-HT on LH secretion in ovariectomized rats, quipazine, a serotonergic agonist, inhibits LH secretion (74, 75). The specificity of the effect is shown by the fact that it is abolished by 5-HT receptor blockers, ketanserin, metergoline, and methysergide (75). Another serotonergic agonist, 5-metoxy *N, N*-dimethyltryptamine does not alter LH secretion (76) but inhibits the release of the hormone in animals pretreated with ketanserin, a 5-HT_2 receptor antagonist (76). Apparently, blockade of 5-HT_2 receptors unmasks the inhibitory response mediated by 5-HT_1 receptors. Therefore, activation of 5-HT_1 or 5-HT_2 binding sites may cause opposite effects on LH secretion in castrated female rats.

D. In vitro experiments

The effect of 5-HT and drugs affecting 5-HT activity on LH secretion in ovariectomized rats may take place in the serotonergic neurons that influence LHRH secretion. In support of such a possibility, superfusion of an area encompassing the POA, SCN, and MBH with 5-HT causes a decrease in the secretion of LHRH into the medium (77). However, alterations of 5-HT metabolism may affect other neurotransmitter systems that in turn modulate LHRH secretion as has been shown for the noradrenergic (74) and the opiate (76) systems. Alternatively, 5-HT could have a direct effect on the AP either modifying the secretory activity of gonadotropes or modulating the activity of LHRH on gonadotropes. An inhibitory effect of 5-HT on LHRH-induced LH secretion was reported by two different studies (78, 79). However, Ryu *et al.* (78) failed to observe any effect of 5-HT on basal LH secretion after a 4-h incubation period. On the other hand, Apfelbaum (79) described an inhibitory effect of 5-HT on basal LH secretion that, interestingly, is only evident during the first hour of incubation.

V. Ovariectomized Rats Treated with E_2 or E_2 + P

Acute treatment of ovariectomized rats with E_2 results in the decrease of blood LH concentrations and in the disappearence of the pulsatile discharge characteristic of gonadectomized animals (80). This consequence of E_2 administration is known as its negative feedback effect on LH release. Chronic treatment with E_2 induces a daily afternoon peak of gonadotropins and this stimulatory action on LH secretions is known as the positive feedback effect of E_2 (81). Treatment of E_2-primed ovariectomized rats with P potentiates the afternoon gonadotropin surge that occurs immediately after administration of P (82). The E_2- and E_2 + P-induced LH surges are similar in timing, duration, and magnitude to the proestrous LH discharge (83). Therefore, they have been widely used as experimental paradigms of the spontaneous preovulatory LH release in the cycling rat. The participation of the serotonergic system in the negative and/or the positive feedback action of E_2 and on its potentiation by P has been studied by employing different experimental approaches.

A. Effect of ovarian steroids on 5-HT metabolism

Low doses of E_2, which do not alter blood LH levels, do not modify 5-HT turnover in estrogen-concentrating areas (84). However, the E_2-induced decrease (negative feedback) or increase (positive feedback) of LH secretion is accompanied by changes in 5-HT metabolism of restricted brain areas. Moreover, P modulates the effect of E_2 on both LH secretion and 5-HT metabolism. Nevertheless, since ovarian steroids regulate several brain-dependent activities, the changes in 5-HT metabolism after E_2 and P treatment could only represent a temporal correlation without necessarily implying a cause and effect relationship.

1. Negative feedback effect. Pharmacological studies suggest that 5-HT neurons mediate the negative feedback of E_2 on LH secretion in gonadectomized rats (75), but those neurons do not seem to be localized in the hypothalamus (68). Acute (3 h) administration of E_2 to ovariectomized rats diminishes circulating LH levels and decreases 5-HT metabolism in the MPOA and rostral AN whereas it increases 5-HT turnover in the caudal AN (61). These findings were interpreted as an indication that the serotonergic system present in rostral areas represents a positive influence whereas serotonergic terminals located in the caudal AN represent an inhibitory influence on the mechanism controlling LH secretion (61). Johnson and Crowley (68) observed that 3 h after E_2 treatment, there is a decrease in plasma LH, an increase in plasma PRL, and a concomitant increase in 5-HT turnover in the cortical amygdala, the MPN, and VMN. 5-HT turnover in other hypothalamic and amygdaloid areas is not affected. However, the damage of serotonergic terminals within those nuclei does not alter the E_2-induced decrease in blood LH concentrations (68). These results strongly suggest that hypothalamic 5-HT is not involved in the negative feedback effect of E_2 upon LH secretion.

2. Positive feedback effect. The circadian rhythm of hypothalamic 5-HT metabolism observed in ovariectomized rats is modulated by chronic treatment with ovarian hormones. However, when these cyclic changes of neuronal serotonergic activity are analyzed, a series of discrepancies between different groups of researchers becomes evident. The contradictions in the experimental data may arise from several factors: the schedule of ovarian hormone treatment (one or several injections, silastic implants), the dissection of the different brain areas, the time of day at which the tissues were collected, and the method used to measure 5-HT turnover (85, 86).

In the AHA, the circadian rhythm of 5-HT metabolism disappears 2 days after the injection of E_2 (64). In agreement with this result, the implantation of a silastic capsule con-

taining E_2 abolishes the daily fluctuation of 5-HT activity within the MPN (63). However, it was also found that E_2 implants do not affect the circadian rhythm of 5-HT turnover in the MPOA (62). The SCN of ovariectomized rats is characterized by a higher 5-HT turnover during the light hours than during the dark period; 2 days after implantation of a silastic capsule containing E_2, a reversal of the rhythm was observed (63). However, 2 weeks after the implantation of a similar silastic capsule, Héry *et al.* (8) still describe a higher 5-HT metabolism in the morning than in the afternoon. In the MBH, the circadian oscillation of 5-HT turnover is not affected by E_2 (62).

An increase in hypothalamic 5-HT turnover takes place during the E_2-evoked LH discharge (87). When 5-HT content or turnover is evaluated in discrete areas, no changes are observed in the POA-AH, posterior hypothalamus (PH), and septal area (88, 89). However, in the ME, where no diurnal rhythm in the accumulation of 5-HT is evident in the ovariectomized rat, E_2 induces an elevation in the metabolism of the indolamine just before the onset of the gonadotropin surges (63). This fact, according to the authors, emphasizes the role of ME 5-HT as a signal for the phasic release of LH.

On the other hand, P modulates the E_2-induced changes in 5-HT activity. In the AH, P restablishes the circadian rhythm of 5-HT metabolism abolished by treatment with E_2 (64). Administration of P to E_2-primed ovariectomized rats increases 5-HT metabolism in the DRN (88), MPN, interstitial nucleus of the stria terminalis (89), and ME (90, 91), while it decreases 5-HT metabolism in the VMN (89, 91) at the time of the LH surge. The E_2-induced daily fluctuation of 5-HT metabolism in the whole hypothalamus is amplified by P (92). It is worth mentioning here the role of P on LH secretion and 5-HT metabolism, since this steroid is involved in the proestrous LH surge (2). Results suggest that the activity of 5-HT neurons within brain areas related to LH secretion is sensitive to P, and that this could be a step in the potentiation of E_2-induced phasic LH secretion by P.

The steroid dependency of other indicators of serotonergic metabolism, such as the enzymatic activities responsible for the biosynthesis or catabolism of the amine or the uptake by serotonergic neurons, has also been evaluated. For instance monoamine oxidase activity in the MBH and cortical amygdala declines after E_2 administration (93). However, it has to be taken into consideration that catecholamines are also deactivated by this enzyme (94). Treatment of ovariectomized animals for 48 h with E_2 or P increases and decreases respectively the *in vitro* rate of 5-HT uptake in the hypothalamus without affecting the saturable uptake (95). This result suggests the existence of a steroid-sensitive mechanism that accelerates the removal of 5-HT from the synapse, an event that could be part of the neural signal controlling phasic LH secretion.

3. *Serotonergic receptors.* Another important aspect of the serotonergic neurotransmission affected by ovarian hormones is the density of brain 5-HT receptors. The hormone-induced alterations of 5-HT binding sites could be one of the factors that modulate the final effect of 5-HT on the LH secretory mechanism. Acute (1 h) treatment of ovariectomized rats with E_2 decreases the number of 5-HT_1 receptors in hypothalamic as well as in nonhypothalamic areas (96). This acute effect of E_2 is due to a decrease in the viscosity of the plasma membrane (96). On the contrary, 72 h exposure to E_2 increases the density of 5-HT_1 receptors only in E_2-concentrating areas: amygdala, POA, AH, and MBH (AN-ME) (96, 97). P has no additional effect and hypophysectomy does not abolish the E_2-induced increase in 5-HT_1 binding sites (96). Alterations in the concentration of 5-HT_1 and 5-HT_2 binding sites in the cerebral cortex have been related to sexual behavior. A 2-week treatment with E_2 or P decreases 5-HT_1 and increases 5-HT_2 binding sites (98). P inhibits the E_2-induced rise in 5-HT_2 receptors but has no effect on 5-HT_1 receptors. Therefore, in the cerebral cortex, the two serotonergic receptors, which mediate opposite effects (99), are also regulated differentially and independently of each other by ovarian steroids.

B. Pharmacological studies

1. *Negative feedback effect.* The administration of 5-HT receptor antagonists, methysergide, metergoline, or ketanserin, diminishes the effectiveness of E_2 in abolishing the pulsatile LH secretion in ovariectomized rats (75). 5-HT receptor blockade interferes with the production of the pulses but not with the E_2-induced decrease in circulating LH levels (75). These results together with the fact that serotonergic denervation of hypothalamic areas does not affect the E_2-induced decrease in LH suggest that the negative feedback effect of E_2 upon LH secretion in ovariectomized rats is not exerted at the hypothalamic level.

2. *Positive feedback effect.* Experimental evidence also emphasizes the sensitivity of the serotonergic system to circulating levels of estrogens and suggests that the balance between the opposite effects of 5-HT on LH release is shifted by chronic treatment with E_2 in favor of the stimulatory influence (99, 67). Accordingly, the administration of 5-HT or 5-HT agonists to chronically E_2-treated ovariectomized rats induces an increase in blood LH concentrations (27, 99, 100). As we have discussed previously, 5-HT has no effect on castrated male rats; however, when males are castrated at birth and given E_2 in adulthood, 5-HT displays an LH-releasing activity (27). Thus, for a stimulatory influence of 5-HT on LH secretion to occur, high levels of E_2 as well as the presence of a feminine differentiated brain are necessary (27).

A decrease in serotonergic neurotransmission, caused by inhibition of 5-HT synthesis, blockade of serotonergic receptors, or denervation with specific neurotoxins, abolishes the E_2-induced LH surges (87, 95, 100–102) and the E_2 + P-induced LH surge (103). Restoration of 5-HT levels with either the serotonergic agonists, 5-HTP and quipazine, or the serotonergic uptake blocker fluoxetine reestablishes LH surges, but only when given at the appropriate time (87, 95, 100–103). These results suggest that the permissive action of 5-HT has to occur during a "critical period" before the onset of the LH surge.

Paradoxically, the elevation of extraneuronal 5-HT also abolishes the steroid-induced LH surge (28, 103, 104). A

possible explanation of such opposite results was provided by Walker (87). He demonstrated that treatments altering 5-HT metabolism inhibit LH secretion regardless of the increase or decrease in hypothalamic 5-HT concentrations. Therefore, the inhibitory effect of those pharmacological manipulations is due to the elimination of the changing pattern of hypothalamic 5-HT turnover normally associated with the E_2-induced LH surge. In agreement with this hypothesis, the loss of the positive feedback of estrogens in rats treated chronically with E_2 is overcome by the administration of pCPA followed by 5-HTP. This pharmacological recovery of the positive feedback may be related to the amplification of signals within the serotonergic system by pCPA and 5-HTP (105).

5-HT may also participate in the positive effect of P on LH release. P-induced secretion of LH in E_2-primed ovariectomized rats is potentiated by the administration of 5-HTP and blocked by the compound 26–921, a 5-HT antagonist (106).

C. Stimulation and lesion of 5-HT neurons

Electrolytic lesions of the MRN do not alter the E_2-induced LH surge. However, larger lesions that destroy the MRN and DRN significantly reduce the magnitude of the E_2-induced LH release (107), suggesting that the integrity of the DRN is more important than that of the MRN for a "normal" phasic release of LH to happen (107). However, in another work, it was shown that the lesion of an area encompassing the DRN and MRN does not affect the E_2 + P-induced LH secretion (108). Moreover, electrical stimulation of raphe nuclei causes an inhibition of the steroid-induced LH secretion (108). These differences could arise from the variability in the extension of the lesioned/stimulated areas, which may alter other neurotransmitter systems also involved in LH secretion.

Specific destruction of serotonergic terminals within the POA-stria terminalis region with 5,7-DHT effectively blocks the E_2 + P-evoked LH surge (89). These results indicate that serotonergic fibers innervating the POA-stria terminalis area, where the cell bodies of LHRH neurons are located, play a facilitatory role on the phasic secretion of LH induced by sex steroids. In agreement with such a possibility, recent results show that electrical stimulation of the DRN significantly increases 5-HT metabolism in the MPN and the periventricular nucleus of the hypothalamus (PeVN) without any effect on the SCN, the AN, or the ME (109). On the other hand, MRN stimulation increases 5-HT metabolism in the AN and the PeVN although it does not affect 5-HT turnover in the MPN or ME (109). Interestingly, the electrical stimulation of the DRN prolongs the LH release in response to the electrochemical stimulation of the MPN (109). In a recent paper, Kitts and Johnson (67) demonstrate that the inhibitory influence of DRN electrical stimulation on LH secretion in ovariectomized rats switches to a permissive effect in the presence of E_2. Therefore, it is possible that the serotonergic neurons located within the DRN may facilitate the phasic secretion of LH in the presence of adequate levels of ovarian hormones.

D. In vitro experiments

The permissive influence of E_2 on the effect of 5-HT on LH secretion in castrated female rats is also confirmed by *in vitro* experiments. Perifusion of the MBH-POA-SCN from ovariectomized rats with pulses of 5-HT decreases the frequency of LHRH release, whereas an increase of the frequency is observed when tissue from ovariectomized E_2-treated rats is perifused (110). It is then possible that the E_2 exposure of the neural mechanism responsible for LH secretion could be a factor in determining the final effect of 5-HT on LH secretion.

VI. The Estrous Cycle

Normal cycling rats exhibit an acute and massive discharge of LH in the afternoon of proestrus (38, 111). A period of neural activity, known as the "critical period" precedes the discharge of LH (40). This critical period is characterized by an increased pulsatile release of LHRH from the ME into the portal blood (112, 113). The ovarian steroid feedback actions are key steps that determine the periodic pattern of LHRH release during the estrous cycle (80).

A. 5-HT neuronal activity during the estrous cycle

5-HT binding sites undergo changes during the estrous cycle of the rat. A 40% decrease in the number of 5-HT_1 receptors occurs in the basal forebrain at proestrous noon with respect to the other stages of the cycle (114). The density of 5-HT_1 binding sites in the frontal cerebral cortex is lowest during the early afternoon of proestrus and highest during the afternoon of estrus (115). These changes can be correlated with the biphasic effect of E_2 on 5-HT_1 binding sites seen in ovariectomized rats (96). The high levels of E_2 on the morning of proestrus induce a decrease in the number of 5-HT_1 binding sites (acute effect), while 24–48 h later when E_2 levels are low again, there is an increase in 5-HT_1 binding sites (chronic effect). The decline in the number of binding sites may cause a decrease in the responsiveness of these tissues to 5-HT at specific moments of the cycle.

5-HT steady state concentrations fall during the afternoon of proestrus in the septum, POA, MBH, and serotonergic nuclei of the mesencephalon (116). A decrease in ME 5-HT content occurs during the onset of the preovulatory LH secretion (Table 1 and Refs. 117–119). [^{3}H]5-HT uptake increases in the SCN just before the peak of the preovulatory surge of LH (120). However, even though these data strongly suggest the involvement of 5-HT in the regulation of LH secretion during proestrus, the physiological significance of changes in the steady state concentration or uptake of neurotransmitters is not yet clear and, several times, the same result has been interpreted as indicating a stimulatory or an inhibitory effect.

Turnover studies give more clear-cut information. Hypothalamic 5-HT turnover is significantly higher at the onset of the LH surge than during its termination (121). Moreover, when the preovulatory secretion of LH is prolonged by exposure of rats to light, the hypothalamic 5-HT turnover

TABLE 1. Pargyline-induced accumulation of 5-HT in the ME at diestrus and proestrus

Experimental groups	5-HT concentration (ng/mg protein)		5-HT turnover rate (ng 5-HT/mg protein)
Diestrus I			
1400 h saline	11.07 ± 0.57	(11)	
pargyline	19.20 ± 1.12[a]	(8)	8.13 ± 1.69
1630 h saline	11.16 ± 0.75	(12)	
pargyline	17.11 ± 1.26[b]	(12)	5.99 ± 2.01
Proestrus			
1400 h saline	11.50 ± 1.59	(11)	
pargyline	16.28 ± 1.25[b]	(12)	4.78 ± 2.84
1630 h saline	6.42 ± 0.61	(16)	
pargyline	26.67 ± 1.59[c]	(15)	20.26 ± 0.98[d]

ME 5-HT turnover was estimated using the pargyline-induced accumulation of 5-HT (134). Pargyline was administered to rats at 1300 h and 1530 h in diestrus I or proestrus; animals not receiving pargyline were injected with saline (1 ml/kg). Rats were killed 60 min after injections. 5-HT concentrations in MEs were measured by the microradioenzymatic assay of Saavedra *et al.* (135). Values represent the mean ± SEM, and the number of animals is shown in parentheses. Data were evaluated by one-way analysis of variance and the differences between groups were determined by the Keuls multiple range test. [Taken from M.L. Vitale *et al. Neuroendocrinology* 39:136–141, 1984 (118) with permission from the publisher, S. Karger AG, Basel.]

[a] $P < 0.001$ compared to saline-treated rats.

[b] $P < 0.05$ compared to saline-treated rats.

[c] $P < 0.0001$ compared to saline-treated rats.

[d] $P < 0.0001$ compared to 1400 and 1630 h on diestrus I and 1400 h on proestrus.

also remains high. Thus, the time pattern of LH secretion during proestrus positively correlates with an increased activity of hypothalamic 5-HT (121). ME serotonergic metabolism also changes during the estrous cycle. An increase in 5-HT activity occurs in the afternoon of proestrus when the preovulatory surge of LH starts (Table 1 and Refs. 118 and 119), suggesting that ME 5-HT can be involved physiologically in the stimulation of LHRH release, perhaps through an action on axon terminals. The spatial relationship between 5-HT and LHRH immunoreactive fibers in the ME (122) provides neuroanatomical support to such a functional association.

These changes in hypothalamic and ME 5-HT turnover may be a consequence of the increased E_2 and P secretion during proestrus. It has been shown that the brain serotonergic system is more active in female than in male rats (35), most likely, as a consequence of the different ovarian hormone milieu. Moreover, ovarian steroids can amplify the morning-afternoon fluctuation of hypothalamic 5-HT (92) and this could be part of the positive feedback effect of steroids on gonadotropin secretion. Particularly, the serotonergic system appears to be very sensitive to circulating P concentrations (85, 92). The administration of an antiprogesterone drug during the critical period for LH secretion reduces serum P, the preovulatory LH secretion, and the oscillation of hypothalamic 5-HT metabolism (92). Moreover, adrenalectomy and ovariectomy performed in the afternoon of proestrus block the afternoon release of LH, even though serum E_2 concentration had already reached the peak value. P administration restores hypothalamic 5-HT fluctuations and the LH surge (92). Therefore, it is possible than during the estrous cycle E_2 and P act synergistically in order to amplify the fluctuations in hypothalamic 5-HT activity and to integrate it with the neural signal for phasic LH secretion.

5-HT metabolism may also influence other neurotransmitter systems known to participate in the regulation of LH secretion during the estrous cycle. For instance it has been reported that the effects of the opiate and noradrenergic systems (123, 124) or the GABAergic system (125) on proestrous LH secretion may involve an interaction with serotonergic neurons.

B. In vivo effects of 5-HT

The effect of exogenous 5-HT on preovulatory LH is controversial and the final result depends on the dose, route, and timing of administration. Systemic injections of large amounts of 5-HT (50–100 mg/kg) have no effect on preovulatory LH surge when injected in the morning of proestrus or during the critical period (126, 127). However, administered on the afternoon of diestrus II, the same dose of 5-HT blocks the preovulatory LH surge and ovulation (126, 127). The inhibitory effect is overcome by pretreatment with either the serotonergic antagonist, methysergide (126), E_2, or a peripheral vasodilator (127). These results suggest that the inhibitory effect of high doses of 5-HT on LH secretion takes place at a peripheral site, probably the ovary. On the other hand, it has also been shown that the administration of low doses of 5-HT (12.5–25 mg/kg) during the day of estrus elicits a dose-dependent increase of serum LH (27).

Intraventricular administration of 5-HT gave conflicting results. The infusion of 4 μg 5-HT into the third ventricle fails to alter LH secretion at any stage of the cycle (128). The injection of larger amounts of 5-HT (200–400 μg) into a lateral ventricle, at different times during proestrus, does not alter the preovulatory LH surge (127). However, it has been recently shown that the intraventricular administration of 15 μg 5-HT during the critical period for LH secretion blocks the preovulatory surge of the hormone, this effect being blocked by the serotonergic antagonist methysergide (125).

C. Pharmacological studies

There is general consensus that pharmacological manipulations of the serotonergic neuronal activity indicate a facilitatory role for 5-HT on the preovulatory LH surge. Administration of quipazine, a serotonergic agonist, during the proestrous afternoon or evening, stimulates LH secretion in such a way that it advances or prolongs, respectively, the preovulatory LH surge (121). Accordingly, depletion of 5-HT concentration or impairment of 5-HT neurotransmission abolished the preovulatory LH surge. Systemic injection of pCPA blocks the preovulatory surge of LH (4, 104), although pCPA also reduces circulating E_2 levels, indicating that it may have deleterious effects on the ovary (129). The 5-HT antagonists, cyproheptadine or methysergide, given during the critical period block the preovulatory LH discharge (121).

The steroidal background of the animals is critical for the final effect of 5-HT or drugs affecting 5-HT activity on LH secretion. Systemic injection of 5-HT (27) or of its agonist, quipazine (121), does not alter serum LH levels when serum E_2 is low. On the contrary, after the increase of serum E_2 concentration, 5-HT exerts a stimulatory influence on LH secretion (27, 121). These results agree with the stimulatory role of 5-HT in ovarian hormone-treated ovariectomized rats in comparison with the inhibitory effect observed in non-treated animals.

D. Effect of stimulation or lesion of 5-HT neurons

The bulk of the results discussed here indicate that within the hypothalamus and specifically the ME, a period of increased serotonergic activity occurs during the preovulatory surge of LH. Earlier experiments reported that the electrochemical stimulation of the dorsal tegmental area induces ovulation, whereas stimulation of the ventral tegmental area, the MRN, and the periaqueductal gray block ovulation (130). It was later shown that the electrochemical stimulation of the DRN does not have any effect on the proestrous release of LH, whereas the stimulation of the MRN blocks the preovulatory LH release (70). It has also been claimed that the effect of MRN stimulation upon LH secretion is a non-specific response to surgical stress (131). The local injection of pCPA into the MRN or the systemic administration of methysergide prevents the inhibitory effect of the stimulation, suggesting the involvement of a serotonergic component (70). Moreover, it was recently reported that the inhibitory effect of MRN stimulation on the preovulatory secretion of LH involves a 5-HT-GABAergic interaction (125). Damage of the MRN does not affect the preovulatory secretion of LH (70).

Two weeks after the electrolytic lesion of the DRN, rats fail to ovulate and preovulatory LH secretion is reduced (70). This effect seems to be mediated by serotonergic neurons, since injection of pCPA into the DRN during diestrus I and II mimics the effect of the lesion (70). Apparently, serotonergic projections originating in the DRN and innervating the locus coeruleus are involved in this event (123). Lesion of the DRN on diestrus I and the analysis of LH secretion and ME 5-HT during the first proestrus immediately after lesioning (short-term lesion) shows an enhanced preovulatory surge of LH and a fall in ME 5-HT content with no extra decline during proestrus (132). The increased LH secretion in short-term lesioned animals may be due to a transient rise in responsiveness to residual 5-HT in the ME. Chronic damage of the rostral and middle thirds of the DRN performed during diestrus I causes a progressive disorganization of the stages of the cycle: there is an increase in the number of diestrus (leucocytic vaginal smears) and a decrease in the number of proestrus (epithelial cells); the rats resume cyclicity within a month (132). At that moment, animals killed during proestrus show a delay and a decrease in the preovulatory surge of LH. In spite of this diminished surge long-term lesioned rats ovulate normally (132). After the chronic lesion of the DRN, ME 5-HT decreases by 50%. The decline in ME 5-HT content that occurs in control and sham animals during the afternoon of proestrus is no longer observed in lesioned animals (132). The recovery of the cyclic pattern of LH secretion following long-term lesions of the DRN may represent a new steady state achieved by the activation of neural compensatory mechanisms. Altogether, results indicate that the ME 5-HT, which fluctuates during proestrus, originates in the DRN. Moreover, the DRN may be involved in the initiation and amplitude of the preovulatory surge of LH. This effect may be partly mediated by serotonergic neurons whose cell bodies are located in the DRN and whose projections innervate the ME (132).

E. In vitro experiments

ME 5-HT may affect LH secretion in several ways. It may evoke the release of LHRH into the portal circulation, it may modulate the effect of LHRH on the pituitary, or it may have an "LHRH-like" activity. The dynamic incubation of ME and AP from proestrous rats in a serial double chamber system shows a stimulatory role of the indolamine on LH secretion (34, 133). This stimulatory effect only takes place when 5-HT is injected into the chamber containing the ME but not when it is injected into the tube connecting both chambers or into the chamber containing the AP (34, 133). The fact that 5-HT has no effect on the AP indicates that is not an LHRH-like factor or a modulator of LHRH activity. It is interesting to point out here that the 5-HT content of the AP does not change during the cycle whereas that of ME falls during the onset of the preovulatory LH surge (118). The addition of 5-HT into the ME chamber evokes the secretion of LHRH in a dose-dependent manner; the effect is blocked by cyproheptadine and methiothepin (Fig. 1 and Ref. 34). The stimulatory effect of 5-HT on *in vitro* LH release is greater in tissues obtained from female than from male rats (34). This datum is in agreement with the observation that 5-HT exerts a stimulatory effect upon LH secretion in the presence of high levels of estrogens (27, 67, 99).

VII. Final Remarks

Successful secretion of LH depends on the balanced interrelationship between the brain, the pituitary, and the gonads. In spite of the efforts made during the past 10 yr to assess the role of 5-HT in LH secretion, experimental results were discordant and no concluding ideas were raised. As discussed in this review, the discrepancies among the experimental data may result from different sources. First, it is evident that the final effect of 5-HT on the release of LH depends on the model of LH secretion. On the other hand, it is also clear that different serotonergic circuits may mediate opposite effects on LH secretion, even within the same experimental model. These arguments are particularly evident when comparing the data obtained in intact rats during the estrous cycle with those obtained in ovariectomized rats primed with ovarian hormones. The effect of 5-HT on the preovulatory LH surge is less conflictive than on the ovarian-induced LH discharge. Probably, in ovariectomized rats, subtle changes in the dose of E_2 or P and/or in the schedule of their administration may alter the response of the LHRH releasing

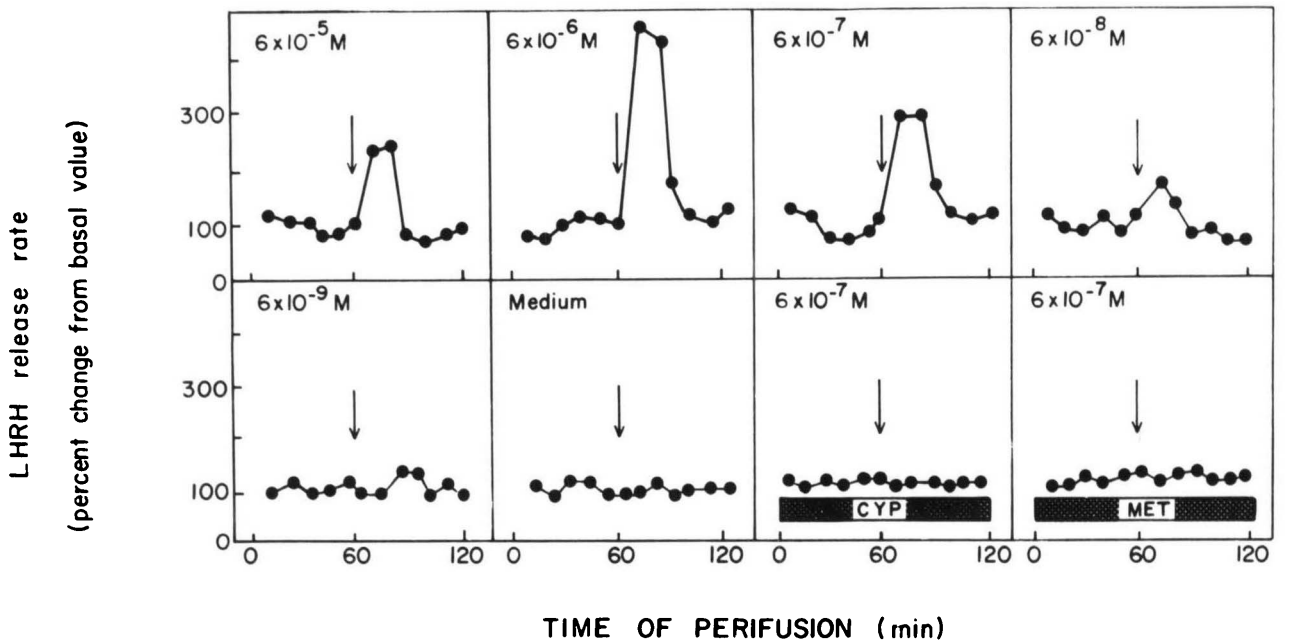

FIG. 1. Effect of 5-HT on the *in vitro* rate of release of LHRH from ME from proestrous rats. Five MEs from rats killed between 1200 and 1300 h on proestrus were incubated together in a perifusion system at 37 C (Vitale *et al.*, 1985). After a period of stabilization a pulse of 5-HT (60, 6.0, 0.6, 0.06, or 0.006 μM, final concentration) was introduced (*arrows*) into the chamber. Vehicle alone was injected as control. Stimulation of LHRH release was abolished when serotonergic receptor antagonists, cyproheptadine [5 μM (CYP)] or methiothepin [5 μM (MET)] were included in the perifusion fluid. The *horizontal bars* represent the period of treatment with the antagonists.
LHRH released into the incubation medium was measured by RIA. Each *point* represents the percent change from mean basal values (first six samples = 100%). [Reproduced with permission from ML Vitale *et al.*: *J Endocrinol* 111:309–315, 1986 (34).]

apparatus to 5-HT. It is obvious then that experimental data must be analyzed and evaluated with caution, taking into account not only the brain area under study but the experimental paradigm of LH secretion as well.

The role of 5-HT in LH secretion in male rats is still uncertain. The bulk of evidence indicates a lack of participation, although recent results suggest that 5-HT may have a weak permissive influence. In any case, much work needs to be done regarding the implication of 5-HT and different serotonergic pathways in the control of LH release in males. Moreover, the involvement of 5-HT in the generation or in the modulation of the pulsatile pattern of LH secretion in males should be investigated.

A stimulatory effect of 5-HT on the phasic release of LH is evident in female rats. This positive effect has a critical period and is linked to blood ovarian steroid levels. In intact rats, 5-HT's facilitatory influence on the proestrous LH surge is exerted, at least in part, within the hypothalamus, and more precisely, at the level of the ME. The fluctuating pattern of neuronal 5-HT activity observed in these areas is likely to be a component of the neurogenic signal for the preovulatory LH-RH release. The fact that serotonergic inputs to the ME modulate LH-RH secretion is important since this structure constitutes the anatomical link between the brain and the pituitary. However, the intrinsic mechanism of the stimulatory influence of 5-HT on LH release in female rats is not known. Further research will clarify whether 5-HT acts directly on the peptidergic neuron or influences other neurotransmitters or neuropetides involved in the control of the preovulatory surge of LH. Moreover, the implication of serotonergic neurons or terminals localized in other hypothalamic or even nonhypothalamic brain areas also awaits additional studies.

VIII. Summary

The involvement of serotonin (5-HT) in the regulation of LH secretion is discussed on the basis of experimental and physiological models. The role of 5-HT on low amplitude pulsatile LH release in male rats is not yet clear, in spite of the fact that recent results suggest a weak permissive role. In ovariectomized rats, 5-HT expresses a negative influence on the increased rate of LH release, which is converted into a stimulatory effect by pretreatment of the animals with E_2. Moreover, in castrated female rats there is a morning/afternoon oscillatory pattern in 5-HT metabolism of brain areas associated with the control of LH secretion including the hypothalamus. In this area, the fluctuation is modulated by E_2 in such a way that the peak of 5-HT neural activity occurs simultaneously with the induced afternoon discharge of LH. On the other hand, P enhances the amplitude of the oscillation of hypothalamic 5-HT metabolism concomitantly with a potentiation of the induced LH surge. This facilitatory role of 5-HT upon phasic LH discharge is also evident in intact female rats. The preovulatory surge of LH is accompanied by an increased hypothalamic and, more precisely, ME 5-HT turnover. Furthermore, 5-HT stimulates *in vitro* LHRH re-

lease from the ME. Serotonergic nuclei located in the brain stem seem to mediate this effect.

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