The Stimulatory Roles of Catecholamines and Acetylcholine in the Regulation of Gonadotropin Release in Ovariectomized Estrogen-primed Rats

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Synopsis

Injections of 2 mg of progesterone into ovariectomized estrogen-primed rats significantly increased serum LH and FSH concentrations 3, 5 and 8 hr later. Receptor blockers of noradrenaline (NA), dopamine (DA) or acetylcholine (ACH), phenoxybenzamine (20 mg/kg body weight), pimozide (1 mg/kg body weight) or atropine (700 mg/kg body weight), respectively, prevented the progesterone-induced gonadotropin release. On the other hand, none of them blocked the gonadotropin release following unilateral electrochemical stimulation (100 μ A for 60 sec) of the medial preoptic area which occurred 0.5 and 1.5 hr later, although pimozide or atropine reduced serum LH concentrations at 4.0 hr after stimulation.

Furthermore, the sites of action of NA, DA and ACH with respect to LH release were examined by intracerebral implantation in ovariectomized estrogen-primed rats. DA or ACH, when implanted unilaterally into the medial preoptic area, induced a significant increase in serum LH 5 hr later, whereas NA decreased LH levels. Implantations of NA or ACH into the bed nucleus of the stria terminalis or the medial amygdala increased serum LH although the effect of NA into the latter was not statistically significant. Only implantations of NA among the three substances into the lateral septum induced LH release.

These results suggest that all of NA, DA and ACH play stimulatory roles in the regulation of gonadotropin secretion, and that there are regional differences of their effectiveness in releasing LH within the limbic-preoptic area.

Since Sawyer *et al.* (1947) presented experimental results suggesting that an adrenergic mechanism plays an important role in the regulation of gonadotropin secretion, it has been long a big subject in the neuroendocrinology of reproduction what chemical substances are involved in the neural transmission for gonadotropin release.

Although there is a general agreement on the distribution of catecholamines in the brain, controversies exist concerning their roles in the regulation of gonadotropin secretion. Sawyer *et al.* (1947) reported

that ovulation following coitus in the rabbit was prevented by prompt intravenous injection of an adrenergic blocking agent, dibenamine. They also demonstrated that intraventricular injection of adrenaline or noradrenaline (NA) induced ovulation in the estrogen-primed rabbit (Sawyer, 1952), but the injection of dopamine (DA) failed to stimulate an ovulatory LH surge and actually inhibited the stimulatory effect of NA (Sawyer *et al.*, 1974). Furthermore, it has been shown that intraventricular injection of adrenaline or NA, but not of DA, was effective in inducing ovulation or LH surge in the proestrous pentobarbital-

blocked rat (Rubinstein and Sawyer 1970), in the ovariectomized steroids-primed rat (Krieg and Sawyer, 1976) and in the rat made anovulatory either by electrolytic lesions in the anterior hypothalamus or by exposure to continuous illumination (Tima and Flerkó, 1974). However, other investigators reported that intraventricular DA injection was effective in stimulating LH secretion in the female (Schneider and McCann, 1970) and male rat (Kamberi *et al.*, 1970), whereas an equivalent amount of NA or adrenaline was ineffective to induce LH release. In addition, such a discrepancy with respect to the stimulatory effect of NA and DA was also observed in *in vitro* studies (Schneider and McCann, 1969; Miyachi *et al.*, 1973; Rotsztein *et al.*, 1977) and in studies using blockers of catecholamines (Kordon and Glowinski, 1969; Kalra and McCann, 1974).

On the other hand, the involvement of a cholinergic mechanism in the regulation of gonadotropin secretion has been suggested in an early experiment in which ovulation was blocked by injection of a cholinergic blocking agent, atropine in the rat (Everett *et al.*, 1949). Furthermore, it was recently shown that intraventricular injection of acetylcholine (ACH) induced a significant increase in serum LH in the male rat (Justo *et al.*, 1975).

In most of the studies cited above, catecholamines or ACH was injected into the third ventricle. Intraventricular injections as well as *in vitro* experiments did not allow any discrete localization of the sites of action of the substances. Consequently, the present study was undertaken in order to examine where NA, DA and ACH are involved in the regulation of gonadotropin release in the limbic-preoptic-hypothalamic system of ovariectomized estrogen-primed rats. The results of the present study seem to partly explain the discrepancies concerning the roles of catecholamines and ACH in the regulation of gonadotropin release.

Preliminary studies have been presented previously (Kawakami *et al.*, 1975 and 1976).

Materials and Methods

Wistar female rats weighing 210–320 g were housed under controlled conditions of light (lights on from 0500 to 1900 hr) and temperature (23–25°C) and given free access to water and laboratory chow. Animals were used two or three weeks after ovariectomy.

Experiment I

In order to examine the involvement of NA, DA and ACH in the regulation of progesterone (P)-induced gonadotropin release, three different receptor blockers were administered to estrogen and P-treated rats. Animals were injected s.c. with 20 µg of estradiol benzoate (EB) at 1200 hr followed by s.c. injections of 2 mg of P 72 hr later. Immediately before the injection of P, an α -adrenergic blocker, phenoxybenzamine hydrochloride (Tokyo Kasei Kogyo Co. Ltd., 20 mg/kg body weight, i.p., in 50% ethanol), a specific dopaminergic receptor blocker, pimozide (Fujisawa Pharmaceutical Co. Ltd., 1 mg/kg body weight, i.p., in 0.1 M tartaric acid), a cholinergic receptor blocker, atropine sulphate monohydrate (Wako Pure Chemical Industries Ltd., 700 mg/kg body weight, s.c., in saline) or saline was administered. Another group was injected with EB but treatments with P and receptor blockers were omitted. Blood samples were taken immediately before injection of blockers and subsequently at 1500, 1700 and 2000 hr for measurement of serum LH and FSH.

Experiment II

In this experiment rats were pretreated with blockers to determine whether NA, DA and ACH participate in the regulation of the gonadotropin release in response to the electrochemical stimulation of the preoptic area. The animals were anesthetized with sodium pentobarbital (30 mg/kg body weight, i.p.). A bipolar concentric stainless steel electrode was stereotaxically oriented into the medial preoptic area (MPO) according to the atlas of Albe-Fessard *et al.* (1966) and fixed to the skull with dental cement. Three weeks after the operative procedures, rats primed with 20 µg EB 70 hr before were administered at 1000 hr with the same receptor blockers as described in Experiment I. Electrochemical stimulation was then performed unilaterally by passing a direct current of 100 µA for 60 sec through stimulating electrode between 1100 and 1200 hr. Blood samples were taken immediately before stimulation and subsequently at 0.5, 1.5 and 4.0 hr after electrochemical stimulation.

Experiment III

This experiment was undertaken in order to determine the effective substance(s) among NA, DA and ACH for stimulating LH release when they are implanted into the various parts of the limbic-preoptic system. The limbic-preoptic system has been shown to be involved in the stimulatory feedback action of estrogen and P on gonadotropin release in ovariectomized EB-primed rats (Kawakami *et al.*, 1978a and b). A double cannula was utilized for the intracerebral implantation of catecholamines and ACH as described elsewhere (Kawakami *et al.*, 1978a). A stainless steel tube as an outer cannula was stereotactically oriented unilaterally into the MPO, the bed nucleus of the stria terminalis (BST), the medial amygdala (m-AMYG) or the lateral septum (1-SEPT) under pentobarbital anesthesia. Three weeks after the implantation of the guiding outer cannula, animals were primed with 20 μ g of EB at 1200 hr and intracerebral implantations of the inner cannula were performed 72 hr later. For packing the substances, the inner cannula was tapped twenty times into a small amount of crystal of NA (1-noradrenaline bitartrate, Wako Pure Chemical Industries Ltd.), DA (dopamine hydrochloride, *ibid*) or ACH (acetylcholine hydrochloride, Dai-ichi Pharmaceutical Co. Ltd.) on a glass slide and thereafter inserted through the outer cannula into the brain without anesthesia. The first blood sample was taken 30 min before implantation, and the remaining sample was taken 5 hr after implantation.

Blood samples of 0.5 ml were taken by heart puncture under light ether anesthesia, and the serum was separated by centrifugation and stored at -20°C until assayed. Serum LH and FSH were measured by radioimmunoassay kits supplied by the NIAMDD, U.S.A. The method was principally based on the double antibody procedure described in the instructions of the kits. The referential standards were NIAMDD rat LH RP-1 and NIAMDD rat FSH RP-1. Serum LH and FSH concentrations were expressed in terms of NIH-LH-SI and NIH-FSH-SI, respectively.

At the time of sacrifice, the brains were perfused with 10% formalin. The sites of implantations and electrochemical stimulation were microscopically identified in tissue slices.

The data were statistically analyzed using Student's *t*-test for comparison of means between different groups and the paired *t*-test for comparison of means of different time within the same group.

Results

Experiment I

As shown in Fig. 1, EB priming induced a slight and transient increase in serum

LH concentration 3 and 5 hr later ($p < 0.01$), whereas in animals injected with 2 mg P following EB priming, there was a marked elevation in serum LH 3 ($p < 0.001$) and 5 hr later ($p < 0.02$). The elevated concentrations declined 8 hr later, but still remained significantly higher levels ($p < 0.02$) when compared with those obtained from the animals without P injection. Injections of phenoxybenzamine or atropine immediately before P injection completely blocked the P-induced LH release ($p < 0.02$). Pimozide also prevented the LH release significantly 3 ($p < 0.01$) and 5 hr later ($p < 0.05$), but the significant difference of LH levels between pimozide-injected group and saline-injected control group could not be observed at 8 hr after P injection ($p > 0.10$).

Fig. 1. Effects of adrenergic, dopaminergic and cholinergic receptor blockers on progesterone (P) induced LH release in ovariectomized estradiol benzoate primed rats. Broken line represents the mean of concentrations of animals primed with estradiol benzoate but not treated with P and any blockers. Phenoxybenzamine (20 mg/kg body weight), pimozide (1 mg/kg body weight), atropine (700 mg/kg body weight) or saline was injected immediately before injections of 2 mg P at 1200 hr. Each point represents mean and vertical bar, SEM. The number of animals is given in parentheses.

Although there was a gradual increase in serum FSH in EB-primed rats, P injections induced a further significant increase in serum FSH 3, 5 and 8 hr later ($p < 0.02$) (Fig. 2). Among injections of receptor blockers, only atropine significantly suppressed the P-induced FSH release 3 hr later ($p < 0.05$). At 5 and 8 hr after injections of blockers, FSH values tended to rise, but they were significantly lower than those of saline-injected control group ($p < 0.05$).

Experiment II

Sham stimulation slightly increased serum LH at 0.5 and 1.5 hr later ($p < 0.05$), but did not alter serum FSH at any time. Electrochemical stimulation of the MPO (100 μ A for 60 sec) in saline-injected control group produced a dramatic rise in serum LH 0.5 and 1.5 hr later ($p < 0.001$) (Table 1). The elevated serum LH concentrations fell 4.0 hr later, but still remained higher than the prestimulation levels ($p < 0.02$). Serum LH levels before stimulation were not statistically different among the groups

Fig. 2. Effects of adrenergic, dopaminergic and cholinergic receptor blockers on P-induced FSH release in ovariectomized estradiol benzoate-primed rats. Each point represents mean and vertical bar, SEM. The number of animals is given in parentheses.

Table 1. Effects of adrenergic, dopaminergic and cholinergic receptor blockers on gonadotropin release induced by electrochemical stimulation of the medial preoptic area in ovariectomized estradiol benzoate-primed rats.

Hr after stimulation	Sham stimulation (4) ^b	Preoptic electrochemical stimulation			
		Salinea (9)	Phenoxy-benzamine (6)	Pimozide (6)	Atropine (5)
Serum LH					
0	1.00±0.18 ^c	1.17±0.25	2.15±0.47	1.12±0.27	0.98±0.15
0.5	2.42±0.21	8.51±1.08	10.81±1.29	10.40±2.02	6.21±0.51
1.5	1.98±0.14	7.16±1.10	10.46±2.12	8.33±2.93	4.79±0.89
4.0	1.25±0.17	4.48±0.96	4.34±1.06	1.55±0.14*	1.64±0.17*
Serum FSH					
0	1.70±0.12 ^d	1.78±0.15	1.71±0.09	1.68±0.24	1.87±0.08
1.5	1.68±0.14	2.40±0.18	2.45±0.19	2.23±0.16	2.36±0.19
4.5	1.73±0.21	2.58±0.22	2.66±0.16	2.20±0.11	2.35±0.10

a Phenoxybenzamine (20 mg/kg body weight), pimoziide (1 mg/kg body weight), atropine (700 mg/kg body weight) or saline was injected between 1 and 2 hr before electrochemical stimulation (100 μ A for 60 sec).

b Number of animals

c Mean ± SEM (serum concentration ng/ml)

d Mean ± SEM (serum concentration μ g/ml)

* $p < 0.05$ vs. saline-injected group

of rats treated with one of the three different blockers, of which the doses were effective in preventing P-induced gonadotropin release. After stimulation of the MPO, serum LH was significantly elevated 0.5 and 1.5 hr later to the levels not different from those of control animals. However, the concentrations of serum LH in animals treated with pimozone or atropine 4.0 hr after stimulation were significantly lower than those in the control group ($p < 0.05$).

On the other hand, rats with electrochemical stimulation of the MPO showed

significantly higher concentrations of serum FSH than those of sham stimulated animals 1.5 and 4.0 hr later ($p < 0.05$) (Table 1). Phenoxybenzamine, pimozone or atropine neither affected the prestimulation levels of serum FSH nor blocked the FSH release following the electrochemical stimulation of the MPO, which occurred 1.5 and 4.0 hr later in control animals.

Experiment III

Although insertions of empty inner cannula into various parts of the brain of EB-primed rats through chronically implanted outer cannula induced a slight increase in serum LH 5 hr later ($p < 0.01$), no significant difference among the sites of implantation could be observed, thus the data were combined into one control group. DA or ACH, when implanted into the MPO at 1200 hr induced a significant increase in serum LH 5 hr later ($p < 0.05$ or $p < 0.01$, respectively), whereas implantations of NA into the same area significantly reduced serum LH concentration as compared to the value obtained from the control group ($p < 0.05$) (Fig. 3). Implantations of NA or ACH, but not of DA into the BST were significantly effective in increasing serum LH ($p < 0.01$), and the concentrations 5 hr later rose above 20 ng/ml. Furthermore, ACH implanted into the m-AMYG induced an LH release ($p < 0.05$), and NA implantations tended to increase LH levels although they were not statistically significant ($p > 0.05$). The LH levels after DA implantations into this area did not differ from those of the control group. Among the implantations of NA, DA or ACH into the 1-SEPT, only NA implantations were effective in increasing serum LH 5 hr later ($p < 0.01$).

The locations of the implantation sites are shown in Fig. 4.

Fig. 3. Effects of intracerebral implantations of noradrenaline (NA), dopamine (DA) or acetylcholine (ACH) on LH release in ovariectomized estradiol benzoate-primed rats. Each bar and the vertical line represent mean and the SEM, respectively. Values were obtained 30 min before implantation (at 1200 hr) and 5 hr after implantation (at 1700 hr). The number of animals is given in the base of each column. Abbreviations used in this figure are BST, bed nucleus of the stria terminalis; m-AMYG, medial part of the amygdala; MPO, medial preoptic area; 1-SEPT, lateral part of the septum.

* $p < 0.05$ vs. control at 1700 hr.

Discussion

Although numerous blockers of catecholamines and ACH are widely used to investigate their effects on brain functions, it should be mentioned, first of all, that none of blockers are completely specific. Indeed, even the receptor blockers employed in the present study have been shown to have central neural actions other than blocking respective receptors (Goodman and Gilman, 1975), but their actions seem to be relatively specific in the dose employed in this study.

The present experiment using monoaminergic and cholinergic blockers demonstrated that NA, DA and ACH, all play, possibly as neurotransmitters, stimulatory roles in the regulation of P-induced gonadotropin release in ovariectomized EB-primed rats. Phenoxybenzamine prevented P-induced gonadotropin release in ovariectomized EB-primed rats. α -Blockers have been shown to inhibit coitus-induced ovulation in the rabbit (Sawyer *et al.*, 1947), spontaneous ovulation in the rat (Everett *et al.*, 1949) and the pulsatile release of LH in the ovariectomized rat (Gnodde and Schuiling, 1976). The same effects have been observed following administration of inhibitors of dopamine- β -hydroxylase, diethylthiocarbamate (Kalra and McCann, 1974) or FLA 63 (Terasawa *et al.*, 1975), which are known to decrease NA levels of the brain without altering DA levels.

On the other hand, pimozide, a potent dopamine receptor blocker, also blocked P-induced LH and FSH release. Among the studies using catecholamine synthesis blockers, there is an apparent disagreement concerning the role of DA in the regulation of gonadotropin secretion. Kordon and Glowinski (1969) reported that blockade of ovulation by the administration of α -methyl-*p*-tyrosine in PMS- and HCG-treated immature rats could be overcome by restora-

Fig. 4. Schematic illustrations showing the positions of intracerebral implantations. Circles, triangles and squares indicate that substances implanted were noradrenaline, dopamine and acetylcholine, respectively. Solid symbols represent effective sites inducing a significant change in serum LH concentration and open ones, ineffective sites. Abbreviations are AC, anterior commissure; ARC, arcuate nucleus; BST, bed nucleus of the stria terminalis; CC, corpus callosum; CD, caudate nucleus; CH, optic chiasma; FX, fornix; 1-SEPT, lateral part of the septum; m-AMYG, medial part of the amygdala; MPO, medial preoptic area; OT, optic tract; PED, cerebral peduncle; VM, ventromedial nucleus; ZI, zona incerta.

tion of both brain NA and DA levels but not by that of NA level only, suggesting the stimulatory involvement of a dopaminergic mechanism in the regulation of LH release. Conversely, other investigators (Kalra and McCann, 1974) have suggested the involvement of NA but not of DA in LH release by applying a similar experimental method to proestrous rats. Furthermore, the inhibitory role of DA in LH release was proposed by other studies using DA release blocker (Gnodde and Schuiling, 1976) and DA receptor stimulants (Fuxe *et al.*, 1975). The results of the present study using receptor blockers support the stimulatory role of DA in the regulation of LH release which is also shown by the present implantation experiments, although the differences of hormonal conditions of animals used should be taken into consideration.

Atropine was shown to completely prevent P-induced gonadotropin release in ovariectomized EB-primed rats in the present study. Blocking effects of atropine on ovulation (Everett *et al.*, 1949) and preovulatory release of LH, FSH and prolactin (Libertun and McCann, 1973) were also observed in proestrous rats. Moreover, it was demonstrated that cholinergic receptor blockers decreased plasma LH in ovariectomized rats (Libertun and McCann, 1973; Gnodde and Schuiling, 1976). Apparently, ACH plays a stimulatory role in the regulation of LH and FSH release.

Monoaminergic or cholinergic blockers, at a dose shown to block P-induced gonadotropin release following electrochemical stimulation of the MPO. On the basis of the present results it would seem most reasonable to propose that none of adrenergic, dopaminergic and cholinergic synapses are involved in mediating the effect of electrochemical stimulation of the MPO to the medial basal hypothalamus, and that electrochemical stimulation activates directly luteinizing hormone-releasing hormone (LH-RH) neurons or LH-RH containing neural

elements in the MPO to induce discharge of LH-RH from the axon terminals in the median eminence into portal vessels. However, Rubinstein and Sawyer (1970) have shown that treatment with reserpine in proestrous female rats elevated the threshold for inducing ovulation by stimulation of the MPO but not of the median eminence-arcuate nucleus region. Furthermore, Kalra and McCann (1973) demonstrated that the elevation in plasma LH following preoptic stimulation of rats was markedly inhibited by pretreatment with α -methyl-*p*-tyrosine or a more specific NA synthesis blocker, diethylthiocarbamate, suggesting that a noradrenergic synapse may lie between the MPO and median eminence. Our results were in disagreement with theirs. It is possible that the discrepancy could be due to the methodological problem, such as the differences in the strength of stimulation of the MPO or the mechanism of action of blockers used. However, the results reported here are consistent with the recent findings on the localization of the cell bodies of LH-RH producing neurons. It was reported that the interruption of connection between the MPO and medial basal hypothalamus was followed by a marked decrease in the content of LH-RH (Weiner *et al.*, 1975; Brownstein *et al.*, 1976) or of immunoreactive LH-RH axons (Sétáló *et al.*, 1976) in the medial basal hypothalamus, suggesting that a considerable portion of the LH-RH neurons terminating in the median eminence have their cell bodies in the extra-hypothalamic regions. Barry *et al.* (1973) demonstrated that cell bodies of LH-RH neurons were found in the septal, supra-chiasmatic and parolfactory region. However, failure of blockers in inhibiting gonadotropin release induced by electrochemical stimulation of the MPO does not completely exclude the possibility of the concurrence of another mediating pathway, which proceeds from the MPO to synapse with LH-RH neurons in the medial basal hypo-

thalamus. And the possible modulatory action of catecholaminergic and cholinergic neurons on the activity of LH-RH neurons by axo-axonic connections in the median eminence (Fuxe and Hökfelt, 1969) is not also excluded.

This is the first study that examined systematically the effects of the administration of NA, DA and ACH into various discrete areas of the rat brain on LH release. Concerning the effects of implantations of catecholamines into the arcuate nucleus-median eminence on ovulation, a few reports are available. Uemura and Kobayashi (1971) have shown that implantations of lower doses of DA into the median eminence, but not into other hypothalamic regions, inhibited ovulation and produced prolonged estrus, and implantations of the higher doses into the same area induced prolonged diestrus. Their suggestion of the inhibitory action of DA at the level of the median eminence was supported by the results of histochemical studies (Fuxe and Hökfelt, 1969; Löfström *et al.*, 1977). In the present study, the inhibitory effect of DA implantations on LH release was not demonstrated in any locations examined, though DA implantation into the arcuate nucleus-median eminence was not attempted, but DA was shown to be effective in inducing LH release when implanted into the MPO. The preoptic anterior hypothalamic region was reported to receive an abundance of fibers from the incerto-hypothalamic dopaminergic system (Björklund *et al.*, 1975). Our results may offer the possible explanation to the controversy concerning the role of DA in the regulation of gonadotropin secretion: Both in *in vitro* experiments and in experiments of intraventricular injection, DA might act on the MPO as well as the median eminence. If neurons in the MPO are mainly exposed to DA, LH surge may occur, and if the arcuate nucleus-median eminence is mainly exposed, LH surge may not be induced or rather sup-

pressed. The concept of this dual regulation of LH release by DA might be also applicable to the explanation of results of Kamberi *et al.* (1970) that intraventricular injections of lower doses of DA induced LH release, but higher doses of DA inhibited the LH release. The site and dosage of administration in *in vivo* experiments and dissected area of hypothalamic fragment in *in vitro* experiments seem essential factors determining whether LH release is stimulated or inhibited.

The present experiment shows that NA implantations into the BST or 1-SEPT have a stimulatory effect on LH release while NA into the MPO has a inhibitory effect. Interestingly, both the BST and 1-SEPT are located within the area indispensable for the stimulatory feedback action of estradiol on LH release in EB-primed ovariectomized rats (Kawakami *et al.*, 1978b). Furthermore, the MPO is a sensitive area to estrone. Thus, estrogen may modulate the responsiveness of NA sensitive neurons in these areas which regulate LH secretion. In addition, the effectiveness of NA implantations into the BST and 1-SEPT should be noted since it may be allowed to postulate that a large dose of NA injected into the third ventricle would have reached these neural structures to release LH in other studies (Sawyer *et al.*, 1974; Kamberi *et al.*, 1970; Tima and Flerkó, 1974). Furthermore, recent histochemical studies have shown that these neural structures are innervated by terminals from the ascending NA pathway originating in the brain stem (Fuxe and Hökfelt, 1969), and that NA terminals in the MPO may be concerned in the regulation of LH release (Löfström *et al.*, 1977). The recent finding that lesions in the ventral NA pathway by microinjections of 6-hydroxydopamine blocked ovulation or P-induced gonadotropin release in ovariectomized rats (Martinovic and McCann, 1977) also suggests that these NA sensitive neural structures including the BST and 1-SEPT

as well as the MPO play important roles in the regulation of gonadotropin secretion.

Effective sites of ACH implantations for inducing LH release were spread extensively. The present results are reasonably explained by the finding that ACH was able to release LH from the anterior pituitary gland incubated *in vitro* in the presence of rat hypothalamic fragments (Fiorindo and Martini, 1975). The same authors also reported that intraventricular injections of ACH induced a significant LH release in male rats (Justo *et al.*, 1975). Additionally, our unpublished data that ACH implantations into the vicinity of one of the circumventricular organs, the organum vasculosum lamina terminalis, in which immunoreactive LH-RH has been found (Zimmerman *et al.*, 1974), significantly increased serum LH suggest that ACH axons may terminate directly on LH-RH containing elements in this region.

The concept is suggested by the present study that the neural regulation of gonadotropin secretion involves several synapses with different neurotransmitters in different areas. This concept may give possible explanations for the discrepancies concerning the role of NA, DA and ACH in the regulation of gonadotropin release. However, in order to clarify at which sites chemical substances are exerting their effects and which substance(s) is finally crucial for inducing LH-RH release, further studies are needed.

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