Comparison of the Effect of Electrochemical Stimulation of the Medial Preoptic Area and the Hypothalamic Arcuate Nucleus upon LH Release in Ovariectomized and Proestrous Rats

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Abstract

The effect of electrochemical stimulation of the medial preoptic area (MPO) and the hypothalamic arcuate nucleus (ARC) on LH release was compared among long-term ovariectomized (OVX), ovariectomized rats treated with estradiol benzoate (OVX-E) and proestrous (PE) rats under pentobarbital anesthesia. The MPO stimulation failed to facilitate LH release in OVX rats whereas the stimulation increased serum LH levels in both OVX-E and PE rats. The ARC stimulation resulted in an elevation of serum LH in OVX and PE rats. The time course of serum LH elevation after the electrochemical stimulation of the MPO or the ARC was different between OVX-E and PE rats. In PE rats serum LH concentrations began to rise 20-30 min after the stimulation of the MPO or the ARC with peak values obtained around 70-90 min. Serum LH started to elevate immediately with peak values in 10-20 min following the ARC stimulation in OVX rats and the MPO stimulation in OVX-E rats. The time courses for changes in serum LH concentrations following LH-RH administration were similar in OVX and PE rats. From these results, the lack of circadian rhythm in serum LH levels in long-term OVX rats may be due to the inability of the MPO to respond to the stimulus generated from circadian pacemaker(s). The cause of the delay observed between the stimulation of the MPO and LH rise in PE rats may not exist in the levels of neural transmission between the MPO and the ARC or the pituitary, but in the level of the mediobasal hypothalamus.

The medial preoptic area (MPO) of the brain is thought to be the center for the cyclic gonadotropin secretion which occurs at a fixed time of day under controlled lighting schedule in female rats (Everett and Sawyer, 1950; Halász and Gorski, 1967). By contrast, the arcuate nucleus (ARC) is thought to be one of the main sites responsible for tonic gonadotropin secretion and its activity is under the influence of the MPO (Halász and Gorski, 1967; Blake and Sawyer, 1974). Serum LH levels increase following ovariectomy and the elevated

levels which are thought to be tonic secretion, exhibit pulsatile patterns, but lack the circadian variation (Blake, 1974; Soper and Weick, 1977). It is necessary to administer estrogen to restore circadian rhythm in LH release in ovariectomized (OVX) rats (Caligaris *et al.*, 1971; Legan and Karsch, 1975). Therefore it may be important to elucidate the function of the MPO in OVX rats for understanding the mechanism by which the cyclic gonadotropin surge occurs.

Electrical or electrochemical stimulation of the MPO or the ARC induce LH release and ovulation in cyclic rats in which spontaneous ovulation has been blocked with

pentobarbital anesthesia (Everett and Radford, 1961; Kawakami *et al.*, 1971; Turgeon and Barraclough, 1973). Although the precise mechanism of action of this procedure is not known, it is generally assumed that electrochemical or electrical stimulation of these areas induce luteinizing hormone-releasing hormone (LH-RH) release by facilitating neural activity of the LH-RH neurons (Terasawa and Sawyer, 1969; Kawakami *et al.*, 1971; Van der School *et al.*, 1978; Higuchi, 1978). There is still dispute on the causal relationship between the stimulated neural activity and the increase in serum LH. Terasawa and Sawyer (1969) observed an immediate increase in the multiunit discharge of the ARC lasting about 30 min after electrochemical stimulation of the MPO. But the serum concentration of LH does not rise until 30 min after electrochemical stimulation of the MPO (Kalra *et al.*, 1971; Turgeon and Barraclough, 1973). The discrepancy between the rapid increase in neural activity and delayed elevation in serum LH levels remained to be explained.

In order to clarify the cause of the delayed increase in serum LH after the MPO stimulation and the function played by the MPO in OVX rats, we attempted to compare the effect of electrochemical stimulation of the MPO and the ARC on LH secretion and LH response to LH-RH between in OVX and proestrous (PE) rats.

Materials and Methods

Female rats of Wistar strain (200–250 g body weight) were raised in a light controlled (lights on 0500 to 1900 hr), air-conditioned ($25 \pm 1^\circ\text{C}$) room and allowed free access to pelleted food (Oriental Kobo, Japan) and tap water. Vaginal smears were checked every morning and rats showing regular 4 day estrous cycles were used for the experiments. Ovariectomy was performed under ether anesthesia 1 to 2 months prior to the electrical stimulation study (OVX). A group of OVX rats were injected s.c. with estradiol benzoate (Teikoku Hormone Mfg. Co.

Japan) 72 hr before the stimulation (OVX-E).

OVX, OVX-E or rats at the proestrous day (PE) were anesthetized with pentobarbital sodium (Abbott, USA; 30 mg/kg BW) administered at 1330 to 1400 hr and about 40–50 min after the pentobarbital injection blood sampling was started at 10 min intervals for 60 min. The animals were then electrochemically stimulated unilaterally through a concentric bipolar stainless steel electrode (inner diameter 0.13 mm, outer diameter 0.4 mm). An anodal current stimulus of 250 or 50 μA was applied to the medial preoptic area (Anterior 8.2, Lateral 0.5, Depth 3.0–3.5; Albe-Fessard *et al.*, 1966) or the arcuate nucleus (Anterior 6.0, Lateral 0.1, Depth 1.0–1.5) for 30 sec using electrical stimulator (Nihon Kohden MSE-3R). Sham stimulated rats were inserted with electrode into the MPO for 30 sec without passing current. The site of stimulation was confirmed histologically from brain sections and distributed within an area 0.5–0.6 mm from the aimed point.

Fifteen PE and 12 OVX rats were treated with either synthetic luteinizing hormone-releasing hormone (Sankyo Pharmaceutical Co., Japan, 0.5 $\mu\text{g}/\text{ml}$ saline solution) or vehicle injected through intra-atrial cannula.

Blood samples were taken through indwelling intra-atrial cannula implanted by 1000 hr in the morning under ether anesthesia. One tenth ml of blood was taken at 10 min intervals and the same volume of physiological saline was replaced. Separated serum was kept at -20°C until assayed for LH. Serum concentration of LH was determined by double antibody radioimmunoassay using anti-ovine LH serum (R-15; generous gift from Dr. G. D. Niswender, Colorado State University, USA) and NIAMDD rat LH radioimmunoassay kit (Niswender *et al.*, 1968). The concentrations of LH in serum were expressed as ng NIH-LH-S1/ml serum. Statistical analysis was performed using one way analysis of variance or Student *t* test.

Results

Effect of electrochemical stimulation of the MPO of the ARC on serum LH in OVX rats

Electrochemical stimulation of the MPO with current strength of 250 μA for 30 sec immediately inhibited pulsatile LH patterns for 20 to min thereafter (Fig. 1). Electrochemical stimulation of the MPO with smaller current strength (50 μA , 30 sec) also induced similar inhibition of the pulsatile LH secretion for 20 to 30 min (data not

Fig. 1. Effect of electrochemical stimulation of the MPO on serum LH levels in three individual OVX rats. Line with vertical bars, which denote standard error of means, represents mean values of 13 rats.

Fig. 2. Effect of sham stimulation of the MPO on serum LH levels in three individual OVX rats. Line with vertical bars, which denote standard error of means, represents mean values of 6 rats.

shown). None of the MPO stimulated rats (13 rats) exhibited an immediate increase of serum LH as those induced by the stimulation of the ARC. The inhibition of LH secretion was terminated by an abrupt resumption of the pulsatile secretory patterns which were of larger amplitude and longer duration compared with those observed during control period (Fig. 1).

Sham stimulated rats also exhibited inhibition of pulsatile patterns of serum LH concentration for 20–30 min in some animals (3 out of 6 rats as indicated individually in Fig. 2). Mean serum LH values at any time following MPO stimulation were not different for the stimulated and sham control groups.

Electrochemical stimulation of the ARC

resulted in a rapid increase in serum LH with maximal levels occurring 10 to 30 min after the stimulation (Fig. 3). The serum LH returned to pre-stimulation levels after 70 min, although some animals showed another small elevation (3 out of 7 rats) about 120 to 170 min after stimulation.

Effect of electrochemical stimulation of the MPO in OVX-E rats

In the OVX rats which had received estradiol benzoate (EB) injection of 2, 10 or 50 μ g, 3 days prior to the stimulation, electrochemical stimulation of the MPO caused an elevation in serum LH with peak values appearing in 20–40 min after the stimulation in all the rats (5–6 rats in each group). More LH was released in the rats treated with 50 μ g of EB than in the rats treated with 2 or 10 μ g EB. This was quantified by comparing sum of LH values in each sample after subtraction of basal values before the stimulation (121.8 ± 13.2 vs. 34.0 ± 5.2 ; $p < 0.001$, Student *t* test). There was no apparent difference in the pattern of LH secretion after the MPO stimulation in the rats treated with 2 and

10 μ g EB. The elevated serum LH concentrations declined more gradually in the rats receiving 50 μ g EB than those receiving 2 or 10 μ g EB (Fig. 4).

Effect of electrochemical stimulation of the ARC or the MPO in the PE rats

In PE rats electrochemical stimulation of the MPO or the ARC with current strength of 250 μ A for 30 sec increased serum levels of LH. LH values began to rise within 20–30 min and continued to increase until 70–90 min after stimulation. Then, the LH levels gradually declined but were still higher than the pre-stimulation levels 3 hr after stimulation (Fig. 5). The time course of the elevation in serum LH concentrations in PE rats after MPO or ARC stimulation was different from those in OVX rats after ARC stimulation or in OVX-E rats after MPO stimulation. The peak values appeared later in PE rats than in OVX rats.

LH response to LH-RH in OVX and PE rats

In order to compare the pituitary res-

Fig. 3. Effect of electrochemical stimulation of the ARC on serum LH levels in OVX rats. Each point and vertical bar represent mean and standard error of means in 7 rats.

Fig. 4. Effect of electrochemical stimulation of the MPO in serum LH levels in OVX-E rats receiving 2 μ g (broken line) or 50 μ g (solid line) estradiol benzoate 72 hr prior to the stimulation. Each point and vertical bar represent mean and standard error of means in 6 rats.

ponsiveness to LH-RH in OVX and PE rats, synthetic LH-RH (500 ng/kg BW) was administered under anesthesia induced by pentobarbital between 1300 and 1345 hr through cannula. Physiological saline (0.2 ml) was administered to similarly treated control rats. Serum LH levels were determined immediately before and at 10 min intervals until 60 min after the LH-RH injection. As shown in Table 1 serum LH levels elevated within 10 min and peaked 10–20 min after LH-RH injection in both OVX and PE rats, but released amount of LH were significantly ($p < 0.01$) greater in OVX than PE rats at every time when blood samples were taken during 60 min after LH-RH injection. In control rats saline induced no apparent changes in serum LH concentrations.

Discussion

Electrochemical stimulation of the MPO failed to induce LH release in OVX rats without estrogen treatment as reported by Clemens *et al.* (1972). This irresponsiveness of the MPO may closely relate to the absence of circadian variation in LH release in spayed rats (Blake, 1974; Soper and Weick, 1977). Estrogen injection restored the ability of the MPO to activate LH release system. This fact is in accordance with the reappearance of phasic LH release which is related to the daily light-dark cycle in estrogen treated OVX rats (Caligaris *et al.*, 1971; Legan and Karsch, 1975).

Fig. 5. Effect of electrochemical stimulation of the MPO (upper panel) or the ARC (lower panel) on serum LH levels in PE rats. Each point and vertical bar represent mean and standard error of means in 6 rats.

Table 1. Change in serum LH concentrations after LH-RH injection

			Serum LH (ng/ml)						
			No. of animals	Time after injection (min)					
				0	10	20	30	40	50
Proestrous	Control	6	0.6±0.2*	0.7±0.3	0.6±0.1	0.4±0.1	0.5±0.2	0.4±0.1	0.5±0.2
	LH-RH	9	0.5±0.1	10.8±1.9	13.2±2.8	10.7±2.4	7.4±1.2	5.3±0.8	5.2±0.7
Ovariectomized	Control	6	8.9±2.0	9.7±2.2	8.7±2.5	8.9±1.9	8.2±2.0	9.0±2.5	8.5±1.7
	LH-RH	6	9.2±1.4	54.5±5.9	52.9±6.9	40.6±5.4	35.3±5.2	26.5±3.4	20.2±2.0

* mean±standard error of means

The suprachiasmatic nucleus is an essential pacemaker of the circadian rhythm in cycle LH release as well as other functions that are influenced by light-dark cycles (Raisman and Brown-Grant, 1977; Moore and Eichler, 1972). The suprachiasmatic nucleus and/or other pacemaker(s) may no longer operate in long-term OVX rats. However, circadian rhythms in other biological systems such as sleep-wakefulness (Kawakami *et al.*, 1978), serum corticosterone rhythm (Hiroshige *et al.*, 1973) are maintained following ovariectomy. Thus it is more possible that the stimulus originating from the neural pacemaker(s) reaches the MPO but this brain region then fails to activate LH-RH release.

There are several possible reasons for the inability of the MPO stimulation to induce LH release in OVX rats which have not been pre-treated with estrogen. Low pituitary responsiveness to LH-RH is one of the possibilities. However, since the sensitivity of the pituitary was not lower in OVX than in PE rats, at least examined with the dose of LH-RH used (500 ng/kg BW), and that electrochemical stimulation of the ARC can induce LH release in OVX rats without estrogen treatment, we assume that MPO stimulation in spayed rats does not increase the amount of LH-RH reaching to anterior pituitary. The inability of the electrochemical stimulation of the MPO to induce LH release in OVX rats may be due to the higher threshold of MPO stimulation required to facilitate ARC neural activity in OVX rats (Kubo *et al.*, 1975). Alternatively, extrahypothalamic area such as hippocampus may increase its inhibitory influence on responsiveness to the stimulation of the MPO (Kawakami *et al.*, 1973) in the absence of ovarian steroids, and the inhibitory stimulus may counteract the facilitatory effect of the electrochemical stimulation of the MPO for LH-RH release. Furthermore, short-loop feedback action by the elevated serum LH on its secretion in

OVX rats may influence on the MPO sensitivity to electrochemical stimulation (Molitch *et al.*, 1976). Another possibility is that there is less readily releasable LH-RH store in the hypothalamus in OVX rats as indicated by lower hypothalamic content of LH-RH in OVX rats than that in intact rats (Araki *et al.*, 1975). But from our study, the mediobasal hypothalamic region seemed to have LH-RH store enough to activate LH release in OVX rats in response to electrochemical stimulation. Further studies are need to clarify which is the main cause of the inability of the MPO stimulation to release LH-RH in OVX rats.

Electrochemical stimulation of the MPO did not facilitate but rather suppressed pulsatile LH release in individual rats. However, mean LH levels following the stimulation were not different for the stimulated and sham control groups. This is probably because that resumed LH pulses following the inhibited period had higher amplitude and longer duration than those observed in prestimulation period. Different periods of inhibition and different time of reappearance of the pulsatile LH release in individual animals make mean LH levels equal to or greater than those in prestimulation period except 10 and 20 min after the stimulation. Moreover, since the inhibition of LH pulse we observed in some sham control rats, this inhibitory effect of the electrochemical stimulation on LH release may be explained by the general stress effect accompanied with the stimulation procedure.

Electrochemical stimulation of the MPO caused an elevation in serum LH in both PE and OVX-E rats, but the time course of the LH rise was not the same for the two groups. Serum LH level began to rise in 20 min and reached its peak 70–90 min after the stimulation in the PE rats, in contrast with OVX-E rats in which it started to increase in 10 min with a peak 20–40 min after stimulation. These results

confirmed relatively long time lag between the stimulation and LH rise in PE rats reported by others (Kalra *et al.*, 1971; Turgeon and Barraclough, 1973). When the larger doses of estrogen were injected, the serum LH concentrations seem to remain at elevated levels for the longer period after the stimulation. But the peak values appeared uniformly within 20–40 min after stimulation irrespective of the estrogen dose. Moreover, ARC stimulation in OVX rats also induced a rapid increase in serum LH, with maximal values occurring 10–30 min after stimulation. In these cases the time course of serum LH elevation following the electrochemical stimulation is similar to that following LH-RH injection, indicating that rapid increase of LH-RH secretion after the stimulation. Electrochemical stimulation in PE rats at the ARC as well as the MPO produced similar delay in increase of LH. Thus the cause of this delay observed following MPO stimulation may not exist in neural transmission between the MPO and the ARC, but rather in the levels from the mediobasal hypothalamus including the ARC to the pituitary. As shown in Table 1 LH-RH injection caused a similar elevation in serum LH levels in both PE and OVX rats indicating that the difference in the time delay may not be due to the difference in the responsiveness of the pituitary. Since there is little elevation in serum LH until LH surge begins (Butcher *et al.*, 1974) in spite of higher pituitary sensitivity to LH-RH before the beginning of LH surge on proestrus (Cooper and McCann, 1975), some active mechanism inhibitory to LH-RH release may exist in the mediobasal hypothalamus.

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