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LHRH Effects on Hippocampal Neurons Are Modulated by Estrogen in Rats

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Abstract. The effect of luteinizing hormone releasing hormone (LHRH) on the neuronal activity of CA1 and CA3 regions of the hippocampus was studied by means of extracellular recordings in the castrated male, intact male, castrated female, and castrated female injected s.c. with 20 μg estradiol benzoate (EB) for 3 days. The basal firing rate of the CA3 neurons of castrated EB-treated female rats was significantly lower than that of the neurons in castrated EB-untreated female rats as well as male rats, either intact or castrated, whereas that of the CA1 neurons was not significantly different from castrated EB-untreated female rats. Iontophoretically applied LHRH predominantly resulted in facilitation of the neuronal firing in most of the animal groups. In the CA3 region of castrated EB-treated female rats, however, inhibition occurred in a large percentage of neurons, and the ratio of facilitation, inhibition and no response was significantly different from that in castrated EB-untreated female rats. In the CA1 region of castrated EB-treated female rats, the predominant effect of LHRH was facilitation and the ratio of facilitation, inhibition and no response was not significantly different from that in castrated EB-untreated female rats. These results suggest that LHRH is involved in increasing the neuronal activity of the CA3 region in the hippocampus, and estrogen reduces this LHRH action.

Key words: Hippocampus, Extracellular recording, LHRH, Estrogen

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RECENT autoradiographic studies have demonstrated that luteinizing hormone releasing hormone (LHRH) has specific receptor binding sites in the rat brain and in its highest concentration in the limbic system, especially the hippocampus, amygdala and septum [1–5]. Quite recently, the expression of LHRH receptor mRNA has also been reported [6]. Electrophysiologically, hippocampal CA1 pyramidal neurons in hippocampal slices were predominantly excited by LHRH [7, 8], suggesting the role of LHRH as a neurotransmitter/neuromodulator in the hippocampus.

On the other hand, many morphological and functional characteristics of the hippocampus are found to be sexually dimorphic or affected by gonadal steroids [9]. Indeed, the presence of relatively high concentrations of estrogen-receptor protein, structurally and functionally indistinguishable from the one found in the hypothalamus, are disclosed by studies utilizing immunoenzymatic and *in situ* hybridization in the hippocampus of adult ovariectomized rats [10–12], although low levels of estrogen receptors have long been reported in this brain region [13–15]. It was observed, further, that hippocampal estrogen receptor concentrations increased during the critical period of neonatal male and female rats, suggesting that the hippocampus is a potential substrate for estrogen-mediated organizational events [16]. There were estradiol effects, for instance, in electrophysiological [17–19] and neurochemical [20–24] functions of the hippocampus. Sex differences in the hippocampal reproductive function have also been reported [25–

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28].

In the present study, to elucidate whether the hippocampal electrophysiological response to LHRH manifests a sex difference or gonadal steroid effects, the basal firing rate and responsiveness to LHRH of hippocampal neurons in the pyramidal cell layer were examined under various hormonal circumstances in both male and female rats.

Materials and Methods

Twenty male and 17 female Wistar rats, weighing 290-440 g (9–14 weeks old) and 240–335 g (9–15 weeks old), respectively, were used. Twelve of the male and all of the female rats were castrated 3–8 weeks before use. Nine of the castrated female rats received a s.c. injection of 20 μg estradiol benzoate (EB) dissolved in 0.1 m*l* sesame oil 3 days before the recording experiment.

For the unit recording, the animals were anesthetized with urethane (1.25 g/kg bw, i.p.), and placed in a stereotaxic apparatus. Extracellular recording in the hippocampus was carried out through a glass micropipette filled with 0.5 M sodium acetate solution containing 2% pontamine sky blue. The recording pipette was attached to a 3-barreled glass micropipette containing solutions of the following substances, LHRH (Peptide Institute Inc., 0.001 M, pH 6.0), sodium L-glutamate (Wako Pure Chemical Industries Ltd., 0.5 M, pH 7.5), and 0.15 M NaCl. The impedance for recording and microiontophoresis barrels was 15–20 and 25–60 MΩ, respectively, and the distance between the tips of the two barrels was about 10 μm. The electrodes were directed toward the CA1 and CA3 regions of the right hippocampus according to the atlas by Albe-Fessard *et al.* [29]. Action potentials were amplified, monitored and recorded by a conventional method. As we intended to record the electrical activity from pyramidal cells, which were shown to be complex-spike cells, neurons having spontaneous activity greater than 10 Hz [30, 31] were excluded. For the microiontophoresis, a constant current supply was used to pass anodal or cathodal currents through at an intensity of 5–50 nA for 30–60 s. To avoid current artifacts, the ejection current was automatically neutralized by passing a balancing counter-current through the NaCl barrel. Sodium glutamate was sometimes applied to facilitate recording from cells having very low spontaneous activity. Changes in the firing rate greater than 30% of that in the preceding period were regarded as significant.

After each recording session, a cathodal current of 20 μA was applied to the recording barrel for 20 min to make a pontamine sky blue deposit. The brain was fixed with 10% formalin and histologically examined in frozen sections 40 μm in thickness. The site of the unit recording was reconstructed on the basis of the dye deposit, trace of the electrode track and microdrive measurements.

The data were statistically analyzed by Duncan's multiple range test following analysis of variance or chi^2-test and differences were considered to be significant at $P<0.05$.

Results

A total of 54, 62, 53 and 66 hippocampal neurons were recorded from intact male, castrated male, castrated EB-untreated female and castrated EB-treated female rats, respectively. Most of the neurons were located in the pyramidal cell layer of CA1 and CA3, and very few were in the granule cell layer of the dentate gyrus area (Fig. 1). They had a low spontaneous firing rate (<5 Hz) and often had a burst-like activity of about 2–10 Hz action potentials (Table 1).

In the male rat, the basal firing rate in either the CA1 or CA3 region was similar in the castrated and intact male rat ($P>0.05$) (Table 1). However, in the female rat, the firing rate in the CA3 region, but not in the CA1 region, was significantly lower in the castrated EB-treated rat than that in the castrated EB-untreated rat. Furthermore, the firing rate in the castrated EB-treated rat was also significantly lower than that in the male rat either castrated or intact ($P<0.05$).

Representative examples of the effect of iontophoretically applied LHRH on spontaneous activity of hippocampal neurons are shown in Fig. 2, and the effects are summarized in Table 2. In either the CA1 or CA3 region of the intact and castrated male rat, the LHRH effect was predominantly facilitation, and the ratio of facilitation, inhibition, and no response was not significantly different ($P>0.05$). LHRH caused predominantly facilitation in the CA3 region of the castrated female rat and

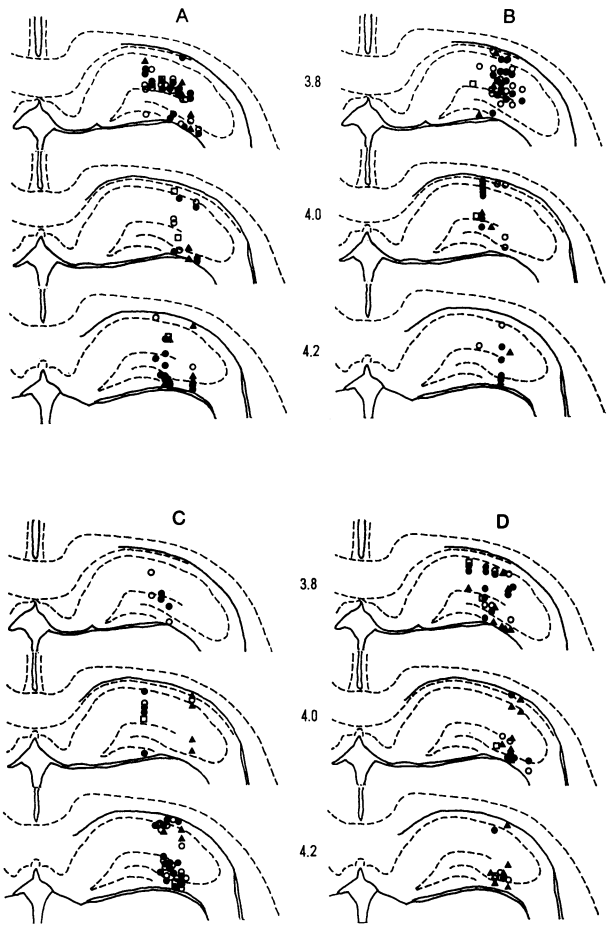

Fig. 1. Schematic illustrations show the site of unit activity tested with microelectrophoresis of LHRH. Neurons were tested in castrated male (A), intact male (B), castrated female (C) and castrated female rats treated with 20 μg estradiol benzoate for 3 days (D). Closed circles, facilitation; open circle, inhibition; closed triangles, no response; open square, only for recording of firing rate, respectively. The numbers 3.8–4.2 refer to the anterior-posterior coordinates [29].

the effect was similar to those in the castrated and intact male rat ($P>0.05$). However, in the CA3 region of the castrated EB-treated female rat, inhibition occurred in a large percentage of neurons, and the ratio of facilitation, inhibition and no response was significantly different from that in the castrated EB-untreated female rat ($P<0.05$). In the CA1 region of the castrated EB-treated female rat, the predominant effect of LHRH was facilitation and the ratio of facilitation, inhibition and no response was not significantly different from that in the castrated EB-untreated female rat ($P>0.05$). However, a considerably large percentage of neurons in the CA1 region showed inhibition, and probably due to this response, the ratio of facilitation, inhibition and no response was significantly different from that in the intact male rat ($P<0.05$).

The hippocampal units have been classified as complex-spike cells and theta cells on the basis of electrophysiological criteria and localization [30, 31]. Complex-spike cells sometimes have a burst of 2–10 action potentials and low (<5 Hz) spontaneous firing rates, whereas theta cells never fire in complex-spike fashion and tend to have high (>10 Hz) spontaneous firing rates. Most complex-spike cells in Ammon's horn are considered to be pyramidal neurons, while the theta cells are complicated. In the present study, most neurons had a low firing rate (<5 Hz) and were located in the pyramidal cell layer of the hippocampus, and therefore it was presumed that we recorded mainly from pyramidal cells.

Discussion

It was observed in the present study that the

Table 1. Basal firing rates of hippocampal neurons

Sex	Treatment	CA1		CA3	
		N[a]	Firing rate (Hz)	N[a]	Firing rate (Hz)
Male	Castrated	31	2.1 ± 0.4	27	2.9 ± 0.5
	Intact	34	1.7 ± 0.3	11	2.8 ± 0.6
Female	Castrated	23	1.9 ± 0.5	27	2.7 ± 0.5
	Castrated+EB[b]	23	0.8 ± 0.3*	42	0.8 ± 0.2**

Data are the mean ± SEM. [a]Number of neurons recorded. [b]Estradiol benzoate injected s.c. for 3 days. *$P<0.05$ *vs.* male rats either intact or castrated. **$P<0.05$ *vs.* castrated untreated female rats, and *vs.* male rats either intact or castrated.

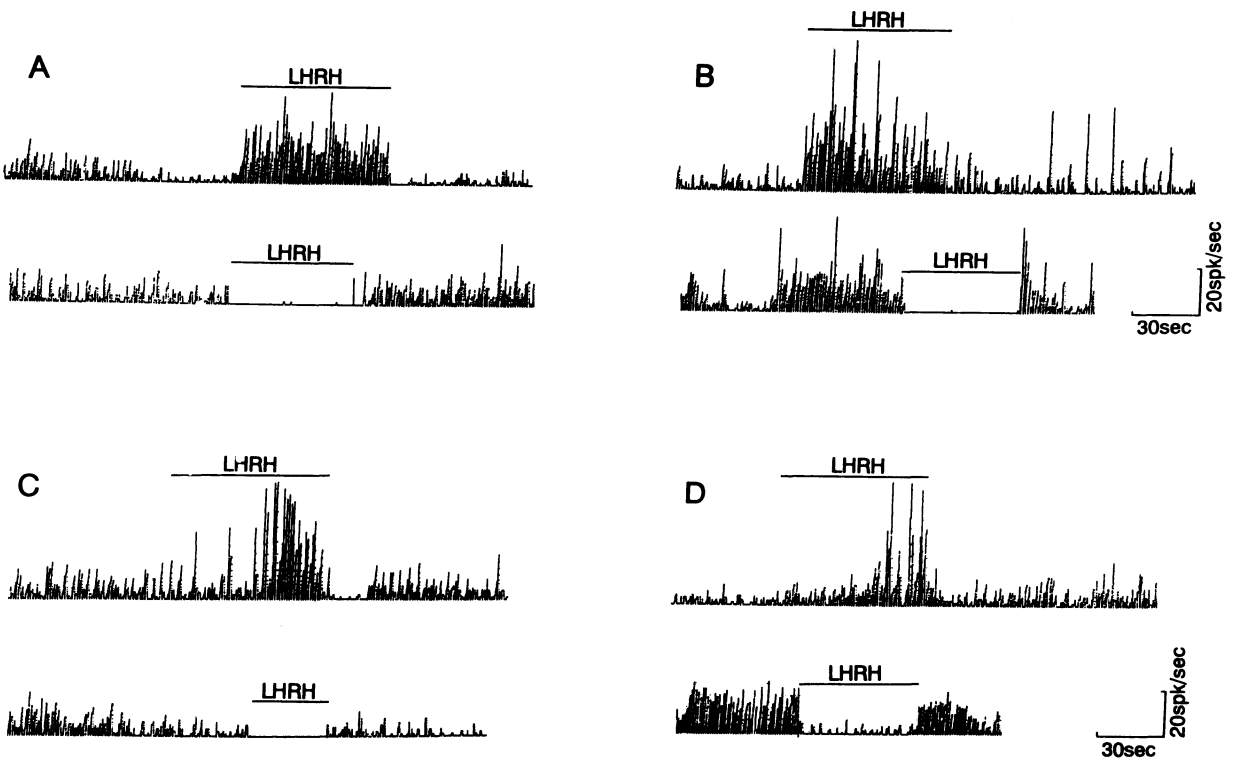

Fig. 2. Firing pattern of neurons in the CA1 and CA3 regions of the hippocampus and LHRH action on it. The bars represent the duration of applications of LHRH. Neurons were recorded in castrated male (A), intact male (B), castrated female (C) and castrated female rats treated with 20 μg estradiol benzoate for 3 days (D).

Table 2. Effects of LHRH on the hippocampal neurons

Sex	Treatment	CA1				CA3			
		N[a]	Facilitation	Inhibition	No response	N[a]	Facilitation	Inhibition	No response
Male	Castrated	32	15 (47%)	4 (13%)	13 (41%)	21	10 (48%)	6 (29%)	5 (24%)
	Intact	34	17 (50%)	2 (6%)	15 (44%)	11	6 (55%)	2 (18%)	3 (27%)
Female	Castrated	22	8 (36%)	5 (23%)	9 (41%)	26	15 (58%)	2 (8%)	9 (35%)
	Castrated+EB[b]	19	10 (53%)*	6 (32%)*	3 (16%)*	32	9 (28%)**	14 (44%)**	9 (28%)**

[a]Number of neurons recorded. [b]Estradiol benzoate injected s. c. for 3 days. *$P<0.05$ *vs.* intact male rats. **$P<0.05$ *vs.* castrated EB-untreated female rats.

firing rate of the CA3 neurons in the hippocampus of the castrated EB-treated female rat was much lower than that in the castrated EB-untreated female rat and the male rat, either intact or castrated. These results provide evidence for the inhibitory modulation by estrogen of the neuronal activity of the CA3 region. In a previous study, however, the inhibitory effect of estrogen on the firing rate of the CA2 and CA3 regions in the castrated EB-treated female rat was not significant when compared to that in the castrated EB-untreated female rat [32]. We did not analyze the estrogen effect on each of the CA2 and CA3 regions separately in this previous study. This is probably the reason why the previous study could not disclose the inhibitory estrogen effect, because the CA3 region seemed specific for this estrogen effect, as seen in the present study. It has been suggested that the firing rate of hippocampal pyramidal cells is controlled by GABAergic inhibitory interneurons that make intensive contacts throughout the pyramidal cell layer [33]. The increase in muscimol binding in the hippocampus after a subcutaneous injection of EB [24] therefore explains due to the

decrease in the basal firing of neurons in these regions by estrogen.

In the present extracellular recording study, LHRH predominantly caused facilitation in CA1 and CA3 neurons in the hippocampus of the male, either intact or castrated, and the castrated EB-untreated female rat. The results were in agreement with those obtained by *in vitro* intracellular studies in which this peptide produced a biphasic, predominantly excitatory response in the hippocampal neurons from the intact male and female rat [7, 8]. Together with the finding of dense LHRH binding sites in the hippocampus [1, 2, 34, 35] and the expression of LHRH receptor mRNA [6], the present *in vivo* results further provide strong evidence that LHRH plays an important role in the regulation of the neuronal activity of pyramidal neurons.

The most interesting finding in the present study is that the effect of LHRH on the CA3 neurons in the hippocampus, but not on the CA1 neurons, is altered in the castrated female rat due to EB treatment. A large percentage of neurons in the CA3 region responded with inhibition by LHRH in castrated EB-treated female rats. It has been shown that estrogen may alter many aspects of hippocampal functions including the number of receptors for LHRH in the ovariectomized rat [20–24, 36]. Further, as observed in the present study, basal activity is lowered by estrogen treatment. Such an alteration in the hippocampal function due to estrogen may underlie the increase in inhibitory response to LHRH, although the precise mechanism has not been determined. In addition, different effects of estrogen on the CA1 and CA3 neurons have been reported; estrogen treatment increased choline acetyltransferase activity [37], progesterone receptor concentration [38], and muscimol binding in the CA1 [23, 24], but not in the CA3 [24, 38]. The results of the present study suggesting a difference between CA1 and CA3 in the hippocampal response to LHRH in the female rat, are therefore understandable.

In conclusion, the present results suggest that there is no sex difference, but there is an estrogen effect, in both the basal firing and the hippocampal response to LHRH. Basal firing rates were very low in castrated EB-treated rats, but high in both male and castrated EB-untreated rats, and major response to LHRH was facilitation in both male and castrated EB-untreated rats. Whether estrogen also reduces the facilitatory effect of LHRH in the castrated male rat was not ascertained in the present study, but it is very likely. It seems that, in the intact female rat, the hippocampal activity is lowered by an increase in estrogen in blood and further in cerebrospinal fluid [2] increasing LHRH just as on the day of proestrus. Considering the inhibitory involvement of the hippocampus in the reproductive functions [39, 40], this alteration in hippocampal neuronal activity is quite reasonable.

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