Sex Differences in the Basal Firing Rate and the Responsiveness to Opioid Peptides of Rat Hippocampal Neurons

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Abstract The basal firing rate and the responsiveness to dynorphin and leu-enkephalin of the neurons in the pyramidal cell layer of the hippocampus were studied in intact male, castrated male, castrated female, and castrated female rats injected s.c. with 20 μ g estradiol benzoate (EB) for 3 days. Most of the neurons studied had low spontaneous firing rate (< 5 Hz) and burst-like activity in all the groups. The basal firing rates in castrated males and females were not different from those in intact males and castrated females treated with EB, respectively. However, neurons in intact and castrated males had significantly higher firing rates than those in castrated females with and without EB treatment, respectively. When effective, both iontophoretically applied dynorphin and leu-enkephalin evoked predominantly excitatory responses. Either castration of males or EB treatment to castrated females did not affect the neuronal responsiveness to these peptides. On the other hand, the responsiveness of neurons to leu-enkephalin, but not to dynorphin, was significantly different between castrated male and female rats. These results suggest that sex steroids do not affect the basal firing rate as well as the responsiveness to opioid peptides of the pyramidal cells in the hippocampus in adult animals. However, sex differences in the basal firing rate and in the responsiveness to leu-enkephalin might account for the differences in hippocampal functions between male and female rats.

Key words: hippocampus, dynorphin, leu-enkephalin, sex difference.

The presence of significant amount of opioid peptides and dense receptor site for them in the hippocampus have been demonstrated (reviewed by STENGAARD-PEDERSEN, 1989). The major opioids found in this brain region are met- and leu-enkephalin and dynorphin (GALL *et al.*, 1981; KHACHATURIAN *et al.*, 1982; ZAMIR *et al.*, 1985). These peptides were found in both cell bodies and nerve

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terminals and suggested to be involved in the neuronal functions of the hippocampus, such as learning, memory and epilepsy, as synaptic neurotransmitters (MAUK *et al.*, 1982; GALLAGHER *et al.*, 1983; SIGGINS *et al.*, 1986).

On the other hand, it has been well documented that hippocampal functions are under the influence of sex steroids. Multiunit activity of the hippocampus shows fluctuations during the estrous cycle in female rats (KAWAKAMI *et al.*, 1973). Seizure threshold in the hippocampus also changes during the estrous cycle and is decreased by estrogen (TERASAWA and TIMIRAS, 1968). Further, there is a sex difference in learning such as radial maze performance (KOBAYASHI *et al.*, 1988). All these studies have suggested that sex steroids affect the excitability and/or chemical sensitivity of hippocampal neurons.

In the present study, to elucidate how the opioid system is involved in the sex steroid-modulation of the hippocampal functions, the basal firing rate and responsiveness to leu-enkephalin and dynorphin of the hippocampal neurons in the pyramidal cell layer were examined under various steroidal circumstances in both male and female rats.

MATERIALS AND METHODS

Twenty-two male and 23 female Wistar rats, weighing 280–510 g (9–17 weeks old) and 280–400 g (12–19 weeks old), respectively, were used. Ten of the male and all of the female rats were castrated 4–10 weeks before use. Eleven of the castrated female rats received s.c. injection of 20 μ g estradiol benzoate (EB) dissolved in 0.1 ml sesame oil 3 days before the experiment.

For the unit recording, animals were anesthetized with urethane (1.2 g/kg, 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 4-barreled glass micropipette containing solutions of the following substances: leu-enkephalin (Peptide Institute Inc., 0.9 mM, pH 4.7), dynorphin (Peptide Institute Inc., 0.3 mM, pH 6.8), sodium L-glutamate (Wako Pure Chemical Industries Ltd., 0.1 M, pH 7.5), and 0.15 M NaCl. The impedance for recording and microiontophoresis barrels was 15–20 and 25–60 M Ω , respectively, and the distance between the tips of the two barrels was about 10 μ m. The electrodes were directed toward the pyramidal cell layer in the CA2 and CA3 areas of the right hippocampus according to the atlas by ALBE-FESSARD *et al.* (1966). Action potentials were amplified, monitored and recorded by means of a conventional method. For the microiontophoresis, a constant current supply was used to pass anodal or cathodal currents at an intensity of 10–50 nA for 30–50 s duration. 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 $20\mu\text{A}$ was given 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 section $40\mu\text{m}$ in thickness. The site of unit recording was reconstructed based on the dye deposit, trace of the electrode track and the microdrive measurements.

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

RESULTS

A total of 72, 64, 67 and 95 neurons were recorded from intact male, castrated male, castrated female, and castrated female rats treated with EB, respectively. In the present study, as we intended to record electrical activity from pyramidal cells, neurons having spontaneous activity greater than 10 Hz (FOX and RANCK, 1981; PANG and ROSE, 1989), or not located in and around the pyramidal cell layer were excluded. Most of the neurons recorded had a low spontaneous firing rate ($< 5\text{ Hz}$) and often showed a burst-like activity of about 2–10 action potentials (Fig. 1).

The basal firing rate of the hippocampal neurons in castrated male rats was not significantly different from that in intact male rats (Table 1). In castrated female rats, EB treatment did not affect the firing rate of the neurons studied. However, the firing rates in intact and castrated males were significantly higher than those in castrated females with and without EB treatment, respectively.

The representative effects of iontophoretically applied dynorphin and leu-enkephalin on spontaneous activity of hippocampal neurons are shown in Fig. 1, and the neuronal responsiveness to these peptides is summarized in Table 2. The majority of neurons in all the groups of animals responded to neither dynorphin nor leu-enkephalin. When effective, either peptides evoked predominantly excitatory responses. Only a small portion of neurons (8–13%) responded to both peptides in all the groups. Castration did not affect the responsiveness of the hippocampal neurons in male rats to dynorphine and leu-enkephalin. Similarly, EB treatment did not affect the neuronal responsiveness in castrated female rats to either peptides. However, the responsiveness of neurons, i.e., the ratio of facilitation, inhibition and no response, to leu-enkephalin, but not to dynorphin, in castrated female rats was significantly different from that in either castrated or intact male rats. Especially, the responsiveness to the inhibitory action of leu-enkephalin was significantly higher in castrated female rats.

DISCUSSION

Due to the heterogeneity of the hippocampal neurons, it is rather difficult to identify and record from the specific population of the neurons *in vivo*. However,

Fig. 1. Firing pattern of neurons in the pyramidal cell layer in the CA2 and CA3 areas of the hippocampus and the effects of dynorphin (DYN) and leu-enkephalin (ENK) on it. The bars and numbers represent the duration and current intensity (nA) of applications of the drugs. Neurons were recorded in intact male (A), castrated male (B), castrated female (C) and castrated female rats treated with 20 μ g estradiol benzoate for 3 days (D).

Table 1. Basal firing rates of hippocampal neurons.

Sex	Treatment	N*	Firing rate (Hz)
Male	Castrated	64	$3.7 \pm 0.2^{a,**}$
	Intact	72	$3.1 \pm 0.2^{a,b}$
Female	Castrated	67	$2.6 \pm 0.3^{b,c}$
	Castrated + EB***	95	2.5 ± 0.2^c

Data are mean $\pm$ S.E.M. * Number of neurons recorded. ** Values having the same superscript are not significantly different ($p > 0.05$). *** Estradiol benzoate injected s.c. for 3 days.

Table 2. Responsiveness of hippocampal neurons to dynorphin and leu-enkephalin.

Sex	Treatment	N*	Facilitation	Inhibition	No response
A. Dynorphin					
Male	Castrated ^{a,***}	57	14 (25%)	5 (9%)	38 (67%)
	Intact ^a	53	14 (26%)	3 (6%)	36 (68%)
Female	Castrated ^a	33	8 (24%)	1 (3%)	24 (73%)
	Castrated + EB ^{a,***}	51	9 (18%)	3 (6%)	39 (76%)
B. Leu-enkephalin					
Male	Castrated ^a	57	14 (25%)	4 (7%)	39 (68%)
	Intact ^a	51	14 (27%)	2 (4%)	35 (69%)
Female	Castrated ^b	57	16 (28%)	13 (23%)	28 (49%)
	Castrated + EB ^{a,b}	66	19 (29%)	10 (15%)	37 (56%)

* Number of neurons recorded. ** Responsiveness of neurons in the treatment group having the same superscript is not significantly different ($p > 0.05$) to each drug. *** Estradiol benzoate injected s.c. for 3 days.

it has been demonstrated that pyramidal cells have low spontaneous activity and sometimes discharge in bursts (complex spikes), while interneurons tend to have high spontaneous firing rates (> 10 Hz) and never discharge in complex spikes (FOX and RANCK, 1981; PANG and ROSE, 1989). Thus, although we cannot exclude the possibility that some of the neurons recorded in the present study were interneurons, the majority of the neurons studied were presumed to be the pyramidal cells on the basis of low spontaneous discharge rates (< 5 Hz), burst-like activity, and also relative localization of the neurons recorded from the dye deposit in the pyramidal cell layer in CA2 and CA3 areas.

Opioid peptides have been shown to act differently on the pyramidal cells in different hippocampal area. For example, met-enkephalin facilitates the excitability of CA1 pyramidal cells: the action is probably indirect by inhibiting the local inhibitory interneurons (HENDERSON, 1983; PANG and ROSE, 1989). On the other hand, it has been reported that enkephalins and dynorphin have both facilitatory and inhibitory effects on CA3 pyramidal cells (MASUKAWA and PRINCE, 1982; IWAMA *et al.*, 1986; CHAVKIN and BLOOM, 1986). In the present study, when effective, both dynorphin and leu-enkephalin had predominantly facilitatory effects on the neurons in CA2 and CA3 areas in all the experimental groups, though it was unknown whether the actions were direct or not. A principal role of opioid peptides in the hippocampus may be the facilitation of the excitability of the pyramidal cells.

Although it has been shown that sex steroids affect the electrical activity and some functions of the hippocampus (TERASAWA and TIMIRAS, 1968; KAWAKAMI *et al.*, 1973; KAWAKAMI and TERASAWA, 1973), castration of males and estrogen treatment of castrated females did not have any effects on the basal firing rates and the responsiveness to opioid peptides of the neurons studied. This may be consistent

with the finding by TEYLER *et al.* (1980) that field potentials recorded *in vitro* from the CA1 pyramidal cells in male and female rats were not affected by testosterone and estradiol, respectively. These observations suggest that sex steroids act on hippocampal neurons other than pyramidal cells or the action of sex steroids on the hippocampus is indirect. In support of the latter notion, the hippocampus has virtually no receptor sites for sex steroids (PFAFF and KEINER, 1973).

The major finding of the present study was that the basal firing rates and the responsiveness to leu-enkephalin of the presumed pyramidal cells in the CA2 and CA3 were significantly different between male and female rats irrespective of the presence of sex steroids. Sex differences in the hippocampus in relation to the reproductive function have been extensively studied. For example, hippocampal stimulation increased serum leuteinizing hormone (LH) in castrated males and androgenized females while it decreased serum LH in castrated females (KAWAKAMI and TERASAWA, 1972, 1973). Hippocampal ablation enhanced LH release in females but abolished the increase in LH in castrated males during immobilization stress (KAWAKAMI *et al.*, 1974). Further, hippocampal lesions delayed puberty in males while it advanced in females (KIMURA *et al.*, 1980). Sex difference in learning activity, which is probably concerned with hippocampal function, has been also reported (KOBAYASHI *et al.*, 1988). Although the mechanism underlying the sex difference in these hippocampal functions is still unknown, the difference in the basal firing rate and the responsiveness to opioid peptides of pyramidal cells shown in the present study could be involved in it, since the brain opioid system has been shown to be closely related to the reproductive function (KALRA and KALRA, 1984; KIMURA *et al.*, 1991) as well as learning and memory (MAUK *et al.*, 1982; GALLAGHER *et al.*, 1983).

Morphologic differences in the hippocampus between male and female rats have been also well documented (LOY and MILNER, 1980; DIAMOND *et al.*, 1982, 1983). Sex differences in hippocampal functions mentioned above may partially be based on these morphologic differences. Although most of the sex differences in the brain functions and structures have been shown to depend on sex steroids during the postnatal period (FLERKO, 1975; GORSKI *et al.*, 1978), it was reported that morphologic sex differences in the hippocampus were not altered by castration at birth (DIAMOND *et al.*, 1982). To clarify how sex differences in the hippocampal functions are produced, the investigation of the firing rate and the responsiveness to opioid peptides of hippocampal pyramidal cells in neonatally castrated males and androgenized females will be needed.

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