Full Paper

Effects of 5-HT_{1A}-Receptor Agonist, 8-OH-DPAT, and GABA_B-Receptor Agonist, Baclofen, on Lordosis in Female Rats With Lesions in Either the Dorsal Raphe Nucleus or Septum

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Received February 22, 2005; Accepted June 10, 2005

Abstract. The inhibitory effects of 8-OH-DPAT, a 5-HT_{1A}-receptor agonist, and baclofen, a GABA_B-receptor agonist, on lordosis were examined in estrogen and progesterone-treated ovariectomized rats with lesions in either the dorsal raphe nucleus (DRN) or septum and in rats with either sham lesions or no lesions. The first behavior test series was carried out 6 days after implantation of the rats with silicon tubes containing estradiol. Four hours after injection with 0.5 mg progesterone, behavioral tests were performed before and 30 min after an injection with 1 mg/kg body weight 8-OH-DPAT. As a result, the mean lordosis quotient (LQ)s were changed from 100 to less than 20 before and after the injection in all groups. These results suggest that 8-OH-DPAT acts on areas other than the DRN and the septum, leading to a decrease in lordosis. Two weeks after implantation with estradiol, the next behavioral test series was carried out after injection with progesterone. Behavioral tests were performed before and after an injection with 10 mg baclofen. The results showed that the mean LQs decreased after the injection in all groups, but the mean LQ in the DRN lesion group was higher than that in the sham groups. These results indicate that baclofen may act partially on the DRN in inhibiting lordosis in female rats.

Keywords: lordosis, 5-HT_{1A}-receptor agonist, GABA_B-receptor agonist, dorsal raphe nucleus lesion, septal lesion

Introduction

The inhibitory role in regulating female sexual behavior of both serotonergic and gamma-aminobutyric acid (GABA) neurones is well known. These two neurons are thought to influence each other in the regulation of lordosis (1, 2). Injection with the serotonin (5-HT)_{1A}-receptor agonists, (±)8-OH-DPAT (3, 4) and (+)8-OH-DPAT, decreases lordosis, and conversely, injection with an antagonist, WAY-100635, facilitates lordosis (5), indicating that the 5-HT_{1A} receptor is involved in the lordosis-inhibiting mechanism. On the other hand, the GABA_B receptor is responsible for inhibition of lordosis behavior by GABA neurons, because intraperitoneal injection of baclofen (a GABA_B-receptor agonist) suppresses lordosis in estrogen-primed

ovariectomized rats (1, 2, 6).

Two prominent lordosis-inhibiting mechanisms have been reported to exist in the septum and dorsal raphe nucleus (DRN) of rats (7). Destruction or cutting of the lateral septum enhanced lordosis and soliciting behaviors in female rats (8–10). On the other hand, destruction or cutting of the DRN also enhanced these female sexual behaviors (1, 11). Conversely electrical stimulation of the DRN suppressed lordosis in rats (12). Reports that lordosis is enhanced by the serotonin synthesis inhibitor, *p*-chlorophenylalanine (PCPA), in rats (13), but not in rats with DRN lesions (14) suggest that lordosis is inhibited by serotonergic neurons in the DRN.

Serotonergic and GABAergic terminals and/or their receptor-containing cells are seen in these nuclei. The septum has been reported to contain the 5-HT_{1A} receptor (15, 16), GABA neurons (17, 18), and the GABA_B receptor (19) and to receive serotonergic projections from the raphe nuclei (20, 21). In the DRN, a large

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number of serotonergic terminals and the 5-HT_{1A} receptor can be seen (21), suggesting the possibility of extranucleous serotonergic control of the inhibition of lordosis. GABA neurons and the GABA_B receptor also have been found in the DRN (19, 21). Our previous report suggested that the role of the GABA_B-receptor system in the DRN is dependent on the function of the serotonergic system in the DRN, because the inhibitory effect of baclofen on lordosis is attenuated by DRN lesions (1). However the mechanisms of inhibition in the DRN and the septum are thought to be different, because inhibition in the lateral septum, but not in the DRN is released by estrogen (22); moreover, inhibition in the DRN appears to be independent of that in the lateral septum (23).

In this study, to examine the role of the 5-HT_{1A} receptor in the DRN and lateral septum and to confirm the role of the GABA_B receptor in these nuclei in regulating female sexual activity, ($\pm$)8-OH-DPAT or baclofen was injected and lordosis behavior and soliciting behaviors were observed after the DRN or the lateral septum was lesioned in female rats.

Materials and Methods

Female Wistar rats (215–300 g) were purchased from Takasugi Animal Farm (Saitama). The rats were housed under conditions of controlled temperature (23–25°C) and photoperiod (14:10 h, light:dark). Food and water were available *ad libitum*. The present experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in the Human Science Department of Waseda University. All female rats received brain surgery and/or ovariectomy under ether anesthesia.

At the same time as the ovariectomy, the DRN or the septum was lesioned by a radiofrequency lesion generator (RFG-4A; Radionics, Inc., Burlington, MA, USA) using a 0.7 mm diameter electrode. For brain surgery, the rats were fixed to a stereotaxic instrument with an incisor bar set at 3.3 mm below the interaural line. In the dorsal raphe nucleus lesion (DRL) group, the electrode was lowered stereotaxically 6.5 mm from the bregma level at a point 8 mm posterior to the bregma on the midline in 8 rats. In 6 rats, to make the septal lesion (SL), the electrode was lowered stereotaxically 5.2 mm from the bregma level at a point 0.6 mm anterior to the bregma at 0.5 mm right and left from the midline. Current was applied and the temperature at the electrode tip was maintained at 55°C for 1 min. In 5 sham-operated rats (Sham group), the electrode was lowered to the same point as in the DRL group, but the current was not applied. For comparison purposes, 7 ovariectomized

rats were prepared without brain surgery (Control group). One week after the surgery, all rats were subcutaneously implanted with two silicon tubes (inner diameter, 1.57 mm; outer diameter, 3.18 mm; length, 30 mm; Kaneka Medix Co., Osaka) containing estradiol-17 β (Sigma Chemical Co., St. Louis, MO, USA).

In the 1st experiment series, the effect of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, Sigma) on female sexual behavior was investigated in female rats with DRL or SL. Six days after the implantation of estradiol, the first behavioral test was performed 4 h after the injection of 0.5 mg progesterone. In the behavioral test, each animal was placed in a plastic observation cage (40 × 60 × 50 cm) with two vigorous male rats. The lordosis quotient (LQ: number of lordosis reflex / 10 mounts × 100) and the incidence of soliciting behaviors (ear-wiggling and hopping) were recorded. Soon after the behavioral test, all females were injected intraperitoneally with 1 mg/kg body weight 8-OH-DPAT (1 mg/ml saline) and the behavioral test was performed again 30 min after the injection.

In the 2nd experiment series, in order to examine the effect of baclofen (Sigma) on female sexual behavior in female rats with DRL or SL, 7 days after the 1st experiment series, 0.5 mg progesterone was injected and the first behavioral test was performed 4 h later. Soon after the behavioral test, 7 control, 4 sham, 7 DRL, and 6 SL females were injected intraperitoneally with 10 mg/kg body weight baclofen (10 mg/ml 0.2 N HCl) and the behavioral test was performed again 30 min after the injection.

At the end of the test, in order to determine the precise localization of the lesion, a histological examination of the brain was performed. Each brain was removed in the rat with brain surgery and fixed in 10% Formalin solution. About one week later, frozen 100- μ m sections were prepared and then stained with cresyl fast violet. The lesions were evaluated according to the brain atlas of Paxinos and Watson (24).

The differences in the mean LQ between before and after injection in each group were analyzed by the Wilcoxon signed rank test. Differences in the mean LQ among the groups were analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney U-test. The data regarding the incidence of soliciting behaviors were evaluated by means of the chi-square test.

Results

Effects of 8-OH-DPAT on lordosis and soliciting behavior in the first experiment series are shown in Fig. 1. In all groups, including the DRN or septal lesion groups, the mean LQ values after the injection of 8-OH-

Fig. 1. The mean LQs $\pm$ S.E.M. and incidence of lordosis before and after injection with 1 mg/kg body weight 8-OH-DPAT in estrogen-treated ovariectomized rats with a dorsal raphe nucleus lesion (DRL) or a septal lesion (SL). * $P < 0.05$ vs before injection in each group.

Fig. 2. The mean LQs $\pm$ S.E.M. and incidence of lordosis before and after injection with 10 mg/kg body weight baclofen in estrogen-treated ovariectomized rats with a dorsal raphe nucleus lesion (DRL) or a septal lesion (SL). * $P < 0.05$ vs before injection in each group. [#] $P < 0.05$ vs Sham.

Table 1. Effects of 8-OH-DPAT (1 mg/kg body weight) on incidence of ear-wiggling (EW) and hopping (HP) in estrogen-treated ovariectomized rats with dorsal raphe nucleus lesions (DRL) or septal lesions (SL)

Groups	Number of rats	Before injection		After injection	
		EW	HP	EW	HP
Control	7	6/7	4/7	0/7**	1/7
Sham	5	5/5	3/5	0/5*	0/5
DRL	8	5/8	1/8	1/8	0/8
SL	6	6/6	6/6	1/6*	2/6

* $P < 0.05$, ** $P < 0.01$ vs before injection.

Table 2. Effects of baclofen (10 mg/kg body weight) on incidence of ear-wiggling (EW) and hopping (HP) in estrogen-treated ovariectomized rats with dorsal raphe nucleus lesions (DRL) or septal lesions (SL)

Groups	Number of rats	Before injection		After injection	
		EW	HP	EW	HP
Control	7	7/7	4/7	2/7*	0/7
Sham	4	4/4	3/4	0/4*	0/4
DRL	7	5/7	4/7	2/7	2/7
SL	6	6/6	6/6	0/6**	0/6**

* $P < 0.05$, ** $P < 0.01$ vs before injection.

DPAT were lower than the values before the injection ($P < 0.05$). There were no statistically significant inter-group differences in LQ either before or after the injection. The incidence of ear-wiggling after the injection with 8-OH-DPAT was also statistically lower than that before the injection in the control ($P < 0.01$), the sham

($P < 0.05$), and SL groups ($P < 0.05$) (Table 1). Although the incidence of hopping was decreased after the injection in all groups, there was no statistical difference in the incidence before and after the injection.

The effects of baclofen on lordosis and soliciting behavior in the second experiment series are shown in

Fig. 3. Schematic representations of frontal sections of the rat brain at three levels showing outlines of the area of the dorsal raphe nucleus (DRL) or the septal (SL) lesions. AC: anterior commissure, CC: corpus callosum, CG: central gray, CPu: caudate-putamen, MR: median raphe nucleus, xscp: decussation of the superior cerebellar peduncle. The drawings are modified from the rat brain atlas of Paxinos and Watson (1998) (Ref. 24).

Fig. 2 and Table 2. As with 8-OH-DPAT, the mean LQs after the injection with baclofen were lower than before the injection in all groups ($P < 0.05$). There was no statistical difference among the groups in the LQs in the test before the injection. After the injection of baclofen, although the mean LQ in the DRL group was higher than in the other group, a statistical difference was seen only between the DRL and the sham groups ($P < 0.05$). The incidence of ear-wiggling behavior after the injection of baclofen was statistically lower than before the injection in the control ($P < 0.05$), sham ($P < 0.05$), and SL groups ($P < 0.01$). A decrease in the incidence of ear wiggling was not seen after the injection in the DRL lesion group only. Furthermore, only 2 DRL rats showed hopping behavior after treatment with baclofen.

Histological examination showed that the main body of the DRN was destroyed in all rats with lesions, but the rostral and caudal parts of the DRN remained intact (Fig. 3). The lateral area close to the main body of the DRN was lesioned partially. Furthermore, the DRN lesions penetrated to the decussation of the superior cerebellar peduncle, but did not reach the median raphe

nucleus. In the SL group, most of the lateral septal area was destroyed in all rats. In this group, some parts of the medial septal nucleus were damaged.

Discussion

As shown in previous reports (4), 1 mg/kg body weight ($\pm$)8-OH-DPAT was effective in suppressing female sexual activity in female rats in the present results. This confirms that 5-HT_{1A} receptor is involved in the lordosis inhibiting system of the serotonergic neurons.

The DRN plays an inhibitory role in regulating lordosis (1, 11, 12). This inhibition is produced by serotonergic neurons in this nucleus (14). Tritium labeled 8-OH-DPAT has been reported to bind the raphe neurons (25). The 5-HT_{1A} receptor is abundant in the DRN (16). These findings indicate that the mechanism of lordosis regulation in the DRN may include an auto-receptor of serotonin or serotonergic afferent from other raphe nuclei. The 5-HT_{1A} receptor is thought to be involved in those mechanisms. However, the results of the present study showed that the lordosis-inhibiting effect of 8-OH-DPAT is not produced by acting in the DRN, because the inhibitory effect was seen even when the DRN was lesioned. This suggests that the 5-HT_{1A} receptor in an area other than the DRN may be responsible for regulating lordosis behavior and soliciting behavior in an inhibitory fashion.

The lateral septum also strongly inhibits lordosis behavior in female rats (26). Although the septal area also contains 5-HT_{1A} (16) and receives serotonergic axons originating from the DRN (20, 21), it has been reported that facilitatory effects of the DRN lesion on lordosis were observed even if the septal outputs and inputs including serotonergic fibers were interrupted (23). In the present experiment, lesions in the septum had no influence on the inhibitory action of 8-OH-DPAT in the induction of both lordosis and soliciting behaviors. Thus, as with the DRN, the septum can be excluded as the site of action of 8-OH-DPAT in inhibiting lordosis and soliciting behaviors.

The 5-HT_{1A} receptor was found in the ventromedial hypothalamic nucleus (VMH), which exerts a lordosis-facilitating influence (27). Direct application of 8-OH-DPAT into the VMH has been reported to suppress lordosis behavior and the effect was antagonized by injection with a 5-HT_{1A} antagonist, WAY-100135 (28). Direct injection with a toxin for serotonergic neurons into the VMH enhances lordosis (29). From these reports and the present experiments, one of the possible sites of action of 8-OH-DPAT may be the VMH.

In the present experiment, 10 mg/kg body weight

baclofen inhibited lordosis and soliciting behaviors in female rats. This suggests that as a 5-HT_{1A} receptor, the GABA_B receptor is involved in the inhibitory mechanisms in regulating not only lordosis, but also soliciting behaviors. GABA_B receptors are found in the septum (30, 31). Suppression of lordosis and ear-wiggling were seen after the injection of baclofen in females with septal lesions in the present results. Thus, the inputs and outputs of the GABAergic neurons or intranuclear connections of the GABA_B receptor-containing neurons in the septum may not be involved in the inhibitory action of baclofen in regulating lordosis.

In female rats with the DRN lesions, baclofen decreased lordotic activity. This indicates that baclofen acts on areas other than the DRN. On the other hand, the inhibitory effect of baclofen is dependent partially on the function of the DRN because there was a higher mean LQ in the DRN group than in the other groups even after an injection with baclofen in this experiment and baclofen did not cause a decrease in the incidence of soliciting behavior in rats with DRL lesions. This phenomenon is in agreement with a previous report of Kakeyama and Yamanouchi (1), which demonstrated that the GABA_B receptor in the DRN is important in regulating lordosis in an inhibitory fashion.

In the DRN, half of the neurons are serotonergic (21). The DRN contains GABA_B receptors and GABAergic neurons (30, 32, 33). Electron microscopic immunostaining evidence suggests that GABAergic axons terminate at the serotonergic perikarya and dendrites (34). The present results of a low suppressive effect of baclofen on female sexual behavior in the DRL rats suggest that one of the possible sites of action of baclofen is the intranuclear circuit of GABAergic neurons with a GABA_B receptor and/or neurons containing the GABA_B receptor which project their axons to the DRN. On the other hand, it has been reported that direct application of baclofen into the DRN decreased the content of extracellular 5-HT in the DRN (35). The serotonergic terminals contain presynaptic GABA_B receptors (30, 33). The suppressive effect of baclofen on lordosis was decreased by a neurotoxic lesion of the serotonergic terminals (2). These indicate another possibility that GABAergic neurons act on the GABA_B receptor of the extranuclear 5-HT neuron terminal and control the function of the DRN. Furthermore, it is important to note that the coexistence of serotonin and GABA in the neurons of the DRN has been reported (see review 21). Further experiments are needed to clarify the relationships among the 5-HT_{1A}-receptor and GABA_B-receptor systems in the DRN in regulating female sexual behavior of rats.

Acknowledgments

This study was supported by a Grant-in-Aid for scientific research from the Japan Society for the Promotion of Science (17570052) and Grants for Special Research Projects of Waseda University (2005A-605) to KY and by the Academic Frontier Project of MEXT.

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