POTENTIATION OF EJACULATORY ACTIVITY BY MEDIAN RAPHE NUCLEUS LESIONS IN MALE RATS: EFFECT OF p-CHLOROPHENYLALANINE

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Abstract. The effect of radiofrequency lesions in the median or dorsal raphe nucleus (MRL or DRL) on copulatory behavior was examined in sexually inexperienced male rats. Three weeks after castration and the brain surgery, all males were subcutaneously implanted with Silastic capsules containing testosterone. In the first behavioral test, the frequency of ejaculation in the MRL group was significantly higher than that in sham and DRL males, but mount and intromission were not. Seven days after the first test, the second test was carried out after treatments with 100 mg/kg p-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor, or saline daily for 4 days in MRL and DRL males. The frequencies of male sexual behavior in PCPA treated DRL males were higher than those in saline treated DRL males. In contrast, even after treatments with PCPA, male sexual activity in MRL males was comparable to those in saline treated MRL males. These results suggest that serotonergic neurons in the median raphe nucleus play an inhibitory role in the regulation of male sexual activity, especially ejaculation. Furthermore, it can be thought that PCPA acts on the median raphe neurons and facilitates ejaculatory behavior.

Key words: Sexual behavior, Ejaculation, Midbrain raphe nuclei, Serotonin, Male rat

SEROTONERGIC neurons in the central nervous system are thought to exert an inhibitory influence in regulating male sexual behavior and erection, because administration of serotonin synthesis inhibitor, p-chlorophenylalanine (PCPA), potentiates male copulatory activity [1, 25, 30, 31]. Direct application of serotonergic neurotoxin, 5,7-dihydroxytryptamine, into the midbrain tegmentum or the medial forebrain bundle also facilitates copulatory behavior [20, 29]. Conversely, intracranial application of a precursor of serotonin [3] or serotonin per se [13, 33] inhibits sexual behavior in male rats.

A large number of serotonergic neuronal cells exist in the raphe nuclei [11]. The medullary raphe nuclei send their serotonergic axons to the spinal cord. Serotonergic neurons in the raphe obscurus have been reported to be involved in the facilitatory mechanisms of intromission and ejaculation in male rats [35]. On the other hand, serotonergic neural fibers of the dorsal and median raphe nuclei in the midbrain send the fibers to the forebrain, such as the preoptic area and the amygdala, which plays an important role in regulating male sexual behavior [14–17]. The inhibitory role of the mesencephalic raphe nuclei in regulation of ejaculation is mentioned in the review of Ahlenius et al. [2] and a recent report [4], but there is no direct evidence of a relationship between serotonergic neurons and function of the midbrain
raphe nucleus. In this experiment, to clarify this point, the effect of PCPA in the male rats with raphe nucleus lesions on the behavior was examined.

**Materials and Methods**

**Subjects**

Thirty-nine sexually inexperienced male Wistar rats (210-310 g) were kept under controlled light (light : dark=14 : 10 h) and temperature (23-24 °C). Food and water were given ad libitum.

**Brain surgery**

All male rats were castrated and subjected to brain surgery. Lesions of the dorsal raphe nucleus (DRL) or of the median raphe nucleus (MRL) were made with a radiofrequency lesion generator (RFG-4A, Radionics Inc., Burlington, MA) under anesthesia with ketamine HCl and xyladine HCl. Having set an incisor bar of the stereotaxic instrument at 3.3 mm below the interaural line, an electrode (0.7 mm) was lowered 6.7 mm (DRL, 14 rats) or 8.5 mm (MRL, 12 rats) from the skull surface at the midline point 7.8 mm posterior to the bregma. A current was applied and the temperature at the electrode tip was kept at 54 °C for 1 min. In 13 castrated males, the electrode was lowered to the same level as the MRL, but no current was applied (sham group).

**Treatments**

Three weeks after the surgery, all animals received a subcutaneous implantation of 2 Silastic tubes (5 cm in length, No. 603-285, Dow-Corning, Midland, MI) containing testosterone (Sigma, T) under ether anesthesia, according to modified methods by Kusaka et al. [19]. Two preliminary behavioral tests were conducted at 7 and 14 days after T-implantation to facilitate sexual experience. Seven days after the preliminary tests, the first behavioral test was carried out. One week after the first test, MRL and DRL males were subjected to the second behavioral test. Six MRL and 7 DRL males received intraperitoneal injections of 100 mg/kg p-chlorophenylalanine methylester HCl (Sigma, PCPA, dissolved in saline) daily for 4 days from 3 days before the test. Instead of PCPA, saline was injected into 6 MRL and 7 DRL rats. The behavioral test was started 4 h after the final injection of PCPA or saline.

**Copulatory behavior tests**

In each behavioral test, an experimental male was placed in a plastic observation cage (60 x 50 x 40 cm). After 5 min adaptation, a highly estrous female, which was ovariectomized and primed with 20 µg estradiol benzoate 48 h before and 0.5 mg progesterone 3-6 h before the test, was introduced into the cage. Male sexual behavior was measured for 30 min after the introduction of the females. The females were replaced by another one every 10 min to eliminate the influence of partner affinity. The following standard measures of male copulatory behavior were recorded: frequency of mounts (MF) and intromissions (IF) during the period from the female introduction to the first ejaculation or during 30 min if no ejaculation occurred, the MF and IF being calculated for numbers per 5 min, the frequency of ejaculations (EF) over 30 min, and latency of mounts (ML), intromissions (IL) and ejaculations (EL) (time in seconds from the female introduction to the first occurrence of each behavior). When the first ejaculation occurred, interval time from the ejaculation to the next mount or intromission (postejaculatory interval, PEI), was also measured.

**Histology**

After the test, all animals were sacrificed by perfusion of saline followed by 10% formalin through the cardiac artery under pentobarbital overdose anesthesia. The brains were kept in 10% formalin solution for several days. Frozen sections 50 µm thick were made and stained with cresylviolet to identify the precise locations of the lesions.

**Statistics**

Data on frequencies of sexual behavior in the first test were analyzed by two way ANOVA with Duncan New Multiple Range test. For values in the second test, factorial ANOVA (2 groups x 2
injections) and t-test were used. Chi-square test was also used for analysis of incidence of each behavior.

Results

Effects of raphe lesions

In the first test, incidences of mount and intromission were similar in sham and groups with lesions. In the latencies and frequencies of mount and intromission, there were no statistical differences among groups although values for means MF and IF in the MRL group were higher than those in the control and DRL groups (Fig. 1). In contrast, mean EF in the first test in the MRL group were higher than those in the DRL and sham groups (F(2, 36)=5.3, P<.01). Mean EL in MRL males tended to be shorter, although no statistical difference from those in the sham and the DRL groups was yielded.

Effects of PCPA treatments

Results of the second test are shown in Fig. 2. In the saline treated MRL group, means MF and IF were comparable to those in the saline treated DRL group. In the means ML and IL, there are no statistical differences between the groups, although these latencies were shorter than those in the saline DRL group. The means MF and IF in PCPA treated DRL males were higher than those in saline treated DRL males (MF: t=2.43, df=12, P<.05, IF: t=2.91, df=12, P<.05). In the MRL group with PCPA, MF and IF were comparable to those in the saline treated MRL group. The IL in the PCPA treated MRL males were shorter than those in the DRL group with PCPA or saline (Fig. 2, t=2.5, df=9, P<.05) but comparable to that in saline treated MRL males. In ejaculatory behavior, mean EF in the PCPA treated DRL group was higher than that in the saline treated DRL group (Fig. 2, t=2.91, df=12, P<.05). On the other hand, even after treatment with PCPA, the mean MF in the MRL group was comparable to that in the saline treated MRL group and PCPA treated DRL males. The PEIs were similar in all groups in both the first and second tests.

Location of lesions

In the histological examination, location of the lesion was determined according to the brain atlas
of Paxinos and Watson [28]. The DRL was located on the midline at the levels from the posterior end of the oculomotor nuclei to the locus coeruleus (Fig. 3A). The DRL damaged most parts of the dorsal raphe and the adjacent areas. The lesions did not penetrate to the median raphe nucleus. The MRL was located on the midline at the same levels in the anteroposterior axis as the DRL. The MRL

Fig. 2. The incidences, mean frequencies and latencies (sec, ± SEM) of each behavior in the second behavioral test. PEI means post ejaculatory period (sec, mean ± SEM). The animals were injected with saline or PCPA daily for 4 days before the test. DRL, dorsal raphe lesion; MRL, median raphe lesion; *P<.05 vs. saline treated groups.

Fig. 3. Photomicrographs of representative coronal sections of the midbrain in each group (cresyl fast violet). A, median raphe lesion (MRL); B, dorsal raphe lesion (DRL). Bars indicate 1 mm.
was located ventral to the decussation of the superior cerebellar peduncle. The median raphe and its surrounding areas were destroyed by the MRL (Fig. 3B). A part of the decussation of the superior cerebellar peduncle was occasionally penetrated by the lesion. The trace of passing the electrode was seen in the midline of the cortex and the dorsal central gray.

**Discussion**

In the present results, the frequency of ejaculation in the MRL males was higher than those in the other males, but the effects of the MRL on MF and IF were not prominent. These suggest that the median raphe nucleus is involved in the inhibitory mechanism of ejaculatory behavior in male rats, but the dorsal raphe nucleus is not. The results agree with the report of Ahlenius *et al.* [2, 4]. The median and dorsal raphe nuclei contain a large number of serotonergic neural cells which send axons to the forebrain. The serotonergic neural system is thought to play an important role in the inhibition of male sexual activity, because treatment with synthesis inhibitor [1, 25, 30, 31], neurotoxin [20, 29] or antagonist [9] enhances male sexual activity and conversely precursor [3] or agonist [9, 34] suppresses it. In this experiment, the facilitatory effect of median raphe lesions on ejaculation is due to destroying the serotonergic cells in the nucleus, because PCPA could not further potentiate ejaculatory activity in MRL rats. This is direct evidence of the function of the serotonergic neurons in the median raphe nucleus sending inhibitory signals for ejaculation. Since many reports indicate strong potentiation of ejaculation by PCPA in male rats [1, 18, 25, 30-32] and the same effect was seen in DRL males in the present series of experiments, it can be thought that PCPA acts on the neurons in the median raphe nucleus and facilitates ejaculatory behavior in male rats.

In the forebrain, the preoptic area (POA) is the most important neural substrate controlling male sexual behavior, since direct implantation of androgen into this region facilitates the behavior [8, 22] and destruction of it causes a severe and permanent loss of male sexual activity [6, 7, 12, 15, 16, 18, 21] including the erectile function of the penis [23]. The POA contains serotonergic terminals from the midbrain raphe nuclei, especially the median raphe nucleus [5]. Direct infusion of serotonin into the POA inhibited copulatory behavior [33]. An increase in the amount of serotonin metabolite, 5-hydroxyindoleacetic acid, has been reported in male rats immediately after ejaculation [26]. Furthermore, PCPA could not enhance ejaculation in male rats with POA lesions [18]. Thus, the POA is thought to be a possible focus of the serotonergic neurons in the median raphe nucleus in the regulation of sexual activity.

The limbic system is also involved in male sexual behavior regulating mechanisms [27]. Especially the medial amygdala is critical in regulating copulatory behavior, because destruction of this area caused severe impairment of it [10, 14, 15, 17, 32]. The amygdala also contains a large number of serotonergic terminals from the mesencephalic raphe nuclei [5, 24]. As well as the POA, PCPA could not enhance copulatory behavior in male rats with medial amygdala lesions [18, 32]. The medial amygdala may therefore be another candidate for the terminating loci of serotonergic neurons of the median raphe nucleus in regulating ejaculatory behavior. Further experiments are needed to clarify the neural network including the median raphe nucleus for the regulation of ejaculatory behavior in male rats.

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**References**

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