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Facilitation of Muricide by Dorsal Norepinephrine Bundle Lesions in Olfactory Bulbectomized Rats

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OISHI, R. AND S. UEKI. *Facilitation of muricide by dorsal norepinephrine bundle lesions in olfactory bulbectomized rats.* PHARMAC. BIOCHEM. BEHAV. 8(2) 133–136, 1978. — The role of the dorsal norepinephrine bundle in the occurrence of muricide was studied in olfactory bulbectomized rats. Although the incidence of muricide was 30% in the olfactory bulbectomized rat, it significantly increased up to 80% and 90% on the 5th and 10th day, respectively, after dorsal bundle lesion. Cortical norepinephrine content decreased by about 50% after dorsal bundle lesions. These results indicate that the dorsal bundle system plays an inhibitory role in the occurrence of muricide in olfactory bulbectomized rats.

Muricide Olfactory bulbectomy Dorsal bundle lesions Norepinephrine

RECENT studies have suggested a role for endogenous monoamines in the inhibitory control of mouse-killing behavior (muricide). Karli *et al.* [12], Sheard [25] and Miczek *et al.* [17] have reported that a large dose of parachlorophenylalanine (PCPA) reliably induced muricide and massive depletion of brain serotonin (5-HT) in non-killer rats. Moreover, Grant *et al.* [7] found a high incidence of muricide after extensive lesions in the raphe nuclei. However, Vergnes *et al.* [30] and Sheard [25] found no significant correlation between 5-HT depletion and the incidence of muricide after raphe lesions. On the other hand, Avis [1] and Barr *et al.* [2] have suggested that brain catecholamines played an inhibitory role in muricide. This hypothesis is based primarily on the facts that drugs which increase the activity of catecholaminergic neurons in the brain such as tricyclic antidepressants, monoamine oxidase inhibitors and psycho-stimulants are potent and selective inhibitors of muricide [8, 9, 26]. Various experiments have pointed to the amygdala as an important part of the development of muricide [9,11] and the site of action of antidepressants [10]. It has been reported that olfactory bulbectomized rats (OB rats) show muricide [27,29], which is selectively inhibited by antidepressants [15]. Moreover, Pohorecky *et al.* [21,22] reported that brain norepinephrine function might be changed by olfactory bulbectomy, without changes in dopamine and 5-HT. Histofluorescence studies have demonstrated that most of the telencephalic norepinephrine terminals are derived from the ascending dorsal bundle from the locus coeruleus [16,28]. These facts suggest that the dorsal norepinephrine bundle with its terminals in the amygdala plays an inhibitory role in muricide. If so, selective destruction of this nerve bundle may produce or facilitate muricide in OB rats. The purpose of this experiment is to determine the role of the dorsal norepinephrine bundle in the occurrence of muricide.

METHOD

Animals

Thirty-two male rats of the Wistar-King A strain, supplied by Kyushu University Institute of Laboratory Animals, were used. Although this strain has a low incidence of spontaneous killer rats, only non-killer rats were used in the experiment. Body weight was 200–250 g at the beginning of the study. The animals were individually housed in an air conditioned room with a 12 hr light/dark cycle (light on at 08:00). They were given food and water ad lib.

Experimental Procedure

The animals were divided into the following 4 groups: Group OB–DB (N = 12), Group OB–S (N = 10), Group S–DB (N = 5) and Group S–S (N = 5). In the first surgery, each rat in Groups OB–DB and OB–S was subjected to olfactory bulbectomy and the rats in Groups S–DB and S–S underwent sham operations. After 7 days, each rat in Groups OB–DB and S–DB was subjected to dorsal bundle lesions, and the others in Groups OB–S and S–S under-

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went sham operations. The muricide test was performed for 5 min between 10:00 and 11:00 over a period of 27 days after olfactory bulbectomy. Statistical significance of the data was determined using Fisher's exact probability test. On the 28th day the animals were killed for norepinephrine assay.

Surgery

The animals were anesthetized with pentobarbital (40 mg/kg, IP) and placed on a stereotaxic instrument. For olfactory bulbectomy, after a suitable hole was made in the skull just above the olfactory bulb, the dura was cut and the main olfactory bulb was bilaterally removed by suction under direct vision. In sham operations, the same surgical procedure was performed with the exception of olfactory bulbectomy. For dorsal bundle lesions, an unipolar electrode made of stainless steel wire of 0.2 mm in diameter and insulated except for the tip was inserted into the brain according to the stereotaxic atlas of König and Klippel [14] (anterior 1.3 mm, lateral 0.7 mm, ventral 6.3 mm deep from the skull), and a 1 mA d.c. current was applied for 10 sec. Sham operations were performed in the same way without applying electric current. After these operations, each animal was given an intramuscular injection of 150,000 units of procaine penicillin.

Norepinephrine Assay and Histology

Rats were sacrificed by decapitation, the brains were quickly removed and placed on ice. Brains were dissected according to the following procedure: First, the cerebral cortex was dissected by separation, from the corpus callosum and cutting at the sulcus rhinus. Then, two cuts in the coronal plane were made from ventral side of the brain. One was made through the optic chiasma just posterior to the anterior commissure and another at the rostral edge of the mammillary body. The hypothalamus was separated by a cut in the horizontal plane at the level of the anterior commissure. Laterally, the remaining parts of the temporal lobes were trimmed away.

Norepinephrine was extracted in 0.4 N perchloric acid, purified by the alumina adsorption method according to our previous report [23], and quantitated by the trihydroxyindole method of Chang [3]. The data obtained were evaluated using Student's t statistics.

The brainstem regions including the lesion site of the dorsal bundle were soaked in a 10% Formalin for several days. They were then sectioned coronally at a thickness of 50 μ on a freezing microtome, and brain sections were stained with cresyl violet to aid in the identification of the site and extent of the dorsal bundle lesions. Figure 1 shows a representative section of typical dorsal bundle lesions.

RESULTS

The incidence of muricide in each group of rats is illustrated in Fig. 2. None of the rats in Groups S–S or S–DB showed any observable signs of muricide for 27 days after the first surgery. The incidence of muricide in Group OB–S was 30% on Day 6 after olfactory bulbectomy, and then slightly increased up to 50% on Days 14–17, but dropped again to 30% on Day 27. However, Group OB–DB showed a significantly higher incidence of muricide than did Group OB–S on Day 12 ($p<0.05$), and the incidence remained high until Day 27 ($p<0.05$ and $p<0.01$).

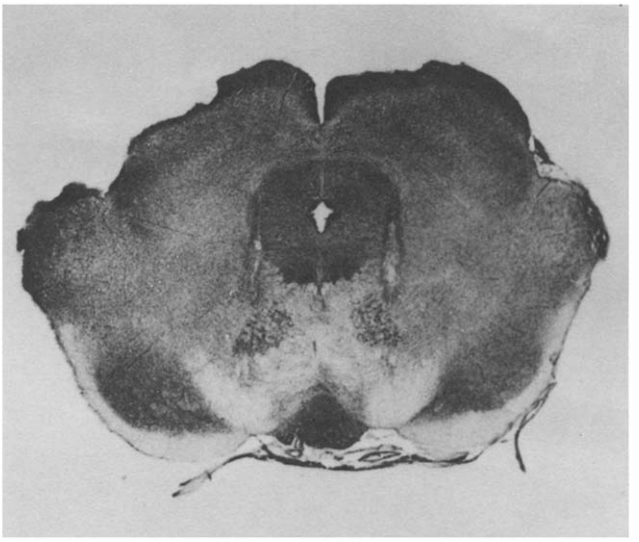

FIG. 1. Representative section of typical dorsal bundle lesions.

The concentrations of norepinephrine in the cerebral cortex and hypothalamus are illustrated in Fig. 3. There was no significant difference in cortical norepinephrine between Group OB–S and Group S–S. But that of Groups S–DB and OB–DB was significantly lower than in the former two groups (t = 6.21, 7.35; $p<0.01$). Hypothalamic norepinephrine was slightly decreased by either OB or dorsal bundle lesions. There were significant differences in hypothalamic norepinephrine concentration between Group S–S and Groups OB–S or OB–DB (t = 3.10, 5.23; $p<0.01$). No significant difference was found between Group S–S and Group S–DB, or among the other three groups.

DISCUSSION

In our preliminary report [19], locus coeruleus lesioned rats showed no significant change in muricide. However, it was reported that dilation of the urinary bladder, urinary retention and hematuria were found after locus coeruleus lesions [20]. These urinary disorders may disturb the role of norepinephrine system in muricide. The results obtained in the present investigation showed that dorsal norepinephrine bundle lesions highly facilitated muricide in OB rats. However, Group S–DB displayed no evidence of muricide during the 20 days after dorsal bundle lesions. Therefore, it is suggested that the dorsal bundle normally plays an insignificant role in the production of muricide, but may become a key factor when muricide is on the verge of development such as in OB rats. DiChiara *et al.* [4] reported that brain 5-HT was an important neurotransmitter for muricide since PCPA increased the incidence of muricide in OB rats and 5-hydroxytryptophan decreased it. However, there may be some qualitative differences between muricide in OB rats and that induced by 5-HT depletion such as after PCPA administration or raphe lesions. Although the rats with brain 5-HT depletion exhibited mouse-killing behavior followed by mouse-eating, most of the OB rats seldom or never showed mouse-eating within the bounds of our observations. The muricide induced by OB and dorsal bundle lesions in this experiment

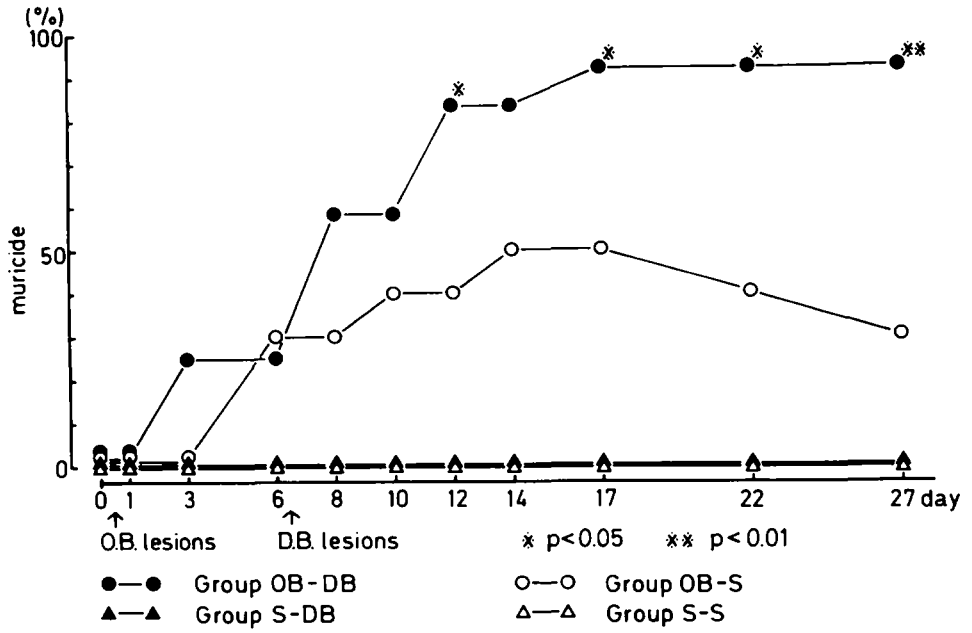

FIG. 2. Effects of olfactory bulb and dorsal bundle lesions on the incidence of muricide in rats. *$p<0.05$, **$p<0.01$ significant difference vs Group OB–S.

was never accompanied by mouse-eating. These facts suggested that muricide in OB rats might be modulated by brain norepinephrine rather than 5-HT.

In a histofluorescence study, it was reported that the major area of termination of the dorsal bundle system in the di- and telencephalon were found to be the thalamus, neocortex and hippocampus; in addition, the ascending locus axons were seen to also project to the tectum, pretectum, metathalamus and amygdaloid-piriform region [16].

These results are in accordance with the autoradiographic study of the projection of the locus coeruleus [24]. Cortical norepinephrine was assayed in this experiment as an index of the extent of dorsal bundle lesions

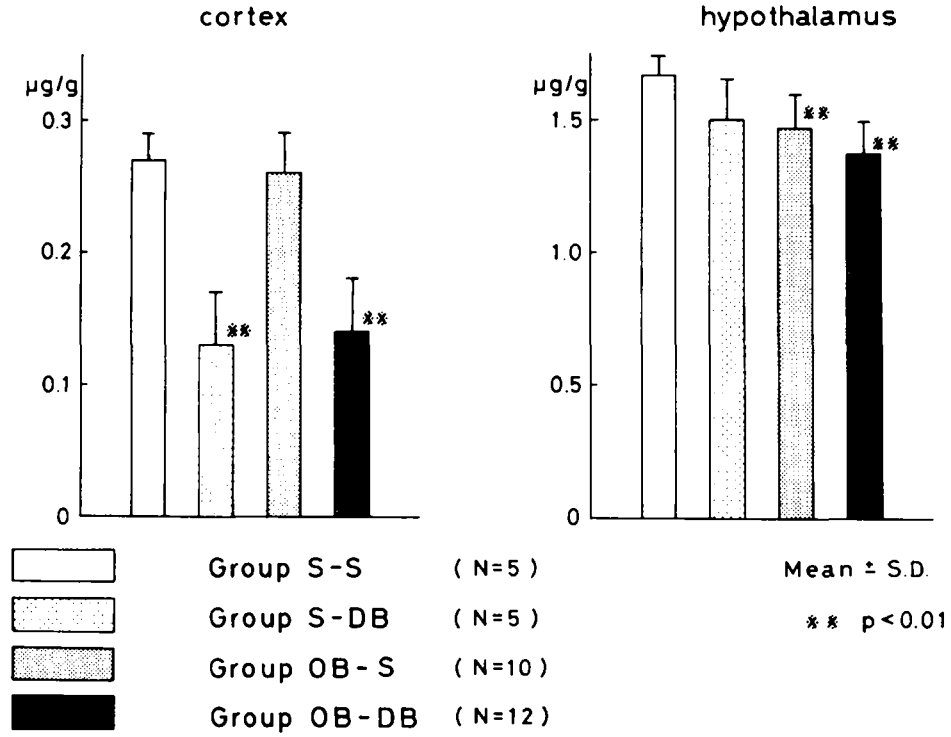

FIG. 3. Effects of olfactory bulb and dorsal bundle lesions on norepinephrine contents in rats. **$p<0.01$ significant difference vs Group S–S.

because of the diffuse innervation associated with this area. Group S–DB and OB–DB showed an approximate 50% decrease in the concentration of cortical norepinephrine. This suggested that more than half of the dorsal bundle was destroyed in this experiment.

Several investigators [6, 13, 21, 22] have reported on the changes in brain norepinephrine after olfactory bulbectomy. Recently, Edwards *et al.* [5] claimed that telencephalic norepinephrine content was minimally affected following the induction of olfactory bulb lesions, but greatly decreased as the region of destruction extended to the olfactory peduncle and olfactory tubercle. The results obtained in the author's experiment showed that cortical norepinephrine content did not change after olfactory bulbectomy, in agreement with the results of Edwards *et al.*

It has been reported that rats showed a high incidence of muricide after the induction of olfactory tubercle lesions [18]. This may be due to widespread damage of olfactory input to the amygdala and a decrease in telencephalic norepinephrine. Since Group S–DB showed no signs of muricide, the olfactory input to the amygdala may be of optimal importance in inhibiting the development of muricide. In addition, since it has been reported that direct injection of antidepressants into the amygdala produced inhibition of muricide [10], the norepinephrine mechanism in the amygdala mediated through the dorsal bundle might play an important role in the antimuricide action of drugs such as antidepressants and amphetamine.

It was concluded that the dorsal norepinephrine bundle may modulate the development of muricide in OB rats.

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