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Regional Changes in Brain Cholinergic Enzyme Activities After Bilateral Olfactory Bulbectomy in Relation to Mouse-Killing Behavior by Rats

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YOSHIMURA, H. *Regional changes in brain cholinergic enzyme activities after bilateral olfactory bulbectomy in relation to mouse-killing behavior by rats.* PHARMAC. BIOCHEM. BEHAV. 15(3) 517–520, 1981.—Bilateral olfactory bulbectomy produced the increased tendency of mouse-killing behavior in nonkiller rats (60% on the 14th day after surgery). Scopolamine hydrobromide (4 and 8 mg/kg, IP) significantly suppressed the killing response in a dose-dependent manner, whereas methylscopolamine nitrate was ineffective. In order to investigate a possible neural mechanisms, choline acetyltransferase (CAT) and acetylcholinesterase (ACh-E) activities were measured in 7 discrete brain areas: cortex, amygdala, hypothalamus, thalamus, tegmentum, hippocampus, and pons plus medulla oblongata. Although the central anticholinergic drug suppressed mouse-killing, no significant difference in either CAT and ACh-E activities was found between the killer and nonkiller rats in any of the brain areas determined in this study. The evidence suggests that the neurochemical findings may not fit the pharmacological findings for supporting a unified cholinergic hypothesis for mouse-killing behavior.

Mouse-killing behavior Olfactory bulbectomy Choline acetyltransferase Acetylcholinesterase Scopolamine Rats

BILATERAL olfactory bulbectomy is known as a strong variable to induce mouse-killing behavior in rats, accompanied by increased irritability and emotionality (e.g. [3,14]). Since these behavioral changes can be selectively suppressed by certain classes of drugs, such as antidepressants and tranquilizing drugs, the killing response and hyperemotionality have been used as an animal model in the preclinical evaluation of psychotropic drugs (e.g. [9,12]); drugs are evaluated for their potential "taming effect." A knowledge of brain pathology following the olfactory bulbectomy is necessary for understanding the drug's effect and also establishing an animal model in psychopharmacological studies. At present, however, the biochemical changes in the brain remain incompletely understood. For the neural mechanisms mediating mouse-killing behavior, pharmacological analyses suggest that cholinergic systems in the brain may participate in the manifestation of the behavior (e.g. [13,16]). Interestingly, drugs which suppressed the killing response, such as antidepressants and tranquilizers, also influence the central cholinergic system [2,4]. The present study was conducted to investigate the correlation between brain cholinergic activity and mouse-killing behavior induced by bilateral olfactory bulbectomy. Choline acetyltransferase and acetylcholinesterase activities were estimated in 7 discrete areas of the brain.

METHOD

Subjects

All animals (n=40) employed were male Wistar rats weighing between 250 and 300 g at the time of surgery. After surgery, the animals were housed individually in conventional metal cages with wire mesh floors, and had free access to food and water. The temperature in the vivarium was maintained at 22 ± 1°C, and a 12 hr light-dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.) was kept constant. Albino mice weighing between 20 and 30 g were used as stimuli for mouse-killing behavior.

Surgical Procedure

Surgery was performed under pentobarbital anesthesia (40 mg/kg, IP). The olfactory bulbs were bilaterally removed by suction through two holes made in the skull just above the bulbs. The sham-operated animals underwent the same procedure as the bulbectomized rats, but without aspiration of the olfactory bulbs. After the experiment, all bulbectomized animals (n=24) were killed and their brains were examined using a binocular dissecting loupe to ensure that the bulbs had been completely ablated. The ablations of the olfactory bulbs in this study were limited to both the main and accessary olfactory bulbs, and did not extend to more central

TABLE 1

BRAIN CHOLINE ACETYLTRANSFERASE ACTIVITY IN RATS AFTER BILATERAL OLFACTORY BULBECTOMY (NMOLES ACETYLCHOLINE SYNTHESIZED/HR/MG PROTEIN)

Brain Areas	Control Group		Bulbectomized Group	
	Intact (n=5)	Sham (n=5)	Killer (n=5)	Nonkiller (n=5)
Cortex	32.44 ± 5.62	32.86 ± 3.00	27.02 ± 2.07	31.49 ± 3.88
Amygdala	95.50 ± 8.61	90.49 ± 3.47	93.80 ± 4.58	90.79 ± 6.28
Hypothalamus	44.32 ± 1.39	43.26 ± 2.97	45.85 ± 9.28	37.35 ± 2.63
Thalamus	58.25 ± 2.01	70.46 ± 4.31	65.87 ± 8.40	73.21 ± 8.28
Tegmentum	74.79 ± 8.97	78.55 ± 4.59	71.66 ± 6.56	74.83 ± 5.91
Hippocampus	47.27 ± 4.65	47.35 ± 2.85	46.76 ± 5.54	49.04 ± 2.96
Pons + Medulla Oblongata	93.93 ± 4.26	94.46 ± 3.81	103.78 ± 3.51	99.86 ± 0.93

Each value is the mean ± S.D.

portions. Sham-operated rats (n=8) were also examined for any possible brain damage.

Behavioral Testing

Mouse-killing behavior was determined by introducing an albino mouse into a rat's home cage. A rat which killed the mouse within 5 min was judged to be a killer, while one which failed to do so was termed a nonkiller rat. The killed mouse was removed immediately from the rat's home cage, and the killer rat was not allowed to eat the prey. All rats were tested for mouse killing behavior before any experimental manipulations. In the present experiments, spontaneous killer rats (n=3) were eliminated from the subjects. After operation, the mouse-killing behavior was tested 3, 7, and 14 days.

Pharmacological Studies

Ten bulbectomized rats which consistently exhibited the killing response within 2 min were used. All animals were tested for mouse-killing twice with an injection of saline before any drug treatment. Then, scopolamine and methylscopolamine at doses of 2, 4, and 8 mg/kg were administered intraperitoneally in a systemically varied sequence; successive drug treatments were separated by at least 3 days. Mouse-killing behavior was retested 30 min before injection, and was tested repeatedly at 15, 30 min, 1, 2, 4, and 24 hr after injection. The pharmacological agents employed in this study were scopolamine hydrobromide (Merck) and methylscopolamine nitrate (Merck).

Biochemical Studies

A separate group of rats were sacrificed by near-freezing method of Takahashi and Aprison [15] 14 days after surgery, using liquid nitrogen. The brain was quickly removed and 7 regions—cortex, amygdala, hypothalamus, thalamus, tegmentum, hippocampus, and pons plus medulla oblongata—were separated on an ice-cold glass plate. In order to assay the enzyme activity, tissues were homogenized in 5 mM Tris buffer containing 0.2% Triton X-100. Acetylcholinesterase (ACh-E) activities were determined at 30°C [11] by the spectrophotometric method of Ellman *et al.* [6] using 10^{-5} M of iso-OMPA as an inhibitor of nonspecific cholinesterase. Choline acetyltransferase (CAT) activities were determined by the radiochemical micromethod of Fonnum [7] using [^{3}H]-acetyl Coenzyme A as a substrate. Protein content was measured according to the method of Lowry *et al.* [10].

Statistical Analysis

Statistical evaluations were performed by means of two-tailed McNemar test for evaluating the drug effects. Biochemical data were evaluated by means of analysis of variance.

RESULTS AND DISCUSSION

The incidence of mouse-killing behavior following olfactory bulbectomy was as follows: 32% on the third day, 52% on the 7th day, and 60% on the 14th day after surgery. Once the killing response developed, it was constant and did not disappear under repeated manipulations. As described elsewhere, the bulbectomized rats were hyperirritable as evidenced by difficulty of handling regardless of whether or not the rat killed a mouse. On the other hand, the intact and sham-operated rats did not exhibit any killing response throughout the experiment.

An intraperitoneal injection of scopolamine hydrobromide significantly suppressed the killing response at dose of 4 and 8 mg/kg ($p<0.05$), but suppressive effect of the drug at 2 mg/kg was not statistically reliable. The peak effect occurred at 30 min after injection; namely, 3 (2 mg/kg), 6 (4 mg/kg), and 7 (8 mg/kg) out of 10 killer rats did not kill mice. The suppressive effect was still observed 2 hr after injection; 1 (2 mg/kg), 3 (4 mg/kg) and 4 (8 mg/kg) out of 10 killer rats did not kill mice. The killing response recovered completely 24 hr after injection of scopolamine. Methylscopolamine nitrate (2, 4, 8 mg/kg, IP) and saline, however, did not show any significant suppression of the behavior. Thus, the evidence indicates that the suppressive effect of scopolamine is due to its central anticholinergic property because methylscopolamine does not cross the blood-brain barrier easily.

The results of the studies on the activity of choline acetyltransferase (CAT) and acetylcholinesterase (ACh-E) in 7 discrete brain areas are shown in Tables 1 and 2, respectively. An overall analysis of variance indicated that there are no significant differences in either synthetic or catabolic enzyme activities among four groups. Ebel *et al.* found that both spontaneous and bulbectomized killer rats exhibited

TABLE 2

BRAIN ACETYLCHOLINESTERASE ACTIVITY IN RATS AFTER BILATERAL OLFACTORY BULBECTOMY (NMOLES ACETYLTHIOCHOLINE HYDROLYZED/MIN/MG PROTEIN)

Brain Areas	Control Group		Bulbectomized Group	
	Intact (n=5)	Sham (n=5)	Killer (n=5)	Nonkiller (n=5)
Cortex	48.76 ± 6.69	52.48 ± 3.90	42.38 ± 3.35	48.44 ± 3.64
Amygdala	138.25 ± 4.70	142.49 ± 7.34	141.56 ± 5.49	135.26 ± 8.09
Hypothalamus	118.86 ± 4.63	118.54 ± 2.79	116.27 ± 12.17	112.14 ± 1.38
Thalamus	121.56 ± 4.50	124.92 ± 6.26	117.32 ± 9.11	119.42 ± 6.09
Tegmentum	160.23 ± 5.35	157.88 ± 5.52	152.15 ± 7.41	154.08 ± 5.02
Hippocampus	86.94 ± 4.43	88.64 ± 4.61	83.75 ± 6.01	90.38 ± 4.40
Pons + Medulla Oblongata	136.11 ± 3.95	140.27 ± 4.00	142.25 ± 8.78	141.84 ± 2.80

Each value is the mean ± S.D.

higher CAT activity in the amygdala without affecting ACh-E activity [5]. The present experiment, however, demonstrated no significant difference in the amygdala between the killer and nonkiller rats in either CAT or ACh-E activities. The reason for this discrepancy may lie in the different degree of degeneration of nerve fiber connecting with olfactory bulbs; Ebel *et al.* [5] sacrificed 2 months after bulbectomy, but we sacrificed 2 weeks after the surgery. It should be noted here that they did not compare the bulbectomized-killer rats with the corresponding bulbectomized-nonkiller rats, and also with the sham-operated rats. It is obscure in their study, therefore, whether or not the observed activation of CAT activity in the amygdala is related to the mouse-killing behavior.

It has been postulated that the release of ACh and activation of cholinoceptive mechanisms may play an important role in the regulation of mouse-killing behavior. Most of the evidence is based on studies using pharmacological manipulations; the facilitation of killing response by cholinomimetic drugs and the suppression of killing by anticholinergic drugs [13]. The present finding that the blockade of central ACh receptor prevented the killing response may support this notion. Biochemical analysis, however, demonstrated no significant difference between killer and nonkiller rats in either CAT or ACh-E activities in any of the brain areas determined in this study. I have also previously found that ACh content in rats which manifested the mouse-killing after olfactory bulbectomy did not differ from that of the bulbectomized-nonkiller rats [17]. Recently, Albert indicates that the high dose of atropine is producing a nonspecific suppression of behavior rather than a specific suppression of mouse-killing [1]. This evidence is interesting in light of the fact that there exists no regional change in both brain ACh levels and enzyme activities specific to killing response, while suppressive effect of scopolamine occurs only at a high dose. On the other hand, Hirsch reported in mice that cholinergic ligand binding in the limbic structures was altered by bilateral olfactory bulbectomy [8], and he proposed that olfactory bulbs exert an inhibitory influence on the limbic cholinergic system. The measurement of the brain choline availability and the receptor binding, which might be a more sensitive indication of modulated cholinergic activity than enzyme activities, may provide a clue to the question whether or not brain cholinergic system participates in the regulation of mouse-killing behavior by rats.

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