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BRIEF COMMUNICATION

Changes in Acetylcholine Content in Rat Brain After Bilateral Olfactory Bulbectomy in Relation to Mouse-Killing Behavior

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YOSHIMURA, H., Y. GOMITA AND S. UEKI. *Changes in acetylcholine content in rat brain after bilateral olfactory bulbectomy in relation to mouse-killing behavior.* PHARMAC. BIOCHEM. BEHAV. **2**(5) 703–705, 1974. – Acetylcholine (Ach) content was measured in 5 discrete areas of rat brain following bilateral olfactory bulbectomy. The rats with olfactory bulbectomy (O.B. rat) exhibited significantly lower Ach levels in the cortex than intact and sham-operated controls. However, there was no significant difference in Ach level of all brain areas between the killer and non-killer O.B. rats. These results may suggest that cortical cholinergic function is activated by bulbectomy, but the change in cortical Ach level is not related to muricide.

Olfactory bulbectomy Mouse-killing Brain acetylcholine

BILATERAL olfactory bulbectomy has been known to induce mouse-killing behavior (muricide) in rats [7,14]. It was also reported that this behavior could be depressed by certain classes of drugs, such as antidepressant and tranquilizing drugs [6,12], and be facilitated by p-chlorphenylalanine instead [4].

There have been, however, a few reports which dealt with biochemical changes in the brain following bulbectomy. The recent study of Eichelman *et al.* [5] indicated that bilateral olfactory bulbectomy in rats resulted in a lower cortical norepinephrine level without altering dopamine and serotonin levels and that there was no difference between the killer and non-killer rats. Similar changes in cortical amines were also observed by Pohorecky *et al.* [8,9], although they did not mention behavioral changes of rats. On the other hand, there was no report concerning changes in brain Ach level, although it was assumed that this substance played an important role in behavior and varied with activity of the brain function. It has also been reported that drugs which depressed muricide, such as antidepressants and tranquilizers, had influences on the central cholinergic system [1, 2, 3].

In this investigation, therefore, the authors attempted to elucidate the correlation between the brain Ach levels and muricide induced by bilateral olfactory bulbectomy. Ach contents were estimated in 5 discrete areas of the rat brain, since a knowledge of regional biochemical changes in the brain was necessary for understanding behavior as described by Weiss and Heller [15].

METHOD

Animals

Male Wistar King-A rats, weighing between 160 and 180 g at the time of surgery, which were supplied from the institute of laboratory animals in Kyushu University, were used in the experiments. All the animals were housed throughout the experimental period in individual cages, 18 × 17 × 17 cm, with wire mesh walls, and were given food and water ad lib. The animals were maintained at a room temperature of 23 ± 1° C and in a relative humidity of 60%.

Procedure

The rats were divided into the intact, sham-operated and bilateral olfactory bulbectomized groups. The surgery was performed under pentobarbital anesthesia (40 mg/kg, i.p.). The olfactory bulbs were bilaterally removed by suction

TABLE 1

EFFECTS OF BILATERAL OLFACTORY BULBECTOMY ON BRAIN ACETYLCHOLINE LEVELS IN RATS

Group	Number of Animals	Acetylcholine content (μg/g tissue)				
		Cortex	Striatum	Amygdala	Diencephalon	Brainstem
Intact	13	1.99 ± 0.22	6.47 ± 0.68	4.61 ± 0.60	4.07 ± 0.51	4.21 ± 0.54
Sham Operated	9	1.85 ± 0.23	6.08 ± 0.84	3.93 ± 0.36*	4.03 ± 0.50	4.08 ± 0.28
Bulbectomized	14	1.43 ± 0.57*	6.09 ± 1.37	3.96 ± 0.83†	4.07 ± 0.42	4.26 ± 0.43

Rats were housed in individual cages throughout the experimental period and sacrificed 14 days after bulbectomy.
Each value is the mean ± S.D.
*Significantly different from intact ($p<0.01$, 2-tailed Student's t-test).
†($p<0.05$, 2-tailed Student's t-test).

TABLE 2

BRAIN ACETYLCHOLINE LEVELS IN THE KILLER AND NON-KILLER GROUPS OF THE RATS WITH BILATERAL OLFACTORY BULBECTOMY

Group	Number of Animals	Acetylcholine content (μg/g tissue)				
		Cortex	Striatum	Amygdala	Diencephalon	Brainstem
Killer	8	1.39 ± 0.58	6.18 ± 1.61	4.09 ± 1.02	4.06 ± 0.47	4.41 ± 0.35
Non-killer	6	1.50 ± 0.55	5.97 ± 0.95	3.78 ± 0.40	4.09 ± 0.36	4.02 ± 0.43

Each value is the mean ± S.D.

through a hole made in the skull just above the bulbs. The sham-operated group underwent the same procedure as the bulbectomized group, but without aspiration of the olfactory bulbs.

Muricide was tested 3, 7 and 13 days after surgery. The rat which killed a mouse within 5 min after introduced into the home cage, was named a killer. The animals were sacrificed by the near-freezing method of Takahashi and Aprison [11] 14 days after the surgery. The brain was dissected into 5 regions of the cortex, striatum, amygdala, diencephalon and brainstem, according to the method of Schubert and Sedvall [10]. Total Ach in each region was estimated on the frog rectus abdominis muscle sensitized with physostigmine.

RESULTS AND DISCUSSION

As can be seen in Table 1, Ach levels of the cortex ($p<0.01$) and amygdala ($p<0.05$) were significantly lower in the bulbectomized group than in the intact group. However, the decrease of Ach in the amygdala was unlikely to be related to bulbectomy, since this change was also found in the sham-operated group. On the other hand, the incidence of muricide after bulbectomy was 8 out of 14 rats (as shown in Table 2) and no significant difference was found in Ach levels of all brain areas between the killer and non-killer groups. However, the brainstem tended to show higher, although insignificant, Ach levels in the killer group.

It is interesting but rather confusing that Ach level is reduced after olfactory bulbectomy only in such a distant brain area as the neocortex which has no direct neural connection with the olfactory bulb. Eichelman *et al.* [5] and Pohorecky *et al.* [8,9] also reported that the change in norepinephrine level was found in the cortex after olfactory bulbectomy. In the cholinergic system, it is generally accepted that a decrease in Ach content can be explained as being due to increased destruction of neuronally active Ach by acetylcholinesterase. The decrease in cortical Ach level found in the present investigation might, therefore, be a reflex of activation of the cortical cholinergic system through the brainstem reticular activating system after olfactory bulbectomy, because it has been observed (in behavioral as well as in electroencephalographic studies in our laboratory) that the arousal level is elevated in the rat with bilateral olfactory bulb ablation [13].

It is still obscure as to whether this change in cortical Ach level is either secondary to the behavioral changes or a transsynaptic alteration via the limbic structures and hypothalamus following olfactory bulbectomy. Further investigations are necessary to elucidate biochemical correlates in muricide.

REFERENCES

1. Benešová, O. The relation of imipramine-like drugs to the cholinergic system. In: *First International Symposium on Antidepressant Drugs,* edited by S. Garattini and M. N. G. Dukes. Milan: Excerpta Medica Foundation, 1966, pp. 247–254.
2. Brimblecombe, R. W. and D. M. Green. Central effects of imipramine-like antidepressants in relation to their peripheral anticholinergic activity. *Int. J. Neuropharmac.* **6:** 133–142, 1967.
3. Consolo, S., H. Ladinsky, G. Peri and S. Garattini. Effect of central stimulants and depressants on mouse brain acetylcholine and choline levels. *Eur. J. Pharmac.* **18:** 251–255, 1972.
4. Di Chiara, G., R. Camba and P. F. Spano. Evidence for inhibition by brain serotonin of mouse killing behavior in rats. *Nature* **233:** 272–273, 1971.
5. Eichelman, B., N. B. Thoa, N. M. Bugbee and K. Y. Ng. Brain amine and adrenal enzyme levels in aggressive, bulbectomized rats. *Physiol. Behav.* **9:** 483–485, 1972.
6. Kumadaki, N., M. Hitomi and S. Kumada. Effect of psychotherapeutic drugs on hyperemotionality of rats in which the olfactory bulb was removed. *Jap. J. Pharmac.* **17:** 659–667, 1967.
7. Myer, J. S. Stimulus control of mouse-killing rats. *J. comp. physiol. Psychol.* **58:** 112–117, 1964.
8. Pohorecky, L. A. and J. P. Chalmers. Effects of olfactory bulb lesions on brain monoamines. *Life Sci.* **10:** 985–998, 1971.
9. Pohorecky, L. A., M. J. Zigmond, L. Heimer and R. J. Wurtman. Olfactory bulb removal: Effects on brain norepinephrine. *Proc. natn. Acad. Sci. U.S.A.* **62:** 1052–1055, 1969.
10. Schubert, J. and G. Sedvall. Accumulation and disappearance of ^{3}H-5-hydroxytryptamine formed in vivo from ^{3}H-tryptophan in various regions of the rat brain. *Eur. J. Pharmac.* **17:** 75–80, 1972.
11. Takahashi, R. and M. H. Aprison. Acetylcholine content of discrete areas of the brain obtained by a near-freezing method. *J. Neurochem.* **11:** 887–898, 1964.
12. Ueki, S., S. Nurimoto and N. Ogawa. Effects of psychotropic drugs on emotional behavior in rats with limbic lesions, with special reference to olfactory bulb ablations. *Folia Psychiat. Neurol. Jap.* **26:** 245–255, 1972.
13. Ueki, S., S. Watanabe, Y. Araki and Y. Gomita. Pharmacological studies on the electrical activity of the limbic system in the rat with olfactory bulb lesion. *Folia Pharamc. Jap.* **64:** 21, 1968.
14. Vergnes, M. and P. Karli. Déclenchement du comportement d'agression interspécifique Rat-Souris par ablation bilatérale des bulbes olfactifs. Action de l'hydroxyzine sur cette agressivité provoquée. *C. r. Soc. Biol.* **157:** 1061–1063, 1963.
15. Weiss, B. and A. Heller. Methodological problems in evaluating the role of cholinergic mechanisms in behavior. *Fedn Proc.* **28:** 135–146, 1969.