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Biochemical Correlates in Mouse-Killing Behavior of the Rat: Prolonged Isolation and Brain Cholinergic Function

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YOSHIMURA, H. AND S. UEKI. *Biochemical correlates in mouse-killing behavior of the rat: prolonged isolation and brain cholinergic function.* PHARMAC. BIOCHEM. BEHAV. 6(2) 193–196, 1977. – After 30 days of isolation, 45% of the rats exhibited mouse-killing behavior. The killing response was suppressed by atropine (5 mg/kg and 8 mg/kg, IP) and scopolamine (8 mg/kg, IP), whereas methylatropine was ineffective. Acetylcholine (ACh) content and acetylcholinesterase (AChE) activity were measured in 5 discrete areas of rat brain. As compared with the aggregated rats only the killer rats exhibited higher ACh levels in the diencephalon. The activity of AChE in all brain areas was unchanged by isolation; no significant difference was found between the killer and nonkiller rats. These results suggest that central cholinergic mechanisms participate in the mediation of mouse-killing behavior in the rat.

Mouse-killing behavior Brain cholinergic function Biochemical correlates

SOCIAL isolation is known to induce various physiological and behavioral changes in rats [9, 10, 20]. Mouse-killing behavior constitutes a distinct example of the latter [6, 12, 15, 27].

Mouse-killing behavior can be elicited in rats also by olfactory bulbectomy [12, 14, 21] or administration of Δ^9-tetrahydrocannabinol [26,30]. In these two cases, isolation is a powerful variable affecting development of killing response [14,26].

Increasing evidence supports the view that central cholinergic mechanisms may play an important role in the mediation of mouse-killing behavior [3, 4, 7, 19, 23, 28]. The killing response was facilitated by the application of carbachol to the lateral hypothalamus [4], while carbachol and neostigmine applied to this site elicited the killing response in rats which did not ordinarily kill mice [23]. Similarly repeat administration of pilocarpine has been shown to convert nonkillers to killers [19,28]. Furthermore, Ebel *et al.* [7] recently found that spontaneous killer rats and rats exhibited mouse-killing behavior after olfactory bulbectomy showed higher choline acetyltransferase activity in the amygdala than did nonkiller rats.

Thus, it can be expected that central cholinergic mechanisms participate in mouse-killing behavior induced by prolonged isolation. This investigation is aimed at elucidating this problem.

METHOD

Animals

Male Wistar King A strain rats, 180–200 g in weight, supplied from the Institute of Laboratory Animals, Kyushu University, were used. Albino mice weighing between 20 and 30 g were used to test the mouse-killing behavior. All the animals were given food and water ad lib.

Apparatus

The isolation cage was constructed of five compartments, 18 × 17 × 17 cm, made of wire mesh wall, the compartments were separated by opaque plastic partitions. Under these conditions, the isolated rats were still exposed to social stimuli such as vocalization and odor. The aggregated animals were housed in a 40 × 33 × 17 cm cage, made of plastic wall, 5 animals per cage. The temperature was maintained at 23 ± 1°C, and light cycle was automatically controlled (lights on at 7:00 a.m., off at 7:00 p.m.).

Procedure

The rats were housed individually for 30 days in the isolation cage. During this period, the mouse-killing behavior was tested at intervals of 5 days; the tests were

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carried out at 9:00 a.m. The rats exhibiting the killing response within 5 min of the introduction of the mouse into the rat's home cages were termed killers; those which failed to do so were termed nonkillers. The nonkiller rats housed communally for 30 days were referred to as controls. One hundred and fifty rats were originally screened for mouse killing; 13 showed naturally-occurring killing response and were discarded.

Pharmacological Studies

These were started on the 31st day after isolation, and two different drugs were administered twice each to each animal. However, one week was intervened between successive experimental treatments. Mouse-killing was retested 30 min before drug administration, and only stable killer rats were used. Drugs were administered intraperitoneally at a fixed time, i.e., 9:00 a.m., and killing response was tested at 30 min, 1, 2, 4, 8 and 24 hr after injection. Pharmacological agents employed in this experiment were atropine sulfate (Merck), scopolamine hydrobromide (Merck), and methylatropine nitrate (Sigma). All drugs were dissolved in distilled water.

Biochemical Studies

On the 30th day of isolation rats were sacrificed 15 min after the last presentation of a mouse. The rats were killed by the near-freezing method of Takahashi and Aprison [25]. The brain was quickly removed and 5 regions – cortex, striatum, amygdala, diencephalon and brainstem – were separated on an ice-cold glass plate [22]. To determine total ACh, tissues were homogenized in ice-cold frog Ringer solution containing 50 μM of physostigmine. ACh is extracted from homogenate by adjusting samples to pH 4 and placing them in a boiling water bath for 10 min [29]. The sample was then neutralized to pH 6.8. ACh was bioassayed on the frog rectus abdominis muscle sensitized with physostigmine [5,24]. For the enzyme assay, tissues were homogenized in phosphate buffer (pH 8.0). AChE activity was determined at 37°C [17] by the spectrophotometric method of Ellman *et al.* [8].

Statistical Analysis

Statistical evaluations were carried out by means of the Fisher Exact Probability test and of Student's *t*-test.

RESULTS AND DISCUSSION

The incidence of mouse-killing behavior following prolonged isolation increased gradually; on the 30th day of isolation approximately 45% of the rats exhibited killing response. The aggregated rats did not show any killing response during this time span.

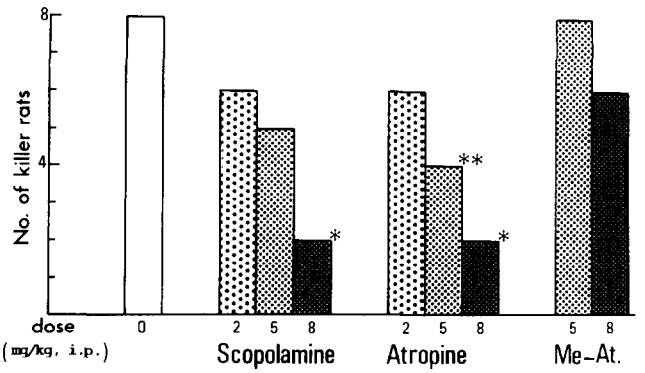

FIG. 1. Effects of cholinolytic drugs on mouse-killing behavior. Data shown are at peak time of drug effect; scopolamine 30 min, atropine 1 hr, methylatropine 1 hr after injection. Each group employed 8 killer rats. *$p<0.01$, **$p<0.05$, significantly different from control (one-tailed Fisher exact probability test).

The effects of cholinolytic drugs on the killing response are shown in Fig. 1. Intraperitoneal injection of either atropine sulfate (5 mg/kg and 8 mg/kg) or scopolaime hydrobromide (8 mg/kg) suppressed significantly the killing response. The peak effect occurred at 1 hr and 30 min respectively; the killing response recovered 8 hr after injection of the drugs. Methylatropine nitrate did not suppress the killing response; this suggests that the suppressive effect of atropine was due to its central action.

The results of the studies on the levels of ACh and AChE activity are shown in Tables 1 and 2 respectively. In the case of the diencephalon ACh content was significantly higher in the killer rats than in the aggregated rats ($p<0.05$), while ACh content of the same area in the nonkiller rats was unchanged as compared with the aggregated rats (Table 1). No significant differences in ACh levels were found between the isolation and the aggregated rats in the case of the other four brain regions.

On the other hand, the activity of AChE in all brain areas was unchanged after isolation in both the killer and nonkiller rats as compared with the aggregated rats (Table 2). There was no significant difference between the killer and nonkiller rats.

Previous research has indicated that brain cholinergic mechanisms participated in the mediation of mouse-killing

TABLE 1

CHANGES IN ACETYLCHOLINE CONTENT IN RAT BRAIN AFTER PROLONGED ISOLATION

Group	n	Cortex	Striatum	Amygdala	Diencephalon	Brainstem
Aggregated	14	2.03 ± 0.30	5.78 ± 0.84	4.33 ± 0.59	4.08 ± 0.52	4.27 ± 0.61
Isolated						
Killer	6	2.32 ± 0.36	6.33 ± 0.65	4.81 ± 0.51	4.70 ± 0.41*	4.38 ± 0.43
Nonkiller	8	2.10 ± 0.22	6.60 ± 1.30	4.53 ± 0.85	4.24 ± 0.34	3.87 ± 0.41

Results shown are at 30th day after isolation.
Values (mean ± SD) are expressed as μg of g wet tissue. n = number of animals.
* Significantly different from aggregated group ($p<0.05$, 2-tailed Student's *t*-test).

TABLE 2

CHANGES IN ACETYLCHOLINESTERASE ACTIVITY IN RAT BRAIN AFTER PROLONGED ISOLATION

Group	n	Cortex	Striatum	Amygdala	Diencephalon	Brainstem
Aggregated	8	7.31 ± 0.57	50.41 ± 2.92	13.27 ± 1.19	13.50 ± 0.64	13.93 ± 1.11
Isolated						
Killer	7	7.26 ± 0.89	50.04 ± 3.54	13.79 ± 1.15	13.37 ± 0.95	13.65 ± 0.95
Nonkiller	7	7.48 ± 0.76	48.10 ± 2.40	14.45 ± 1.04	13.75 ± 0.51	13.83 ± 0.85

Results shown are at 30th day after isolation.
Values (mean ± SD) are expressed as μmoles acetylthiocholine hydrolyzed/min/g of wet tissue.
n = number of animals.

behavior in the rats [1, 2, 4, 7, 23]. Indeed, it was shown at present that cholinolytic drugs suppress the killing response induced by prolonged isolation, in agreement with the data obtained in the spontaneous killer rats [3], carbachol-induced killer rats [4], or killer rats induced by olfactory bulbectomy [18].

Smith *et al.* [23] and Bandler [3,4] suggested that the release of ACh and the activation of cholinoceptive mechanisms at the lateral hypothalamus, were essential in the manifestation of killing response. The present finding that ACh content of the diencephalon increased only in the killer rats is interesting but rather confusing, because the increased availability of ACh may, via a negative feedback, decrease levels of ACh. The mechanism of the observed increase in ACh content, is uncertain. The possibility of a decreased rate of destruction of the neurotransmitter may be ruled out, because no change was found in the activity of AChE in the diencephalon. Recently, Ebel *et al.* [7] reported increased activity of choline acetyltransferase in the amygdala of spontaneous- and bulbectomized-killer rats. In addition, amygdala seems to exert a facilitating influence upon the killing response [7, 11, 12, 13, 16], being conveyed to the diencephalic and mesencephalic structures via the ventral amygdalofugal fibre system [13]. Hence it is possible that the increased levels of ACh in the diencephalon may be attributable to the activation of this neural pathway.

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