Pharmacology Biochemistry & Behavior, Vol. 34, pp. 395–398. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Inhibition of Mouse-Killing Behavior by S-Adenosyl-L-Methionine in Midbrain Raphe-Lesioned and Olfactory-Bulbectomized Rats

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Received 8 August 1988

YAMAMOTO, T., S. YATSUGI, M. OHNO AND S. UEKI. *Inhibition of mouse-killing behavior by S-adenosyl-L-methionine in midbrain raphe-lesioned and olfactory-bulbectomized rats.* PHARMACOL BIOCHEM BEHAV **34**(2) 395–398, 1989.—The effect of S-adenosyl-L-methionine (SAM), a methyl donor, on mouse-killing behavior in rats with lesions of the midbrain raphe nuclei and in olfactory-bulbectomized rats was investigated. Systemic administration of SAM at doses of 180 and 320 mg/kg IP caused a significant inhibition of both kinds of mouse-killing behavior. The inhibitory effects of SAM on both types of mouse-killing behavior were almost equipotent. Microinjection of SAM at 10–100 μg/rat into the lateral ventricle also inhibited mouse-killing behavior induced by raphe lesions in a dose-dependent manner. The ED_{50} value of SAM for this effect was 38.6 (18.4–81.4) μg/rat. It is concluded that SAM has inhibitory effects on mouse-killing behavior in both raphe-lesioned and olfactory-bulbectomized rats through a site of action in the central nervous system.

S-Adenosyl-L-methionine (SAM) Mouse-killing behavior Raphe lesions Olfactory bulbectomy

S-ADENOSYL-L-METHIONINE (SAM) is the major methyl donor involved in numerous important transmethylation reactions in the brain (8,13). Administration of SAM has been shown to increase the turnover rate of both noradrenaline (NA) and serotonin (5-HT) in some regions of the rat brain (2,5). In clinical settings, a deficiency of catecholamines and 5-HT in the CNS has been implicated in the etiology of human depression and therapeutic efficacies of typical antidepressants have been attributed to activation of monoamine neurotransmission (4, 17, 18). SAM has been reported to have antidepressant effects in depressive patients (1, 3, 6, 15).

On the other hand, mouse-killing behavior has been used as an animal model of depression for preclinical evaluation of antidepressant drugs because this behavior appears to be selectively inhibited by treatment with antidepressants (19, 24, 27). Mouse-killing behavior has been shown to be induced by olfactory bulbectomy in rats (14, 19, 24). Lesions of the midbrain raphe nuclei, which contain 5-HT cell bodies, also induce a high incidence of muricide in rats (7, 26, 27). Biochemical studies have shown that the reduced NA and 5-HT function may be related to the induction of mouse-killing behavior in both olfactory-bulbectomized and raphe-lesioned rats, respectively (9, 10, 23).

The present study was designed to investigate the effect of SAM on mouse-killing behavior in rats with olfactory bulbectomy and midbrain raphe lesions.

METHOD

Subjects

Subjects were male Wistar King A strain rats supplied by Seiwa Laboratory Animal Center, weighing 230–260 g at the time of surgery. After surgery, the rats were housed in individual wire-mesh cages (18 × 17 × 18 cm) and given food and water ad lib throughout the experiment. Room temperature was maintained at 23 ± 2°C with a 12-hr light-dark cycle (light on 0700–1900).

Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg IP) and fixed in a stereotaxic instrument (Narishige: SR-5). For

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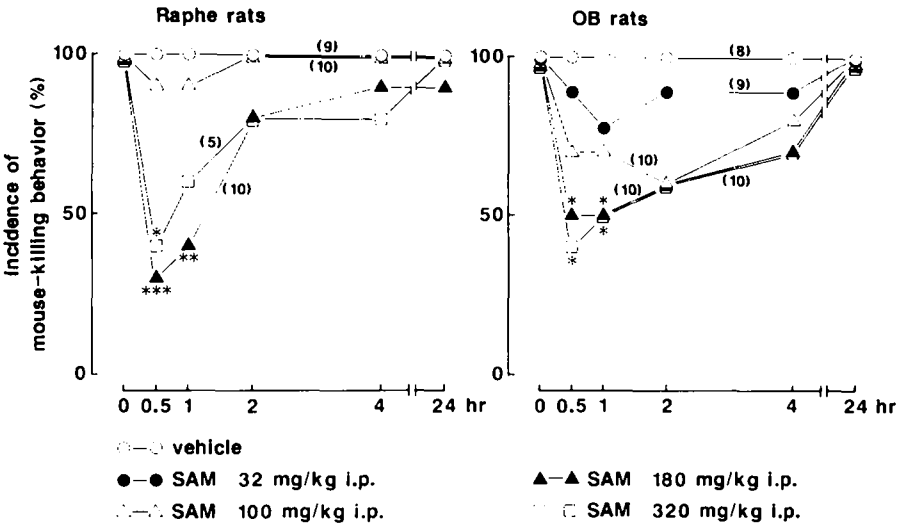

FIG. 1. Effect of S-adenosyl-L-methionine (SAM) on mouse-killing behavior in midbrain raphe-lesioned rats (Raphe rats) and olfactory-bulbectomized rats (OB rats). Significant differences from the vehicle-treated group were determined using the Fisher's exact probability test. $*p<0.05$, $**p<0.01$, $***p<0.005$. Numbers in parentheses designate the number of animals used for each dose.

raphe lesions, a monopolar electrode (0.4 mm in diameter, insulated stainless steel wire with bare tip) was inserted into the midbrain raphe nuclei. A direct current of 3 mA was applied for 15 sec to induce lesions of both the medial raphe nucleus (anterior (A) from the interaural line: 1.2 mm, lateral (L) from the midline: 0 mm, horizontal (H) from the surface of the skull: 9.2 mm) and dorsal raphe nucleus (A: 1.2, L: 0, H: 7.0), located according to the rat brain atlas of Paxinos and Watson (16). Following raphe lesions, 10 rats were bilaterally implanted with guide cannulae (0.7 mm in external diameter, stainless steel) aimed 1 mm above the lateral ventricle (A: 8.2, L: 1.7, H: 3.2). The remainder of raphe-lesioned rats were used for systemic drug administration experiments. For olfactory bulbectomy, a hole approximately 1 mm in diameter was made in the skull of rats under pentobarbital anesthesia and the olfactory bulbs were bilaterally removed by suctioning. At least one week was allowed for recovery from the surgery before starting the experiment.

Experimental Procedures

Rats were tested for mouse-killing behavior immediately before drug administration and only rats exhibiting mouse-killing behavior within 3 min were used for the tests with drug. In case of systemic administration, tests for mouse-killing behavior were performed 0.5, 1, 2, 4 and 24 hr after drug treatment. In case of microinjection into the lateral ventricle, drug solutions of 2 μl were bilaterally injected through a stainless steel injection cannula (0.35 mm in external diameter) extending 1.0 mm below the tip of the guide cannula. Injection rate was 1 μl per minute, with the cannula left in the brain for an additional one minute after termination of the injection. Tests for mouse-killing behavior were performed 5, 15, 30 min and 1, 4 and 24 hr after drug microinjection. Mouse-killing behavior was assessed as positive if the rat bit and killed a mouse within 3 min after it was introduced into the rat's home cage.

The drug used in this study was S-adenosyl-L-methionine sulfate tosylate (SAM: Fuji). SAM was dissolved in phosphate buffer solution and the pH was adjusted to about 4–6 with an appropriate amount of NaOH solution. The drug was intraperitoneally injected in a volume of 0.1 ml per 100 g body weight and microinjected bilaterally into the lateral ventricle in a volume of 2 μl per side. The dose of drug was expressed in terms of the salt.

Histology

After completion of the experiment, the animal was anesthetized with ether and perfused with 10% formalin solution through the left cardiac ventricle. After the brain was removed, sectioned slices were stained with cresyl violet. The site of the cannula tips was verified histologically by visual inspection.

Statistical Analysis

The significance of the drug effect on mouse-killing behavior was determined using the Fisher's exact probability test. The ED_{50} values and their 95% confidence interval were calculated by the method of Litchfield and Wilcoxon (12).

RESULTS

Systemic injection of SAM at doses of 180 and 320 mg/kg IP caused a significant inhibition of mouse-killing behavior in midbrain raphe-lesioned rats, the inhibition rates at the time of peak effect, 0.5 hr after injection, being 70 and 60%, respectively (Fig. 1). SAM at 180 and 320 mg/kg IP also significantly inhibited mouse-killing behavior in olfactory-bulbectomized rats, the inhibition rates at 0.5 hr after administration were 50 and 60%, respectively. When SAM at doses over 100 mg/kg was administered, the rats showed sedation without ataxia or muscle relaxation in both raphe-lesioned and olfactory-bulbectomized rats. The inhibition of mouse-killing behavior progressively weakened with time with almost complete recovery 24 hr postinjection.

As shown in Fig. 2, SAM (10–100 μg/rat) microinjected into the lateral ventricle inhibited mouse-killing behavior induced by raphe lesions in a dose-dependent manner. The ED_{50} value of SAM for this effect 15 min after microinjection was 38.6 (18.4–81.4) μg/rat. Intracerebroventricular injection of SAM caused no behavioral change such as marked sedation and ataxia. Mouse-

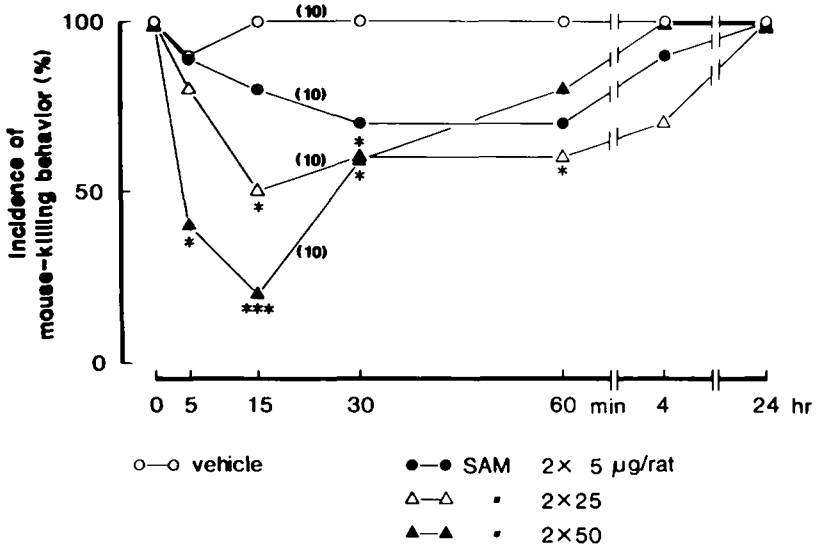

FIG. 2. Effect of S-adenosyl-L-methionine (SAM) microinjected into the lateral ventricle on mouse-killing behavior in midbrain raphe-lesioned rats. Significant differences from the vehicle-injected group were determined using the Fisher's exact probability test. $*p<0.05$, $***p<0.005$. Numbers in parentheses designate the number of animals used for each dose.

killing behavior gradually recovered thereafter.

DISCUSSION

In the present study, systemic administration of SAM resulted in a significant inhibition of mouse-killing behavior in raphe-lesioned and olfactory-bulbectomized rats. SAM microinjected into the lateral ventricle also inhibited mouse-killing behavior induced by raphe lesions. Stramentinoli *et al.* (21,22) reported that SAM can penetrate the blood-brain barrier and SAM concentrations in the rat brain were increased after a systemic administration of SAM. These findings suggest that SAM exerts its suppressive effect on mouse-killing behavior through a site of action in the central nervous system.

Mouse-killing behavior induced by olfactory bulbectomy and raphe lesions are selectively inhibited by antidepressant drugs (19, 24, 27) which are potent blockers of the specific reuptake process of 5-HT and/or NA. Although SAM does not appear to affect the 5-HT or NA reuptake mechanism, it caused significant inhibition of both types of mouse-killing behavior. From behavioral studies with amygdaloid lesioning (20), locus coeruleus stimulation (25), electroconvulsive shock (11) and antidepressants (24,27), it is suggested that the mechanism underlying the exhibition of mouse-killing behavior seems to be different depending upon the method of induction. Biochemical studies show that the turnover rate of NA in the lateral hypothalamus and medial amygdala is decreased in olfactory-bulbectomized killer rats (9,10). In raphe-lesioned rats, both 5-HT and its metabolite, 5-hydroxyindoleacetic acid, concentrations are decreased in almost all brain regions (23). SAM has been found to accelerate the turnover of 5-HT and NA in some regions of the rat brain (2,5). From these results, it is concluded that SAM has inhibitory effects on mouse-killing behavior in both raphe-lesioned and olfactory-bulbectomized rats through the activation of the central 5-HTergic and NAergic nervous system, respectively.

Several clinical studies have suggested that SAM is significantly more effective than placebo (1,3) and is as effective as tricyclic antidepressants (clomipramine or amitriptyline) (6,15) in the treatment of depression. It has been demonstrated that a deficiency of catecholamines and 5-HT in the CNS may be implicated in the etiology of human depression (4, 17, 18). Although the catecholamine and 5-HT theories were often considered to be in competition, further research has indicated that both may be involved, creating the concepts of a 'catecholamine depression' or a '5-HT depression.' From the present results showing that SAM inhibits both types of mouse-killing behavior with equal potency, it is anticipated that SAM has some beneficial effect on both a 'catecholamine depression' and a '5-HT depression.'

ACKNOWLEDGEMENTS

The authors wish to thank Fuji Chemical Industry Co., Ltd. (Toyama, Japan) for the generous supply of SAM and Miss H. Hitaka for typing the manuscript.

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