Pharmacology Biochemistry & Behavior, Vol. 18, pp. 203–207, 1983. Printed in the U.S.A.

Effects of Adrenergic Blockers on the Inhibition of Muricide by Desipramine and Noradrenaline Injected into the Amygdala in Olfactory Bulbectomized Rats

SHIBENOBU SHIBATA, SHIGENORI WATANABE, SHYH YUH LIOU AND SHOWA UEKI

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan

Received 9 August 1982

SHIBATA, S., S. WATANABE, S. Y. LIOU AND S. UEKI. *Effects of adrenergic blockers on the inhibition of muricide by desipramine and noradrenaline injected into the amygdala in olfactory bulbectomized rats.* PHARMACOL BIOCHEM BEHAV **18**(2) 203–207, 1983.—Muricide in olfactory bulbectomized rats (OB rats) is readily inhibited by systemic administration of desipramine (DMI) or microinjection of DMI and noradrenaline (NA) into the medial amygdaloid nucleus. The present experiment investigated whether the muricide inhibition produced by these forms of drug treatment was mediated by alpha- or beta-noradrenergic receptors in the central nervous system. Muricide inhibition produced by systemic administration of DMI was antagonized by an alpha-blocker phenoxybenzamine but unaffected by a beta-blocker sotalol, although administration of these adrenergic blockers alone had no effect on muricide. Muricide inhibition induced by the microinjection of DMI and NA into the medial amygdaloid nucleus was similarly antagonized by pretreatment of phenoxybenzamine injected into the same site, but sotalol had no effect. Injection of phenoxybenzamine or sotalol alone into the medial amygdaloid nucleus did not elicit any changes in muricide. These findings suggest that mechanisms mediated by brain noradrenergic alpha-receptor play an important role in muricide inhibition by tricyclic antidepressants in rats and that the medial amygdaloid nucleus is an important site of action of these drugs.

α-Adrenergic blocker Antidepressant Microinjection Amygdala Olfactory bulbectomy Muricide

BILATERAL olfactory bulbectomy in the rat is well known to elicit characteristic hyperemotionality including mouse-killing behavior (muricide). Since hyperemotionality in olfactory bulbectomized rats (OB rats) is suppressed by various psychotropic drugs, this procedure has been employed for evaluating the taming effect of psychotropic drugs [9,20]. Among the types of hyperemotionality, muricide is selectively inhibited by tricyclic antidepressants and this inhibition is particularly marked with desipramine (DMI) known to have a potent blocking activity on noradrenaline (NA) uptake at synapses [1,15]. Muricide is similarly inhibited by destruction of the amygdala [6,19] and microinjection of NA or DMI into the amygdala [21]. Moreover, the incidence of muricide is augmented by destruction of the noradrenergic dorsal bundle [13]. These facts suggest that the mechanisms eliciting muricide in OB rats involves the central noradrenergic system, particularly that in the amygdala. Previous experiments have demonstrated that activation of the NA system in the amygdala resulted in muricide suppression. However, whether this muricide suppression is mediated by alpha- or beta-receptors remains unknown.

The present experiment was therefore designed to investigate effects of alpha- and beta-adrenergic blockers on muricide inhibition produced by systemic administration of DMI and microinjection of DMI and NA into the medial amygdaloid nucleus.

METHOD

Subjects and Surgery

Three-hundred-ninety-six male Wistar King A rats supplied by the Kyushu University Institute of Experimental Animal were used. Body weights at the initiation of the experiment ranged from 250 to 300 g. Before olfactory bulbectomy, all rats underwent one muricide test. Only animals not showing muricide (363 rats) were selected for the experiment. The olfactory bulbs were removed bilaterally by suction as described previously [18]. Immediately after olfactory bulbectomy, isolated housing was commenced. Only rats displaying muricide within 7 days after olfactory bulbectomy (291 rats) were used in the following part of the experiment. One hundred rats were subjected to surgery for

chronic cannula implantation for microinjection of drugs into the medial amygdaloid nucleus. The remainder was used for the experiment of systemic drug administration. Under anesthesia with pentobarbital sodium 40 mg/kg IP, guide cannulae (stainless steel, external diameter 0.7 mm) were chronically implanted into the medial amygdaloid nucleus of both sides according to the rat brain atlas of König and Klippel [8] (A: 5.7 mm, L: 3.5 mm, V: 8.2 mm deep from the surface of skull) as described in our previous reports [21]. Throughout the experiments both the animal room and experimental room were maintained at a controlled temperature of 23±2°C. Illumination was provided on a 12-hr light-dark cycle (lights on at 7:00). Food and water were supplied ad lib.

Experimental Procedure

Muricide tests were performed immediately before and 0.5, 1, 2, 4 and 24 hr after the drug treatment in the case of systemic administration, and immediately before and 10, 20, 30 and 60 min and 24 hr after treatment in the case of bilateral microinjection into the medial amygdaloid nucleus. Muricide was assessed as positive if the rat bit and killed a mouse within 3 min after introducing it into the rat's home cage. All experiments were performed between 13:00 and 18:00. The following drugs were used in this study: desipramine hydrochloride (DMI) (CIBA-GEIGY, Pertfran), phentolamine methylate (CIBA-GEIGY, Regitine), phenoxybenzamine hydrochloride (Tokyo Kasei, Dibenzyline), propranolol hydrochloride (Sumitomo, Inderal), satalol hydrochloride (Mead-Johnson), yohimbine hydrochloride (Sigma), L-arterenol hydrochloride (Sigma, L-noradrenaline), L-epinephrine bitartrate (Sigma, adrenaline), 5-hydroxytryptamine creatinine sulfate (Sigma, serotonin), DL-isoproterenol hydrochloride (Sigma), dopamine hydrochloride (Sigma) and γ-aminobutyric acid (Sigma).

For systemic administration, all of the drugs were dissolved in physiological saline, and were subcutaneously given at 0.1 ml per 100 g rat body weight. For microinjection into the medial amygdaloid nucleus, drugs were dissolved in distilled water, and the final solution was made isotonic by the addition of appropriate amount of NaCl and the drug solution of 2 μl each was bilaterally injected into the medial amygdaloid nucleus. When adrenergic blockers were examined on the action of DMI, blockers were administered simultaneously with DMI. In the intra-amygdaloid injection experiments, adrenergic blockers were injected 10 min before DMI or NA injection.

Histology

After completion of experiments the animals were anesthetized with ether and their brains were perfused with saline and 10% Formalin through the carotid. The brain was then removed, frozen sections, 50 μ thick, were made and stained with cresyl violet. The extent of olfactory bulbectomy and the placement of guide cannulae were verified histologically. If the extent of olfactory bulbectomy and the placement of guide cannulae were not appropriate, the results in these rats were discarded from the data.

RESULTS

Effects of Systemically Administered Drugs on Muricide

Subcutaneous administration of 10 and 20 mg/kg of DMI produced a dose-dependent inhibition of muricide. At the time of peak effect, 1 hr after administration, the incidence of muricide was about 60% at 10 mg/kg and 30% at 20 mg/kg (Fig. 1A). Almost complete recovery of muricide was seen after 24 hr. Even when muricide was inhibited by DMI, rats only displayed mild signs of sedation without showing ataxia or muscle relaxation.

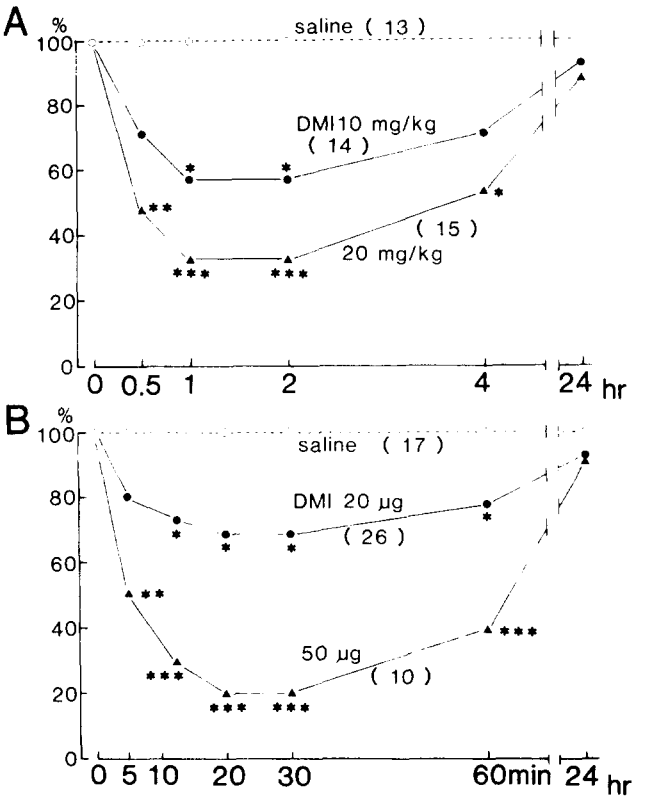

FIG. 1. Effects of systemic administration (A) and microinjection into the medial amygdaloid nucleus (B) of desipramine on the muricide of olfactory bulbectomized rats. Significant differences from the values of saline-treated animals (Fisher's exact probability test): $*p<0.05$, $**p<0.01$ and $***p<0.005$. Abscissa: incidence of muricide (%). Numbers in parentheses designate the number of animals used.

Table 1 summarizes the effects on muricide of systemically administered DMI and various alpha- and beta-blockers given alone and in combination with DMI.

The incidence of muricide at 1 hr after administration of 10 and 20 mg/kg of DMI was 57 and 33%, respectively. Single administration of phentolamine 10 mg/kg SC, phenoxybenzamine 20 mg/kg SC, yohimbine 5 mg/kg SC, and propranolol 20 mg/kg SC inhibited muricide; the incidence of muricide at 1 hr after administration was 88, 89, 63 and 67%, respectively. Concurrently with muricide inhibition by yohimbine and propranolol, the rats exhibited remarkable muscle relaxation and ataxia. The emotional response to external stimuli such as a rod touching the back was also extremely suppressed. In contrast, administrations of phenoxybenzamine 10 mg/kg SC or sotalol 20 mg/kg SC alone, did not have any effect on muricide. Ataxia, muscle relaxation and other behavioral changes were not observed.

TABLE 1

EFFECTS OF ALPHA- AND BETA-BLOCKERS ON MURICIDE INHIBITION INDUCED BY SYSTEMIC ADMINISTRATION OF DESIPRAMINE (DMI)

Blockers (mg/kg, SC)		Saline	DMI (mg/kg, SC) 10	20
Saline		13/13*†	8/14	5/15
Phentolamine	10	7/8	5/8	—
Phenoxybenzamine	10	9/9	9/9‡	—
	20	8/9	—	7/9‡
Yohimbine	3	9/9	6/9	—
	5	5/8‡	4/8	—
Propranolol	10	8/8	6/8	—
	20	6/9	2/9	—
Sotalol	20	8/8	5/8	—

*Number of rats exhibiting muricide/the number of rats tested.
†Muricide was tested 1 hr after drug administration.
‡Significant difference from the values in saline-treated animals: $p<0.05$.

Concomitant administration of DMI with yohimbine 5 mg/kg or phentolamine 10 mg/kg produced muricide inhibition in almost the same degree as with the administration of DMI alone. Moreover, remarkable muscle relaxation and ataxia were seen concurrently with muricide inhibition.

When DMI 10 and 20 mg/kg was administered concomitantly with phenoxybenzamine 10 and 20 mg/kg, SC, respectively, the incidence of muricide were 100 and 78% and were significantly higher than that obtained by administration of DMI alone. There was no sign of muscle relaxation or ataxia. The incidence of muricide after concomitant SC administration of DMI 10 mg/kg and sotalol 20 mg/kg was similar to that produced by DMI alone. Behavioral change such as muscle relaxation were not seen.

Effects of Drugs Injected into the Amygdala

Injection of DMI, 20 and 50 μg, into the medial amygdaloid nucleus elicited a dose-dependent inhibition of muricide. Peak effect was obtained 20–30 min after the injection (Fig. 1B). Muricide gradually recovered thereafter. Complete recovery of muricide was seen after 4 hr. Several minutes after DMI injection, mild paralysis of the hindlimbs was transiently observed, but complete recovery took place 10 min after the injection and no remarkable behavioral changes were seen thereafter.

Table 2 summarizes the effect on muricide of various amines and γ-aminobutyric acid (GABA), injected into the medial amygdaloid nucleus. Injection of NA produced a dose-dependent muricide inhibition. The incicence of muricide at 30 min after injection was 50% at 20 μg and 24% at 50 μg, respectively. Injection of adrenaline (AD), 50 μg, similarly inhibited muricide. However, muricide was not appreciably inhibited by isoproterenol, dopamine and GABA, and no other remarkable behavioral changes were noted. Serotonin, 50 μg, slightly inhibited muricide and the incidence was 78%. There were no noteworthy behavioral changes with these drugs.

The effects of alpha- and beta-blockers were studied only using rats in which muricide was suppressed by DMI, 20 μg or NA, 20 μg, injected into the medial amygdaloid nucleus. The results of NA were shown in Fig. 2 and Table 3 and those of DMI were summarized in Table 3. Muricide was not inhibited by the single administration of 20 μg of phenoxybenzamine or sotalol. No other noteworthy behavioral changes were observed.

TABLE 2

EFFECTS ON MURICIDE OF VARIOUS BIOGENIC AMINES AND GABA INJECTED INTO THE MEDIAL AMYGDALOID NUCLEUS

Drug	Dose (μg/2 μl)	The number of rats	Incidence of muricide (%)
Saline		17	100*
Noradrenaline	20	20	50†
	50	17	24†
Adrenaline	50	10	30†
Isoproterenol	50	12	93
Dopamine	50	9	100
Serotonin	50	9	78
GABA	50	9	100

*Muricide was tested 30 min after drug injection.
†Significant differences from the values of saline-treated animals: $p<0.001$.

NA-induced muricide inhibition was markedly antagonized by phenoxybenzamine, 20 μg, injected 10 min before NA treatment (Fig. 2A). Although muricide was inhibited in all rats receiving NA alone, muricide was suppressed in only 30% of the rats pretreated with phenoxybenzamine (Fig. 2A). On the other hand, pretreatment with sotalol, 20 μg, did not affect NA-induced muricide inhibition (Fig. 2B). Mild paralysis of the hindlimbs was seen for several minutes immediately after NA administration, in rats concomitantly treated with NA and phenoxybenzamine or sotalol, but no other noteworthy behavioral changes were seen subsequently.

DISCUSSION

Muricide in OB rats was inhibited by systemic adminis-

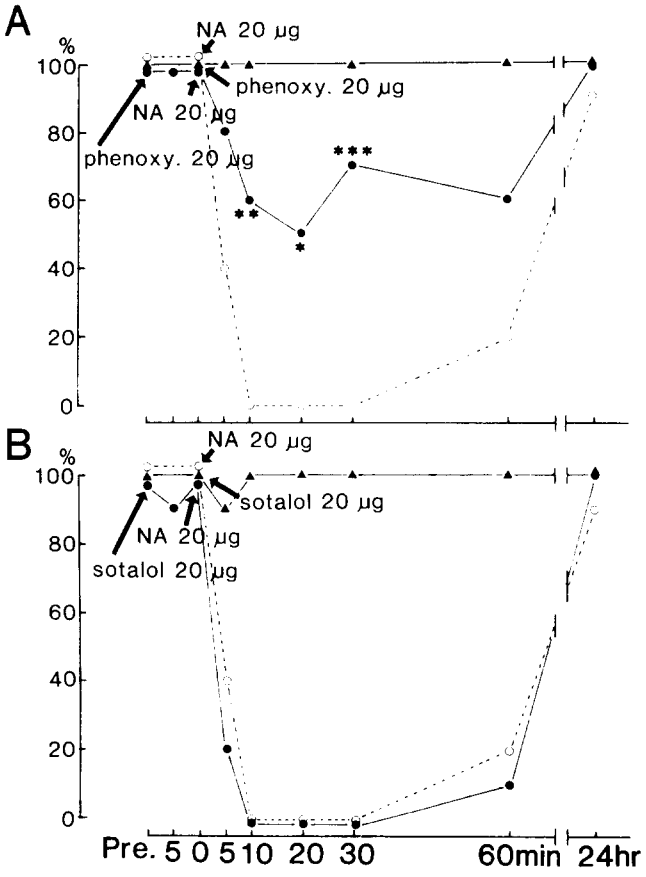

FIG. 2. Effects of phenoxybenzamine (A) and sotalol (B) on muricide inhibition induced by the microinjection of NA 20 μg into the medial amygdaloid nucleus. Ten rats were used in each group. Pre: before drug administration, phenoxy: phenoxybenzamine. Significant differences from the values of NA-treated animals: $*p<0.05$, $**p<0.01$ and $***p<0.005$.

tration of DMI without any remarkable signs of ataxia or muscle relaxation. Among tricyclic antidepressants, DMI is known to predominantly inhibit the uptake of NA into nerve endings [1,15]. DMI-induced muricide inhibition is therefore thought to be due to increased activity of the NA neurons in the central nervous system. Since both alpha- and beta-receptors are known to exist in the central nervous system [2,4], we have studied as to whether DMI-induced muricide inhibition was mediated by alpha- or beta-receptors. DMI-induced muricide inhibition was not antagonized by yohimbine and phentolamine. Moreover the administration of yohimbine alone significantly inhibited muricide. Yohimbine and phentolamine preferentially block alpha$_2$-receptors [3] whereas phenoxybenzamine is more effective at alpha$_1$- than alpha$_2$-receptors [3,10]. Since excitation of alpha$_2$-receptors is claimed to inhibit NA release via a feedback mechanism, blockade of these alpha$_2$-receptors should promote NA release, resulting in elevated NA nerve function [10]. Therefore, it is likely that yohimbine and phentolamine did not antagonize DMI-induced muricide inhibition because of their blocking action on alpha$_2$-receptor.

Muricide inhibition induced by DMI was antagonized markedly by phenoxybenzamine without causing muscle relaxation or ataxia. The administration of phenoxybenzamine alone had almost no influence on muricide. The antagonism of DMI-induced muricide inhibition by phenoxybenzamine is therefore attributable to a blocking action on the alpha$_1$-receptor.

When DMI was coadministered with beta-blockers, it was interesting to note that DMI-induced muricide inhibition was augmented by propranolol, causing remarkable muscle relaxation and ataxia, but was unaffected by sotalol, without muscle relaxation or ataxia.

Microinjection of NA, AD and DMI into the medial amygdaloid nucleus dose-dependently inhibited muricide in OB rats in agreement with our previous reports [21]. However, this muricide was completely unaffected by isoproterenol, dopamine or GABA, and only mildly inhibited by serotonin. All of these results strongly suggest that NA- or DMI-induced muricide inhibition in OB rats is produced by the mechanism mediated by central noradrenergic alpha-receptors, especially by alpha-receptors in the medial amygdaloid nucleus.

Recently we reported that muricide inhibition by electroconvulsive shock was antagonized by phenoxybenzamine but unaffected by sotalol [18]. The duration of immobility in forced swimming rats was reduced by antidepressants, and this reduction was antagonized by phenoxybenzamine [7]. Histochemical [11], biochemical [14] and autoradiographic [5] studies have indicated that the NA content of the amygdala is quite high. Furthermore, the study of receptor binding assays employing ^{3}H-WO1101 [4] and ^{3}H-dihydroalprenolol

TABLE 3

EFFECTS OF AN ALPHA- AND BETA-BLOCKER ON MURICIDE INHIBITION INDUCED BY MICROINJECTION OF DESIPRAMINE (DMI) AND NORADRENALINE (NA) INTO THE MEDIAL AMYGDALOID NUCLEUS

Blockers	Saline (2 μl)	DMI (20 μg/2 μl)	NA (20 μg/2 μl)
Saline (2 μl)	17/17	8/26*†	10/20*†
Phenoxybenzamine (20 μg/2 μl)	10/10	6/8‡	7/10‡
Sotalol (20 μg/2 μl)	10/10	0/8	0/10

*Effects of alpha- and beta-blockers were studied only using rats in which muricide was suppressed by DMI 20 μg or NA 20 μg.

†Number of rats showing muricide/the number of rats tested.

‡Significant differences from the values of DMI- or NA-tested animals: $p<0.005$.

[2] and electrophysiological studies [16,17] suggest that both alpha- and beta-receptors exist in the amygdala. Moreover, there is a report that the mechanism mediated by alpha-receptors in the amygdala is involved in the manifestation of anticonflict activity in the rat [12], indicating that it is highly probable that this mechanism in the amygdala plays an important role in the regulation of emotional behavior in animals.

The aforementioned facts and results of our present experiment at least indicate that inhibition of muricide in OB rats by DMI and NA injected into the medial amygdaloid nucleus is produced by activating the mechanisms mediated by alpha-receptors in this area.

ACKNOWLEDGEMENT

This study was supported by Grant-in-Aid for Scientific Research from Japanese Ministry of Education, Science and Culture. The authors are also grateful to CIBA-GEIGY Co. for the kind supply of desipramine and phentolamine.

REFERENCES

1. Bunney, W. E. and J. M. Davis. Norepinephrine in depressive reactions: A review. *Arch Gen Psychiatry* **13:** 483–494, 1965.
2. Bylund, D. M. and S. H. Snyder. Beta adrenergic receptor binding in membrane preparations from mammalian brain. *Mol Pharmacol* **12:** 568–580, 1976.
3. Doxey, J. C., C. F. C. Smith and J. M. Walker. Selectivity of blocking agents for pre- and postsypantic α-adrenoceptors. *Br J Pharmacol* **60:** 91–96, 1977.
4. Greenberg, D. A., P. C. U'Prichard and S. H. Snyder. Alpha noradrenergic receptor binding in mammalian brain: differential labeling of agonist and antagonist states. *Life Sci* **19:** 69–76, 1976.
5. Jones, B. E. and R. Y. Moore. Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Res* **127:** 23–53, 1977.
6. Karli, P., M. Vergnes, F. Eclancher, P. Schmitt and J. P. Chaurand. Role of the amygdala in the control of "mouse-killing" behavior in the rat. *Advances in Behavioral Biology*, vol. 2, edited by B. E. Efeftheriou. New York: Plenum Press, 1972, pp. 553–580.
7. Kitada, Y., T. Miyauchi, Y. Kanazawa and S. Satoh. Effects of antidepressants in the rat forced swimming test (report 2). *Jpn J Pharmacol* **31:** 160P, 1981.
8. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore, MD: Williams and Wilkins, 1963.
9. Kumadaki, N., M. Hitomi and S. Kumada. Effect of psychotherapeutic drugs on hyperemotionality of rats in which the olfactory bulb was removed. *Jpn J Pharmacol* **17:** 659–667, 1967.
10. Langer, S. Z. Presynaptic receptors and their role in the regulation of transmitter release. *Br J Pharmacol* **60:** 481–497, 1977.
11. Lindvall, O. and A. Björklund. The organization of the ascending catecholamine neuron systems in the rat brain. *Acta Physiol Scand* (Suppl) **412:** 1–48, 1977.
12. Margules, D. L. Alpha and beta adrenergic receptor in amygdala: Reciprocal inhibitions and facilitations of punished operant behavior. *Eur J Pharmacol* **16:** 21–26, 1971.
13. Oishi, R. and S. Ueki. Facilitation of muricide by dorsal norepinephrine bundle lesions in olfactory bulbectomized rats. *Pharmacol Biochem Behav* **8:** 133–136, 1978.
14. Ross, R. A. and D. J. Reis. Effects of lesions of locus coeruleus on regional distribution of dopamine-β-hydroxylase activity in rat brain. *Brain Res* **73:** 161–166, 1977.
15. Schildkraut, J. J. The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am J Psychiatry* **122:** 509–522, 1965.
16. Shibata, S., N. Hori and S. Ueki. Amygdaloid field potential evoked by stimulation of the lateral olfactory tract in brain slice with relation to muricide of olfactory bulbectomized rats. *Jpn J Pharmacol* **29:** Suppl. 42P, 1979.
17. Shibata, S., R. Oishi and S. Ueki. Effect of desipramine on noradrenergic inhibition of amygdaloid evoked potential. *Jpn J Pharmacol* **29:** 489–492, 1979.
18. Shibata, S., S. Watanabe, H. Nakanishi and S. Ueki. Effects of electroconvulsive shock on mouse-killing behavior (muricide) in olfactory bulbectomized rats. *Jpn J Pharmacol* **31:** 275–280, 1981.
19. Shibata, S., T. Yamamoto and S. Ueki. Differential effects of medial, central and basolateral amygdaloid lesions on four models of experimentally-induced aggression in rats. *Physiol Behav* **28:** 289–294, 1982.
20. Ueki, S., S. Murimoto and N. Ogawa. Effects of psychotropic drugs on emotional behavior in rats with limbic lesions, with special reference to olfactory bulb ablations. *Folia Psychiatr Neurol Jpn* **26:** 246–255, 1972.
21. Watanabe, S., M. Inoue and S. Ueki. Effects of psychotropic drugs injected into the limbic structures on mouse-killing behavior in the rat with olfactory bulb ablations. *Jpn J Pharmacol* **29:** 493–496, 1979.