Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 1, pp. 13–18, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0082-S

β_2- But Not β_1-Adrenoceptors Are Involved in Desipramine Enhancement of Aggressive Behavior in Long-Term Isolated Mice

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Received 27 September 1993

MATSUMOTO, K., K. OJIMA, H. OHTA AND H. WATANABE. *β_2- but not β_1-adrenoceptors are involved in desipramine enhancement of aggressive behavior in long-term isolated mice.* PHARMACOL BIOCHEM BEHAV **49**(1) 13–18, 1994.—The effects of several β-adrenoceptor antagonists on the desipramine-induced increase in aggressive behavior in long-term isolated mice were examined. Desipramine HCl (10 mg/kg, IP) significantly increased the duration of aggressive behavior in isolated mice but did not significantly change the latency to the first attack consistent with our previous reports. Intraperitoneal administration of (±)propranolol HCl (2.5–10 mg/kg), a nonselective β-adrenoceptor antagonist, dose dependently attenuated the desipramine-induced enhancement of aggressive behavior without significantly affecting the basal aggressive responses. ICI118,551 HCl (1.25–5 mg/kg, IP), a selective β_2-adrenoceptor antagonist, also blocked the desipramine-induced enhancement of aggressive behavior in a dose-dependent manner, whereas metoprolol tartrate (5–20 mg/kg, IP), a selective β_1-adrenoceptor antagonist, did not affect it. Moreover, clenbuterol HCl (0.1–0.5 mg/kg, IP), a lipophilic β_2-adrenoceptor agonist, significantly increased the duration of basal aggressive behavior. Taken together with our previous finding that the desipramine-induced enhancement of aggressive behavior can be blocked by yohimbine, an α_2-adrenoceptor antagonist, the present results indicate that not only α_2- but also β_2-adrenoceptor stimulation plays important roles in modulation of aggressive behavior in long-term isolated mice.

Mice Aggressive behavior Isolation Desipramine Noradrenaline Propranolol β_1-Adrenoceptors β_2-Adrenoceptors Metoprolol ICI118,551 Clenbuterol

LONG-TERM social isolation can induce aggressive behavior in nonaggressive laboratory mice (2,25). Such behavioral changes in mice have been extensively studied and used as an animal model to elucidate drug actions on aggression and/or social behavioral deficits (10,23). We have previously reported that antidepressant drugs with the ability to inhibit noradrenaline uptake increase aggressive behavior in socially isolated mice and that these effects can be modulated by drugs acting on α_2- but not α_1-adrenoceptors (5,15). These data suggest that functional changes in the central noradrenergic system, especially changes in the sensitivity of α_2-adrenoceptors, may be induced in mice by long-term social isolation, and that such plastic changes in the central noradrenergic system may be closely related to the antidepressant-induced enhancement of aggressive behavior in isolated mice (5,15).

Recent studies have shown that central β-adrenoceptor stimulation exhibits properties related to antidepressant effect in animal models (8,29). For example, downregulation of β-adrenoceptors by chronic antidepressant drug administration appears to play an important role in antidepressant drug efficacy (1,3). So far, however, enhancement of aggressive behavior by β-adrenoceptor stimulation has not been demonstrated.

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In the present study, to clarify the involvement of the β-noradrenergic system in the desipramine-induced enhancement of aggressive behavior in socially isolated mice, we examined the effects of several β-adrenoceptor antagonists with different selectivity against β_1- and β_2-adrenoceptor subtypes. We also tested whether clenbuterol, a lipophilic β_2-adrenoceptor agonist that penetrates the blood-brain barrier to interact with central β_2-adrenoceptors, enhances isolation-induced aggressive behavior or not.

METHOD

Isolated Housing

Animals were housed in isolation as described in our previous report (5,15). Briefly, male ddY mice, weighing approximately 18-20 g (SLC Co., Shizuoka, Japan) were obtained at the age of 28 days. Mice were isolated in 24 × 17 × 12 cm cages for 6-7 weeks before the experiments. Our preliminary data indicated that this isolation period induces a stable aggressive behavior in nonaggressive ddY strain mice. Housing conditions were thermostatically maintained at 24 ± 1°C, with a 12L : 12D cycle. Food and water were given ad lib.

Measurement of Aggressive Responses

When testing aggressive behavior between isolated mice, two isolated mice were placed in a neutral cage (24 × 17 × 12 cm) of the same size as their home cages. The total duration of biting attacks, wrestling, or both observed during a 20-min period was measured. The latency to the first attack was also recorded using an event recorder connected to a computer (PC 9801NS, NEC). Aggressive behavior was also videotape-recorded (camera and videorecorder, National, Japan) for later analysis.

Drugs

When testing the aggressive behavior, drugs were intraperitoneally injected at a constant volume (0.01 ml/g body weight) 60 min before the experiments. The two isolated mice in the same pair received the same drug. The test drugs were dissolved in saline just before the experiments. Drugs used were as follows: desipramine HCl, (±)-propranolol HCl, metoprolol tartrate, clenbuterol HCl (Sigma, St. Louis, MO), and ICI118,551 (Cambridge Research Biochemicals, Wilmington, DE).

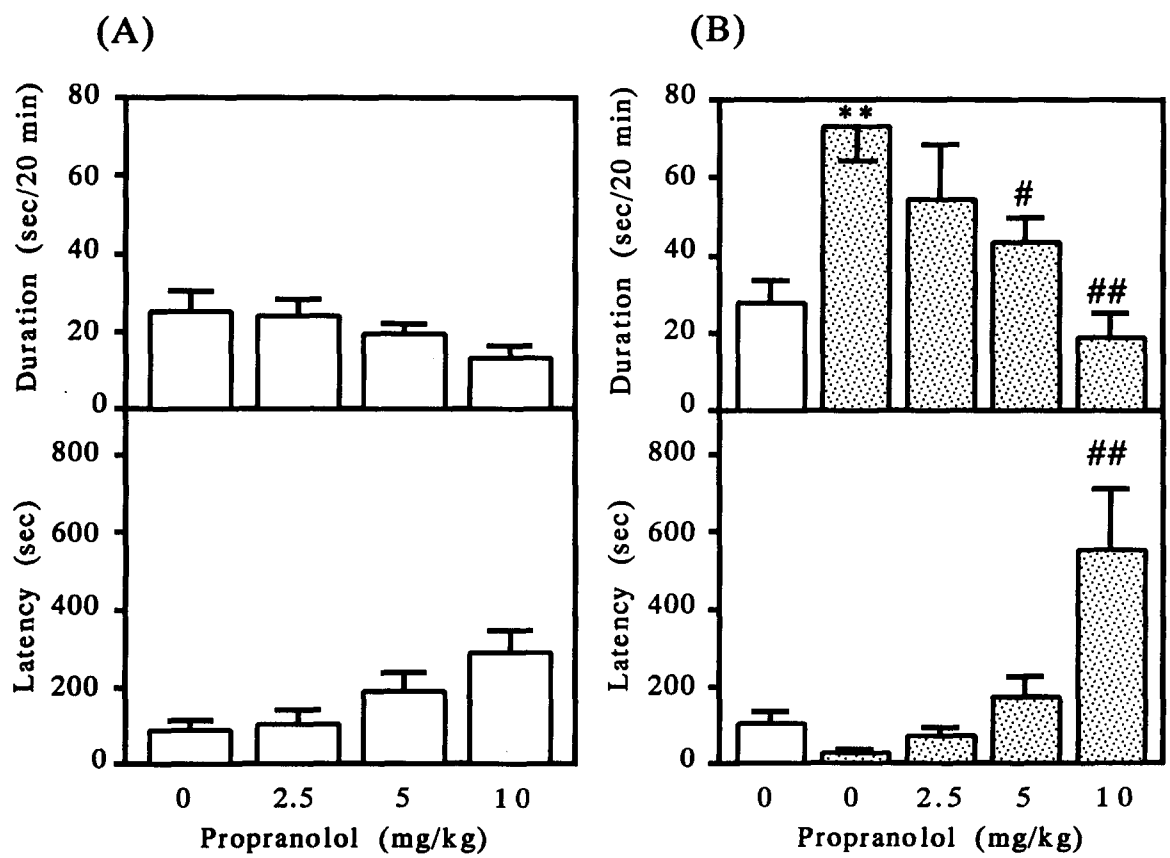

FIG. 1. Effects of (±)-propranolol on basal aggressive behavior (A) and desipramine-induced increase in aggressive behavior (B) in isolated mice. (A) Either saline or (±)-propranolol HCl was injected (IP) 60 min before the experiments. (B) Either saline or desipramine HCl (10 mg/kg, IP) was injected 60 min before the experiments. (±)-Propranolol HCl or saline was injected (IP) immediately after desipramine. Dotted columns represent the animals injected 10 mg/kg desipramine. Each datum represents the mean of seven to eight pairs of animals, with SEM indicated. $**p < 0.01$ compared to animals given saline alone. $\#p < 0.05$ and $\#\#p < 0.01$ compared to animals given desipramine alone.

Statistics

Nonparametric data were analyzed with the Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test for multiple comparisons between groups. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effects of β-Adrenoceptor Antagonists on Desipramine-Induced Enhancement of Aggressive Behavior in Isolated Mice

Intraperitoneal administration of 10 mg/kg desipramine significantly increased the duration of aggressive behavior in isolated mice ($U = 1$, $p < 0.01$). Consistent with our previous data, the desipramine-induced increase in the duration of aggressive behavior did not accompany significant changes in the latency to the first attack. (±)-Propranolol did not significantly change these parameters at doses of 2.5 to 10 mg (Fig. 1A). However, when coadministered with desipramine (10 mg/kg, IP), (±)-propranolol at doses of 5 and 10 mg/kg decreased the duration of aggressive behavior increased by desipramine in a dose-dependent manner ($U = 5$, $p < 0.05$; $U = 0$, $p < 0.01$, respectively). This antagonist significantly prolonged the latency to the first attack at a dose of 10 mg/kg ($U = 0$, $p < 0.01$) (Fig. 1B).

As shown in Fig. 2, a selective β_1-adrenoceptor antagonist, metoprolol, at doses of 5–20 mg/kg, did not significantly affect the duration of aggressive behavior prolonged by 10 mg/kg desipramine. On the other hand, ICI118,551 (1.25–5 mg/kg, IP), a selective β_2-adrenoceptor antagonist, significantly and dose dependently decreased the duration of aggressive behavior prolonged by 10 mg/kg desipramine without affecting the latency to the first attack ($U = 10$, $p < 0.05$; $U = 10$, $p < 0.05$; $U = 5$, $p < 0.01$, respectively) (Fig. 3B). This antagonist did not change these parameters by itself, at doses of 1.25 to 5 mg/kg (Fig. 3A).

Effects of Clenbuterol, a β_2-Adrenoceptor Agonist, on Isolation-Induced Aggressive Behavior in Mice

To further confirm whether β_2-adrenoceptor stimulation causes an enhancement of aggressive behavior or not, we examined effect of clenbuterol, a selective β_2-adrenoceptor agonist, on isolation-induced aggressive behavior. As shown in Fig. 4, clenbuterol, at 0.1 and 0.5 but not 0.02 mg/kg, significantly increased the basal duration of aggressive behavior in isolated mice ($U = 0$, $p < 0.05$; $U = 0$, $p < 0.05$; $U = 27.5$, $p > 0.05$, respectively).

DISCUSSION

We previously found that desipramine administration increased the duration of aggressive behavior but did not significantly change the latency to the first attack in socially isolated mice (15,16). This increase in aggressive behavior caused by desipramine has been shown to be sensitively blocked by yohimbine, an α_2-adrenoceptor antagonist (15). The present findings further support the hypothesis that the adrenergic system is involved in the desipramine-induced increase in aggressive behavior, and provide strong evidence that the β_2-adrenoceptor as well as α_2-adrenoceptor participates in the effect of desipramine on aggressive behavior.

In the present study, (±)-propranolol dose dependently attenuated desipramine-induced increase in aggressive behavior without significantly affecting the basal aggressive responses, suggesting that noradrenaline stimulation of β-adrenoceptors is involved in the desipramine-induced increase in aggressive behavior in socially isolated mice. However, previous in vivo and in vitro studies have shown that (±)-propranolol acts not only as a β-adrenoceptor antagonist but also as a 5-HT receptor antagonist (17,22,26). Moreover, in vivo administration of desipramine, a selective noradrenaline uptake blocker, also has some ability to inhibit 5-HT uptake into brain synaptosomes (14,27). Therefore, it is possible that serotonergic systems play a role in the antagonism between desipramine and (±)-propranolol in isolation-induced aggressive behavior. This possibility, however, is unlikely, since our preliminary data demonstrated that DSP-4–induced lesion of noradrenergic terminals projecting mainly from the locus coeruleus almost completely attenuated the desipramine-induced enhancement of aggressive behavior in isolated mice (16).

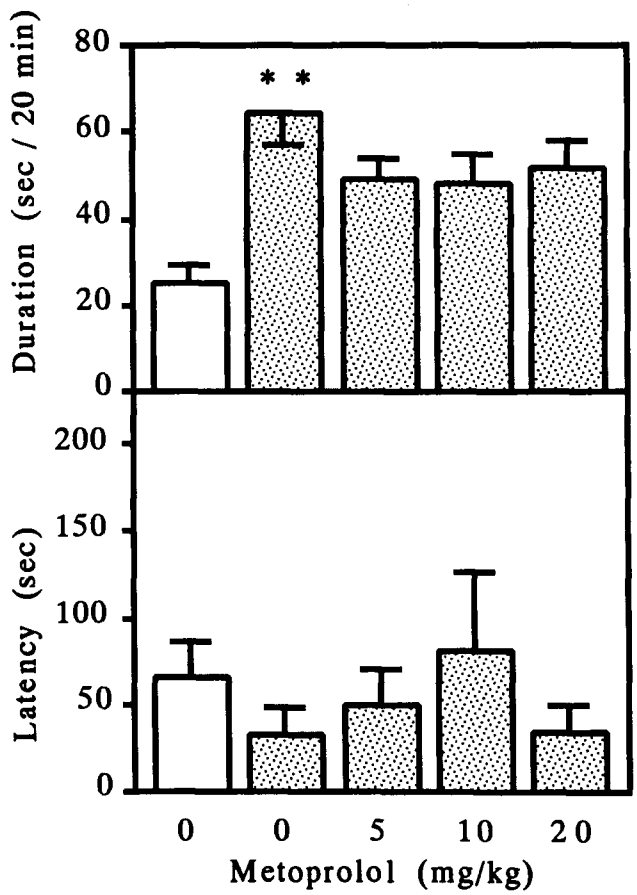

FIG. 2. Effects of metoprolol, a selective β_1 adrenoceptor antagonist, on desipramine-induced increase in aggressive behavior in isolated mice. Either saline or desipramine HCl (10 mg/kg, IP) was injected into isolated animals 60 min before the experiments. Metoprolol tartrate was injected (IP) immediately after desipramine. Dotted columns represent the animals injected with 10 mg/kg desipramine. Each datum represents the mean of seven to eight pairs of animals, with SEM indicated. $^{**}p < 0.01$ compared to animals given saline alone.

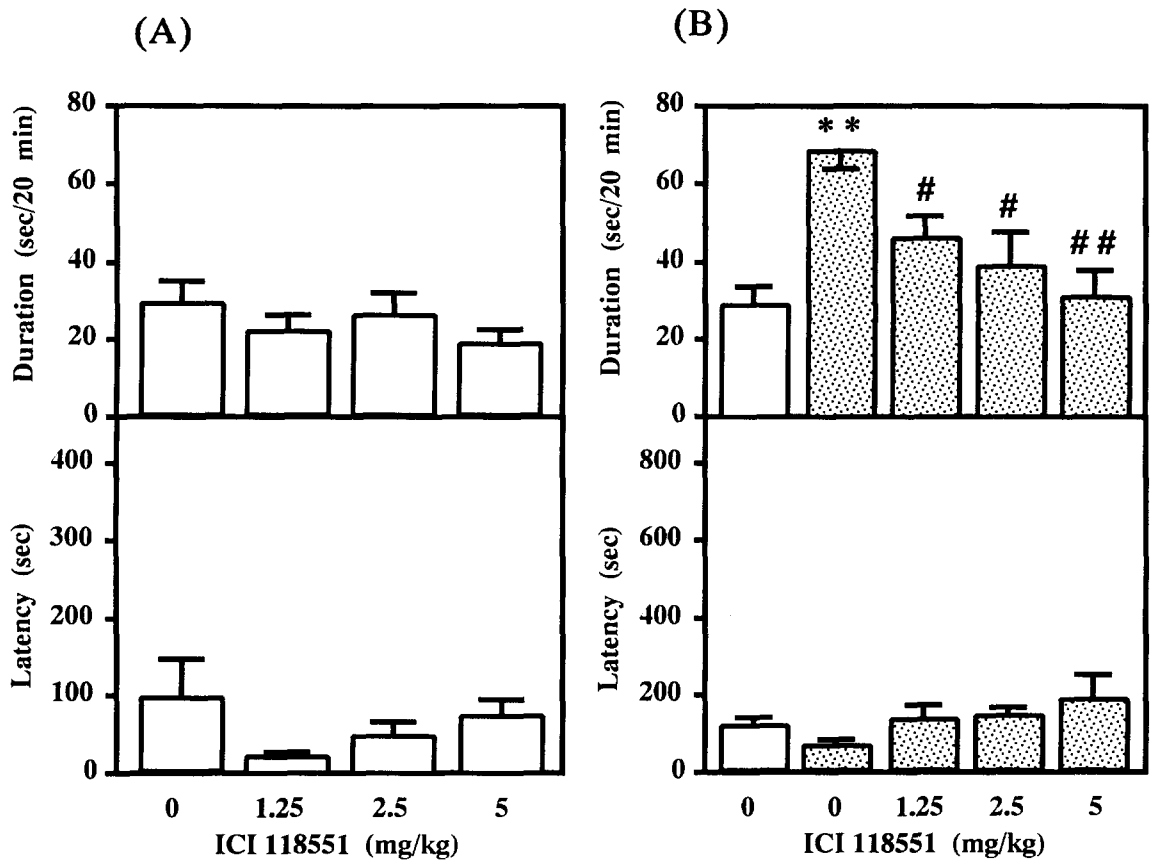

FIG. 3. Effects of ICI118,551, a selective β_2 adrenoceptor antagonist, on basal aggressive behavior (A) and desipramine-induced increase in aggressive behavior (B) in isolated mice. (A) ICI118,551 HCl or saline was intraperitoneally injected 60 min before the experiments. (B) Either saline or desipramine HCl (10 mg/kg, IP) was injected 60 min before the experiments. ICI118,551 HCl was intraperitoneally injected immediately after desipramine. Dotted columns represent the animals injected with 10 mg/kg desipramine. Each datum represents the mean of six to eight pairs of animals, with SEM indicated. $^{**}p < 0.01$ compared to animals given saline alone. $\#p < 0.05$ and $\#\#p < 0.01$ compared to animals given desipramine alone.

Metoprolol and ICI118,551 have been used as highly selective and specific antagonists for the central β_1- and β_2-adrenoceptor, respectively (4,6,21). Moreover, (±)-propranolol is known to interact with β_1- and β_2-adrenoceptors with equal affinity. In the present study, ICI118,551 and (±)-propranolol both attenuated the desipramine-induced enhancement of aggressive behavior in a dose-dependent manner without changing the basal aggressive response, whereas metoprolol did not affect it. Taken together, these results indicate that of the two β-adrenoceptor subtypes, β_2 subtype of adrenoceptor, in particular, participates in the desipramine-induced increase in aggressive behavior in socially isolated mice. The β_2-adrenoceptor-mediated modulation of aggressive behavior is further supported by the fact that clenbuterol, a lipophilic β_2-adrenoceptor agonist, significantly increased the basal duration of aggressive behavior in isolated mice. The effect of clenbuterol was relatively smaller than that of desipramine. The reason for this difference remains unclear but both β_2- and α_2-adrenoceptor stimulation may be necessary to full enhancement of aggressive behavior in isolated mice. Nevertheless, the present finding suggests that long-term social isolation of mice induces functional changes in β_2-adrenoceptor as well as α_2-adrenoceptor (5,15). This speculation is supported by the finding that isolated-housing increases the effect of β-adrenoceptor stimulation on hypothermia induced by oxotremorine and apomorphine in mice (9).

It is of interest that isolation-induced aggressive behavior could be enhanced by β_2- but not β_1-adrenoceptor stimulation, because most of the adaptive changes of β-adrenoceptors following chronic administration of antidepressant drugs and chemical denervation with 6-hydroxydopamine have been ascribed to changes in the β_1- but not the β_2-adrenoceptor subtype (1,7,18). Involvement of different β-adrenoceptor subtypes has also been reported in other behavioral models such as corticotropin releasing factor-induced defensive withdrawal, which can be modulated by β_1- but not β_2-adrenoceptor subtype, and 5-hydroxytryptophane-induced head twitch behavior, which can be modulated by both β_1- and β_2-adrenoceptor subtypes (11,28). Moreover, quantitative autoradiographic studies have revealed a marked difference in the ratio of β_1- to β_2-adrenoceptors among different brain regions (12,21). Therefore, the present results give further evidence

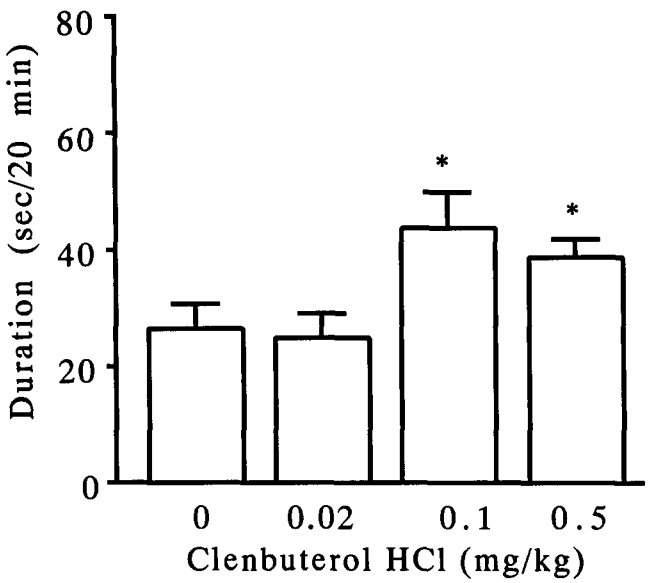

FIG. 4. Effects of clenbuterol on basal aggressive behavior in isolated mice. Either saline or clenbuterol HCl (0.02–0.5 mg/kg) was intraperitoneally injected into isolated animals 60 min before the experiments. Each datum represents the mean of nine pairs of animals, with SEM indicated. *$p < 0.05$ compared to saline control.

that β-adrenergic subtypes may play different roles in neuronal function (21).

Because β_2-adrenoceptor is more sensitive to adrenaline than to noradrenaline (19), it is unclear whether β_2-adrenoceptor-mediated modulation of aggressive behavior is due to direct stimulation of β_2-adrenoceptor by noradrenaline. In the peripheral nervous system, adrenaline stimulation of presynaptic β_2-adrenoceptor facilitates noradrenaline release from nerve terminals (13). Such presynaptic β_2-adrenoceptors also have been demonstrated in the central nervous system (20,24). Therefore, it can be speculated that stimulation of presynaptic β_2-adrenoceptors by clenbuterol may enhance noradrenaline release, resulting in an enhancement of aggressive behavior as desipramine does, and that blocking of such receptors by ICI118,551 may decrease noradrenaline release, producing an apparent antagonistic effect on desipramine-induced enhancement of aggressive behavior in isolated mice. The exact mechanism of β_2-adrenoceptor-mediated modulation of aggressive behavior in isolated mice remains to be clarified.

ACKNOWLEDGEMENT

This work was supported in part by Grant-in-Aid #04671346 for Scientific Research from the Ministry of Education, Science and Culture, Japan, and by the Foundation for the Promotion of Higher Education in Toyama Prefecture.

REFERENCES

1. Banerjee, S. P.; Kung, L. S.; Riggi, S. J.; Chanda, S. K. Development of β-adrenergic receptor subsensitivity by antidepressants. Nature 268:455–456; 1977.
2. Bell, R.; Hepper, P. G. Catecholamine; Aggression in animals. Behav. Brain Res. 23:1–21; 1987.
3. Bergstrom, D. A.; Kellar, K. J. Adrenergic and serotonergic receptor binding in rat brain after chronic desmethylimipramine. J. Pharmacol. Exp. Ther. 209:256–261; 1979.
4. Bilski, A. J.; Halliday, S. E.; Fitzgerald, J. D.; Wale, J. L. The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118,551). J. Cardiovasc. Pharmacol. 5:430–437; 1983.
5. Cai, B.; Matsumoto, K.; Ohta, H.; Watanabe, H. Biphasic effects of typical antidepressants and mianserin, an atypical antidepressant, on aggressive behavior in socially isolated mice. Pharmacol. Biochem. Behav. 44:519–525; 1993.
6. Davies, D. C.; Payne, J. M. Ammnesia of a passive avoidance task due to the β_2-adrenoceptor antagonist ICI118,551. Pharmacol. Biochem. Behav. 32:187–190; 1989.
7. Dooley, D. J.; Hauser, K. L.; Bittiger, H. Differential decrease of the central beta-adrenergic receptor in the rat after subchronic infusion of desipramine and clenbuterol. Neurochem. Int. 5:333–338; 1983.
8. Duteil, J., Rambert, F. A.; Pointeau, A. M.; Mangiameli, P.; Assous, E. Flerobuterol: A new antidepressant drug related to beta-adrenergic agonists. Experimental profile in mice. Psychopharmacology (Berlin) 96(Suppl. S276); 1988.
9. Frances, H.; Lecrubier, Y.; Simon, P. Isolation increases the response to β-adrenergic stimulation in mice. Biol. Psychiatry 15: 965–968; 1980.
10. Frances, H.; Lienard, C. Isolation-induced social behavioral deficit test: Effect of tranquilizing drugs. Pharmacol. Biochem. Behav. 34:293–296; 1989.
11. Handley, S. L.; Singh, L. The modulation of head-twitch behavior by drugs acting on beta-adrenoceptors: evidence for the involvement of both $beta_1$- and $beta_2$-adrenoceptors. Psychopharmacology (Berlin) 88:320–324; 1986.
12. Kalaria, R. N.; Andorn, A. C.; Tabaton, M.; Whitehouse, P. J.; Harik, S. I.; Unnerstall, J. R. Adrenergic receptors in aging and Alzheimer's disease: Increased β_2-receptors in prefrontal cortex and hippocampus. J. Neurochem. 53:1772–1781; 1989.
13. Majewski, H. Modulation of noradrenaline release by prejunctional beta adrenoceptors. J. Autonom. Pharmacol. 3:47–60; 1983.
14. Manias, B.; Taylor, D. A. Inhibition of in vitro amine uptake into rat brain synaptosomes after in vivo administration of antidepressants. Eur. J. Pharmacol. 95:305–309; 1983.
15. Matsumoto, K.; Cai, B.; Satoh, T.; Ohta, H.; Watanabe, H. Desipramine enhances isolation-induced aggressive behavior in mice. Pharmacol. Biochem. Behav. 39:167–170; 1991.
16. Matsumoto, K.; Ojima, K.; Cai, B.; Ohta, H.; Watanabe, H. Noradrenergic denervation and chronic desipramine decrease acute desipramine-induced enhancement of aggressive behavior in isolated mice. Jpn. J. Pharmacol. 61(Suppl. I):191p; 1993.
17. Middlemiss, D. N.; Blakeborough, L.; Leather, S. R. Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. Nature 267:289–290; 1977.
18. Minneman, K. P.; Dibner, M. D.; Wolfe, B. B.; Molinoff, P. B. β_1- and β_2-Adrenergic receptors in rat cerebral cortex are independently regulated. Science 204:866–868; 1979.
19. Minneman, K. P.; Hegstrand, L. R.; Molinoff, P. B. The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. Mol. Pharmacol. 16:21–33; 1979.
20. Misu, Y.; Kubo, T. Presynaptic beta adrenoceptor. Med. Res. Rev. 6:197–225; 1986.
21. Rainbow, T. C.; Parsons, B.; Wolfe, B. B. Quantitative autoradiography of β_1- and β_2-adrenoceptors in rat brain. Proc. Natl. Acad. Sci. USA 81:1585–1589; 1984.
22. Schechter, Y.; Weinstock, M. β-Adrenoceptor blocking agents

and responses to adrenaline and 5-hydroxytryptamine in isolated rat stomach and uterus. Br. J. Pharmacol. 52:283–287; 1974.
23. Sofia, R. D. Effects of centrally active drugs on four models of experimentally induced aggression in rodents. Life Sci. 8:705–716; 1969.
24. Ueda, H.; Goshima, Y.; Kubo, T.; Misu, Y. Involvement of epinephrine in the presynaptic beta adrenoceptor mechanism of norepinephrine release from rat hypothalamic slices. J. Pharmacol. Exp. Ther. 232:507–512; 1984.
25. Valzelli, L. The isolation syndrome in mice. Psychopharmacologia 31:305–320; 1973.
26. Weinstock, M.; Weiss, C.; Gitter, S. Blockade of 5-hydroxytryptamine receptors in the central nervous system by β-adrenoceptor antagonists. Neuropharmacology 16:273–276; 1977.
27. Wyllie, M. G.; Wood, M. D.; Moser, P. C. Assessment of noradrenaline and 5-hydroxytryptamine uptake in intact rat brain. J. Neurochem. 45:456–460; 1985.
28. Yang, X.-M.; Dunn, A. J. Central β_1-adrenergic receptors are involved in CRF-induced defensive withdrawal. Pharmacol. Biochem. Behav. 36:847–851; 1990.
29. Zini, R.; Morin, D.; Martin, P.; Puech, A. J.; Tillement, J. P. Interactions of flerobuterol, an antidepressant drug candidate, with beta adrenoceptors in the rat brain. J. Pharmacol. Exp. Ther. 259:414–422; 1991.