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Chronic forced swim stress of rats increases frontal cortical 5-HT_2 receptors and the wet-dog shakes they mediate, but not frontal cortical β-adrenoceptors

Katsuyuki Takao [a,b], Tadashi Nagatani [b,*], Yoshihisa Kitamura [b], Koh Kawasaki [b], Hiroshi Hayakawa [a], Shigeto Yamawaki [a]

[a] *Department of Psychiatry and Neurosciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan*
[b] *First Laboratory for Pharmacological Research, Institute for Life Science Research, Asahi Chemical Industry Co., Ltd., 632-1 Mifuku, Ohito, Tagata, Shizuoka 410-23, Japan*

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Abstract

We studied the effects of chronic forced swim stress on 5-HT_2 receptors and β-adrenoceptors in the rat frontal cortex. The number of 5-HT_2 receptors was increased immediately after the last chronic stress, but not after an acute stress. In vivo, the number of wet-dog shakes induced by a 5-HT_2 receptor agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), was increased 24 h after the last chronic stress. However, the concentrations of 5-HT and 5-hydroxyindole acetic acid (5-HIAA), measured by high pressure liquid chromatography (HPLC), were not altered by this stress. Binding sites for [^{3}H]CGP-12177, i.e., β-adrenoceptor sites, were unchanged after both the acute and the chronic stress. These results suggest that, in the rat, the chronic forced swim stress increases the number of frontal cortical 5-HT_2 receptors and the number of wet-dog shakes mediated by these receptors, while the number of frontal cortical β-adrenoceptors is not increased by this treatment.

Keywords: Forced swim test; Stress; 5-HT_2 receptor; Wet-dog shake; β-Adrenoceptor

1. Introduction

Stress is thought to be an important etiological factor in many psychiatric diseases, including affective disorders. It is well known that stressful manipulation produces neurochemical and hormonal changes in both the central and the peripheral nervous systems. In the central nervous system (CNS), monoaminergic systems have been the main target of stress research, and stress has been reported to affect the function of brain monoaminergic receptors. In particular, 5-HT receptors and adrenoceptors have been postulated to play an important role in the pathogenesis of affective disorders, based on the following clinical findings. Several studies have shown that the number of 5-HT_2 receptors and β-adrenoceptors is increased in the postmortem brain of suicide victims and depressed subjects (Arango et al., 1990; Mann et al., 1986). These receptors are also increased in the platelets of depressed patients (Biegon et al., 1987; Pandey et al., 1990; Healy et al., 1983).

In animal studies, the number of 5-HT_2 receptors and β-adrenoceptors is decreased in the rat frontal cortex following the chronic administration of antidepressants (Banerjee et al., 1977; Bergstrom and Kellar, 1979; Blackshear and Sanders-Bush, 1980). These findings indicate that the down-regulation of these receptors may be related to the antidepressant activity of antidepressants (Mizuta and Segawa, 1988).

On the other hand, it has become apparent from a number of studies, as noted below, that the capacity of antidepressants to down-regulate 5-HT_2 receptors and β-adrenoceptors is not a property shared by all antide-

* Corresponding author. Tel.: (0558) 76-7081; fax: (0558) 76-7147.

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pressants. A number of antidepressants, such as maprotiline (Barbaccia et al., 1986), citalopram (Hyttel et al., 1984), and fluoxetine (Wong et al., 1985) fail to reduce the number of β-adrenoceptors in rat cerebral cortex. Chronic electroconvulsive seizure (ECS) therapy, one of the most efficacious treatments for depression, is reported to increase 5-HT_2 receptors in the rat frontal cortex (Green et al., 1983; Kellar et al., 1981), an effect opposite to that of antidepressants.

It is not known whether down-regulation of these receptors following the chronic administration of various antidepressants is necessary for their antidepressant activity. Moreover, the reason for the increase in the number of 5-HT_2 receptors and β-adrenoceptors in the postmortem brain of suicide victims and depressed subjects is still not known. One possible explanation for the pathogenesis of affective disorders is that it may represent up-regulation of these receptors, due to lowered levels of serotonergic and noradrenergic activity.

In this study, we examined the influence of acute and chronic forced swim stress, which may model some depressive conditions in animals, on 5-HT_2 receptors and β-adrenoceptors in the synaptic membranes of rat brain. We also carried out a study of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced wet-dog shakes in rats to evaluate the effect of the forced swim stress on 5-HT_2 receptor function in vivo.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 150–200 g (Seiwa Experimental Animals, Fukuoka, Japan), were used. They were housed in groups of five under conditions of controlled temperature (23 ± 3°C) and lighting (dark period 20:00–8:00 h) and had free access to food and water.

2.2. Forced swim stress

For the acute test, the rats were individually forced to swim for 6 min in plastic cylinders (height 24 cm, diameter 10 cm), containing water, to a height of 19 cm, at 25°C. For the chronic tests, they were forced to swim once daily for 14 days. Since the primary aim of the study was to examine the effects of acute and chronic forced swim stress on the 5-HT_2 receptors and β-adrenoceptors themselves, we measured the binding of these receptors according to the same time schedule, immediately after the last chronic stress and after the acute stress. For biochemical studies, receptor binding assay, and measurement of 5-HT and 5-HIAA contents, the rats were removed from the cylinders and decapitated immediately after the last stress. The frontal cortex was dissected and stored at −80°C. However, for the behavioral study, the observations could not be carried out on the wet rats immediately after the swim stress, since the animals lick their wet bodies and the wet-dog shake behavior is interrupted. We therefore observed the wet-dog shake behavior 24 h after the last stress. The biochemical and behavioral experiments were thus performed on different animals.

2.3. Receptor binding assay

2.3.1. Preparation of membranes

The frozen tissues were thawed and homogenized in 0.32 M sucrose in a Polytron (setting 7, 10 s). The homogenate was centrifuged at $1000 \times g$ for 10 min and the supernatant was collected and recentrifuged at $50\,000 \times g$ for 20 min. The resultant pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4 at 25°C) and incubated at 37°C for 15 min. The tissue suspension was recentrifuged at $50\,000 \times g$ for 20 min. The pellet was resuspended in 50 mM Tris-HCl buffer (about 1 mg/ml protein) and stored at −80°C until assayed.

2.3.2. 5-HT_2 receptors

5-HT_2 receptors were measured, using [^{3}H]ketanserin, according to the method of Leysen et al. (1982). Aliquots (200 μl) of the tissue suspension were incubated in duplicate at 37°C for 15 min with 100 μl of 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing [^{3}H]ketanserin (0.06–2.0 nM) and 700 μl of buffer. Non-specific binding was defined with 1 μM methysergide.

2.3.3. β-Adrenoceptors

β-Adrenoceptors were measured, using [^{3}H]CGP-12177, according to the method of Riva and Creese (1989). Aliquots (400 μl) of the tissue suspension were incubated in duplicate at 37°C for 20 min with 100 μl of 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 0.2 nM [^{3}H]CGP-12177 and 500 μl of buffer. Non-specific binding was defined with 1 μM alprenolol.

2.3.4. General methods

The incubation was terminated by rapid filtration through Whatman GF/B filters under reduced pressure. The filters were washed three times with ice-cold Tris-HCl buffer (pH 7.4) and transferred to vials to which scintillation cocktail was added; radioactivity was determined with a liquid scintillation counter. The dissociation constant (K_d) and maximal number of binding sites (B_{max}) were calculated using the EBDA/LIGAND program (McPherson, 1985). The protein content of membranes was determined by the method of Bradford (1976).

Table 1
Effects of acute and chronic forced swim stress on 5-HT_2 receptor binding sites in rat frontal cortex

	K_d (nM)	B_{max} (fmol/mg protein)
Control	0.464±0.020	137.7±4.3
Acute stress	0.518±0.026	145.9±7.8
Chronic stress	0.512±0.028	160.2±5.0 [a]

[^{3}H]Ketanserin, at six concentrations (0.06–2.0 nM), was used for saturation binding experiments and the results were calculated using the EBDA/LIGAND program. Values are means±S.E. for six to seven rats. [a] $P < 0.05$ compared to control rats.

2.4. Measurement of 5-HT and 5-HIAA content

The brain samples were homogenized in 0.1 M perchloric acid sodium containing $Na_2S_2O_5$ (1 g/l) and EDTA 2Na (0.1 g/l) and then centrifuged at 4000 rpm for 20 min at 0°C. The resultant supernatant was analyzed for 5-HT and 5-hydroxyindole acetic acid (5-HIAA) content by high pressure liquid chromatography (HPLC) with electrochemical detection (ECD), as described previously (Takao et al., 1991).

2.5. Measurement of (±)-DOI-induced wet-dog shakes

For the behavioral experiments, the rats were placed in individual clear polycarbonate home cages (35 × 30 × 17 cm). They were treated with (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (1 mg/kg, s.c.) and returned to their cages. Immediately after injection, the number of wet-dog shakes was recorded over a 30-min period, as reported previously (Bedard and Pycock, 1977).

2.6. Chemicals

[^{3}H]Ketanserin hydrochloride (specific activity, 60.1 Ci/mmol), [^{3}H]CGP-12177 (57.2 Ci/mmol) was purchased from New England Nuclear. Alprenolol hydrochloride and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) hydrochloride were purchased from Sigma and RBI, respectively. Other compounds were obtained from commercial sources.

Table 2
Effects of acute and chronic forced swim stress on 5-HT and 5-HIAA content in rat frontal cortex

	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)
Control	3.741±0.254	1.129±0.087
Acute stress	4.644±0.695	1.177±0.189
Chronic stress	3.314±0.399	0.888±0.115

Values are means±S.E. for eight rats.

2.7. Statistics

The biochemical data were analyzed with Dunnett's test and the behavioral study data were analyzed with Wilcoxon's test.

3. Results

3.1. Changes in 5-HT_2 receptors

Table 1 shows the K_d and B_{max} values of frontal cortical 5-HT_2 receptors labeled by [^{3}H]ketanserin, calculated using the EBDA/LIGAND program (McPherson, 1985). Chronic, but not acute, forced swim stress significantly increased the maximal number (B_{max}) of 5-HT_2 receptors, without causing changes in their affinity (K_d).

3.2. Changes in 5-HT and 5-HIAA content

The content of 5-HT and 5-HIAA following the chronic forced swim stress is shown in Table 2. There was no significant difference between the experimental and control groups for the frontal cortical content of either compound.

3.3. Changes in 5-HT_2 receptor-mediated behavior

24 h after the final exposure to the chronic (14-day) stress, the number of wet-dog shakes exhibited during the 30-min period following the injection of (±)-DOI

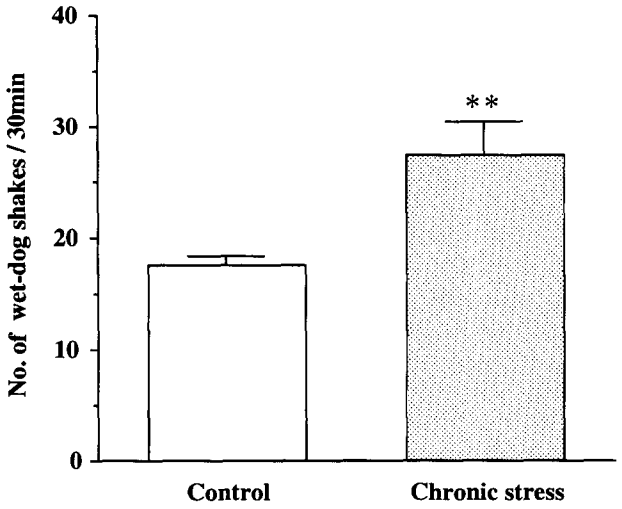

Fig. 1. Effects of chronic forced swim stress on number of wet-dog shakes induced by (±)-DOI. Wet-dog shakes are expressed as the number of responses during the 30-min period following the injection of (±)-DOI (1 mg/kg, s.c.). Values are means±S.E. for eight rats. ** $P < 0.01$ compared to control rats.

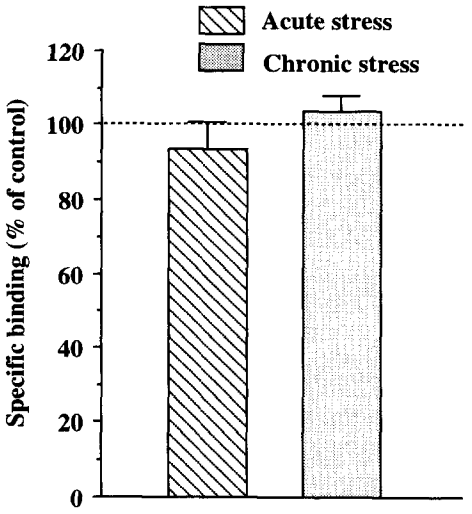

Fig. 2. Effects of acute and chronic forced swim stress on β-adrenoceptors. We used 0.2 nM [^{3}H]CGP-12177 to label β-adrenoceptor binding sites. Values, which are means ± S.E. for six to seven seven rats, represent the percentage specific binding in control rats (27.3 ± 1.3 fmol/mg protein).

(1 mg/kg) was significantly increased compared with the control value (Fig. 1).

3.4. Changes in β-adrenoceptors

Neither acute nor chronic forced swim stress changed the number of β-adrenoceptors labelled by [^{3}H]CGP-12177 (27.3 ± 1.3 fmol/mg protein), in the frontal cortex (Fig. 2).

4. Discussion

In this study, we demonstrated that chronic, but not acute, forced swim stress increased the number of 5-HT_2 receptors in the rat frontal cortex. Previous reports have shown increases in these receptors in some types of depressive models related to chronic stress, such as forced running (Mayeda et al., 1989) and learned helplessness (Martin et al., 1990) models. These models, as well as the forced swim model in the present study, in all of which up-regulation of 5-HT_2 receptors occurs, are useful for the screening of antidepressants. Clinically, up-regulation of 5-HT_2 receptors has been reported for the platelets of depressed patients (Biegon et al., 1987; Pandey et al., 1990) and the frontal cortex of suicide victims (Arango et al., 1990; Arora and Meltzer, 1989; Mann et al., 1986). In view of the results from these depressive stress models and the clinical findings cited above, the present results suggest that chronic forced swim stress may reflect the up-regulation of 5-HT_2 receptors in some depressive conditions in animals.

The precise mechanism of the up-regulation of 5-HT_2 receptors in depressive patients is, thus far, unclear. One explanation for this mechanism is that lower presynaptic serotonergic activity may increase the numbers of 5-HT_2 receptors. In the present study, we measured the concentrations of both 5-HT and 5-HIAA, both of which may reflect presynaptic serotonergic activity to some extent. The chronic forced swim stress did not alter the concentration of either compound. Kuroda et al. (1992) have reported that chronic adrenocorticotrophic hormone (ACTH) treatment, which up-regulates the hypothalamic-pituitary-adrenal axis and may induce some depressive conditions, increases 5-HT_2 receptors without causing any modification of the 5-HT and 5-HIAA concentration. However, we did not investigate either the rate of synthesis of 5-HT or the rate of 5-HT release from the presynaptic nerve terminals. Since it is possible that the rate of synthesis matched the rate of release, the lack of change in the concentration of 5-HT dose may not reflect presynaptic 5-HT activity. Further investigations of the effect of chronic stress on 5-HT release from presynaptic nerve terminals have been carried out with a microdialysis system.

The mechanisms of up-regulation of 5-HT_2 receptors are very complicated. Further evidence that regulation of this receptor system is somewhat atypical is provided by studies that demonstrate that treatment with 5-HT_2 receptor antagonists induces down-regulation, but not up-regulation, of 5-HT_2 receptors (Blackshear and Sanders-Bush, 1980; Conn and Sanders-Bush, 1987). The up-regulation of postsynaptic 5-HT_2 receptors is due directly to changes in presynaptic 5-HT release.

In our behavioral study, the number of wet-dog shakes induced by (±)-DOI was increased 24 h after the last chronic forced swim stress. This behavior is now generally accepted to be 5-HT_2 receptor-mediated (Heaton et al., 1988). However, since wet-dog shakes are thought to be spinal in origin (Fone et al., 1989, 1991), the behavioral change cannot have been due directly to changes in cortical receptor density. Although it is difficult to investigate cortical 5-HT_2 receptor function at the behavioral level, there may be some connection between the increase of cortical 5-HT_2 receptors and the increase in the number of wet-dog shakes after the chronic forced swim stress.

Dey (1994) also reported an enhanced sensitivity of 5-HT_2 receptors, assessed by wet dog shakes induced by quipazine and 5-methoxy DMT, 48 h after 4 weeks of swimming exercise in rats. In our study, the observations could not be carried out in the wet rats immediately after the swim stress, since the animals lick their wet bodies and the wet-dog shake behavior is interrupted. Therefore, in our study, it is likely that the binding and behavioral data may not be directly con-

nected, since the timing of the binding assay was different from that of the behavioral study. It is possible that the up-regulation of 5-HT_2 receptors at the receptor level may have recovered 24 h after the last chronic stress. Further studies are needed to examine whether the increase in the number of 5-HT_2 receptors, as well as the increase in the wet-dog shakes, last for 24 h after the last chronic stress.

The present findings failed to demonstrate changes in β-adrenoceptors following either acute or chronic swim stress. Our results are in agreement with those of Paul et al. (1988) and Duncan et al. (1985), who showed that 2-day forced swim stress failed to alter the cortical binding of [^{3}H]dihydroalprenolol, which labels β-adrenoceptors. Accordingly, these receptors may not be involved in the neurofunctional changes that occur in stressful conditions, including those investigated in the present study. However, as stated above, clinically, up-regulation of β-adrenoceptors has been reported in the frontal cortex of suicide victims (Banerjee et al., 1977; Bergstrom and Kellar, 1979).

In our study, as in the experiments cited above, β-adrenoceptors were investigated at the receptor level. It is possible that a subcellular signal transduction system, beyond the receptors and their second messengers, including the G-protein and adenylate cyclase system, may change after the swim stress. Further studies are required to elucidate the mechanisms responsible for the regulation of β-adrenoceptors, including the subcellular signal transduction system, under stressful conditions.

In summary and conclusion, the present findings demonstrated that chronic forced swim stress increased the number of 5-HT_2 receptors in the frontal cortex and influenced 5-HT_2 receptor-mediated behavior, in terms of increasing the number of (±)-DOI-induced wet-dog shakes. The same stress failed to change the numbers of β-adrenoceptors in the frontal cortex. Overall, the results suggest that 5-HT_2 receptors, but not β-adrenoceptors, may be closely related to responses to the chronic forced swim stress in rats, which test may model some depressive conditions in animals.

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References

Arango, V., P. Ernsberger, P.M. Marzuk, J.-S. Chen, H. Tierney, M. Stanley, D.J. Reis and J.J. Mann, 1990, Autoradiographic demonstration of increased serotonin 5-HT_2 and β-adrenergic receptor binding sites in the brain of suicide victims, Arch. Gen. Psychiatry 47, 1038.

Arora, R.C. and H.Y. Meltzer, 1989, Serotonergic measures in the brains of suicide victims: 5-HT_2 binding sites in the frontal cortex of suicide victims and control subjects, Am. J. Psychiatry 146, 730.

Banerjee, S.P., L.S. Kung, S.J. Riggi and S.K. Chanda, 1977, Development of beta adrenergic receptor subsensitivity by antidepressants, Nature 268, 455.

Barbaccia, M.L., L. Ravizzia and E. Costa, 1986, Maprotiline: an antidepressant with an useful pharmacological profile, J. Pharmacol. Exp. Ther. 236, 307.

Bedard, P. and C.J. Pycock, 1977, "Wet-dog" shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity, Neuropharmacology 16, 663.

Bergstrom, D.A. and K.J. Kellar, 1979, Adrenergic and serotonergic receptor binding in rat brain after chronic desmethylimipramine treatment, J. Pharmacol. Exp. Ther. 209, 256.

Biegon, A., A. Weizman, L. Karp, A. Ram, S. Tiano and M. Wolff, 1987, Serotonin 5-HT_2 receptor binding on blood platelets – a peripheral marker for depression?, Life Sci. 41, 2485.

Blackshear, M.A. and E. Sanders-Bush, 1980, Serotonin receptor sensitivity after acute and chronic treatment with mianserin, J. Pharmacol. Exp. Ther. 221, 303.

Bradford, M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dey binding, Anal. Biochem. 72, 248.

Conn, P.J. and E. Sanders-Bush, 1987, Central serotonin receptors: effector systems, physiological roles and regulation, Psychopharmacology 92, 267.

Dey, S., 1994, Physical exercise as a novel antidepressant agent: possible role of serotonin receptor subtypes, Physiol. Behav. 55, 323.

Duncan, G.E., I.A. Paul, T.K. Harden, R.A. Mueller, W.E. Stumpf and G.R. Breese, 1985, Rapid down regulation of beta adrenergic receptors by combining antidepressant drugs with forced swim: a model of antidepressant-induced neural adaptation, J. Pharmacol. Exp. Ther. 234, 402.

Fone, K.C.F., J.V. Johnson, G.W. Bennett and C.A. Marsden, 1989, Involvement of 5-HT_2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats, Br. J. Pharmacol. 96, 599.

Fone, K.C.F., A.J. Robinson and C.A. Marsden, 1991, Characterization of the 5-HT receptor subtypes involved in the motor behaviours produced by intrathecal administration of 5-HT agonists in rats, Br. J. Pharmacol. 103, 1547.

Green, A.R., P. Jhonson and V.L. Nimgaonkar, 1983, Increased 5-HT receptor number in brain as a probable explanation for the enhanced 5-hydroxytryptamine-mediated behavior following repeated electroconvulsive shock administration to rats, Br. J. Pharmacol. 80, 173.

Healy, D., P.A. Carney and B.E. Leonard, 1983, Monoamine related markers of depression, J. Psychiat. Res. 17, 251.

Heaton, J.C.P., K. Njung'e and S.L. Handley, 1988, Behavioral profile of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective 5-HT_2 agonist, Br. J. Pharmacol. 94, 388.

Hyttel, J., K.E. Overo and J. Arnt, 1984, Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT uptake inhibitor, citalopram, Psychopharmacology 83, 20.

Kellar, K.J., C.S. Cascio, J.A. Butler and R.N. Kurtzke, 1981, Differental effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain, Eur. J. Pharmacol. 69, 515.

Kuroda, Y., M. Mikuni, T. Ogawa and K. Takahashi, 1992, Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT_2 receptor binding sites in neocortex of rat forebrain and 5-HT_2 receptor-mediated wet-dog shake behaviors, Psychopharmacology 108, 27.

Leysen, J.E., C.J. Niemegeers, J.M. Van Nueten and P. Laduron, 1982, [^{3}H]-Ketanserin (R-41 468) a selective [^{3}H]-ligand for

serotonin$_2$ receptor binding sites: binding properties, brain distribution and functional role, Mol. Pharmacol. 21, 301.

McPherson, G.A., 1985, Analysis of radioligand binding experiments. A collection of computer programs for IBM PC, J. Pharmacol. Meth. 14, 213.

Mann, J.J., M. Stanley, P.A. McBride and B.S. McEwen, 1986, Increased serotonin$_2$ and β-adrenergic receptor binding in the frontal cortices of suicide victims, Arch. Gen. Psychiatry 43, 954.

Martin, J.V., E. Edwards, J.O. Johnson and F.A. Henn, 1990, Monoamine receptors in an animal model of affective disorder, J. Neurochem. 55, 1142.

Mayeda, A.R., J.R. Simon, J.N. Hingtgen, J.R. Hofstetter and M.H. Aprison, 1989, Activity-wheel stress and serotonergic hypersensitivity in rats, Pharmacol. Biochem. Behav. 33, 349.

Mizuta, T. and T. Segawa, 1988, Chronic effects of imipramine and lithium on postsynaptic 5-HT$_{1A}$ and 5-HT$_{1B}$ sites and on presynaptic 5-HT$_3$ sites in rat brain, Jpn. J. Pharmacol. 47, 107.

Pandey, G.N., S.C. Pandey, P.G. Janicak, R.C. Marks and J.M. Davis, 1990, Platelet serotonin-2 receptor binding sites in depression and suicide, Biol. Psychiatry 28, 215.

Paul, I.A., G.E. Duncan, K.R. Powell, R.A. Mueller, J.-S. Hong and G.R. Breese, 1988, Regionally specific neural adaptation of beta adrenergic and 5-hydroxytryptamine$_2$ receptors after antidepressant administration in the forced swim test and after chronic antidepressant drug treatment, J. Pharmacol. Exp. Ther. 246, 956.

Riva, M.K. and I. Creese, 1989, Reevalution of the regulation of β-adrenergic receptor binding by desipramine treatment, Mol. Pharmacol. 36, 211.

Takao, K., T. Nagatani, K. Kasahara and S. Hashimoto, 1991, Role of the central serotonergic system in the anticonflict effect of d-AP159, Pharmacol. Biochem. Behav. 43, 503.

Wong, D.T., L.R. Reid, F.P. Bymaster and P.G. Threlkeld, 1985, Chronic effects of fluoxetine, a selective inhibitor of serotonin uptake, on neurotransmitter receptors, J. Neural Transm. 64, 251.