Monoamine Interaction —Effects of Long-Term Treatments with L-tryptophan and Setiptiline Maleate on Rat Brain α_2- and β-adrenergic Receptor—

Yoshiaki KATSUDA

Department of Psychiatry, Kawasaki Medical School, Kurashiki 701-01, Japan

Accepted for publication on November 12, 1991

ABSTRACT. The role of serotonin precursor L-tryptophan and the new tetracyclic antidepressant Setiptiline Maleate in the regulation of β- and α_2-adrenergic receptor was examined by Radio Receptor Assay method. Treatment with either L-tryptophan (300 mg/kg, twice, daily) or Setiptiline Maleate (3 mg/kg, twice, daily) for 14 days caused a significant decrease in the number of β-adrenergic receptors (B_{max}) measured 12 hours after the last dose. Treatment with co-administration of both L-tryptophan and Setiptiline caused no significant difference from that of either L-tryptophan or Setiptiline alone. Treatment with Setiptiline caused a significant decrease in the number of α_2-adrenergic receptor (B_{max}), but treatment with L-tryptophan caused no change in it. Treatment with co-administration of both Setiptiline and L-tryptophan potentiated the decrease in the number of α_2-adrenergic receptors compared with single administration of Setiptiline alone.

Key words: L-tryptophan — Setiptiline Maleate — β-down regulation — α_2-down regulation — monoamine interaction — long-term treatment

In the treatment of affective disorders, especially in the difficult cases, it is often necessary to prescribe different kinds of drugs, such as tricyclic antidepressants, tetracyclic antidepressants, clonazepam, calbamazepine, and antipsychotics. Comparing the metabolites in the central spinal fluid of both pre-treatment and post-treatment patients, John H. Hsiao *et al.*[1] reported that in the responders 5-HIAA, MHPG, and HVA were all normalized in the same way, but in non-responders no relation was seen between these three. From those findings, we can suppose that there is interaction between different kinds of monoamines. To clarify these interactions, we carried out chronic administration of 1) L-tryptophan, a serotonin precursor 2) Setiptiline Maleate, a tetracyclic antidepressant or 3) both of them, and examined the effects on adrenergic receptors using the Radio Receptor Assay method.

MATERIALS AND METHODS

1) Animals

Adult male Wistar rats, initially weighing 250-350 g, were used. They were

勝田吉彰

bled under the same condition for temperature and the photic cycle. Water and feed were given freely.

2) Administration
   a) Group 1 (5 rats) was given L-tryptophan (300 mg/kg), twice, daily for two weeks.
   b) Group 2 (5 rats) was given setiptiline (3 mg/kg), twice, daily for two weeks.
   c) Group 3 (5 rats) was given both drugs, twice, daily for two weeks.
   d) To the control groups (5 rats), distilled water was administered.

3) Membrane preparations

The rats were decapitated 12 hours after the final administration of the drugs. The brains were rapidly removed and the cerebral cortex was rapidly dissected out at 0°C following König & Klippel atlas.[2] Cortical tissues were homogenated in 25 volumes of 50 mM Tris HCl Buffer (pH 7.8 at 25°C) by using Polytron PT-10 homogenizor (Dial set 7, 10s×2). The homogenates were centrifuged at 49000 g for 20 minutes and resuspended in the same buffer and centrifuged again in the same way. The final pellet was resuspended in the same buffer.

4) Drugs

The following drugs were generously donated by the companies below: Setiptiline Maleate, Mochida Pharmaceutical Co.: L-tryptophan, Kyowa Hakko Co.

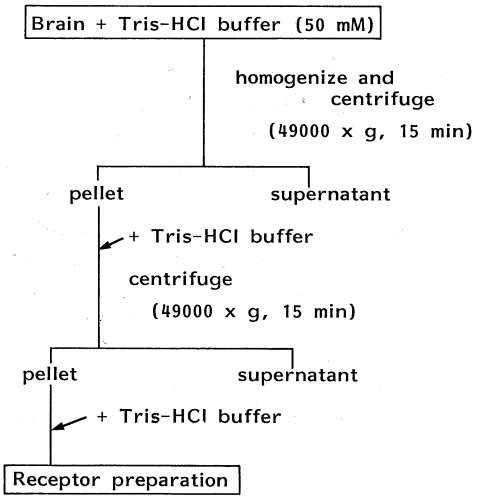

Fig. 1. Tissue preparation. Cortical tissues were homogenated in 25 volumes of 50 mM Tris HCl Buffer (pH 7.8 at 25°C) by using polytron PT-10 homogenizer. The homogenates were centrifuged at 49,000× g for 20 minutes and resuspended in the same buffer and centrifuged again in the same way. The final pellet was resuspended in the same buffer.

5) Binding assays

Binding assays were performed using a PHD cell harvester (Cambridge Technology Inc.) following Harris *et al.*'s method.[3]

a) β-binding assays

A β-adrenergic receptor binding assay using [^{3}H] CGP12177 (Amerscham) was performed by a modification of the method of Asakura *et al.*[4] Namely, cortical membranes (0.2 mg Protein/ml) were incubated for 40 minutes at 30°C with six concentrations of [^{3}H] CGP12177 (0.25-2.0 nM). Just after incubation, the mixture was filtrated by vacuum through a Whatmann GF/B filter, which was presoaked in 3% polyethyleneimine. Next, each filter was washed with 15 ml of Tris-HCl Buffer (0°C) and mixed with 4 ml of a PCS cocktail (Amerscham) in a vial and counted by a scintillation counter. Non-specific binding was determined by concurrent incubations with 100 μM Alprenolol. Specific binding was determined as the difference between total and non-specific bindings. Proteins were measured by Bicinichoninic acid method.[5]

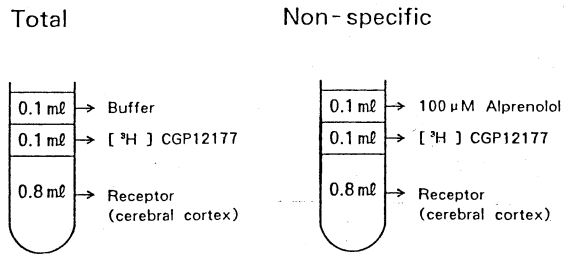

Fig. 2. Binding method (β-adrenergic receptor). Cortical membranes (0.2mg protein/ml) were incubated for 40 min at 30°C with six concentrations of [^{3}H] CGP12177. Non-specific binding was determined by concurrent incubations with 100 nM Alprenolol. Specific binding was determined as the difference between total and non-specific bindings.

b) α_2-binding assay

An α_2-adrenergic receptor binding assay using [^{2}H] Yohimbine was performed by a modification of the method of D. J. Kahn *et al.*[6] Namely,

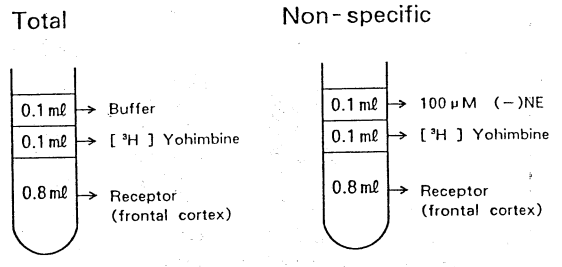

Fig. 3. Binding method (α_2-adrenergic receptor). Cortical membranes were incubated for 50 min at 25°C with six concentrations of [^{3}H] Yohimbine. Non-specific binding was determined by concurrent incubations with 100 μM norepinephrine. Specific binding was determined as the difference between total and non-specific bindings.

cortical membranes were incubated for 50 minutes at 25°C using the same procedure. Non-specific binding was determined by concurrent incubation with 100 μM norepinephrine. Specific binding was determined as the difference between total and non-specific bindings. Proteins were measured by the Bicinichoninic acid method.[5)]

c) Analysis

Each sample was surveyed in triplicate or quadricate. Scatchard analysis was applied to the data for both specific bindings.

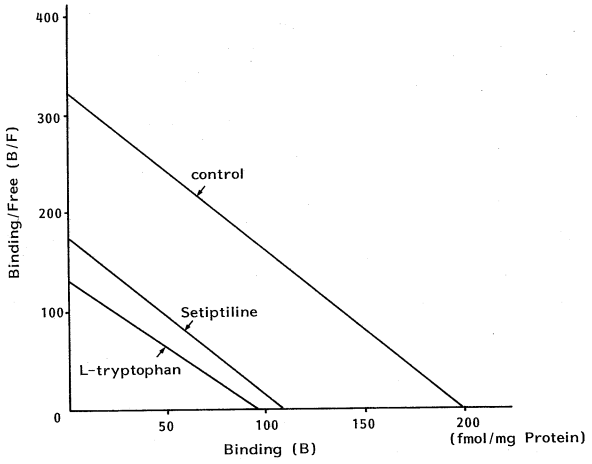

Fig. 4. Scatchard analysis of [^{3}H] CGP 12177 saturation data in rat cerebral cortex. Rats were sacrificed after 12 h of the last injection of L-tryptophan and/or Setiptiline. Membranes were incubated at 30°C 40 min with [^{3}H] CGP 12177 concentration ranging from 0.25 nM to 2.0 nM as described in the methods.

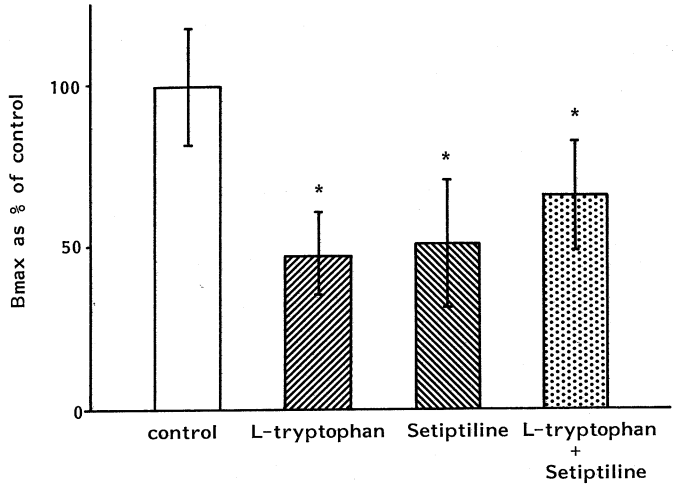

Fig. 5. Effects of L-tryptophan and Setiptiline on β-adrenergic receptor down regulation. Results shown are the mean$\pm$S.E. of B_{max} value for 3-4 determinations and were derived from Scatchard analysis. * Significantly different from control B_{max}, $p<0.01$

TABLE. 1. Effects of L-tryptophan and Setiptiline on β-adrenergic receptor binding

B_{max} (fmol/mg protein)	mean±S.E
Control	188.4±34.8
L-tryptophan	89.9±23.6*
Setiptiline	96.3±37.8*
L-tryptophan+ Setiptiline	124.6±32.6*

* Significantly different from control B_{max}, $p<0.01$

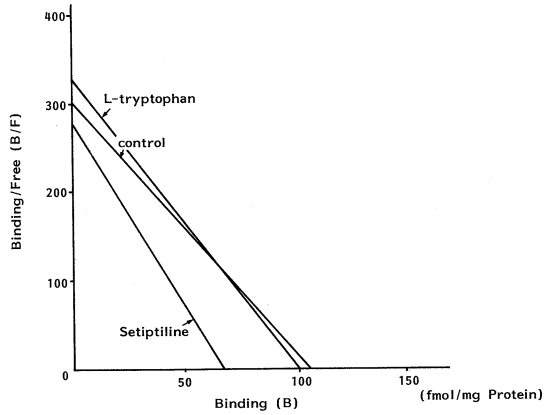

Fig. 6. Scatchard analysis of [^{3}H] Yohimbine saturation data in rats cerebral cortex. Rats were sacrificed after 12 h of the last injection of L-tryptophan and/or Setiptiline. Membranes were incubated at 25°C 50 min with [^{3}H] Yohimbine concentration ranging from 0.25 to 2.0 nM as described in the methods.

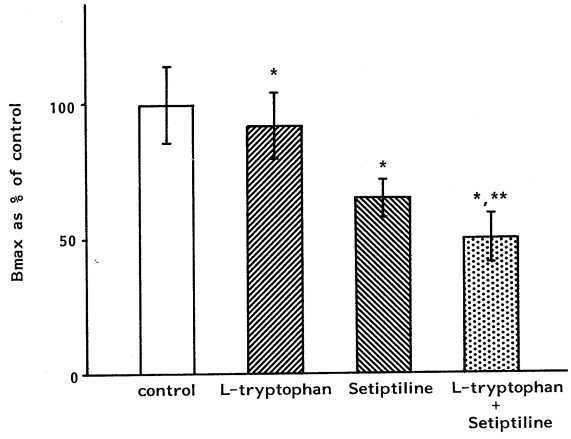

Fig. 7. Effects of L-tryptophan and Setiptiline on α_2-adrenergic receptor down regulation. Results shown are the mean±S.E. of B_{max} value for 3-4 determinations and were derived from Scatchard analysis. * Significantly different from control B_{max}, $p<0.01$ ** Significantly different from Setiptiline administered group B_{max}, $p<0.05$

TABLE. 2. Effects of L-tryptophan and Setiptiline on α_2-adrenergic receptor binding

B_{max} (fmol/mg protein)	mean±S.E
Control	113.4±16.3
L-tryptophan	104,9±13.6
Setiptiline	74.0± 7.9*
L-tryptophan+ Setiptiline	56,2±10.0*,**

* Significantly different from control B_{max}, $p<0.01$
** Significantly different from Setiptiline administered group B_{max}, $p<0.05$

RESULTS

The results of the β-adrenergic receptor binding assay are shown in Table 1. In comparison with the control group, the number of β-adrenergic receptor binding sites (B_{max}) was significantly decreased in all three of the drug-treated groups ($p<0.01$). No significant difference was observed between Group 1, the L-tryptophan-treated group and Group 3, the L-tryptophan/ Setiptiline treated group, or between Group 2, Setiptiline-treated group and Group 3. No significant change in affinity (K_d) was noted among the groups.

The results of the α_2-adrenergic receptor binding assay are shown in Table 2. In comparison with the control group, the number of α_2-adrenergic receptor binding sites (B_{max}) was significantly decreased in Group 2 and Group 3, but it was unchanged in Group 1. In comparison with Group 2, the Setiptiline-treated group, B_{max} was also significantly decreased in Group 3. No significant change in affinity (K_d) was observed among the groups.

DISCUSSION

Recently, there have been a number of reports on the interaction between serotonergic and adrenergic systems. D. J. Heal *et al.*[7] investigated the behavioral changes in pituitary-dissected rats and proposed that the 5-HT and α_2-adrenergic systems are related to each other through pituitary function. Asakura *et al.*[4] reported that co-administration of Fluoxetine or 5-HTP and Mianserin or Maprotiline prolonged the duration of β-adrenergic down regulation, compared with single administration of Mianserin or Maprotiline. In pharmaco-behavioral research, 5-HT_{1A} agonist 8-OHDPAT induced hyperthermia and 5-HT_{1B} agonist RU24969 induced hypermotionality have been reported.

H. Frances and P. Simon *et al.*[8] reported that the administration of the β-adrenergic agonist clenbuterol prevented the hypothermia induced by 8-OHDPAT and promoted the hypermotionality induced by RU24969. They also noted that the chronic administration of β-agonist magnified the effect of 5-HT_{1A} agonist and promoted the 5-HT_2 oriented head-twitch, but on the other hand, attenuated the effect of the 5-HT_{1A} agonist. The number of 5-HT_{1A} receptors increased (50%) at the same time. A.S. Eisen, F.D. Yocca *et al.*[9] observed Quipazine-induced head twitch using DSP_4, β-agonist Clenbutanol,

β-antagonist Proplanolol and indicated that change in the number of β-adrenergic receptors influenced on 5-HT_2 induced behavior, but had no effect on the number of 5-HT_2 receptor itself.

In this study, we used Setiptiline Maleate, which is a tetracyclic antidepressant synthesized in 1974 and used clinically in Japan since 1989. It blocks presynaptic α_2-adrenergic receptors and promotes the release and metabolism of adrenaline. In pharmaco-behavioral studies, it has shown the same effects as Mianserin on Muricide and in forced swimming tests. At the same time, it has blocked the effect of Reserpine and Haloperidole and magnified the effect of Metamphetamine, which has the same effects as tricyclic antideressants.[10-13]

The relationship between pharmacotherapy for depression and the number of receptors has been investigated by a number of researchers. As for β-adrenergic receptors, down-regulation has been reported with Electro Convulsive Therapy by Bergstrom, Keller[14] and by Gillespie,[15] with MAO inhibitor by Sulcer,[16] Sellinger-Barnette *et al.*[5] However, with regard to tricyclic antidepressants, Benerjee,[17] Bergstrom and Keller[14] reported that down-regulation was caused by Desipramine. There have also been some reports indicating that Amitriptyline and Clomipramine have this effect. [24,18] In tetracyclic antidepressant, at first, β-down regulation was not observed.[19] Asakura *et al.*[4] however, reported that although no down regulation was seen 24 hours after the last administration, it was seen 6 hours after. Moreover, down regulation was prolonged to 24 hours after the last administration by the addition of 5HTP or Fluoxetine. In our study, although the condition was slightly different (we made our analysis 12 hours after the last administration), we obtained almost the same result. Down regulation has also continued with the simple administration of Setiptiline. It is supposed that this is because the half time ($T_{1/2}$) of Setiptiline is 105.9 hours, which is longer than that of Mianserin.

On the other hand, with α_2-adrenergic receptors, traditional tricyclic antidepressants such as Amitriptyline, Desipramine, and Imipramine have no effect.[16] Only the tetracyclic antidepressant Mianserin has effect on them.[4] Mianserin blocks presynaptic α_2-receptors and then promotes the release of noradrenaline. This effect is similar to that of Setiptiline, so for Setiptiline also, the same effect could be expected.

In daily practice, we are frequently obliged to prescribe plural drugs which have different mechanisms when treating difficult cases of depression. G. Chouinard[20] proposed the priority of prescriptions for bipolar affective disorders as 1) Lithium 2) Tryptophan 3) Clonazepam or atypical antipsychotics 4) typical antipsychotics 5) tricyclic antidepressants. And a case report on a rapid cycling affective disorder[21] noted improvement as a result of the combination therapy of L-tryptophan and Carbamazepin. In the present investigation, the potentiation of α_2-down regulation by co-administration of two drugs was clarified. co-administration is expected to have an effect on clinical practice. Therefore, further investigation is desirable.

SUMMARY

1) The binding sites of α_2- and β-adrenergic receptors decreased in number (B_{max}) and continued to do so for at least 12 hours as a result of chronic

administration of setiptiline maleate.

2) The binding sites of β-adrenergic receptors decreased in number (B_{max}) and continued to do so for at least 12 hours with chronic administration of L-tryptophan. No effect was seen on α_2-adrenergic receptors.
3) The decrease in the number of α_2-adrenergic receptors was potentiated by co-administration of setiptiline maleate and L-tryptophan. But co-administration had no effect on β-adrenergic receptors.
4) There was also no effect on sensitivity (K_d).

REFERENCES

1) Hsiao, J.K., Agren, H., Bartko, J.J., Rudorfer, M.V., Potter, W.Z., and Linnoila, M: Monoamine neutrotransmitter interactions and the prediction of antidepressant response. Arch. Gen. Psychiatry **44**: 1078-1083, 1987
2) König, J.F.K. and Klippel, R.A.: The Rat Brain — A stereotaxic atlas. The Williams and Wilkins Company, Baltimore, 1963
3) Harris, D.W. and Barsuhn, C.: Use of a cell harvester for the collection of tissue fragments in receptor binding assays. J. Pharmacol. Methods **10**: 207-214, 1983
4) Asakura, M., Tsukamoto, T., Kubota, H., Imafuku, J., Ino, M., Nishizaki, J., Sato, A., Shinbo, K. and Hasegawa, K.: Role of serotonin in the regulation of β-adrenoceptors by antidepressants. Eur. J. Pharmacol. **141**: 95-100, 1987
5) Sellinger-Barnette, M.D., Mendels, J. and Frazer, A.: The effect of psychoactive drugs on beta-adrenergic binding sites in rat brain. Neuropharmacology **19**: 447-454, 1980
6) Kahn, D.J., Mitrius, J.C. and U'Prichard: Alpha2-adrenergic receptors in neuroblastoma×glioma hybrid cells. characterizations with agonist radio-ligands and relationship to adenylate cyclase. Mol. Pharmacol. **21**: 17-26, 1981
7) Heal, D.J., O'shanghnessy, K.M. and Smith, S.L.: Hypophysectomy alters both 5-Hydroxytryptamine and α_2-adrenoceptor-mediator behavioral changes in the rat. Eur. J. Pharmacol. **89**: 167-171, 1983
8) Frances, H. and Simon, P.,: The effect of a beta-adrenergic agonist on behaviors mediated by 5-HT_{1A} and $5HT_{1B}$ receptors. Biol. Psychiatry **21**: 1073-1077, 1986
9) Eisen, A.S., Yocca, F.D. and Gianutsos, G.; Noradrenergic denervation alters serotonin 2-mediated behavior but not serotonin 2 receptor number in rats: Modulatory role of Beta adrenergic receptors. J. Pharmacol. Exp. Ther. **246**: 571-577, 1988
10) Mizota, M., Oikawa, Y. and Nakayama, K.,: Pharmacological studies of MO-8282, a new antidepressant. (No.2) Folia pharmacol. Japon **88**: 457-466, 1986 (in Japanese)
11) Niho, T., Ito, C., Shibutani, Y., Hashizume, H., Yamaguchi, K., Hashizume, H. and Yamaguchi, K.: Behavioral Pharmacology of MO-282, a novel antidepressant. Jpn. Pharmacol. Ther. **14**: 4611-4623, 1986 (in Japanese)
12) Niho, T., Ito, C. and Shibutani, Y.: General pharmacological studies of MO-8282, a novel antidepressant. Jpn. Pharmacol. Ther. **14**: 4625-4649, 1986 (in Japanese)
13) Niho, T., Ito, C. and Shibutani, Y.,: Pharmacological properties of MO-8282, a novel antidepressant. (No. 1) Folia pharmacol. Japon **88**: 309-320, 1986 (in Japanese)
14) Bergstrom, D.A. and Keller, K. J.; Adrenergic and serotonergic receptor binding sites in rat brain after chronic desmethylimipramine treatment. J. Pharmacol. Exp. Ther. **209**: 256-261, 1979
15) Gillespie, D.D., Manier, D.H. and Sulcer, F.: Electroconvulsive treatment; rapid subsensitivity of the norepinephrine receptor coupled adenylate cyclase system in brain linked to down-regulation of beta-adrenergic receptors. Commun. Psychopharmacol. **3**: 191-195, 1979
16) Sugrue, M.F.: A study of the sensitivity of rat brain alpha 2-adrenoceptors during chronic antidepressant treatments. Naunyn-Schmiedeberg's Arch. Pharmacol. **320**: 90-96, 1982
17) Banerjee, S.P., Kung, L.S., Riggi, S.J. and Chanda, S.K.: Development of Beta-adrenergic subsensitivity by antidepressants. Nature **268**: 455-456, 1977
18) Sulcer, F.: New perspectives on the made of action of antidepressant drugs. Trends Pharmacol. Sci. **1**: 92-94, 1979
19) Clements-Jewery, S.: The development of cortical beta-adrenoceptor subsensitivity in the rat by chronic treatment with trazodone, doxepine, and mianserine. Neuropharmacology

17: 779-481, 1978
20) Chouinard, G.: The use of benzodiazepines in the treatment of Manio-Depressive illness. J. Clin. Psychiatry **49**: 15-19, 1988
21) Baba, S., Sasano, H., Shinkado, H. and Watanabe, S.: Clonazepam in the maintenance treatment of rapid cycling affective disorder. Suppl. Biol. Psychiatr. J. Psychiatr. Res. **29**: 346S, 1991