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Action of Chronically Administered Antidepressants on the Serotonergic Postsynapse in a Model of Depression[1]

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NAGAYAMA, H., J. AKIYOSHI AND M. TOBO. *Action of chronically administered antidepressants on the serotonergic postsynapse in a model of depression.* PHARMACOL BIOCHEM BEHAV **25**(4) 805–811, 1986.—A theory of excessive transmission of serotonin (5-HT) in depression has been previously proposed. The purpose of the present study was to test this theory further by using the model of depression in rats induced by L-5-hydroxytryptophan (5-HTP), the precursor of 5-HT. The drug effects on 5-HTP (25 mg/kg) induced behavioral depression were tested by chronic administration using methysergide which is a postsynaptic blocker of 5-HT, or by comparable clinical doses of antidepressant drugs. Methysergide (2 mg/kg) blocked 5-HTP induced depression on days 8 and 22 after initiation of medication by 70% and 83%, respectively. Among antidepressants, mianserin (2 mg/kg) was the first to produce an effect, displaying a 38% effect as early as 1 day after the start of medication and having blocking effects of 52% and 72% on days 8 and 22. Desipramine (5 mg/kg), doxepine (5 mg/kg), imipramine (5 mg/kg) and trazodone (10 mg/kg) showed no significant effect on days 1 and 8, and on day 22, 64, 36, 33 and 32% blocking, respectively. Amitriptyline had an initial effect of 41% at a dose of 10 mg/kg. Clomipramine (5 mg/kg), zimelidine (6 mg/kg) and chlorpromazine (2.5 mg/kg), which is a neuroleptic, showed no effect. Considering these results in light of recent data reported on the 5-HT synapse, it was suggested that 5-HTP induced depression may be induced by excessive transmission of 5-HT and that some antidepressant drugs may produce their effect by blocking this postsynaptic transmission. Based on these results, the mechanisms of human depression were discussed.

Model of depression Serotonergic receptors 5-Hydroxytryptophan Postsynaptic
Chronic antidepressant treatment Methysergide Mianserin Desipramine Doxepine Trazodone
Amitriptyline Clomipramine Chlorpromazine Zimelidine

CONVENTIONAL opinion regarding depression has centered on intracerebral amine deficiency, as claimed in the amine theories [9, 14, 26]. According to these amine based theories, depression is considered to be induced by amine deficiency in the synaptic cleft and antidepressants produce their antidepressive effect by blocking uptake and thus increasing the amine level. Recently, however, a number of results have emerged which appear to contradict these theories. Specifically, it has been shown that mianserin [8] and iprindole [24], which have little uptake blocking action, display antidepressive effects, while on the other hand, cocaine [22] and FG-4963 [11], which do possess uptake blocking action, scarcely show any antidepressive action. Although uptake blocking action is observed from the time immediately following administration, chronic administration is required to obtain an antidepressive effect.

A theory was proposed [4–6, 16, 17, 27], contrary to the conventional theories, that at least in some types of depression, excessive transmission of intracerebral serotonin (5-HT) occurs. The present study was undertaken in order to obtain further evidence for this theory.

In the 5-hydroxytryptophan (5-HTP) induced depression model, methysergide, a 5-HT blocker and several antidepressants (mianserin, amitriptyline, imipramine) at a comparable clinical dose reduced behavioral depression and promoted normalization of behavior of rats. However, fluoxetine known to be a specific uptake blocker of 5-HT, enhanced depression widely [16,17]. The 5-HTP induced depression was temporally correlated with an increase of 5-HT in the telencephalon and diencephalon [1, 3, 19], but not with changes in catecholamine levels [2]. In nerve endings isolated from these areas of the brain, the effect of such an administration of 5-HTP produced an increase in the level of 5-HTP, 5-HT and 5-hydroxyindoleacetic acid. Further, in vivo experiments using these nerve ending fractions indicated that 5-HTP caused a release of preloaded ^{3}H-5HT which could be blocked by a decarboxylase inhibitor [15,20]. The results of these studies suggest that behavioral depression in the present model is induced by excessive 5-HT in the synaptic cleft and that antidepressant drugs, like methysergide, improve behavior by blocking postsynaptic receptors of 5-HT. Furthermore, based on line of reasoning, it was suggested that antidepressants produce their antidepressive effect by inhibiting excessive 5-HT transmission in human depression as well [6, 16, 17].

Only one previous study of the 5-HTP model of depres-

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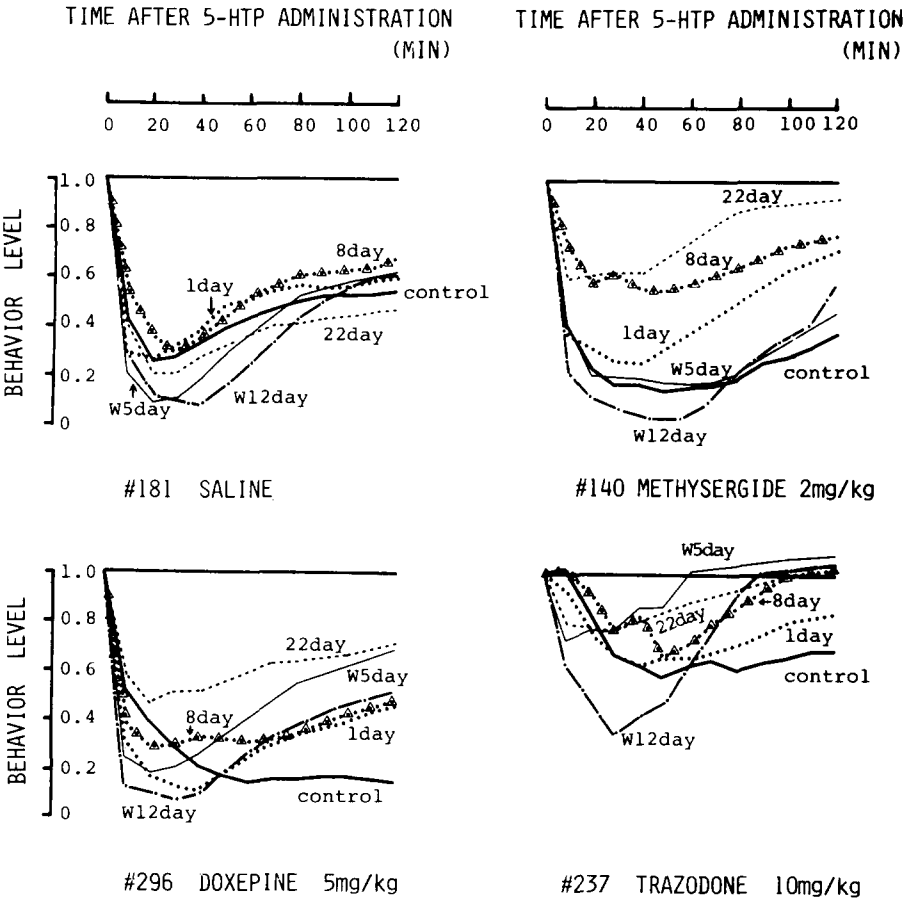

FIG. 1. Behavior level as a function of time following chronic pretreatment with saline, methysergide, mianserin or trazodone to a rat administered 5-HTP while working on a variable interval schedule of milk inforcement. "W" means days after drug withdrawal.

sion has used chronic administration of antidepressant drugs [12], despite the consensus that antidepressants produce their clinical effect through chronic administration. The present study was therefore performed to clarify the effect of chronic administration of antidepressants using the 5-HTP model.

METHOD

Male Wistar rats were used that had been raised in individual cages for more than 4 weeks in a semi-sound-proof room. The room was maintained at 24°C, 50% relative humidity. Artificial light only was given for 12 hours per day from 18:00 to 06:00; during the remaining 12 hours, the animals were kept in complete darkness. Feed was supplied from 16:00 to 16:30 to maintain body weight within the range of 280–290 g and water was available ad lib.

For the experiment, the animals were placed in a small sound-proof room completely isolated from the raising room and a sound-proof Skinner box was employed. The room was without light and the temperature and humidity as in the rearing room were maintained; the experiment was carried out according to the following protocol.

1. Animals were trained for lever-pressing conditioning (VI 1) using milk as the reinforcement. When the rat pressed the lever repeatedly, 0.1 ml of sweetened milk was given automatically at random at a rate of once per minute. The number of times the lever was pressed in 10 minute periods under this schedule of reinforcement was used as the index of activity.

2. The present experiment was performed by using rats conditioned for the lever-pressing procedure. Ten minutes after the start of lever-pressing behavior, 25 mg/kg of L-5-HTP was administered intraperitoneally; the rats were returned again to the experimental box and the behavior measured for the following 120 minutes (5-HTP test). As a result of 5-HTP administration, the level of activity began to drop within several minutes and the behavior level (normal behavior 1, complete inhibition 0) was calculated from the ratio of the levels of activity every 10 minutes after the administration to the "expected levels of activity" of respective periods. "Expected levels" refer to measurements without 5-HTP. The behavior response was lowest 20–60 minutes after administration; for convenience the value indicating the greatest effect (the minimum value) was used as the behavior level. The expected level of activity was calculated according to a linear equation for each rat each day using the level of activity in 10 minute period before administration of 5-HTP. This is based on the observation that there is a high correlation between lever pressing activity counts for 10 minutes before and for each 10 minute-period after administration of the solvent vehicle, $\gamma=0.908$–0.940, $p<0.001$.

TABLE 1

CHANGES OF BEHAVIOR LEVEL BEFORE, DURING AND AFTER CHRONIC ADMINISTRATION (FOR 24 DAYS) OF ANTIDEPRESSANTS, METHYSERGIDE AND CHLORPROMAZINE

Drug	Dose (mg/kg)	Before	1 day	8 day	22 day	W5 day	W12 day
Saline		34.4 ± 3.7	34.2 ± 7.2	19.2 ± 6.9	15.2 ± 4.3	8.2 ± 2.4	7.4 ± 2.9
Methysergide	2	34.4 ± 5.2	62.4 ± 13.9	75.4 ± 12.3†	85.2 ± 8.7‡	45.4 ± 14.6	33.6 ± 13.3
	0.5	37.2 ± 7.5	34.8 ± 6.0	22.6 ± 6.9	17.8 ± 8.6	8.6 ± 4.8	9.6 ± 5.3
Mianserin	2	34.4 ± 9.9	57.2 ± 10.1*	58.0 ± 20.1*	73.6 ± 13.4†	39.2 ± 17.7*	18.8 ± 30.6*
	1	19.2 ± 9.4	34.8 ± 7.9	22.4 ± 7.2	69.8 ± 14.7†	49.4 ± 15.2*	31.4 ± 6.7†
Desipramine	5	41.6 ± 4.5	54.2 ± 9.8	39.8 ± 11.5	70.4 ± 15.0*	51.6 ± 12.5*	65.2 ± 11.5†
Doxepin	5	28.0 ± 9.1	24.2 ± 4.9	31.8 ± 5.9	40.6 ± 11.1*	22.2 ± 8.6*	31.4 ± 13.9†
	2.5	41.2 ± 6.0	46.0 ± 6.5	26.8 ± 7.8	29.2 ± 9.6	13.0 ± 6.0	17.0 ± 6.4
Imipramine	5	23.7 ± 8.4	38.8 ± 8.4	21.0 ± 5.0	34.2 ± 12.5*	26.6 ± 13.3*	47.2 ± 18.4*
	2.5	27.0 ± 7.8	25.2 ± 10.0	31.8 ± 13.3	26.6 ± 7.6	15.4 ± 7.1	16.0 ± 8.4
Trazodone	10	43.6 ± 9.0	36.2 ± 12.5	44.6 ± 17.6	47.0 ± 11.2*	35.0 ± 13.2	15.8 ± 6.2
	5	26.0 ± 5.2	33.8 ± 9.9	22.4 ± 6.4	29.6 ± 6.2	21.4 ± 8.1	10.8 ± 4.1
Amitriptyline	10	41.6 ± 9.0	47.6 ± 17.9	28.8 ± 10.7	54.6 ± 13.4*	39.0 ± 16.5	20.2 ± 11.1
	5	45.4 ± 9.4	44.6 ± 14.2	38.4 ± 13.8	37.6 ± 17.9	28.8 ± 10.3	31.8 ± 14.8
	2.5	43.0 ± 7.9	42.0 ± 8.3	34.4 ± 12.5	34.4 ± 11.5	23.6 ± 8.9	35.2 ± 11.8
Clomipramine	5	13.0 ± 3.7	15.2 ± 2.6	4.6 ± 1.1	11.8 ± 6.0	3.8 ± 0.8	3.0 ± 0.3
Chlorpromazine	2.5	30.6 ± 8.7	24.8 ± 5.5	25.6 ± 7.1	21.2 ± 4.7	8.8 ± 3.5	6.2 ± 1.1
Zimelidine	6	35.4 ± 7.5	21.2 ± 5.5	9.2 ± 2.1	13.0 ± 4.3	8.0 ± 2.4	6.4 ± 2.1
	3	46.6 ± 4.4	29.8 ± 3.0	32.6 ± 9.9	28.4 ± 9.9	25.0 ± 9.2	21.6 ± 8.5

The numbers indicate the behavior level (mean ± SE).
Significant differences from saline control by analysis of covariance *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

The conditioning training was also continued for 3 periods of 30 minutes per week after commencement of the experiment. The training was given during the period from 9:00–16:00 and the experiment was performed so 5-HTP could be administered between 9:30–10:00. To prevent establishment of a conditioned reflex to 5-HTP, additional injections of the vehicle were given to rats once or twice a week during training.

3. Antidepressants and reference agents were administered intraperitoneally for 24 consecutive days. One, 8 and 22 days after initiation of the administration and 5 and 12 days after discontinuation of medication, the 5-HTP test was performed to determine the behavior level. The drugs and doses employed were as follows: antidepressants, mianserin 2, 1 mg/kg, desipramine 5 mg/kg, doxepin 5, 2.5 mg/kg, imipramine 5, 2.5 mg/kg, trazodone 10, 5 mg/kg, amitriptyline 5, 2.5 mg/kg, clomipramine 5 mg/kg, zimelidine 6, 3 mg/kg, and as references, methysergide 2, 0.5 mg/kg and chlorpromazine 2.5 mg/kg. Human clinical doses of the antidepressants were used except for amitriptyline, for which a supplementary dose of 10 mg/kg was added.

Physiological saline was given to a control group. In order to exclude the acute effect which would be observed immediately following individual medications in a chronic procedure, drugs were administered between 16:30–17:00. A 17-hour interval then elapsed between the 5-HTP test and the medication immediately before the 5-HTP test. Furthermore, before and 15 days after initiation of chronic administration, the test was carried out using the solvent vehicle only instead of 5-HTP and this test was used as a control of behavior level for each rat, i.e., the former is the control for tests on 0, 1 and 8 days, and the latter is the control for tests on day 22 and withdrawal period. "1 − behavior level at each test/at control" was the index used for 5-HTP induced behavioral depression.

After dissolution of 5-HTP with 1 N HCl, the solution was adjusted to pH 7.0 with 1 N NaOH and the solution was used within 20 minutes. Other drugs were dissolved in physiological saline solution. 5-HTP was administered using an interval of more than one week in each case. Five animals were used in each group.

RESULTS

5-HTP-induced behavioral depression was blocked following chronic administration of methysergide and some types of antidepressants (Fig. 1, Table 1). The ratio of decrease in behavioral depression indicated in the tests for each period after initiation of medication relative to the periods of 5-HTP testing before initiation of chronic administration in each rat was expressed as percent blockade of 5-HTP-induced depression. Since a certain increase in behavior level was observed also in the group chronically administered physiological saline solution, this amount of increase was deducted from the value of the medicated group beforehand. Results are shown in Fig. 2. Covariance analysis was used for the test and comparison was made between the value before initiation of administration and that on the appropriate post-administration day by using the physiological saline group as a control. For this calculation, original data were used, rather than percentages.

The effect of chronic administration of 2 mg/kg of methysergide, a 5-HT blocker, from a level of 44% after 1

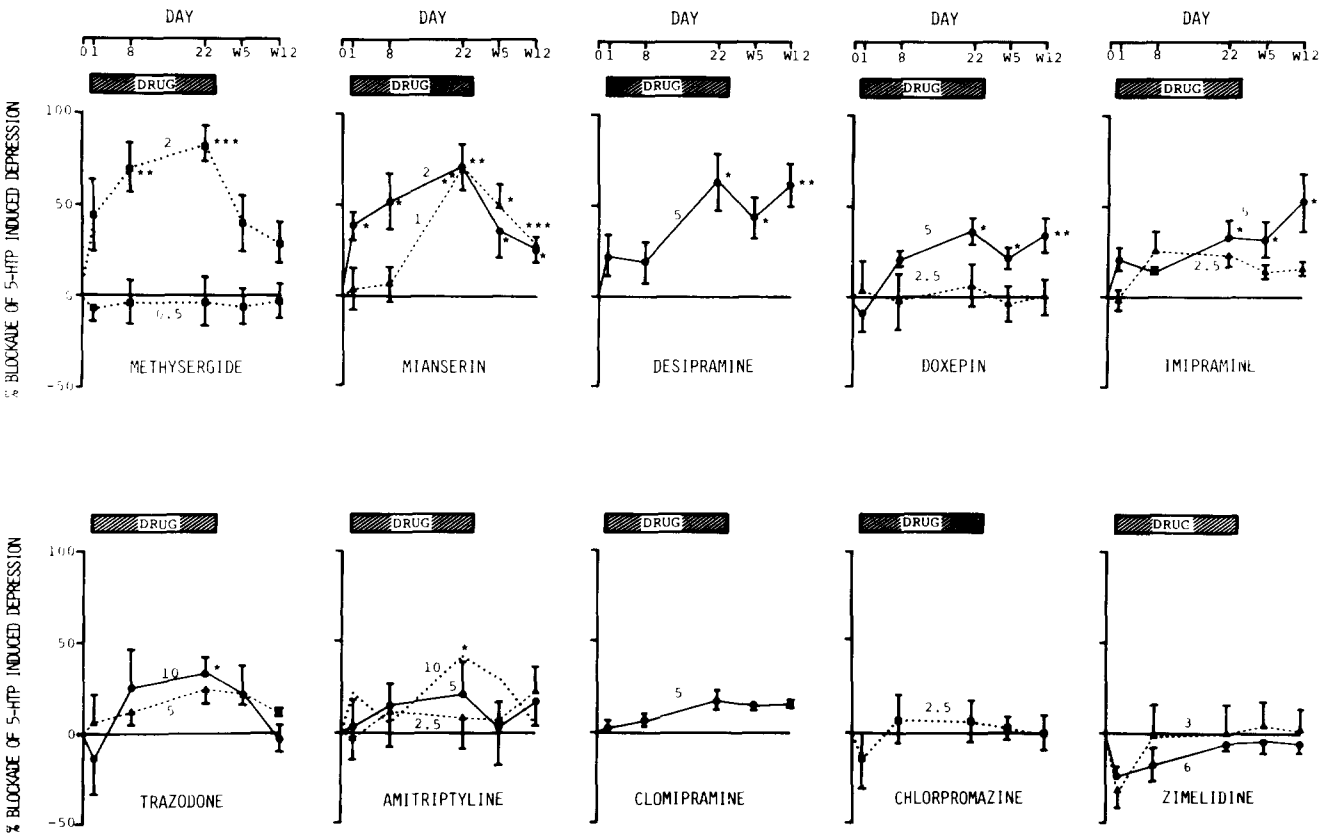

FIG. 2. Percent blockade of 5-HTP induced depression by chronic administration (for 24 days) of comparable clinical doses of eight antidepressants, methysergide and chlorpromazine. Numbers on the curve indicate the dose (mg/kg). Significant differences from saline control by analysis of covariance $*p<0.05$, $**p<0.01$, $***p<0.001$. For statistical comparison, original data rather than percentages were used.

day to 83% after 22 days occurred. However, as early as 5 days after discontinuing medication, this significant effect was lost.

Among the antidepressants, clinical doses of mianserin (2 mg/kg, 72%), desipramine (5 mg/kg, 64%), doxepin (5 mg/kg, 36%), imipramine (5 mg/kg, 33%), and trazodone (10 mg/kg, 32%) showed a significant blocking action at 3 weeks. Amitriptyline showed an effect (41%) for the first time at a slightly higher dose (10 mg/kg). Clomipramine (5 mg/kg, 17%) and zimelidine (6 mg/kg, −6%), which are considered to be specific uptake blockers of 5-HT, showed no significant effect. Chlorpromazine (2.5 mg/kg, 6%), a neuroleptic, was also ineffective.

Those chronically administered drugs which displayed a chronic effect showed enhanced effect with continued chronic administration of the drug; for example, the effect after 22 days was higher than that after 8 days, and the effect after 8 days was higher than that after 1 day.

A dose response effect was also shown. Significant correlation was observed between the effect on day 1 (acute administration) and that on day 22 (chronic administration), $\gamma=0.81$, $p<0.02$ (Fig. 3). After discontinuing drug administration, the effect lasted for an unexpectedly long period of time and many drugs (mianserin, desipramine, doxepin, imipramine) even continued to display significant effects 12 days after discontinuation. This was in contrast to what was observed for methysergide.

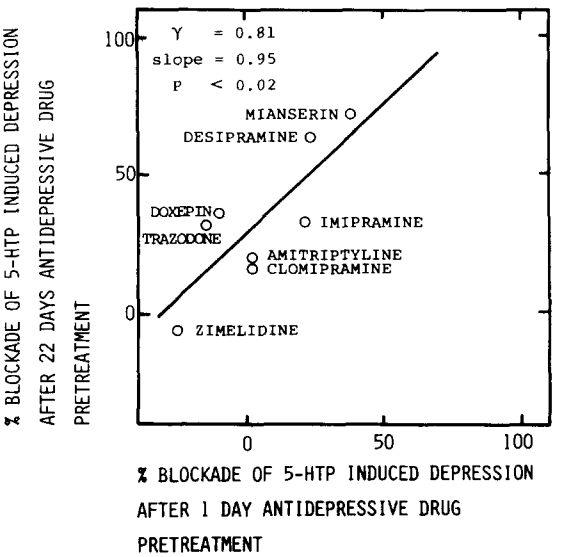

FIG. 3. Relationship of percent blockade of 5-HTP induced depression between acute (1 day) and chronic (22 days) administration of various antidepressants. The doses employed are as follows: mianserin 2 mg/kg, desipramine, doxepine, imipramine, amitriptyline and clomipramine 5 mg/kg, trazodone 10 mg/kg, zimelidine 6 mg/kg.

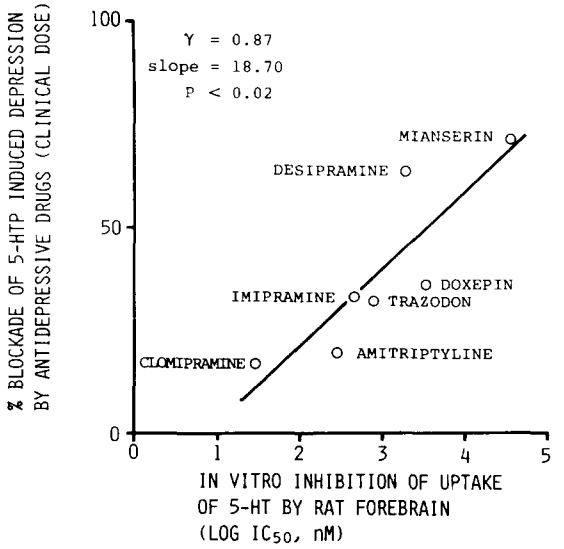

FIG. 4. Relationship between percent blockade of 5-HTP induced depression by chronic (22 days) administration of comparative clinical doses (see the legend to Fig. 2) of antidepressants and in vitro inhibition of uptake by acute administration [7].

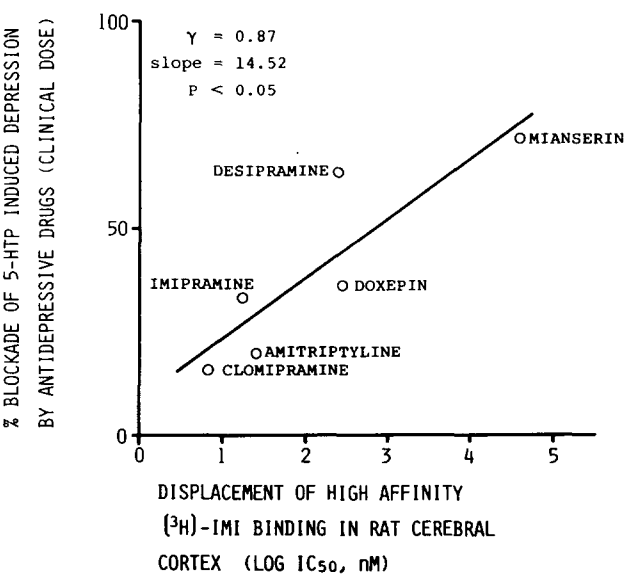

FIG. 5. Relationship between percent blockade of 5-HTP induced depression by chronic (22 days) administration of comparable clinical doses (see the legend to Fig. 2) of antidepressants and displacement of high affinity [^{3}H]-IMI binding in rat cerebral cortex [13].

DISCUSSION

Behavioral depression is used here as a model of human depression. This seems appropriate since in an analogous way, antidepressants reversed the behavior toward normality. It is said that many antidepressants have no effect when acutely administered but display an effect for the first time when chronically administered. The fact that many antidepressants showed an initial effect only after 3 weeks of administration in the present model also suggests close analogy to the clinical situation in depression. It is also important that the dose given experimentally is similar to that usually given clinically because in studies carried out with drugs significantly in excess of the clinical dose, it is possible that actions other than antidepressive effects are being measured.

It has been observed that antidepressants exert a 5-HT postsynaptic receptor blocking action after chronic administration. Many kinds of antidepressants displayed blocking action on 5-HTP-induced depression with a time course similar to that of methysergide, a postsynaptic blocker of 5-HT. These data suggest that at least some types of depression may be induced by excessive transmission of 5-HT at the synapse, with antidepressants exerting their antidepressive effect by blocking the excessive transmission postsynaptically. The differences of behavior effect of each drug may be due to the degree of the relationship with 5-HT systems. It may be necessary to test whether methysergide has an antidepressive effect. The results agree with that of the recent paper [12] with trazodone, amitriptyline and mianserin.

It would be interesting to observe the association between some indicators emphasized in the 5-HT deficiency theory and the result obtained in our present study. We would therefore like to make comparisons with data offered from sources other than ours. As shown in Fig. 4, significant correlation is observed, $\gamma=0.87$, $p<0.02$, between the behavioral effect at 3 weeks and the uptake blocking action [7]. This effect at 3 weeks also indicates a significant correlation

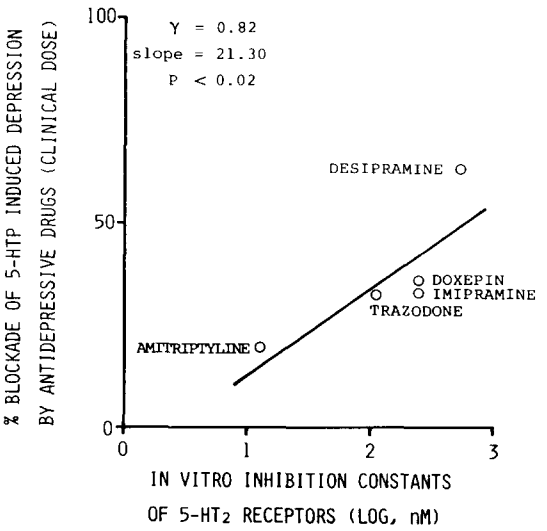

FIG. 6. Relationship between percent blockade of 5-HTP induced depression by chronic (22 days) administration of comparable clinical doses (see the legend to Fig. 2) of antidepressants and in vitro inhibition constants of 5-HT$_2$ receptors [7].

with imipramine (IMI) binding [13] which is considered to be strongly associated with uptake block, $\gamma=0.87$, $p<0.05$ (Fig. 5). That is to say, the stronger the uptake block, or the stronger the complexing with IMI binding sites, the weaker the behavioral effect of the drug. The action of increasing 5-HT at the synapse produces a decrease in the behavioral effect on the model.

A positive, significant correlation is observed between the effect at 3 weeks and 5-HT$_2$ binding [7], $\gamma=0.82$, $p<0.02$

(Fig. 6). The easier the binding with the 5-HT_2 receptor, the lower the behavioral effect of the drug. This phenomenon can be understood by considering the fact that 5-HT has excitatory and inhibitory action [23,25] and that the 5-HT_2 receptor suggests an association with excitatory action [21].

Thus, some inhibitory factors of behavioral effects of antidepressants have been clarified. As a factor enhancing the effect, a blocking action at the postsynaptic receptors is possible, as mentioned above. It is however, suggested that 5-HT_1 receptors can be divided into more than two classes [10,18]. It must be specified which postsynaptic 5-HT receptor has the main role in the action.

It has been suggested that the blocking action appears soon after acute administration [16,17], but shortly (by at least 17 hours after administration) it disappears, as shown in the present data. With many antidepressants, it took more than two weeks before the behavioral effect became continuous and stable. Once it was established, many antidepressants maintained their effect for a long period of time after discontinuation of medication, while methysergide lost its effect at an early stage. This suggests that antidepressant drugs produce a comparatively long-lasting effect on the receptors.

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REFERENCES

1. Aprison, M. H., M. A. Wolf, G. L. Poulos and T. L. Folkerth. Neurochemical correlates of behavior III. Variation of serotonin content in several brain areas and peripheral tissues of the pigeon following 5-hydroxytryptophan administration. *J Neurochem* **9:** 575–584, 1962.
2. Aprison, M. H. and J. N. Hingtgen. Neurochemical correlates of behavior IV. Norepinephrine and dopamine in four brain parts of the pigeon during period of atypical behavior following the injection of 5-hydroxytryptophan. *J Neurochem* **12:** 959–968, 1965.
3. Aprison, M. H. and J. N. Hingtgen. Neurochemical correlates of behavior V. Differential effects of drugs of approach and avoidance behavior in rats with related changes in brain serotonin and norepinephrine. *Recent Adv Biol Psychiatry* **8:** 87–100, 1966.
4. Aprison, M. H., R. Takahashi and K. Tachiki. Hypersensitive serotonergic receptors involved in clinical depression—A theory. In: *Neuropharmacology and Behavior*, edited by B. Haber and M. H. Aprison. New York: Plenum Press, 1978, pp. 23–53.
5. Aprison, M. H. and J. N. Hingtgen. Hypersensitive serotonergic receptors: A new hypothesis for one subgroup of unipolar depression derived from an animal model. In: *Serotonin: Current Aspects of Neurochemistry and Function*, edited by B. Haber, S. Gabay, S. Alivisatos and M. Issidorides. New York: Plenum Press, 1981, pp. 627–656.
6. Aprison, M. H., J. N. Hingtgen and H. Nagayama. Testing a new theory of depression with an animal model: neurochemical behavioral evidence for postsynaptic serotonergic receptor involvement. In: *New Vistas in Depression*, edited by S. Z. Langer, R. Takahashi, T. Segawa and M. Briley. Oxford: Pergamon Press, 1982, pp. 171–178.
7. Baldessarini, R. J. *Biomedical Aspects of Depression and its Treatment*. Washington, DC: American Psychiatric Press, 1983, pp. 85–125.
8. Brogden, R. N., R. C. Heel, T. M. Speight and G. S. Avery. Mianserin: A review of its pharmacological properties and therapeutic efficacy in depressive illness. *Drug* **16:** 273–301, 1978.
9. Bunney, W. E., Jr. and J. M. Davis. Norepinephrine in depressive reactions. *Arch Gen Psychiatry* **13:** 483–494, 1965.
10. Fillion, G., D. Beaudoin, M.-P. Fillion, J.-C. Russelle, C. Robaut and Y. Netter. 5-Hydroxytryptamine receptors in neurones and glia. *J Neural Transm [Suppl]* **181:** 307–317, 1983.
11. Ghose, K., R. Gupta, A. Coppen and J. Lund. Antidepressant evaluation and the pharmacological action of FG 4963 in depressive patients. *Eur J Pharmacol* **42:** 31–37, 1977.
12. Hingtgen, J. N., H. C. Hendrie and M. H. Aprison. Postsynaptic serotonergic blockade following chronic antidepressive treatment with trazodone in an animal model of depression. *Pharmacol Biochem Behav* **20:** 425–428, 1984.
13. Hrdina, P. D. Tricyclic antidepressants: differences in high-affinity binding of [^{3}H]-desipramine and [^{3}H]-imipramine in the brain. In: *New Vistas in Depression*, edited by S. Langer, R. Takahashi, T. Segawa and M. Briley. Oxford: Pergamon Press, 1982, pp. 125–132.
14. Lapin, I. P. and G. H. Oxenkrug. Intensification of the central serotonergic processes as a possible determinant of the thymoleptic effect. *Lancet* **1:** 132–136, 1969.
15. McBride, W. J., M. H. Aprison and J. N. Hingtgen. Effects of 5-hydroxytryptophan on serotonin in nerve endings. *J Neurochem* **23:** 385–391, 1974.
16. Nagayama, H., J. N. Hingtgen and M. H. Aprison. Pre- and postsynaptic serotonergic manipulations in an animal model of depression. *Pharmacol Biochem Behav* **13:** 575–579, 1980.
17. Nagayama, H., J. N. Hingtgen and M. H. Aprison. Postsynaptic action by four antidepressive drugs in an animal model of depression. *Pharmacol Biochem Behav* **15:** 125–130, 1981.
18. Pedigo, N. W., H. I. Yamamura and D. L. Nelson. Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J Neurochem* **36:** 220–226, 1981.
19. Penn, P. E., W. J. McBride, J. N. Hingtgen and M. H. Aprison. Differential uptake, metabolism and behavioral effects of the D and L isomers of 5-hydroxytryptophan. *Pharmacol Biochem Behav* **7:** 515–518, 1977.
20. Penn, P. E. and W. J. McBride. The effects of injections of D,L-5-hydroxytryptophan on the efflux of endogenous serotonin and 5-hydroxyindoleacetic acid from a synaptosomal fraction. *J Neurochem* **28:** 765–769, 1977.
21. Peroutka, S. J., R. M. Lebovitz and S. H. Snyder. Two distinct central serotonin receptors with different physiological functions. *Science* **212:** 827–829, 1981.
22. Post, R. M., J. Kotkin and F. K. Goodwin. The effects of cocaine on depressed patients. *Am J Psychiatry* **131:** 511–517, 1974.
23. Roberts, M. H. T. and D. W. Straughan. Excitation and depression of cortical neurones by 5-hydroxytryptamine. *J Physiol* **193:** 269–294, 1967.

24. Ross, S. B., A. L. Renyi and S. O. Ogren. A comparison of the inhibitory activities of iprindole and imipramine on the uptake of 5-hydroxytryptamine and noradrenaline in brain slices. *Life Sci* **10:** 1267–1277, 1971.
25. Sastry, B. S. R. and J. W. Phillis. Metergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. *Can J Physiol Pharmacol* **55:** 130–133, 1977.
26. Schildkraut, J. J. The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am J Psychiatry* **122:** 509–522, 1965.
27. Takahashi, R., T. Tateishi, H. Yoshida, H. Nagayama and K. H. Tachiki. Serotonin metabolism of animal model of depression. In: *Serotonin: Current Aspects of Neurochemistry and Function*, edited by B. Haber, S. Gabay, M. R. Issidorides and S. G. A. Alivisatos. New York: Plenum Press, 1981, pp. 603–625.