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Postsynaptic Action by Four Antidepressive Drugs in an Animal Model of Depression[1]

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NAGAYAMA, H., J. N. HINGTGEN AND M. H. APRISON. *Postsynaptic action by four antidepressive drugs in an animal model of depression.* PHARMAC. BIOCHEM. BEHAV. 15(1)125–130, 1981.—To further test the new hypersensitive postsynaptic serotonin (5-HT) receptor theory of depression based on our animal model, it was necessary to demonstrate that some of the currently used antidepressive drugs can block D,L-5-hydroxytryptophan (5-HTP) induced depression acting through postsynaptic rather than presynaptic mechanisms. Rats working for milk reinforcement and exhibiting behavioral depression following administration of 5-HTP (IP) were pretreated (1 hour before the 5-HTP injection) with fluoxetine (5 mg/kg IP) or methysergide (5 mg/kg IP) to establish a behavioral basis for distinguishing between pre- and postsynaptic events, respectively. Fluoxetine, a known specific uptake blocker of 5-HT, potentiated the depressive effect of 12.5 mg/kg 5-HTP by 200%. Methysergide, a postsynaptic blocker of 5-HT, almost completely (93%) abolished the depressive effect of 50 mg/kg 5-HTP. Since acute pretreatment with comparable clinical doses of the antidepressive drugs, mianserin, amitriptyline, imipramine, or iprindole, resulted in blockade of the 5-HTP induced depression by 70, 50, 40, and 20% respectively, these drugs can act as antagonists of 5-HT at the postsynaptic serotonin receptor. When these results are viewed in terms of recent data reported from CNS binding studies, the therapeutic effects of some antidepressants may be explained by their postsynaptic rather than presynaptic effects at central serotonergic receptors.

Serotonergic receptors Model of depression 5-Hydroxytryptophan Methysergide Fluoxetine
Amitriptyline Mianserin Imipramine Iprindole Postsynaptic Presynaptic

IN SPITE of their generally accepted inadequacies in explaining important clinical and animal data, two of the most prominent biochemical theories of human depression have been the serotonin deficiency theory [17] and the catecholamine deficiency theory [13,26]. According to these theories, depression is related to a lack of appropriate levels of these amines in the synaptic cleft; thus, the postulated therapeutic mechanism of antidepressive drugs is to increase amine levels by means of uptake blockade. However, recent biochemical evidence has made these theories even less tenable. Cocaine [24] and a new phenylpiperidine derivative, FG-4963 [16] block the uptake of norepinephrine (NE) and serotonin (5-HT), respectively, but they have little or no effect on depression. In addition, it has been demonstrated that there is no correlation between the degree of NE uptake blockade and the clinical effect [15]. Iprindole is a very weak uptake blocker of 5-HT and NE [25] but is effective as an antidepressive drug. Mianserin, another antidepressant, has been reported to effectively block 5-HT at the postsynaptic receptors in experiments with the rat [19]. Finally, the most recent binding studies have found that many of the widely used antidepressive drugs have high affinity for 5-HT, D-LSD [11, 12, 22] and α-NE [29] receptors in brain.

In the period from the early 1960's to the current time, animal experiments undertaken in our laboratories have provided data which suggest strongly that some types of depression may be related to an *excess* of free 5-HT in the synaptic cleft. In these studies, D,L-5-HTP was found to produce periods of behavioral depression when injected into animals working on food-reinforced operant schedules. Behavioral depression is defined here as a significant lowering of control rates of responding, and is not necessarily related to human depression as seen in psychiatric disorders. However, decreased rates of responding are seen in some patients who suffer from some types of depression. It is possible that in certain types of depression, malfunctioning biochemical systems occurring in the brains of these individuals are similar to those seen in our animal model. The period of lowered responding in our studies [1–3] was temporally correlated with an increase of 5-HT in specific brain areas [4–6, 10] but not with changes in catecholamine levels [3]. Based on these neurochemical/behavioral data, as well as *in vitro* and *in vivo*

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studies of nerve ending fractions [20,23], it was suggested that the excess 5-HT in the synaptic cleft following 5-HTP administration lowered behavioral responding through a predominantly postsynaptic phenomenon in our animal model of depression [5–9]. Using a different animal model, Takahashi and co-workers also were led to a similar conclusion [27,28].

In order to reconcile the animal data (which indicates that suppression of animal behavior is caused by an *increase* in 5-HT in the synaptic cleft) with the clinical data (which suggests that human unipolar depression is associated with a *deficiency* of the cerebral serotonin system) a new theory was developed by Aprison, Takahashi, Hingtgen and Tachiki [7,9]. This theory suggests that in some types of human depression, a hypersensitive postsynaptic receptor develops due to a decreased release of 5-HT. This theory was named the "hypersensitive serotonergic receptor theory of depression."

The new theory predicted that drugs which can reverse some types of unipolar depression in man should block the 5-HTP effect in our animal model. Moreover, if one of the drugs commonly used in treating depression could be shown to act postsynaptically, this would provide some indirect evidence for this new theory. Thus, in an initial study, amitriptyline's effect on 5-HTP induced depression was investigated. Two drugs were used to establish a basis for distinguishing between pre- and postsynaptic events. Methysergide, a known postsynaptic blocker of serotonin, almost completely abolished the depressive effect of 5-HT, whereas, fluoxetine, a known specific uptake blocker of 5-HT, potentiated the 5-HTP induced depression. When amitriptyline was tested, it was found to reduce the depression 50%, but had no effect when given alone; these data strongly suggest that this antidepressive drug acted as an antagonist of 5-HT at the postsynaptic receptor [21]. Thus, the effects of some antidepressive drugs might be explained by their postsynaptic rather than presynaptic properties at central serotonergic receptors, as predicted by our new theory. Based on the results of this first study on rats, additional experiments are reported in this paper compairng three other effective antidepressants (mianserin, imipramine and iprindole) to amitriptyline on our animal model of depression.

METHOD

Subjects and Apparatus

Male, adult Wistar rats, maintained at approximatley 75% of their free-feeding weights, were trained to press a lever for sweetened condensed milk in an operant chamber. The conditioning apparatus had interior dimensions of $20 \times 15 \times 12$ cm and was constructed of 0.3 cm Plexiglas. A dipper-feeding device was mounted on the front panel of each box; an aluminum and brass lever was positioned to the right and just above the dipper. Parallel brass rods spaced at 1.5 cm intervals made up the grid floor of the apparatus. The whole unit was enclosed in a specially designed sound-insulated chamber. When the rats were not working in their daily sessions, they were housed in individual home cages and were kept on a constant light-dark cycle (light: 0600 to 1800).

Behavioral and Injection Procedures

Following establishment of the lever-pressing response, the rats received three daily sessions per week on a variable interval 1 (VI 1) schedule of reinforcement in which 0.15 ml of milk was presented to the responding animal on the average of once per minute. During training, each session was 30 to 120 minutes in duration. After a stable baseline pattern of responding was established, the rats were intraperitoneally (IP) injected with D,L-5-HTP (12.5 or 50 mg/kg) or placebo after the first 10 minutes of the VI sessions and immediately returned to the operant chamber until the session was terminated, usually after 90 to 120 minutes had elapsed. All 5-HTP or placebo injections were made between 0930 and 1030.

Methysergide, fluoxetine, mianserin, amitriptyline, imipramine and iprindole were dissolved in saline and injected IP into the rats one hour before the administration of 5-HTP or placebo on the days when these particular drugs were being tested. When either 5-HTP or one of the six drugs or placebo was injected repeatedly into the same rat, at least one week elapsed between successive injections. Each drug was injected at a level comparable to the human clinical dose and at twice the clinical dose. In the case of imipramine, one-half the clinical dose was also used. For methysergide and fluoxetine, which do not have established clinical doses, the amounts injected were based on previously published levels used in animal studies in this laboratory or others. The dose of 5-HTP was 50 mg/kg following methysergide, mianserin, amitriptyline, imipramine and iprindole pretreatment, but only 12.5 mg/kg following fluoxetine pretreatment. This smaller dose was used so that the total duration of the VI session would not exceed 120 minutes for control comparison purposes.

From an inspection of the control data, it was noted that although there was some day-to-day variation (10.5%) in the slopes of the curves, the ratios of cumulative responses for any one session, compared to any other session for similar 10 minute periods, were constant. Since this variation in baseline performance occurred, and in order to use each rat as its own control, a method of calculation was devised in which the behavioral effect of an injection of 5-HTP, with or without placebo or drug pretreatment, was expressed as "depth of depression." The method of calculating this measure has been previously described [21]. The data for each rat were subjected to individual analyses of variance to assess the behavioral effect of each drug on the 5-HTP induced depression.

RESULTS

Acute treatment with either of two doses (clinical dose and twice the clinical dose) of mianserin and amitriptyline, or a clinical dose of imipramine, significantly blocked the 5-HTP induced behavioral depression (40 to 70%) in rats working on a VI schedule of reinforcement (See Fig. 1). Of these three antidepressive drugs, the most effective blocker was mianserin (70% at 2 mg/kg; $p<0.001$), followed by amitriptyline (50% at 5 mg/kg; $p<0.05$) and finally imipramine (40% at 2.5 mg/kg; $p<0.05$). When imipramine was given at a dose of 1.25 mg/kg, the blockade was 10% (n.s.) whereas at 5 mg/kg, the effect was less than that seen at 2.5 mg/kg. A fourth antidepressant, iprindole, showed only a 20% blockade of the animal model of depression (n.s.) with the clinical dose of 1.5 mg/kg; when this dose was doubled, no behavioral effect was observed. The pretreatment effect of these drugs on 5-HTP induced depression was compared to that of the postsynaptic serotonergic blocker, methysergide, and the presynaptic serotonergic blocker, fluoxetine. In the case of methysergide, the blockade was 70 to 93% for the two

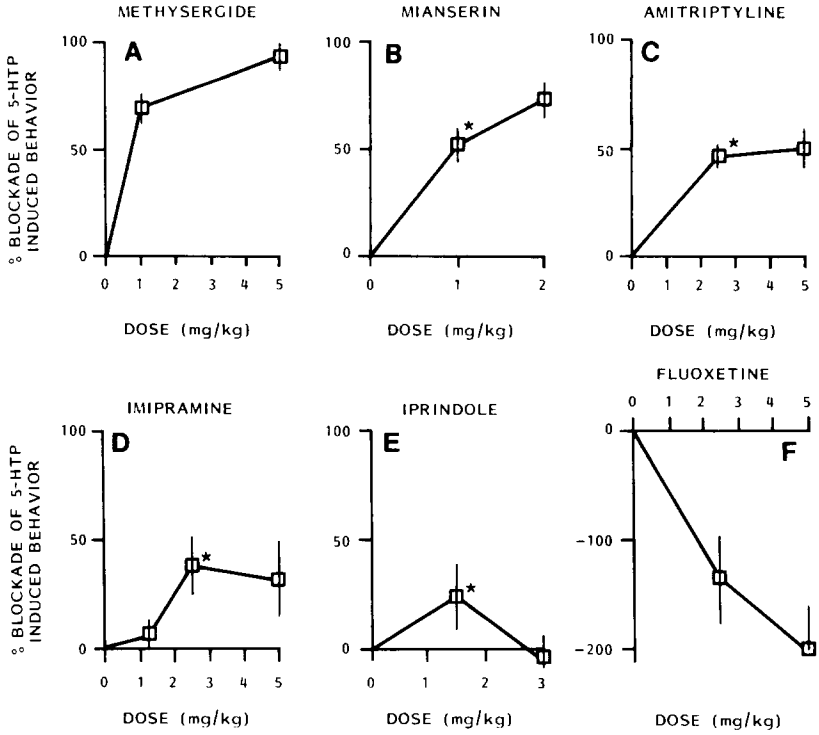

FIG. 1. Percent blockade of 5-HTP induced depression in rats following acute pretreatment with a postsynaptic serotonergic receptor blocker, methysergide, a presynaptic serotonergic receptor blocker, fluoxetine, or one of four clinically used antidepressive drugs, mianserin, amitriptyline, imipramine or iprindole. In the case of fluoxetine, potentiation of 5-HTP induced depression is indicated rather than blockade. A star indicates a dose comparable to the human clinical dose. No established human clinical dose exists for methysergide or fluoxetine. Each mean value (N=5 or 6 per group) is significantly different ($p<0.05$; analysis of variance) from control (placebo plus 5-HTP) except for the 5 mg/kg and 1.25 mg/kg dose of imipramine and both doses of iprindole. The dose of D,L-5-HTP used in each group was 50 mg/kg except in the case of the fluoxetine group in which 12.5 mg/kg was used so that the experimental session would be of the same duration as the control session.

doses, 1 and 5 mg/kg respectively, whereas with fluoxetine, a 140 to 200% potentiation of depression occurred at 2.5 and 5 mg/kg dose level.

Representative data for individual rats under each drug condition are presented in terms of depth of depression during the complete period of the VI sessions in Fig. 2. When any of the drugs was given one hour before placebo administration (no 5-HTP), no behavioral disruption was observed in any of the rats. Several curves reflecting depth of depression are shown in the six panels of Fig. 2: (a) one curve illustrates the behavioral effect of an injection of 50 mg/kg (or 12.5 mg/kg D,L-5-HTP in the case of fluoxetine) into each rat, and (b) two additional curves show the behavioral effect of drug pretreatment (at two different dose levels) when 5-HTP is injected. It is apparent that in some cases only the depth of depression, not the duration is affected, for the single animals presented in Fig. 2. The data in panel A show that for methysergide (1 and 5 mg/kg), both duration and depth of depression were attenuated. This is also true in the case of the most effective dose of mianserin (See panel B). In the case of amitriptyline and imipramine (for those animals that were affected, See Table 1), only the depth of depression is decreased by drug pretreatment (See panels C and D for representative rats).

In Table 1, data are presented to show the number of rats in each drug pretreatment group exhibiting one of three different effects on behavioral depression: blockade, no effect, or potentiation. An overall consistency of behavioral effect is apparent in the case of methysergide, mianserin, amitriptyline (blockade) and fluoxetine (potentiation). There is a greater variability of drug effect in the rat when treated with imipramine or iprindole.

DISCUSSION

Based on the data reported in this study, four clinically used antidepressive drugs, mianserin, amitriptyline, imipramine and iprindole have, to varying degrees of effectiveness, the ability to partially block behavioral depression as measured in a 5-HTP induced animal model in rats working

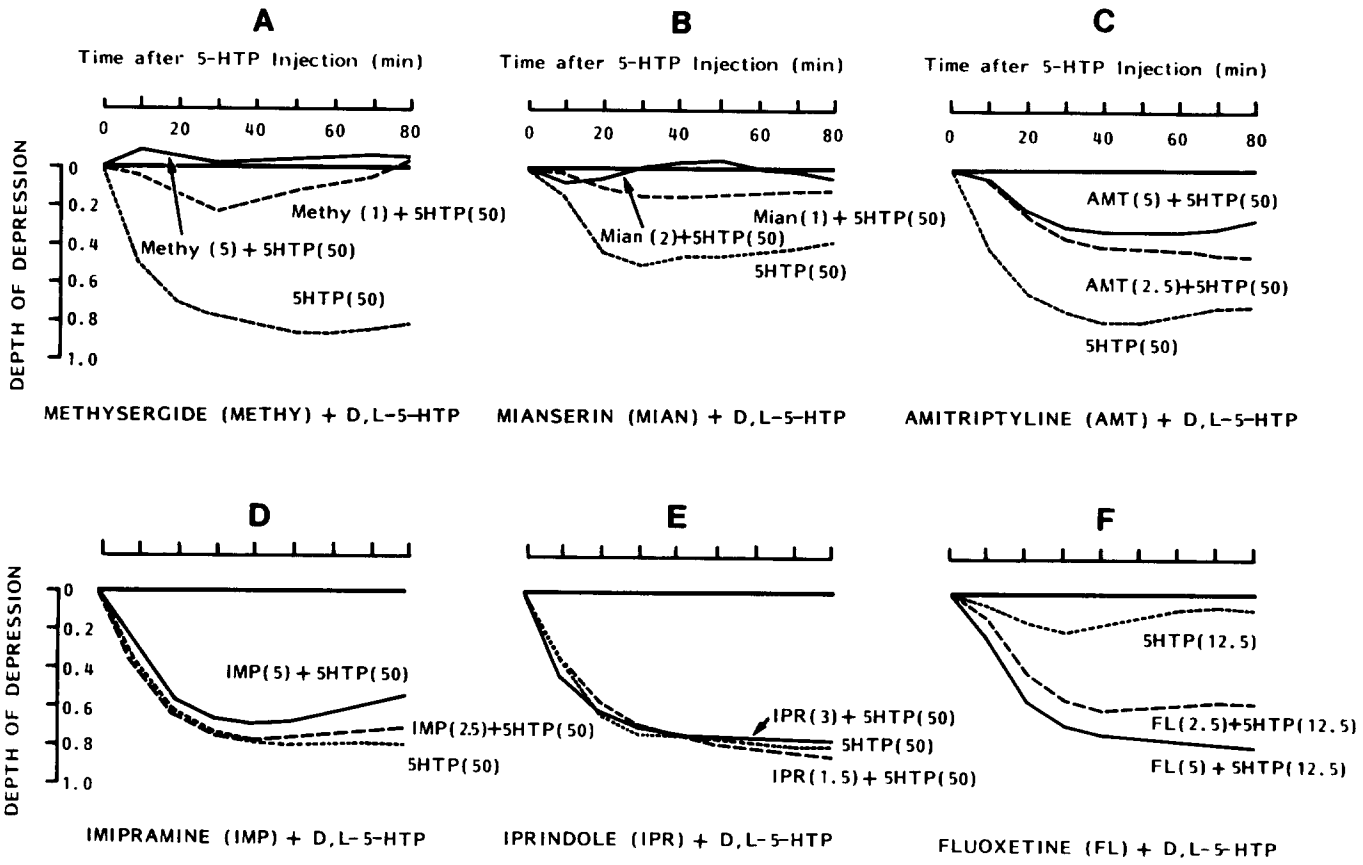

FIG. 2. Depth of depression as a function of time following pretreatment with either a postsynaptic serotonergic receptor blocker, methysergide (panel A), one of four antidepressive drugs, mianserin (panel B), amitriptyline (panel C), imipramine (panel D), iprindole (panel E), or a presynaptic serotonergic receptor blocker, fluoxetine (panel F) to rats administered 5-hydroxytryptophan while working on a variable interval schedule of milk reinforcement. Each panel represents the data from a single rat from each drug group. The dose (mg/kg) of each drug in the figure is given in parentheses. To calculate the depth of depression for each 10 minute period in the case of the 5-HTP session, a control session in which placebo was administered was used in the comparison. In the case of the drug pretreatment plus 5-HTP session, the comparison control session was based on the drug pretreatment plus placebo. A smaller dose of 5-HTP was used for the fluoxetine injections so that the experimental session would be of the same duration as the control session.

on a VI schedule of reinforcement. In previous studies (see [7] and [9] for reviews), we have established that the effect of injecting 5-HTP into pigeons or rats is to cause a type of hypoactivity. Others have shown this effect in primates [18]. Further, our neurochemical data indicate that after an injection of 5-HTP or L-tryptophan, the content of 5-HT increases in serotonergic nerve endings in the telecephalon until it is released into the synapse. The 5-HT in the synapse interacts with the receptor in the postsynaptic membrane [7,14]. This behavioral effect can be completely blocked by a postsynaptic serotonergic receptor blocker, methysergide, or enhanced by a presynaptic serotonergic receptor blocker, fluoxetine [21]. Since mianserin, amitriptyline, imipramine and iprindole have no effect on the model of depression when injected alone, one can conclude that these antidepressive drugs used in our experiments can affect these postsynaptic serotonergic receptors so as to block the 5-HT effect in varying degrees.

The studies referred to above are based on further testing of our ideas in the general area of neurochemical correlates of behavior, a project which was designed to provide insight into some possible cerebral mechanisms of depression and other functional illness. Other lines of investigation with similar goals, (clinical studies, binding studies, etc.) have also appeared in the literature. The data from animal studies, as well as clinical studies, did not fit into the predominant serotonin deficiency theory. In order to reconcile the conflicting animal data and clinical data, i.e. suppression of animal behavior is caused by an increase in the 5-HT in the synaptic cleft, whereas human depression is associated with a deficiency of the cerebral serotonin system, we recently developed a new theory [7,9]. This theory suggests that in some types of human depression, a hypersensitive postsynaptic receptor develops in these individuals due to a decreased release of 5-HT. Thus, if such a subgroup of patients is identified, their serotonergic synapses may be functioning close to normal levels since the postsynaptic receptors compensate for the decreased release of 5-HT by becoming hypersensitive. Then, at some later date, a stress factor causes an increased release of 5-HT which now inter-

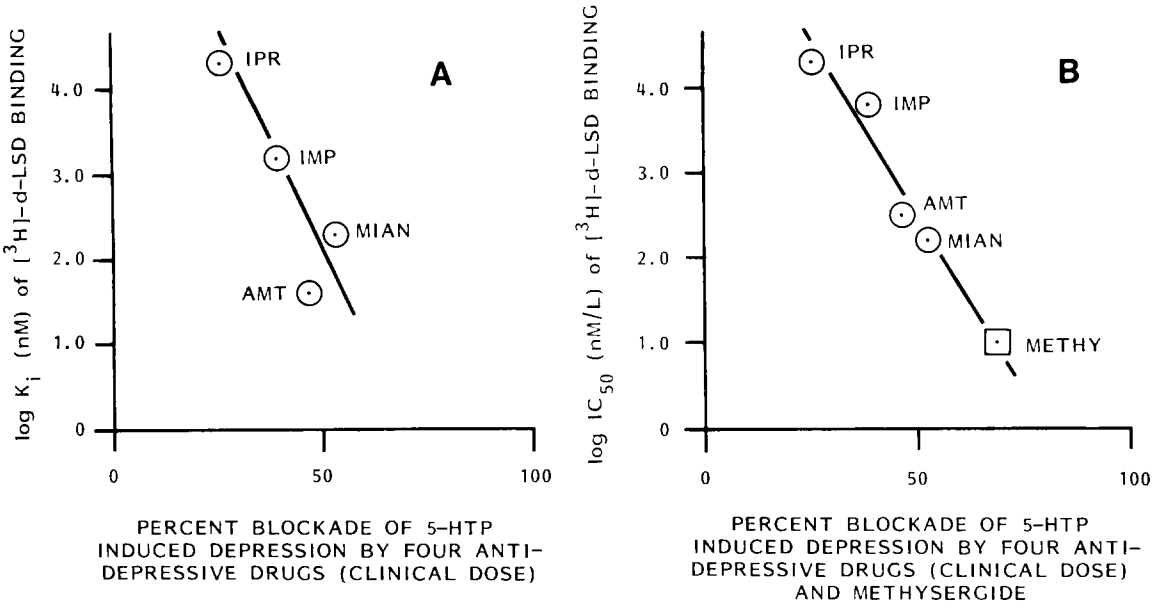

FIG. 3. Relationship of *in vitro* [^{3}H]-d-LSD binding values to percent blockade of 5-HTP induced depression by four antidepressive drugs (clinical dose), mianserin, amitriptyline, imipramine, iprindole, and a postsynaptic serotonergic receptor blocker, methysergide (no established clinical dose available.) The binding data in panel A is derived from data using membranes isolated from the dorsal neocortex of the rat (Ogren *et al.* [22]). The binding data in panel B is derived from IC_{50} data using rat brain (Fuller, personal communication). When these data are subjected to linear regression analyses, the line in panel A can be expressed as $Y=6.54-0.09X$ ($r=-0.89$) whereas in panel B, $Y=6.49-0.08X$ ($r=0.98$). The abbreviations in this figure refer to the same drugs as noted in Fig. 2.

TABLE 1

BEHAVIORAL EFFECT OF METHYSERGIDE, FLUOXETINE AND FOUR ANTIDEPRESSANT DRUGS ON 5-HYDROXYTRYPTOPHAN INDUCED DEPRESSION IN INDIVIDUAL RATS

Drug	Dose mg/kg	N	Blockade*	No Effect	Potentiation*
Methysergide	5	6	6		
	1	6	6		
Mianserin	2	6	6		
	1	6	5	1	
Amitriptyline	5	5	4	1	
	2.5	5	5		
Imipramine	5	6	3	3	
	2.5	6	4	2	
	1.25	6	1	4	1
Iprindole	3	5		4	1
	1.5	5	3	2	
Fluoxetine	5	6			6
	2.5	6		1	5

*Determined by individual analyses of variance ($p<0.05$). Although the group data indicated that the 5 and the 1.25 mg/kg doses of imipramine and the 1.5 dose of iprindole did not significantly block the 5-HTP induced depression (see Fig. 1), significant blockade was demonstrated in individual rats as presented in this Table. For more details on behavioral measures used see Nagayama *et al.* [21].

acts with the supersensitive postsynaptic receptors. In the animal model, excess 5-HT from its precursor also increases in the cleft. In both cases, an increased release of 5-HT occurs when compared to the normal state. Thus, this theory predicted that in at least one group of depression, the clinically effective drugs should interact with the serotonergic receptors in the postsynaptic membrane.

Other data in the literature support our theory. A recent report [22] described the effect of a number of antidepressants on [^{3}H]-d-LSD binding *in vitro* to membrane sites in the dorsal neocortex of rats. We chose to compare their binding data with our behavioral data. When the log K_i(nM) of [^{3}H]-d-LSD binding in the presence of iprindole, imipramine, mianserin and amitriptyline was plotted against the percent blockade of 5-HT induced depression by the same four drugs (given at clinical dose levels), an inverse linear relationship was found (see panel A, Fig. 3). Experiments from a third independent laboratory (Dr. R. Fuller, Lilly Research Laboratories, personal communication) provided IC_{50} data of [^{3}H]-d-LSD binding in the presence of the same four drugs as well as for methysergide. The data expressed as log IC_{50} (nM/L) of [^{3}H]-d-LSD binding was plotted against our behavioral data; the results are shown in panel B of Fig. 3. Again, a direct inverse linear relationship was found. In fact, the slopes of the curves in panels A and B are parallel (see Legend of Fig. 3).

It is not our purpose to discuss the data on the effect of these drugs on the uptake of 5-HT and other neurotransmitters. No doubt, some presynaptic events are occurring in

addition to perhaps other secondary events. However, we call attention to the fact that if presynaptic events and postsynaptic events are occurring simultaneously, in the case of amitriptyline and mianserin, it appears that the postsynaptic effect is the likely predominant effect and it may be this latter action that characterizes these drugs as antidepressants. In the case of imipramine, and to an even greater extent, iprindole, although the data support a postsynaptic action, the results suggest that the serotonergic system may not be the predominant one. Since these drugs are less potent behaviorally and as blockers of [^{3}H]-d-LSD binding (see Fig. 3), perhaps pre- and postsynaptic sites of additional neurotransmitters are also involved in their clinical effectiveness (see Table 1). The data on imipramine and iprindole support the suggestion of multiple subgroups in depression.

It should be noted that although the present animal model, as used in our studies, meets a number of criteria for an animal model of depression [21], there is no general agreement on the validity of animal models. In addition, the present study used acute doses of the four antidepressants, whereas clinical usage involves chronic treatment. Thus, it is important to study the effects of these drugs under chronic conditions using our animal model of depression. Finally, other effective drugs used clinically in depression should be tested with our model, especially drugs thought to act through transmitter systems other than the serotonin system.

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