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Pre- and Postsynaptic Serotonergic Manipulations in an Animal Model of Depression[1]

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NAGAYAMA, H., J. N. HINGTGEN AND M. H. APRISON. *Pre- and postsynaptic serotonergic mainipulations in an animal model of depression*. PHARMAC. BIOCHEM. BEHAV. 13(4) 575–579, 1980.—Rats working on a food-reinforced operant schedule and exhibiting behavioral depression following administration of D,L-5-hydroxytryptophan (5-HTP) were pretreated with one of three drugs: methysergide, fluoxetine, or amitriptyline. The former two drugs were used to establish a basis for distinguishing between pre- and postsynaptic events. We found that methysergide, a known postsynaptic blocker of serotonin, almost completely abolished the depressive effect of 5-HTP, whereas fluoxetine, a known specific uptake blocker of serotonin, potentiated the depressive effect of the 5-hydroxytryptamine (5-HT) precursor. Amitriptyline, one of the commonly prescribed antidepressive drugs, reduced the behavioral depression following 5-HTP by approximately 50%. These data indicate that amitriptyline can act as an antagonist of 5-HT at the postsynaptic receptor. The results of this study, as well as those recently reported from CNS membrane binding studies, suggest that the therapeutic effects of some antidepressive drugs may be explained by their postsynaptic rather than presynaptic properties at central serotonergic receptors. Thus, these studies support the hypothesis that some types of human depression may be primarily due to an excess of free 5-HT acting at postsynaptic receptors.

5-Hydroxytryptophan Model of depression Methysergide Fluoxetine Amitriptyline Serotonin 5-Hydroxytryptamine receptors

RECENTLY, a theory has been proposed which suggests that some types of human depression are due to an excess of free serotonin in the synaptic cleft which can interact with postsynaptic hypersensitive serotonin receptors [9,11]. This theory was based, in part, on previous studies from our laboratories in which D,L-5-HTP was injected into pigeons and rats working on food-reinforced operant schedules. This serotonin precursor produced a period of behavioral depression [2, 3, 27] that was temporally correlated with an increase of 5-HT in the telencephalon and diencephalon [4, 6, 27], but not with changes in catecholamine levels [5]. In nerve endings isolated from these areas of the brain, the effect of such an injection of 5-HTP produced an increase in the level of 5-HTP, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). Further, *in vivo* experiments using these nerve ending fractions indicated that L-5-HTP caused a release of preloaded (^{3}H)-5-HT which could be blocked by a decarboxylase inhibitor [23,26]. Accordingly, it was suggested that lowered behavioral responding following 5-HTP administration is elicited by an excess of 5-HT in the synaptic cleft, and in the animal model of depression is a predominantly postsynaptic phenomenon [7–11].

One way to test this new theory of depression [9,11] is to use a clinically proven antidepressive drug with one known specific action, i.e., a postsynaptic serotonin receptor antagonist. Unfortunately the psychiatrist does not have such a drug at present. However, if one of the drugs used in treating depression could be shown to act postsynaptically, this would provide some indirect evidence for the concept that some types of depression could be due mainly to postsynaptic phenomena. Therefore, the research strategy in this study was to use two drugs to establish specific pre- and postsynaptic effects as seen in our animal model of depression and then compare these effects with those following a commonly used antidepressive drug, amitriptyline [16,21]. This latter drug was compared with fluoxetine, a drug developed as a specific presynaptic uptake blocker of serotonin [18, 31, 32], and methysergide, a drug whose postsynaptic antagonist action against serotonin is well established [17,29].

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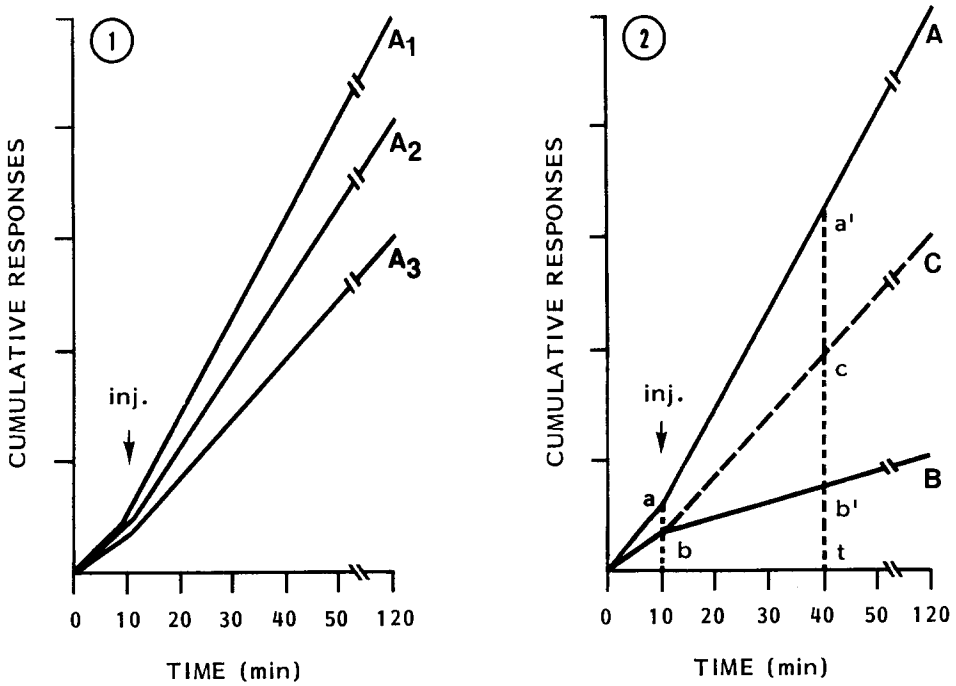

FIG. 1. Method for calculating depth of depression. In Part 1, cumulative responses for three control sessions (A_1, A_2, A_3) are diagramatically shown. The lower response rate as seen in the first 10 min period represents the warm-up effect. If a placebo injection is to be made in any specific session, this is done at the time indicated by the arrow. Part 2 shows how the parameter, depth of depression, was calculated. Line A is the control data on one day, whereas line B is the data showing the effect of a 5-HTP injection (at 10 min) on another day. Line C is the reconstructed line which represents the control for that day if no 5-HTP had been injected. This construction is based on two pieces of data: (a) the data for line A and (b) the knowledge that the ratio at the first 10 min is maintained for the whole time span. As noted in the text, the ratios of cumulative responses for any one session compared to any other session for similar 10 min periods were approximately constant or (b/a)=(c/a') and c=(a'b)/a. The depth of depression (DD) was defined as (c-b')/c and it can be shown that DD=1-[(b'/b)(a/a')]. The latter term can easily be calculated from the behavioral data. The units of this measurement vary from 0 (control or zero depression) to 1 (maximum depression).

METHOD

Subjects and Apparatus

Male, adult, Wistar rats were maintained at approximately 75% free-feeding weight. They were allowed to adapt to individual home cages and were kept on a constant light-dark cycle (light: 0430 to 1630 hr). Rats were trained to press a lever for a sweetened milk reinforcer in an operant conditioning chamber. The conditioning apparatus was constructed of 0.3 cm plastic with interior dimensions of 20×15×12 cm. On the front panel of each box a dipper-feeding device and lever were mounted. The grid floor was made of brass rods spaced at 1.5 cm intervals. The whole unit was placed in a specially designed sound-insulated chamber.

Behavioral and Injection Procedures

Once the lever-pressing response was established, the rats were given 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 ran 30 min. After a stable baseline pattern of responding was established, animals were adapted to placebo (saline or distilled water) injections. The rats were intraperitoneally (IP) injected with D,L-5-HTP (either 12.5, 25 or 50 mg/kg) or placebo after the first 10 min of the VI session, and immediately returned to the operant chamber until the session was terminated, usually after 120 min had elapsed. All 5-HTP or placebo injections were made between 0930 and 1030 hr.

Methysergide, fluoxetine and amitriptyline were each dissolved in saline and injected IP into rats to yield a 5 mg/kg dose. Each drug was individually injected 1 hr before the administration of placebo or D,L-5-HTP (50 mg/kg following methysergide or amitriptyline pretreatment and 12.5 mg/kg following fluoxetine pretreatment).

Calculation of Behavioral Data

From an inspection of the control data (i.e., cumulative responses versus time after injections of distilled water into the same rat), it was noted that although the slopes of the curves were not identical on different days, the ratios of cumulative responses for any one session compared to any other session for similar 10 min periods were constant (see Fig. 1). Since this variation in baseline performance occurs, 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 was expressed as "depth of depression'. This index of degree of depression was calculated as shown

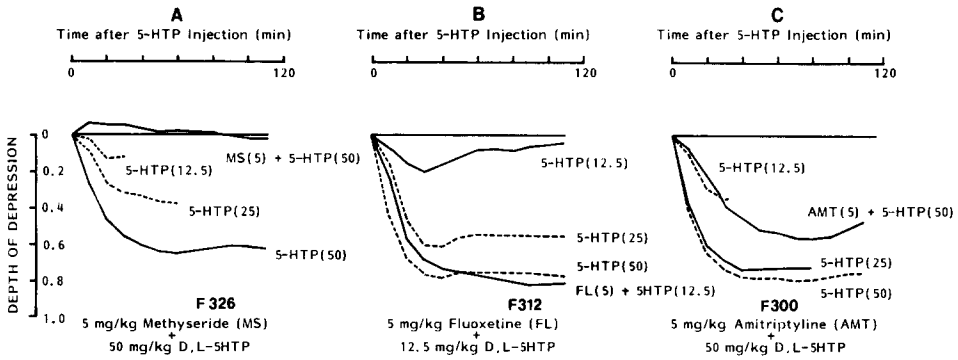

FIG. 2. Depth of depression as a function of time following pretreatment of either methysergide (panel A), fluoxetine (panel B) or amitriptyline (panel C) in rats administered 5-hydroxytryptophan while working on a variable interval schedule of reinforcement. Rats F326, F312 and F300 are representatives from a group of five or six rats given each drug. The dose (mg/kg) of each drug in the figure is given in parenthesis. To calculate the depth of depression for each 10 min 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 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.

in Fig. 1. The data for each rat were subjected to individual analyses of variance comparing the 5-HTP behavioral effect with and without the drug pretreatment.

RESULTS

Pretreatment with 5 mg/kg methysergide 1 hr before the injection of 50 mg/kg D,L-5-HTP almost completely blocked the behavioral effect (see A in Fig. 2 for representative data from one rat). This effect was seen in all six rats ($p<0.005$). On the other hand, 5 mg/kg fluoxetine was found to significantly potentiate the depressive effect of 12.5 mg/kg D,L-5-HTP (B, Fig. 2). This interaction was also seen in all six rats ($p<0.005$). The antidepressive drug, amitriptyline, when given as a 5 mg/kg pretreatment dose, significantly reduced the depressive effect of 50 mg/kg 5-HTP (C, Fig. 2). This effect was seen in all five rats ($p<0.005$). When the drugs were given 1 hr before placebo, no behavioral disruption was observed in any of the rats.

A single parameter was sought which could be used in comparison of the drug data. We found that the lowest point on any of the curves varied as a function of the dose of 5-HTP. Thus, this parameter was used to reflect the depth of depression for each rat in each experimental situation.

In Table 1, group data are presented to show that the effect of 5 mg/kg amitriptyline is to reduce the behavioral effect of 50 mg/kg D,L-5-HTP by approximately 50% whereas 5 mg/kg methysergide almost completely blocked it (93%). To test the effect of 5 mg/kg fluoxetine, a lower dose of D,L-5-HTP was used to reduce the behavioral effect (i.e., 12.5 mg/kg), thus keeping the experimental session within the same 120 min period as the control sessions. The behavioral effect of this low dose of D,L-5-HTP was enhanced (about 200%) when fluoxetine was given 1 hr earlier.

TABLE 1

BLOCKADE OR POTENTIATION OF 5-HTP INDUCED BEHAVIORAL DEPRESSION FOLLOWING PRETREATMENT WITH METHYSERGIDE, FLUOXETINE OR AMITRIPTYLINE IN RATS WORKING ON A VARIABLE INTERVAL 1 SCHEDULE FOR FOOD REINFORCEMENT

Drug Pretreatment	N	Blockade (%)	Potentiation (%)
Methysergide (5 mg/kg)	6	93	—
Fluoxetine (5 mg/kg)	6	—	207
Amitriptyline (5 mg/kg)	5	49	—

To calculate the percent blockade or potentiation, the lowest depth of depression was determined for both injection sessions in which 5-HTP was administered with or without drug pretreatment. The percent change for each rat was calculated and used to determine the mean change for each group. Methysergide and amitriptyline were administered 1 hr prior to 5-HTP (50 mg/kg) which was given 10 min after the start of the VI session. Fluoxetine was administered 1 hr prior to 5-HTP (12.5 mg/kg) which was given 10 min after the start of the VI session. This latter dose of 5-HTP typically yields only mild behavioral depression, and was used so that fluoxetine potentiation of the 5-HTP effect would occur during the same 120 min period as the control sessions.

DISCUSSION

In order to test whether a clinically proven antidepressive drug such as amitriptyline had postsynaptic actions in addition to known presynaptic actions, our first goal was to establish in our animal model a behavioral method for differentiating between serotonergic pre- and postsynaptic effects of injected drugs. This was accomplished by using fluoxetine and methysergide, known pre- and postsynaptic blockers, respectively. Using this testing system, we found that the data for amitriptyline administration support an antagonistic postsynaptic effect (Fig. 2, Table 1). The experimental design was based on our desire to test a new theory for a subgroup of human depression [9,11].

The fact that amitriptyline reduced the depression in our animal model is very interesting, since this finding does not support the generally accepted view that tricyclic antidepressants such as amitriptyline have their antidepressive effect due to their ability to block uptake of 5-HT [1, 12–15,

20, 28, 30]. If the uptake blockade of 5-HT is the main action of amitriptyline, the drug should have increased depression rather than decreased it in our model. Therefore, one conclusion from this study is that although amitriptyline may block uptake of 5-HT at the presynaptic side, it also has an antagonistic action towards 5-HT at the receptors on the postsynaptic side. Both processes are likely taking place at effective concentrations of the drug in regions of 5-HT synapses, but postsynaptic blockade appears to be the predominant effect, and it may be this latter action that characterizes this drug as an antidepressant. However, it should be noted that the present study used an acute dose of amitriptyline, whereas clinical usage involves chronic treatment. Thus, it is important to study the effects of chronic amitriptyline (as well as other tricyclic antidepressants) on our animal model of depression.

Since amitriptyline and its metabolites have effects on neurotransmitter systems other than serotonergic (e.g., noradrenergic), the possible role of these systems in the blocking of 5-HTP induced depression should be considered. As the dose of amitriptyline used in this study had no behavioral effect when used in control sessions with placebo, simple enhancement of response rates, through the action of some neurotransmitter other than 5-HT, does not appear likely. In addition, the predominant role of 5-HT in 5-HTP induced depression in our behavioral model has been clearly established [9–11]. Although the role of other neurotransmitter systems cannot be ruled out by the data presented in this study, postsynaptic 5-HT blockade by amitriptyline is strongly suggested as the mechanism to account for this drug's antidepressive effect on our animal model.

The data presented in this paper as well as that reviewed by Aprison and coworkers [9,11] support the concept that some kinds of human depression may be caused by an excess of 5-HT in the synaptic cleft. Furthermore, the results suggest that tricyclic antidepressants, such as amitriptyline, may improve some kinds of depression in patients by reducing the effect of 5-HT at postsynaptic serotonergic receptors. This hypothesis is strengthened by two recent findings. First, amitriptyline and other tricyclic antidepressants have been found to block, differentially, the binding of (^{3}H)-LSD as well as (^{3}H)-5-HT to central receptors [19,25]. Secondly, amitriptyline has been shown to inhibit the stimulatory action of 5-HT in the flexor reflex preparation of the spinal rat [22].

It is instructive to ask whether the 5-HTP induced depression may be considered as a model of human depression. Although there is no consensus on validity of animal models, it should be noted that it generally meets McKinney and Bunney's criteria of an animal model of depression [24]. Thus, our animal model (a) has some of the properties which are analogous to those seen in human depression, e.g., a period of hypoaction; (b) includes an observable behavioral change which can be objectively evaluated; (c) demonstrates that a treatment method used with humans is also effective in reversing the depression noted in our animals; and (d) can be reproduced by other investigators. Therefore, further studies of the present animal model, in conjunction with appropriate neurochemical measures, could yield important data leading to the clarification of the role of cerebral mechanisms in human depression.

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