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Postsynaptic Serotonergic Blockade Following Chronic Antidepressive Treatment with Trazodone in an Animal Model of Depression[1]

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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**(3) 425–428, 1984.—Acute pretreatment with clinically equivalent doses of antidepressive drugs has been observed to block D,L-5-hydroxytryptophan (5-HTP) induced behavioral depression in rats working on a food-reinforced operant schedule. Data from studies designed to distinguish presynaptic from postsynaptic events, indicated that the antidepressants were acting in part as blockers of postsynaptic serotonergic receptors. Using the same 5-HTP model of depression, we studied both the chronic and acute effects of a recently introduced antidepressant, trazodone, a triazolopyridine compound. Rats working for milk reinforcement and exhibiting behavioral depression following administration of 50 mg/kg 5-HTP were pretreated (one hr before 5-HTP) with 1, 2, or 4 mg/kg trazodone with resulting blockade of 5-HTP induced depression of 35, 62 and 70% respectively. Chronic administration of trazodone (2 mg/kg trazodone/day) also resulted in a significant blockade of the 5-HTP effect (75%). Neither 2 mg/kg or 4 mg/kg trazodone was found to potentiate the shorter period of depression following 25 mg/kg 5-HTP. Chronic treatment with the antidepressant drugs, amitriptyline or mianserin also blocked 5-HTP depression. Thus, as in our earlier studies, these data suggest an important postsynaptic mechanism associated with chronic administration of trazodone, amitriptyline and mianserin which could be implicated in the therapeutic effectiveness of these drugs. The potency of trazodone in relation to other antidepressant drugs in our behavioral model of depression paralleled their potency in displacing radioligand binding to 5-HT receptors, and gives additional support for the new hypersensitive postsynaptic serotonin receptor theory of depression.

Serotonergic receptors Model of depression Trazodone 5-Hydroxytryptophan Amitriptyline Mianserin Chronic antidepressant treatment Presynaptic Postsynaptic

PREVIOUS data from our studies testing predictions from a new theory of depression [1, 3, 4, 12] have indicated that acute pretreatment with clinically comparable doses of the antidepressive drugs, mianserin, amitriptyline, imipramine and iprindole results in varying degrees of blockade of D,L-5-hydroxytryptophan (5-HTP) induced depression in rats working on a food reinforced operant schedule [17,18]. To distinguish between presynaptic and postsynaptic events, these drug effects were compared to those of fluoxetine, a known specific uptake blocker of serotonin (5-HT), which potentiates the 5-HTP induced depression, and to methysergide, a postsynaptic blocker of 5-HT, which almost completely abolishes the depressive effect of 5-HTP. Since mianserin, amitriptyline and imipramine significantly blocked 5-HTP depression, those drugs appeared to be acting as antagonists of 5-HT at the postsynaptic serotonin receptor as one would predict from the new theory. When these results were viewed in terms of supporting data for postsynaptic actions reported from CNS binding studies [19], it was strongly suggested that the therapeutic effects of some antidepressants may be explained in part by their postsynaptic rather than presynaptic effects at central serotonergic receptors [1, 2, 3, 12].

The antidepressants used with our animal model have been reported to have *in vitro* pre- as well as postsynaptic serotonergic effects [15,19]. If an antidepressant with more selective *in vivo* postsynaptic serotonergic receptor blocking action was used with our model, this drug should be very effective in blocking the 5-HTP induced depression in rats. Trazodone, 2-[3-[4-(m-chlorophenyl)-l-piperazinyl]propyl]-s-triazolo[4,3-a]pyridin-3(2H)one, is a recently introduced antidepressant of significant clinical effectiveness [6, 9, 11,

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14]. Although originally characterized as having serotonin uptake effects, trazodone is now known to have weak inhibitory effects on serotonin uptake even at high doses of 10 and 20 mg/kg in rats and 32 mg/kg in mice [10]. In contrast to its weak inhibition of uptake, trazodone appears to be a very potent serotonin receptor antagonist. This drug (a) antagonizes binding of tritiated spiperone to 5-HT_2 receptors in the frontal cortex [5,21], (b) blocks 5-HT_2 receptor-mediated contractions of the rat jugular vein [10], (c) antagonizes a 5-HT receptor-mediated response of elevating serum corticosterone by a 5-HT agonist, quipazine, in rats [10], and (d) antagonizes the increase in serum prolactin produced by 5-HTP [16].

To further test the hypersensitive postsynaptic serotonin receptor theory of depression, and to further elucidate the possible postsynaptic blocking effects of antidepressants, we studied trazodone's effect on D,L-5-HTP induced depression in our animal model following both acute and chronic administration of the drug. (D,L-5-HTP was administered to allow for comparisons to our previous studies. For data on differential uptake, metabolism and behavioral effects of the D and L isomers of 5-HTP with our animal model see [20].) For comparison purposes, we also have studied the previously established postsynaptic blockade following acute administration of amitriptyline or mianserin now using a chronic treatment schedule. As predicted, both acute and chronic treatment with trazodone, as well as chronic treatment with amitriptyline and mianserin, significantly blocked 5-HTP induced depression.

METHOD

Subjects/Apparatus

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

Behavioral/Injection Procedures

Once the lever-pressing response was established, rats received three sessions per week on a variable interval (VI 1) schedule of reinforcement in which 0.15 ml sweetened condensed milk was presented to the responding animal on the average of once per minute. During training each session was 30 to 120 min in duration. After a stable baseline pattern of responding was established (variation did not exceed 15%) the rats were intraperitoneally (IP) injected with (50 mg/kg) D,L-5-HTP or placebo after the first 10–15 min of the VI session, and then immediately returned to the operant chamber until the session was terminated, usually after 90 to 120 min had elapsed. All 5-HTP or placebo injections, as well as other drug injections were made between 0930 and 1130 hr.

Trazodone (1, 2, or 4 mg/kg IP) was injected one hour before subsequent additional injections of 5-HTP or placebo. At least one week elapsed between successive injections of 5-HTP into the same rat. Other rats received chronic administration of trazodone, 2 mg/kg per day for eight consecutive days, followed at the end of the period by an injection of 50 mg/kg D,L-5-HTP. Additional rats received 2 mg/kg trazodone during the VI session following the initial 15 min period of the session. Finally, other rats received a smaller injection of D,L-5-HTP (25 mg/kg) following pretreatment with either 2 mg/kg or 4 mg/kg trazodone.

In another study, rats received daily injections of amitriptyline, mianserin or placebo (IP) for 28 days. This longer period was used to make treatment conditions comparable to the clinical procedures. (Trazodone has been reported to produce a change in depression with a shorter lag time.) During this 28 day period the rats continued receiving at least three VI sessions per week. Each drug was injected at a level comparable to human clinical dose levels (2.5 mg/kg amitriptyline; 1.0 mg/kg mianserin). At 7, 14, 21 and 28 days of chronic antidepressant treatment, the rats also received an injection of 50 mg/kg D,L-5-HTP during a daily VI session according to the procedure described above.

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 treatment, was expressed as "depth of depression" [17]. Thus, the effect of the antidepressive drug was expressed as percent blockade of depression [18].

TABLE 1

PERCENT BLOCKADE OF 5-HTP INDUCED BEHAVIORAL DEPRESSION IN RATS FOLLOWING ACUTE AND CHRONIC PRETREATMENT WITH TRAZODONE (IP)

	Acute	Chronic
1.0 mg/kg Trazodone	35%	
2.0 mg/kg Trazodone	62%	75%
4.0 mg/kg Trazodone	70%	

Mean percent based on groups of 5–6 rats. Acute administration schedule: Drug given one hour prior to D,L-5-HTP (50 mg/kg IP) injection which was administered 10 minutes following start of VI session. Chronic administration schedule: Drug given once each day for eight days with last pretreatment given one hour before 5-HTP administration. Each mean value is significantly different ($p < 0.01$; analysis of variance) from control (placebo plus 5-HTP); 2 mg/kg (acute and chronic) and 4 mg/kg values are significantly different ($p < 0.05$; analysis of variance) from 1 mg/kg value. Further description of behavioral measures used can be found in [17,18].

RESULTS

Acute treatment with trazodone significantly blocked 5-HTP induced depression in rats working on a VI session for milk reinforcement. Doses of 1, 2 or 4 mg/kg resulted in a corresponding blockade of behavioral depression at 35%, 62% and 70% respectively (see Table 1). When 2 mg/kg trazodone was given each day for eight consecutive days to a group of VI responding rats, the 5-HTP depression was effectively blocked (75%). Thus, chronic pretreatment with trazodone was as effective as acute pretreatment in blocking 5-HTP depression (see Table 1).

Rats receiving 2 mg/kg trazodone during the daily VI session showed no period of decreased responding other than an initial 1–2 min disruption associated with injection procedures. The period of depression in those rats receiving

TABLE 2

PERCENT BLOCKADE OF 5-HTP BEHAVIORAL DEPRESSION IN RATS FOLLOWING CHRONIC PRETREATMENT WITH AMITRIPTYLINE, MIANSERIN OR PLACEBO (IP)

Drug	7 Days Pretreatment	14 Days Pretreatment	21 Days Pretreatment	28 Days Pretreatment
Placebo	6%	10%	9%	13%
Amitriptyline (2.5 mg/kg)	48%	55%	47%	64%
Mianserin (1.0 mg/kg)	61%	54%	72%	73%

Mean percents based on groups of 3–4 rats. Amitriptyline and mianserin were administered daily at levels comparable to clinical dose. D,L-5-HTP (50 mg/kg IP) was given once each week during a VI session following the procedure described in methods. Each mean value is significantly different ($p<0.01$; analysis of variance) from control (placebo plus 5-HTP).

a lower dose of 5-HTP (25 mg/kg) following trazodone pretreatment (either 2 or 4 mg/kg) indicated no potentiation effects; in fact, the period of depression was 95% blocked for both dose levels.

Chronic treatment with amitriptyline using the clinically comparable dose of 2.5 mg/kg indicated a significant increase in effective blockade of depression from 48% after 7 days of pretreatment to 64% blockade of 5-HTP depression after 28 days of amitriptyline treatment (see Table 2).

Mianserin maintained, but did not increase its effectiveness in blockading 5-HTP depression during the 28 day period. Using a 1.0 mg/kg daily dose, mianserin showed a 61% blockade at 7 days pretreatment and a non-significant increase to 73% blockade at 28 days of treatment (Table 2).

Chronic treatment with either amitriptyline or mianserin did not have an observable effect on baseline performance of rats performing at least three times a week on VI sessions. Placebo injections over the same period of chronic drug treatment had no significant effect on either the baseline responding or the period of depression following the administration of the standard dose of 5-HTP.

DISCUSSION

The most important results of this study are (a) administration of trazodone (1, 2, or 4 mg/kg) results in the blockade (35, 62, 70% respectively) of 50 mg/kg D,L-5-HTP induced depression in rats working on a food-reinforced operant schedule; (b) this effect appears to be a postsynaptic action rather than a presynaptic one since it is similar to methysergide effects with our model [17,18]; (c) chronic administration of 2 mg/kg trazodone per day also resulted in a significant blockade of the 5-HTP effect (75%); and (d) the blockade of 5-HTP induced depression following both acute and chronic treatment with trazodone was similar to that found with mianserin and amitriptyline.

Previous data from our laboratories have indicated that (a) acute pretreatment with clinical doses of the antidepressive drugs mianserin, amitriptyline, imipramine and iprindole results in varying degrees of blockade of 5-HTP induced depression in rats working on a food-reinforced operant schedule [18] and (b) the log IC_{50} values of (^{3}H)LSD binding for these four drugs is negatively correlated with their percent blockade of the 5-HTP induced depression [3,18]. When we plotted the IC_{50} and behavioral data for 2 mg/kg trazodone on the same plot, the trazodone point fell on the line (Fig. 1). Thus, the binding data for trazodone from an independent laboratory (Dr. R. Fuller, Lilly Research Laboratories, personal communication) supports the concept we have suggested previously, namely that postsynaptic receptor blockade is an important feature contributing to therapeutic effectiveness of the antidepressants.

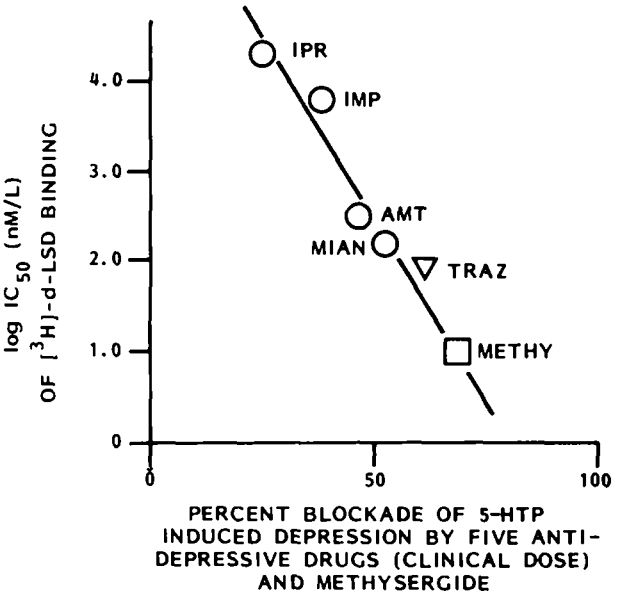

FIG. 1. Relationship between the percent blockade of 5-HTP induced depression by the following five antidepressive drugs, 2 mg/kg trazodone (TRAZ), 1.0 mg/kg mianserin (MIAN), 2.5 mg/kg amitriptyline (AMT), 2.5 mg/kg imipramine (IMP), and 1.5 mg/kg iprindole (IPR), and 5 mg/kg methysergide (METHY), and their IC_{50} (nM/L) values obtained in (^{3}H)-LSD binding studies using rat brain (Fuller, personal communication). See [18] for similar data where the K_i values also were used for four of these drugs. The antidepressants were administered in a single dose within the range comparable to a daily clinical dose.

This concept does not require denial of the existence of presynaptic as well as other secondary events occurring simultaneously. However, in the case of the antidepressive

drugs tested with our animal model of depression as well as with other reported models [22,23], it appears that the postsynaptic receptor effect would be predominant with this latter action leading possibly to the antidepressive features of this class of drugs.

It is also important to note that an inspection of our data suggests that the postsynaptic action remains constant for both the acute and the chronic treatment condition (see Tables 1 and 2) for trazodone as well as mianserin and amitriptyline. These three drugs are quite dissimilar in structure with amitriptyline representative of the older class of tricyclic antidepressants. Yet, they are clinically similar in effectiveness [8].

These latest data obtained from our animal model strongly indicate that an even more sensitive clinical research design using a number of antidepressants would further demonstrate the postsynaptic serotonergic theory of depression. We predict that if clinical measures were taken at shorter intervals (i.e., every few hours during the first few days rather than after seven or more days), trazodone would act sooner (i.e., less than 10 to 14 days) than imipramine in alleviating depression by blocking the action of the depression-related released serotonin on the hypersensitive serotonergic receptor [1, 3, 4, 12]. Amitriptyline effects should be either no different than trazodone or should fall between these drugs [2]. If a drug with additionally potent and selective serotonergic receptor blocking effects were used, that particular drug might prove to be the most effective antidepressant. This possibility has recently been suggested in our model using a new potent and selective 5-HT_2 receptor antagonist [13]. Data from our animal model having provided an early insight as to possible postsynaptic rather than presynaptic deficit in depression, now yields predictions regarding differential therapeutic effectiveness of currently used and developing drugs [1, 2, 3].

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