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Potentiated 5-Hydroxytryptophan Induced Response Suppression in Rats Following Chronic Reserpine

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BRUGGE, K. L., J. N. HINGTGEN AND M. H. APRISON. *Potentiated 5-hydroxytryptophan induced response suppression in rats following chronic reserpine.* PHARMACOL BIOCHEM BEHAV **26**(2) 287–291, 1987.—Since reserpine precipitates depression in some hypertensive patients, we tested this drug on our animal model of depression. The present experiment was designed to measure the effects of chronic reserpine treatment on 5-hydroxytryptophan (5-HTP) induced behavioral depression in rats trained on a food reinforcement operant schedule. Based on the Aprison *et al.* model of depression involving the serotonergic system, we predicted the development of receptor supersensitivity of postsynaptic serotonin (5-HT) receptors due to the decreased release of this neurotransmitter as a consequence of chronic reserpine treatment. Rats were trained on a VI 1 reinforcement schedule and then divided into 3 chronic treatment groups. One received daily injections of a placebo, another 0.025 mg/kg reserpine and the third 0.05 mg/kg reserpine. We found that 5-HTP induced behavioral depression was potentiated in rats chronically treated with reserpine, thus suggesting the development of supersensitive 5-HT receptors. These results support the hypothesis that in some types of human depression a decreased release of 5-HT occurs of sufficient duration to permit the subsequent development of supersensitive 5-HT receptors.

Reserpine Depression 5-Hydroxytryptophan (5-HTP) 5-HT receptors

FOLLOWING a series of studies [1–4, 10] beginning in the early 1960's, Aprison *et al.* [9] proposed a hypothesis concerning the neurochemical basis of depression in a subgroup of patients. They suggested that supersensitive receptors develop in some patients if there is a prolonged decreased release of 5-HT in the brain. This hypothesis is in agreement with clinical data on many depressed patients in which a deficiency rather than an excess of 5-HT appears to exist [11, 16, 17, 24, 29]. The animal model of depression first developed by Aprison *et al.* [9] and then expanded [4, 6, 19], consists of inducing a decrease or depression of behavioral responding in rats and pigeons trained on a food reinforcement operant schedule following an injection of different doses of D,L-5-HTP or L-5-HTP. This behavioral depression [As used in our animal studies, the term "behavioral depression" refers to a significant suppression of response rates. The authors are aware of the fact that this phenomenon is not necessarily related to human depression [6]. However, decreased rates of responding (i.e., psychomotor retardation) are seen in some patients who are diagnosed as having a depressive disorder.] was found to be correlated with increases of 5-HT in the telencephalon and diencephalon but not with changes on catecholamine levels [3, 4, 10]. In further support of the proposed hypothesis of depression, Fleischer *et al.* [13] showed that chronic administration of the inhibitor of tryptophan hydroxylase, p-chlorophenylalanine (PCPA), to rats results in a potentiation of the 5-HTP induced depression. In addition, they reported an increase in receptor affinity of [^{3}H]-5-HT in crude membrane fractions of the cerebral cortex of these rats. These data support the suggestion that chronic depletion of cerebral 5-HT brought about by inhibiting its synthesis, and hence reducing its release, results in supersensitive 5-HT receptors.

One could also expect that supersensitive 5-HT receptors would develop following chronic administration of reserpine, a drug known to deplete cerebral 5-HT and other monoamines by preventing their storage [14, 25, 30]. While several studies show that supersensitive DA and NE receptors develop after chronic reserpine administration [12,31] it is not clear if the same development occurs with 5-HT receptors since little, if any, data exist on this subject. Reserpine is of particular interest to us since it is known to precipitate depression in as high as 26% of hypertensive patients receiving it daily as indicated by a review of the English language literature [15]. In the present study, the development of

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hypersensitive 5-HT receptors following chronic reserpine administration to rats was supported by our psychopharmacological data.

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 frontal 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). Rats were trained to press a lever for 0.15 ml sweetened condensed milk in an operant chamber while working on a VI 1 min reinforcement schedule.

Behavioral/Injection Procedures

After the lever pressing response was established, the rats received a 90–120 min VI 1 min session three times a week. When baseline responding was obtained, each rat received an IP injection of a small dose of D,L-5-HTP (25 mg/kg) 15 min after the start of a 90 min VI session to obtain data on the 5-HTP induced behavioral depression. On the following day the rats were divided into 3 treatment groups. Two groups received daily IP injections of 0.025 (A) or 0.05 mg/kg (B) reserpine while the third group received daily placebo injections. The doses of reserpine were chosen because these are comparable on a mg/kg basis to that used in the clinical treatment of hypertension, and are known to precipitate depression in some patients. Reserpine and placebo injections were always administered at a designated time each day such that on days the rats received a VI session, the injection for that day would occur within two hours after the session ended. Therefore the injection on the previous day would occur 21–23 hr before the VI session.

Two of the three 90 min VI sessions that were given during each week of the experiment were control sessions. During these sessions the rats received a placebo injection 15 minutes after the start of the session, or were given no injection. The remaining session of each week was the experimental session in which the rats received 5-HTP 15 minutes into the VI session. After 34 daily injections of reserpine the rats were pretreated with 2.5 mg/kg amitriptyline or placebo 45 minutes prior to the onset of the 5-HTP session. Following this session the daily placebo and reserpine injections were discontinued. For four additional weeks the rats were continued on the 3 session/week schedule to observe changes of the 5-HTP induced depression during withdrawal of reserpine or placebo treatment.

Three rats (one from the 0.025 mg/kg group and two from the 0.05 mg/kg group) stayed within 2 standard deviations from the mean 5-HTP depression obtained with the placebo rats. As a group, these three rats were not statistically different from the placebo group after six and 13 daily injections of 0.05 or 0.025 mg/kg reserpine. These rats were therefore changed to a higher daily dose of 0.10 mg/kg reserpine on day 14 of the experiment and subsequently considered a separate treatment group—Group C. Since this left three rats in the 0.05 mg/kg treatment group and four rats in the 0.025 mg/kg treatment group, the experiment was repeated with five additional rats. These rats were subdivided into treatment groups as follows: two rats in the 0.05 mg/kg group, two in the 0.025 mg/kg group and one in the placebo group. The 5-HTP induced behavioral depression during the pretreatment session was not statistically different from that of the original placebo group. Therefore the data of these rats were pooled with the data of the original subject pool.

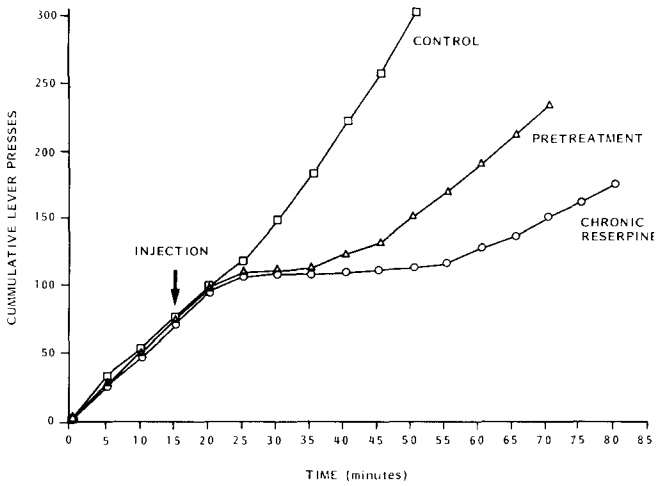

FIG. 1. Cumulative number of lever presses during control (□), pretreatment (△) and chronic reserpine (○) VI 1 sessions in a representative rat. The control session occurred after two daily injections of reserpine (0.05 mg/kg IP) with the rat receiving placebo injection 15 min after the start of the reserpine. The pretreatment session occurred before commencement of reserpine treatment with the rat receiving 25 mg/kg D,L-5-HTP IP at the injection time. The chronic reserpine session took place after 6 daily injections of 0.05 mg/kg reserpine IP with the rat receiving 25 mg/kg 5-HTP at the injection time.

Recordings of the cumulative number of leverpresses over each 90 minute session were obtained for each rat. Behavioral depression was measured by counting the total number of leverpresses occurring in the first 15 minutes of a 5-HTP session. The length of time (min) after the 5-HTP injection required by the rat to double the 15 minute baseline response output was determined. Fifteen minutes was then subtracted from this value to get the amount of behavioral depression (min) induced by the 5-HTP injection in a given session. Statistical comparisons were made for each group during pretreatment, treatment and withdrawal conditions of reserpine or placebo administration.

RESULTS

The data in Fig. 1 show the cumulative number of responses for a single rat on three different days: (a) CONTROL: a session in which a placebo was injected, (b) PRETREATMENT: a 5-HTP session before commencement of the chronic reserpine treatment, and (c) CHRONIC RESERPINE: a 5-HTP session after commencement of daily

TABLE 1

PERIOD OF 5-HTP (25 mg/kg IP) INDUCED BEHAVIORAL DEPRESSION (MIN) PRIOR TO, DURING TREATMENT WITH, AND FOLLOWING WITHDRAWAL OF CHRONIC RESERPINE OR PLACEBO (IP) TO RATS WORKING ON A VI 1 SCHEDULE OF MILK REINFORCEMENT

	Period of Depression (Min)*			
Group:	Placebo	A	B	C
Dose of Reserpine (mg/kg):	0	0.025	0.05	0.10
Pretreatment	5.87 ± 1.87 (6)	4.45 ± 2.14 (5)	5.68 ± 2.94 (5)	1.53 ± 3.04 (3)
Treatment				
Day 6	5.50 ± 2.88 (6)	20.12 ± 7.83 (5)†	23.96 ± 5.16 (5)‡	
Day 13	5.93 ± 4.65 (6)	20.24 ± 2.33 (5)§	16.48 ± 7.01 (5)†	
Day 20	6.60 ± 2.15 (4)	23.90 ± 7.99 (4)†	15.24 ± 4.63 (5)†	
Day 27	3.57 ± 2.27 (6)	19.32 ± 8.63 (5)†	15.68 ± 3.15 (5)†	37.53 ± 4.29 (3)§
Withdrawal				
Day 7	4.28 ± 2.18 (5)	16.13 ± 11.44 (3)	15.60 ± 7.68 (3)	11.87 ± 10.59 (3)
Day 14	3.12 ± 3.04 (5)	16.47 ± 6.87 (3)	3.60 ± 4.06 (3)	4.73 ± 2.15 (3)
Day 21 Day 28 ¶	5.13 ± 3.95 (5)	13.30 ± 6.42 (3)	10.53 ± 2.78 (3)	2.68 ± 2.31 (3)

*Means ± SEM and (N). Levels of significance determined by t-tests: †$p<0.05$; ‡$p<0.025$; §$p<0.001$, compared to the pretreatment session. No statistical differences were found between groups A, B and C for the pretreatment session.

¶Mean of withdrawal Days 21 and 28.

reserpine treatment. After commencement of chronic reserpine treatment, no significant change in the number of responses per unit of time was observed during the control session. When 5-HTP was administered during the pretreatment session (before beginning reserpine treatment) one sees a decrease in cumulative responses over time. Note that the response rate of this rat returns to baseline after about 45 minutes after the experiment had begun. After chronic reserpine treatment the 5-HTP induced depression occurs over a long period of time. Baseline responding does not return until approximately 60 minutes after the start of the experiment. The period of depressed response rate in the chronic reserpine group appears to be approximately twice that of the pretreatment group.

Potentiation of the 5-HTP induced behavioral depression was observed for each treatment group of rats. This effect occurred after 6, 13, 20 and 27 daily injections of 0.025 mg/kg or 0.05 mg/kg reserpine (see Table 1). Three individual rats who failed to show potentiation of 5-HTP depression following 6 and 13 days of reserpine treatment did show much potentiation after 13 additional days of administration of a higher dose (0.10 mg/kg) of reserpine.

During withdrawal from reserpine treatment, the 0.025, 0.05 and 0.10 mg/kg groups (A,B,C) showed a return to pretreatment levels of behavioral depression such that no significant difference occurred after 7 days of withdrawal (see Table 1). The latter continued at this level for subsequent sessions up to 28 days.

Amitriptyline (2.5 mg/kg) given one hour prior to 5-HTP successfully blocked the reserpine potentiated depression. The mean period of 5-HTP induced behavioral depression in five rats that received amitriptyline pretreatment (11±6.0 min) was significantly less ($p<0.01$) than that of five rats which received placebo pretreatment (36±8.7 min).

The weights of the rats of each treatment group were not significantly different from that obtained during the post treatment 5-HTP sessions. No significant differences were observed in the weights of rats between treatment groups as well.

DISCUSSION

This study revealed that chronic treatment with reserpine can produce a potentiation of 5-HTP induced behavioral depression in rats responding on a food-reinforced operant schedule (see Fig. 1 and Table 1). In addition, the data suggest that chronic reserpine administration produces similar psychopharmacological effects to those of chronic PCPA [13], in that pretreatment with reserpine also is associated with an increased period of depression following a low dose of D,L-5-HTP. However, the neurochemical mechanisms are different with these two agents. While PCPA decreases the release of 5-HT and hence the amount available to the postsynaptic site by inhibiting the biosynthesis, reserpine decreases its release and the amount available to the postsynaptic site by depleting the firmly bound storage pool of this important neurotransmitter [5,8].

The study of Haggendal and Lindquist [18] does not support the possibility that this potentiated depression is a reflection of the side effects of reserpine such as sedation, weight loss, or hypokinesia. They showed that the occurrence of side effects with 0.10 mg/kg reserpine given SC within four hours post injection disappears within 20 hours after its administration. They also showed that animals developed a tolerance to the side effects within five daily injections. In the present study, side effects such as ptosis, diarrhea or anorexia (as reflected by a change in weight) were not observed at the small doses used. Furthermore, the 5-HTP session did not occur until 21–23 hr after the last reserpine injection with the first 5-HTP session occurring after 6 daily injections.

In the present study chronic reserpine treatment did not appear to alter baseline VI responding rate during control

sessions in which a placebo or no injection was administered during the session (see Fig. 1). Concerning the possible effect that reserpine induced hypokinesia has on learned behavior, studies [26,27] show that changes in locomotor activity occur independently from changes in learned behavior during chronic reserpine treatment. While chronic reserpine produces a decrease in locomotor activity initially, this depression becomes diminished and is then followed by a rebound effect in which locomotor activity paradoxically increases as daily injections continue [20, 26–28]. Using the same dose (generally larger than the doses used in the present study) the effect of chronic reserpine treatment on learned behavior is only a depression (without a subsequent diminishment or rebound effect on behavior). Even if treatment is extended to 60 days learned behavior remains depressed. Leith and Barrett [21] used doses comparable to that employed in the present study (0.05 mg/kg) and found no effect in locomotor activity at this lower dose.

In previous studies from this laboratory [22,23], amitriptyline blocked the 5-HTP depression by approximately 50% (using 50 mg/kg D,L-5-HTP). The present study (using a lower D,L-5-HTP dose of 25 mg/kg) showed an even greater blockade (>70%) with amitriptyline in reserpine treated rats with a potentiated 5-HTP depression. Since amitriptyline blockade of 5-HTP induced depression was previously associated with its effect in serotonin receptor binding studies [22] our results with chronic reserpine reflect involvement of the serotonergic system.

Since reserpine is known to affect catecholamine levels as well as serotonin levels, the possible influence that the catecholamines may have on reserpine related depression in humans cannot be ruled out. However, previous studies from this laboratory [3,4] show that 5-HTP induced depression in animals is correlated with changes in serotonin and not with changes in catecholamines. Therefore, the effect of reserpine on 5-HTP induced depression in our rats appears to be a reflection of changes in serotonin which fits the predictions based on the hypersensitive postsynaptic serotonin receptor model of depression described by Aprison and Hingtgen and others [6, 7, 9]. This suggests that similar serotonin mechanisms may be associated with the depression observed in some patients who were treated with reserpine for hypertension.

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