Pharmacology Biochemistry & Behavior, Vol. 4, pp. 407–409.

Blockade of the Behavioral Effects of 5-HTP by the Decarboxylase Inhibitor Ro 4-4602[1]

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(Received 21 November 1975)

CARTER, R. B. AND J. B. APPEL. *Blockade of the behavioral effects of 5-HTP by the decarboxylase inhibitor Ro 4-4602.* PHARMAC. BIOCHEM. BEHAV. 4(4) 407–409, 1976. – Twenty male rats were trained to press a bar on a fixed-ratio (FR 32) schedule of water reinforcement. The behavioral effects of 5-HTP (50 mg/kg) were studied following pretreatment with small (50 mg/kg) and large (400 mg/kg) doses of the decarboxylase inhibitor Ro 4-4602. It was found that both doses of the pretreatment agent blocked the disruptive effects of 5-HTP. This result suggests that at least some of the effects of 5-HTP may be mediated peripherally.

5-HTP Ro 4-4602 Fixed-ratio responding

THE behavioral effects of 5-hydroxytryptophan (5-HTP) have been assumed to be the result of drug-induced increases in the concentration or metabolism (turnover) of the neurotransmitter, serotonin (5-HT), of which 5-HTP is the immediate precursor [1]. Moreover, these effects have been said to be mediated centrally [4]. For example, Aprison and Ferster observed a quantitative relationship between dose of 5-HTP and amount of behavioral disruption of responding maintained by a Multiple FR 50 FI 10 min schedule of food reinforcement in pigeons [1,2]. This behavioral effect was most closely correlated with changes in 5-HT concentrations in the telencephalon and diencephalon although correlations also were found with 5-HT concentrations in other parts of the brain as well as in heart, liver, and lungs. There were, however, no correlations between amount of disruption and concentrations of other biogenic amines such as dopamine or norepinephrine [3,4]. It has also been demonstrated that under similar conditions, 5-HTP leads to increases in concentrations of 5-HT in nerve endings isolated from the telencephalon and diencephalon [8].

Recently, data which suggest that peripheral serotonergic mechanisms may be either partially or exclusively involved in the mediation of the behavioral effects of 5-HTP have been obtained. Thus, the peripheral 5-HT antagonist, xylamidine tosylate, has been found to partially block the disruptive effect of 5-HTP in rats working on a FR 20 schedule of milk reinforcement, although cinanserin, a potent 5-HT antagonist both centrally and peripherally, is able to block the 5-HTP effect to a much greater extent [11]. In the present study, we present data which show that the drug Ro 4-4602 which, in small doses (e.g., 50 mg/kg), is supposed to inhibit peripheral but not central 5-HTP decarboxylase (the enzyme required for the synthesis of 5-HT from 5-HTP) and, in large doses (e.g., 400 mg/kg), to inhibit both peripheral and central decarboxylase [6,10], effectively antagonizes the effects of 5-HTP at both dosage levels.

METHODS

Animals

Twenty experimentally naive male rats, weighing between 290–300 g, were obtained from ABS Sprague-Dawley, Madison, Wisconsin. They were housed in individual cages in a room of constant temperature (75°F) and humidity (40–50%) with a 12 hr (8:00 a.m.–8:00 p.m.) day–night cycle. Food was always available ad lib but water was given only in the experimental situation. Experiments were conducted at approximately the same time every day, 7 days a week.

Apparatus

Four commercially available experimental chambers (BRS/LVE, Model No. 143-24) contained in sound- and light-attenuating, ventilated enclosures (BRS/LVE, Model No. 132-02) were used for training and testing. Each box contained one lever on the left side of the front panel with a liquid feeder in the center of that panel which delivered a reinforcer consisting of 0.01 ml of tap water. Sessions were 30 min long and were conducted in the presence of a dim house light. Solid state programming and recording equipment were located in an adjacent room.

Training Procedure

After a 1 week period of adaptation to their home cages, all animals were deprived of water for a period of 36 hr.

[1] This research was supported by USPHS Research Grants MH-24333 and MH-24593 from the National Institute of Mental Health.

They were then placed into the experimental chambers and trained by the method of successive approximations (shaping) to press a lever on a continuous schedule of water reinforcement (FR 1). On succeeding days the schedule was gradually raised from FR 1 to a fixed-ratio of 32 (FR 32). When a stable rate of bar-pressing was obtained after about three weeks of training (± 5% change in rate), daily intraperitoneal (IP) injections of 0.35 ml of isotonic saline (NaCl) were begun. Immediately following the experimental session on the first day of NaCl injections all animals were given 400 mg/kg of Ro 4-4602.

Testing Procedure

One week after the start of NaCl administration, the animals were randomly divided into 1 or 4 groups of 5 animals each: 1) those which were to receive 400 mg/kg of Ro 4-4602 as the pretreatment and 5-HTP vehicle as the treatment; 2) those which were to receive Ro 4-4602 vehicle as the pretreatment and 50 mg/kg of 5-HTP as the treatment; 3) those which were to receive 400 mg/kg of Ro 4-4602 as the pretreatment and 50 mg/kg of 5-HTP as the treatment; and 4) those which were to receive 50 mg/kg of Ro 4-4602 as the pretreatment and 50 mg/kg of 5-HTP as the treatment. Each treatment was given one time.

Pharmacological Procedure

Injections of Ro 4-4602 were given 4 hr prior to and injections of 5-HTP were given 15 min prior to the start of the experimental session.

Ro 4-4602 (N-(DL-serl)-N′-(2,3,4-trihydroxybenzyl) hydrazine) was generously provided by Dr. W. E. Smith of Hoffmann-LaRoche (Nutley, N. J.); it was dissolved to concentrations of 25 mg/ml for the 50 mg/kg of Ro 4-4602 or 200 mg/kg for the 400 mg/kg of Ro 4-4602 group in 0.9% NaCl solution acidified in 0.001 N HCl. D,L5-hydroxytryptophan was purchased from Regis Chemical Company (Chicago, Ill.). 5-HTP in pure crystalline form, was placed in a small amount of distilled-deionized water; dilute HCl and NaOH were used to obtain a solution with a pH of 2.0. Distilled deionized water was then added to this solution to produce a concentration of 50 mg/ml. Dosage and time parameters were selected on the basis of previous research [9].

Analysis of Data

Results are presented in terms of group mean percent control response rates. This percent for each animal is obtained by dividing the total number of responses on the test day by the average number of responses during the 3 days immediately preceding that day (baseline) and multiplying by 100. A 1-way analysis of variance with a Scheffé test for comparison of group means was used to determine the significance of the data.

RESULTS

Figure 1 shows that animals which received 400 mg/kg of Ro 4-4602 plus 5-HTP vehicle (Group 1) exhibited almost no decrement in bar-pressing behavior (98.3% of control), i.e., 400 mg/kg of Ro 4-4602 does not disrupt behavior at the time parameters employed. Conversely, animals which received Ro 4-4602 vehicle plus 5-HTP (Group 2) showed a marked decrement in overall response rate (15.5% of control). This decrement was caused by a drug-induced period of non-responding on the FR schedule rather than by changes in local response rates.

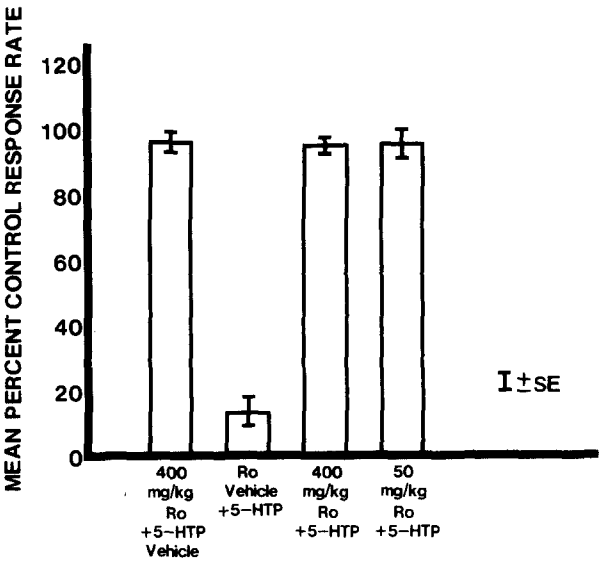

FIG. 1. Effects of Ro 4-4602 on 5-HTP (50 mg/kg) induced disruption of fixed-ratio responding maintained by water reinforcement. Control rates in responses/min for each group were as follows: 1) 126.3 resp/min (N = 5); 2) 118.5 resp/min (N = 5); 3) 115.5 resp/min (N = 5); and 4) 120.7 resp/min (N = 5).

The figure shows that pretreatment with either a small (50 mg/kg) or a large (400 mg/kg) dose of Ro 4-4602 completely blocked the rate-decreasing effect of 5-HTP (50 mg/kg). A 1-way analysis of variance showed that differences among groups were highly significant ($F(16) = 90.55$, $p<0.001$). A post hoc Scheffé comparison revealed that while there were no differences among Groups 1, 3, or 4, these groups were significantly different from Group 2, ($p<0.0001$).

It is interesting to note that pretreatment with Ro 4-4602 also blocked the effects of 5-HTP on the days immediately following drug treatments. As may be seen in Table 1, the mean response rates for the 3 days following drug treatment did not markedly differ from those for the three days preceding drug treatment for any group except Group 2, which received Ro 4-4602 vehicle plus 5-HTP.

TABLE 1

CONTROL RESPONSE RATES* BEFORE AND AFTER DRUG TREATMENT

Group	Response Rate *Before* Drug Treatment	Response Rate *After* Drug Treatment
400 mg/kg Ro 4-4602 50 mg/kg 5-HTP	126.3	121.6
50 mg/kg Ro 4-4602 50 mg/kg 5-HTP	115.5	116.3
400 mg/kg Ro 4-4602 5-HTP Vehicle	118.5	117.6
Ro 4-4602 Vehicle 50 mg/kg 5-HTP	120.7	97.1

*Mean responses/minute for the three days prior to and three days following treatment.

DISCUSSION

The results of this experiment confirm the hypothesis that the behaviorally disruptive effects of 5-HTP are due to the action of 5-HT [8]. If these effects were caused by some other, nonspecific action of 5-HTP, as has been suggested [5], we would not have been able to block them with a decarboxylase inhibitor.

The far more interesting aspect of this experiment, however, was the attempt to determine whether 5-HTP induced disruption is mediated centrally or peripherally. If these effects are mediated centrally [4], then we would expect a large dose of Ro 4-4602 to block the effect of 5-HTP administration, while a small dose would not [6,10]. If these effects are mediated centrally and peripherally [11], then we would expect a large dose to completely block the effects of 5-HTP and a small dose to partially block these effects. As was the case, however, both the large and small dose of Ro 4-4602 completely blocked 5-HTP induced behavioral disruption. Thus, the results tend to support the notion that the effects of 5-HTP are mediated entirely in the periphery. However, recent data of Hyttel and Fjalland [7] suggest that 50 mg/kg of Ro 4-4602 does in fact have central as well as peripheral inhibiting properties on 5-HTP decarboxylase, at least in mice. Further neurochemical study of the effects of relatively small doses of Ro 4-4602 on cerebral 5-HT metabolism is therefore required before any conclusive statements can be made.

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