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Blockade of 5-HTP Reduction of Ethanol Drinking with the Decarboxylase Inhibitor, Ro 4-4602

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GELLER, I., R. J. HARTMANN AND F. S. MESSIHA. *Blockade of 5-HTP reduction of ethanol drinking with the decarboxylase inhibitor, Ro 4-4602.* PHARMAC. BIOCHEM. BEHAV. 15(6) 871–874, 1981.—5-Hydroxytryptophan (5-HTP) reduced ethanol intake in laboratory rats. The reduction of ethanol intake was blocked when Ro 4-4602, the decarboxylase inhibitor, was given in combination with the 5-hydroxytryptophan. These observations provide further support for the involvement of brain serotonin in voluntary ethanol drinking by the rat.

Ethanol 5-HTP 5-HT Ro 4-4602

A NUMBER of investigators have shown that increasing brain serotonin (5-HT) of ethanol drinking rats will reduce their intake of ethanol. This has been accomplished by administering 5-hydroxytryptophan (5-HTP), the 5-HT precursor, systemically to rats [7,14] or to hamsters [8] as well as through intraventricular administration of 5-HT to rats [11]. Several β-carbolines, reported to decrease ethanol intake of rats [9,10] also have been reported to raise brain 5-HT of rats [2, 3, 10, 13]. The increases in brain 5-HT levels were shown to occur through MAO inhibition as well as through inhibition of 5-HT uptake [4]. Increasing brain 5-HT through inhibition of MAO with pargyline also resulted in a reduction of ethanol intake of rats [18]. More recently Rockman *et al.* [17] demonstrated that treatment of ethanol-preferring rats with zimelidine, an inhibitor of 5-HT uptake, reduced ethanol intake of rats through an increase of brain 5-HT.

The availability of a substance which might alter the effects of a 5-HTP-induced increase of brain 5-HT permitted a further investigation of the role of brain 5-HT in voluntary ethanol consumption of rats. Ro 4-4602 has been reported to inhibit decarboxylation of aromatic amino acids [5], a rate limiting step in the conversion of 5-HTP to 5-HT [16]. It has also been shown to block the disruptive effects of 5-HTP on fixed ratio behavior of laboratory rats [6]. These investigators found that small doses of 50 mg/kg or large doses of 400 mg/kg of Ro 4-4602 given prior to administration of 50 mg/kg 5-HTP blocked the reduction of lever pressing that resulted from administration of 50 mg/kg 5-HTP alone. Since Ro 4-4602 blocked the effects of 5-HTP on behavior, it was decided to administer it to ethanol-drinking rats in combination with a dose of 5-HTP known to reduce voluntary ethanol intake [7].

METHOD

The animals were 24 Holtzman Sprague-Dawley rats 75 days old at the start of the experiment. They were housed individually in 9×15×18 inch cages (Wahman LC-28) in a laboratory with ambient temperatures of 70°–76°F, and were maintained on a diet of Wayne Lab Blox ad lib. The laboratory was maintained in total darkness for 23½ hrs per day; ½ hr was required for recording of data and routine animal care. Water and a 6% ethanol solution were available at all times in 100 ml drinking tubes mounted on the back or either side of the cages so that the drinking spouts protruded into the cages approximately 1½ inches above floor level. The 2-choice, 3-bottle method as previously described [15] was used to prevent the rats from selecting a fluid based on a position preference.

The cages contained a tube of ethanol solution (6% v/v) prepared from 95% ethanol, a tube of water and an empty tube. Twenty-four hr fluid intakes were recorded each day at 10:00 a.m. The drinking tubes were washed, refilled and put back on the cages and their positions were rotated daily.

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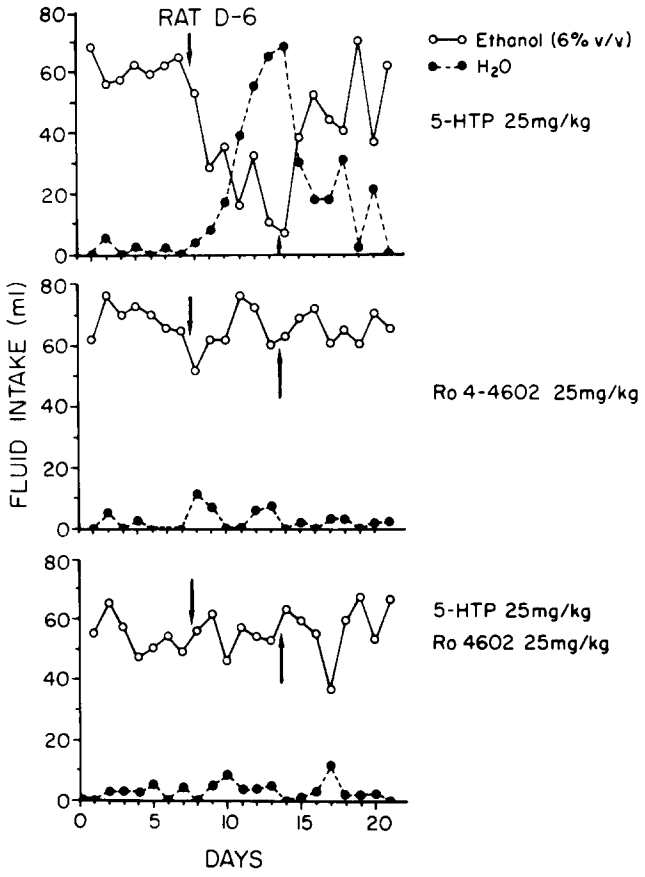

FIG. 1. The effects of 25 mg/kg 5-HTP and 25 mg/kg Ro 4-4602, administered alone or in combination, on ethanol preference in the rat. Treatment onset is indicated by the downward arrows and termination of treatment by the upward arrows.

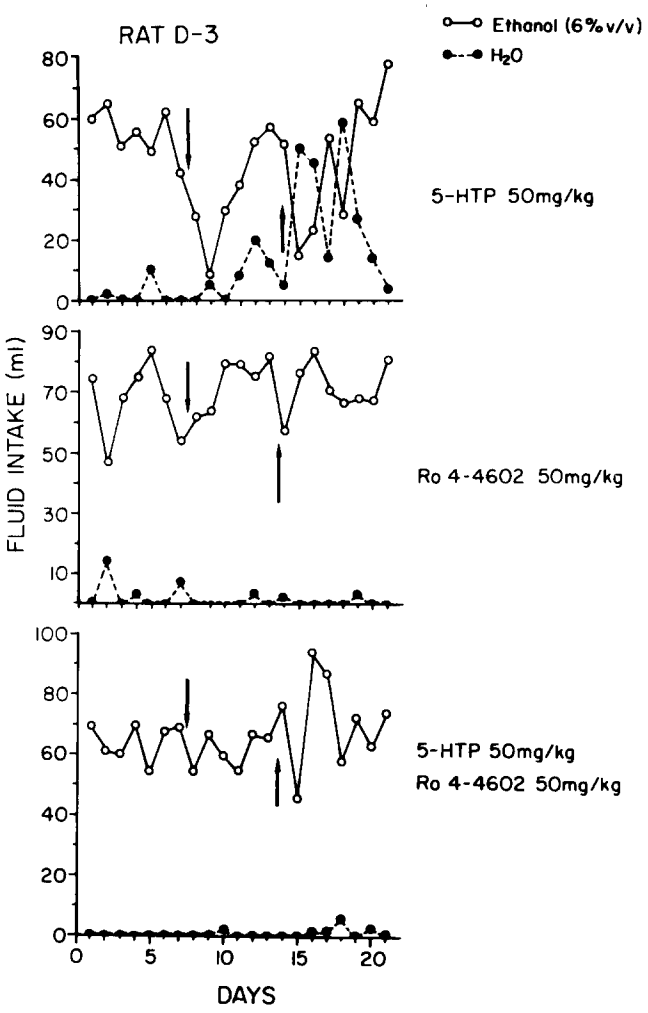

FIG. 2. The effects of 50 mg/kg 5-HTP and 50 mg/kg Ro 4-4602, administered alone or in combination, on ethanol preference in the rat. Treatment onset is indicated by the downward arrows and termination of treatment by the upward arrows.

After a period of 80 days, 12 rats with a high preference for ethanol solution were selected for this study.

Drug Preparation and Administration

The DL form of 5-HTP (Aldrich Chemical) was prepared as a saline solution in a concentration of 10 mg/ml. Ro 4-4602 (Roche) was prepared as a saline solution in a concentration of 25 mg/ml. They were administered intraperitoneally, alone or in combination, once a day for 7 days in doses of 25 or 50 mg/kg. The drugs were given in a mixed order so that six of the animals received 5-HTP first and six animals received the combination (5-HTP plus Ro 4-4602). Three weeks after the first series of injections the groups were switched so that the 5-HTP rats received the combination and the combination rats received 5-HTP. In a few cases where the decarboxylase inhibitor did not block the effects of 5-HTP, the animals were given the same dose of 5-HTP with a higher dose of Ro 4-4602. Three weeks after all animals had received a series of 5-HTP or the combination of injections, they were administered Ro 4-4602 alone in the dose that was effective in preventing 5-HTP effects on ethanol intake.

RESULTS

In Fig. 1 are shown the effects on ethanol preference of 25 mg/kg 5-HTP and 25 mg/kg Ro 4-4602 administered alone and in combination. The solid lines show the daily intake of a 6% ethanol solution and the broken lines the daily intake of water. During a 7-day period of 5-HTP administration, a reduction of ethanol intake occurred with a concomitant increase of water intake. Administration of Ro 4-4602 in combination with 5-HTP blocked the 5-HTP reduction of ethanol drinking. Ro 4-4602 when administered alone also had no effect on ethanol intake.

In Fig. 2 are shown qualitatively similar effects for another rat administered the compounds at 50 mg/kg. For this animal 5-HTP produced a reduction of ethanol intake during the first 4 days of drug administration. Ethanol intake approximated baseline control values during the last 3 treatment days. The preference for the ethanol solution was then

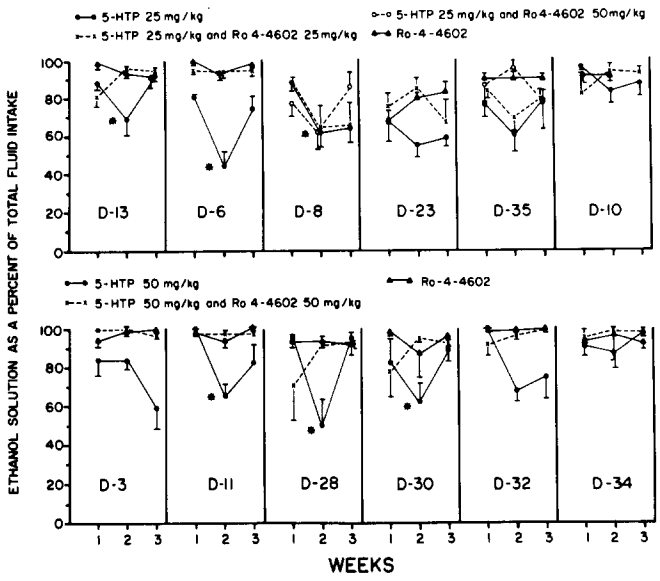

FIG. 3. The effects of 5-HTP and Ro 4-4602, administered alone or in combination, on ethanol preference in the rat. Weekly averages and SEM's of ethanol intake, shown for each rat, are expressed as percent of total fluid intake. The second week represents the treatment period and weeks 1 and 2 the pre- and post-treatment control weeks. The doses administered are indicated on the charts; asterisk denotes significance at $p<0.05$ (Student's t-test).

diminished for the next 5 post-drug days after which there occurred a rebound effect above the original baseline control level. As with the lower doses of the compounds, the 50 mg/kg dose of Ro 4-4602 alone or in combination with 5-HTP had virtually no effect on ethanol or water intake.

Fig. 3 shows the averaged weekly intake of ethanol solutions for each animal under each of the treatment conditions. The intake of ethanol is expressed as a percent of the total fluid consumed. Weeks 1 and 3 represent the data for the pre- and post-week control periods and week 2 shows the data for the 7-day treatment period. The top panels (solid lines) show the effects of 25 mg/kg and the bottom panels (solid lines) show the effects of 50 mg/kg 5-HTP on average ethanol consumed. For almost every animal with the exception of rat D-3 ethanol intake was reduced. For rat D-3 ethanol intake was reduced during the post-treatment week. During the post-treatment week, ethanol intake returned toward baseline-control levels for 7 of the rats while the 5-HTP effect persisted for 4 of the rats. When Ro 4-4602 was administered in combination with 5-HTP (broken lines) the reduction of ethanol intake was blocked for all but 2 of the rats; administration of a higher dose (50 mg/kg) of Ro 4-4602 in combination with 25 mg/kg 5-HTP blocked the reduction of ethanol intake for rat D-35 but not for D-8. It was not possible to increase the dose of Ro 4-4602 further for this rat since the ethanol preference did not remain at the baseline control-level following the last treatment and the animal died two weeks later. The effects of Ro 4-4602 alone administered at the dose level that effectively blocked the effects of 5-HTP did not reduce ethanol intake. The post-treatment data for rat D-10 are missing since the animal was sacrificed immediately after the treatment period.

DISCUSSION

The findings of this study are in agreement with those of Carter and Appel [6] who reported that Ro 4-4602 blocked the effects of 5-HTP on operant behavior of rats. The data strongly support a 5-HT involvement in voluntary ethanol drinking by rats. The action of Ro 4-4602 has been reported to be primarily that of peripheral decarboxylase inhibition since extremely large doses are required for inhibition of decarboxylase centrally [1]. This would support the speculation that the 5-HTP reduction of ethanol intake and its blockade by Ro 4-4602 may be mediated peripherally. However, several reports in the literature do indicate that Ro 4-4602 may act centrally as well as peripherally. Pletscher and Gey [16] found that intraperitoneal administration of Ro 4-4602 to rats resulted in a dose-dependent reduction in brain levels of 5-HT which was maximal at 1–2 hr after injection; control levels were restored after 4–6 hr. In rats, the dose-effect curve was very shallow with approximately 25% decrease in brain 5-HT at 840 mg/kg. Based on their findings, it is likely that the doses used in the present study could have produced a 5–10% reduction in brain 5-HT after 1–2 hr. Pletscher and Gey also demonstrated that the increase of 5-HT in brains of rats administered 75 mg/kg 5-HTP was diminished when Ro 4-4602 was given with the 5-HTP. Maximum inhibition in the rise of brain 5-HT occurred with doses of Ro 4-4602 above 50 and below 75 mg/kg. Hyttel and Fjalland [12] reported that the increase in brain 5-HT which occurred in mice given an intravenous injection of 100 mg/kg 5-HTP was reduced by 67% when an intraperitoneal injection of 50 mg/kg Ro 4-4602 was given prior to the 5-HTP. Although supportive brain biochemical studies have not yet been carried out for the present investigation, it seems reasonable to assume that the blockade of the 5-HTP reduction of ethanol intake with Ro 4-4602 was due to a reduction of available brain 5-HT.

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