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BRIEF COMMUNICATION

Effects of Para-Chlorophenylalanine and 5-Hydroxytryptophan on Alcohol Intake in the Rat[1]

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GELLER, I. *Effects of para-chlorophenylalanine and 5-hydroxytryptophan on alcohol intake in the rat.* PHARMAC. BIOCHEM. BEHAV. 1(3)361–365, 1973.–Para-chlorophenylalanine, the tryptophan hydroxylase inhibitor that depletes serotonin selectively, increased alcohol intake in rats. 5-Hydroxytryptophan, the serotonin percursor, reduced alcohol intake. These findings suggest that alcohol preference in the rat may be related to the brain tryptaminergic system.

Alcohol 5-HTP pCPA Serotonin

Attempts to establish a relationship between alcohol action and the tryptaminergic system of the brain have resulted in controversial findings. Para-chlorophenylalanine (pCPA), the tryptophan hydroxylase inhibitor which depletes serotonin selectively [7,8], "significantly reduced or totally abolished" a preference for ethyl alcohol in rats. However, subsequent experiments open to question the suggested relationship between depletion of endogenous 5-HT and aversion to alcohol. Cicero and Hill [2] found that the pCPA effect was obtained when absolute rather than 95% alcohol was used to prepare the test solutions, while Nachman *et al.* [12] provided most convincing evidence that pCPA-induced aversion to alcohol was due to a conditioned aversion established through pairing of alcohol or sacharrin drinking with the administering of pCPA or other noxious substances.

We have reported an increased intake of ethanol by rats kept in total darkness [4], an observation which suggested to us a possible relationship of the rat-brain serotonergic system to alcohol preference. This speculation was based on previous reports that rats kept in a totally dark environment have an increased pineal hydroxyindole-O-methyltransferase (HIOMT) activity and a concomitant increased conversion of n-acetylserotonin to melatonin [16]. If brain 5-HT levels of rats are lowest in periods of darkness, it seems reasonable to assume that reduction of brain 5-HT might increase alcohol preference while elevation of brain 5-HT might decrease alcohol preference of rats. The present investigation deals with the effects of experimental manipulation of brain 5-HT on the intake of ethanol by laboratory rats. This was accomplished by the administration of pCPA or 5-hydroxytryptophan (5-HTP), the serotonin precursor which penetrates the central nervous system of the rat and is converted to 5-HT [5,13].

METHOD

The animals were eighteen male Sprague-Dawley rats 90 days old at the start of the experiment. They were housed individually in 9 x 15 x 18 in. 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 kept in a normal light–dark photoperiod consisting of nine hr of darkness during each 24-hr period. Water or an ethanol solution was available at all times in 100 ml drinking tubes mounted on the back or on either side of the cages so that the drinking spouts protruded into the cages approximately 1¼ in. above floor level. The two choice, three bottle method as previously described [10] was used to prevent the rats from selecting a fluid based on a position preference.

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The cages contained a tube of alcohol solution prepared from 95% ethanol, a tube of water and an empty drinking tube. At 10:00 each morning, the experimenter recorded the amounts of fluid consumed during the preceding 24 hr. Drinking tubes were washed, refilled and put back on the cages and their positions were rotated from day to day.

First, ethanol preference curves were established as previously described [9]. The concentration at which a rat drank 50% of its total fluid as ethanol solution, the selection threshold, was used to determine the ethanol concentration to be used for each animal in the experiment. For the six rats to be given 5-HTP, the concentrations were maintained at or below the selection threshold. For the remaining twelve rats, ethanol concentrations were assigned at selection threshold (3 rats), five percent above selection threshold (7 rats) and three percent below the selection threshold (2 rats).

Once the ethanol preference curves were established, the rats were allowed access to only water for a sixteen day period. The three drinking tubes were then placed on each cage as described above and a record was kept of 24 hr ethanol and water intake for the next 60 days.

Drug Preparation and Administration

The DL form of pCPA (Pfizer) was prepared as a 0.5% carboxymethylcellulose (CMC) suspension in a concentration of 25 mg/ml. The DL form of 5-HTP (Aldrich Chemical) was prepared as a saline solution and administered intraperitoneally in a concentration of 10 mg/ml.

Six rats were given chronic oral administrations of pCPA each day at 10 a.m. until a change in ethanol drinking occurred. The time period approximated twelve days as previously determined in preliminary exploratory experiments. Five rats received pCPA at 150 mg/kg while the sixth was given pCPA at 100 mg/kg. A second group of six rats received as control, chronic daily oral administrations of 0.5% CMC suspension over an eleven day period in volumes equivalent to those administered for pCPA.

Sixteen days after completion of this drug treatment, three of the pCPA rats were given CMC over the same period that they had been administered pCPA. Three of the CMC rats were given pCPA chronically beginning with 100 mg/kg and the dose was varied periodically during the treatment period in order to obtain a maximum effect.

Of the remaining six rats, three were administered 50 mg/kg 5-HTP and one was given saline chronically at 10 a.m. each day during a five day period. Two weeks later, the three 5-HTP rats received saline and the saline rat received 50 mg/kg 5-HTP administered over the same time period. All injections were intraperitoneal and volumes of saline and 5-HTP were equivalent for each animal. Treatment of the two remaining animals consisted of a single intraperitoneal administration of saline or 50 mg/kg 5-HTP. The saline rat was given a 100 mg/kg dose of 5-HTP seven days after the saline injection while the 5-HTP rat was administered saline 20 days after the 5-HTP injection. Volumes of saline and 5-HTP were equivalent for each rat.

RESULTS

In Fig. 1 are shown data for six rats that received only pCPA and three rats (42, 46, 49) that received 0.5% CMC several weeks prior to pCPA treatments. Ethanol intake is expressed as a percentage of total fluid consumed during a 24 hr period. Chronic administration of pCPA resulted in increased ethanol consumption by all rats. Prior to drug treatments, Rats 59 and 47 drank little or no alcohol. During pCPA administration at the doses indicated, ethanol intake increased gradually reaching a maximum of 100% after nine days for Rat 59, who drank 10% ethanol v/v. Ethanol intake for Rat 47, the 14% animal, reached a maximum of 56% after 16 days of treatment. Upon discontinuance of pCPA administration, a rather precipitous drop of ethanol intake to baseline level occurred. With the exception of a single day for Rat 35, the pre-pCPA control levels of ethanol intake for Rats 41, 61, and 35 ranged between 25 and 50%. pCPA produced an initial reduction of ethanol intake followed by a gradual increase to 100% for Rats 61 and 35 and to 62% for Rat 41, the rat drinking the highest concentration of ethanol. Stopping pCPA treatments produced an almost immediate reduction of ethanol intake to control values.

The concentration of ethanol assigned Rat 58, 3 percent below the selection threshold, resulted in an ethanol intake during the predrug control period ranging from 56–82%. This animal reached a maximum ethanol intake of 100% after 12 days of pCPA. The postdrug data show that the effect persisted for as long as 12 days. For Rat 42, the predrug control values for ethanol intake ranged from 17–57% and for Rat 46, from 22–45%. The initial administration of pCPA at the indicated dose produced a slight reduction of ethanol intake followed by a steady, gradual increase of ethanol drinking above predrug control levels. Cessation of pCPA treatment resulted in a drop in ethanol intake below predrug control levels. This occurred immediately for Rat 42 and after three days for Rat 46. Data for Rat 49 show an initial drop of ethanol intake followed by a gradual increase up to 100% during pCPA treatments. After pCPA was discontinued, the effect persisted for 16 days when ethanol intake once again approximated the predrug control range. Control administrations of 0.5% CMC were virtually without effect.

Figure 2 shows the effects of chronic or acute administration of 5-HTP or saline on ethanol intake. In contrast to pCPA, chronic administration of 50 mg/kg 5-HTP produced a reduction of ethanol intake which persisted for as long as four days in Rats 33, 51, and 45. Acute administration of 50 mg/kg 5-HTP resulted in a reduction of ethanol drinking similar to that obtained after chronic dosing. A similar effect following a single 100 mg/kg dose of 5-HTP lasted for two weeks postdrug. Acute or chronic dosing with saline had very little effect on ethanol drinking. We have found that if 5-HTP is given over a period as long as two weeks, a similar decrease in ethanol preference is obtained.

DISCUSSION

The results of this study show that pCPA, the serotonin depletor, increases alcohol intake in rats and that 5-HTP, the serotonin precursor, decreases alcohol intake.

Our findings with pCPA are not in agreement with previous reports that pCPA reduced ethanol preference in rats [11,15]. The lack of agreement may be due to the fact that different doses of pCPA were used in these studies. Myers and Veale [11] and Frey *et al.* [3] used 300 mg/kg of pCPA, a dose which we have found makes animals sick when administered daily over an eleven day period. Other investigators have suggested that the reported pCPA induced aversion to ethanol is probably due to the toxic action of the drug rather than to its serotonin depleting activity [12].

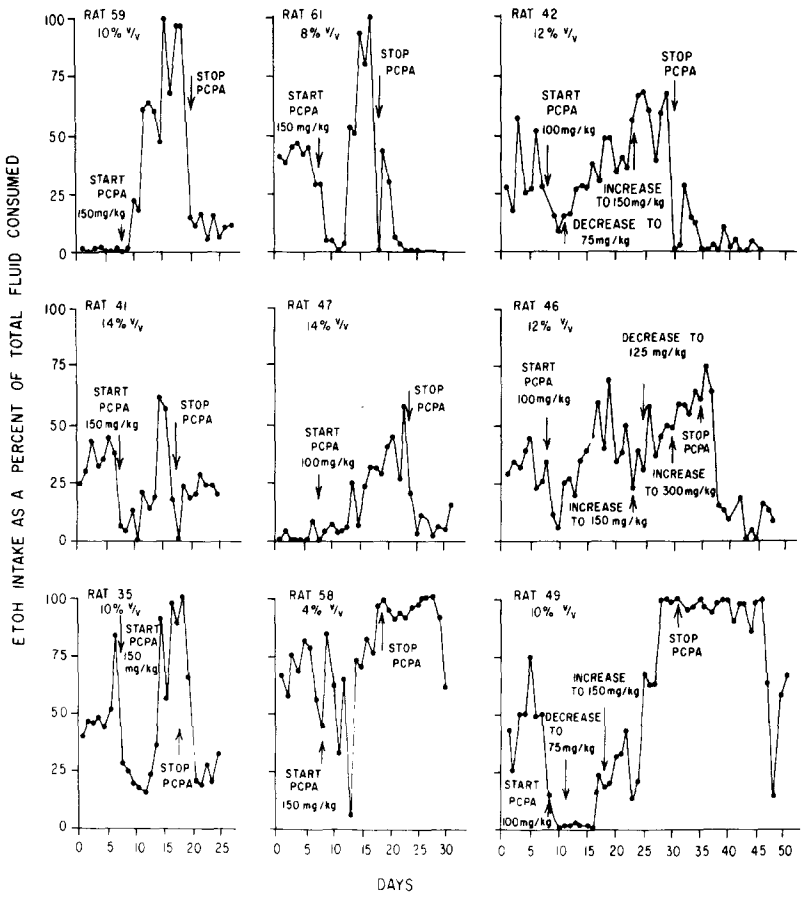

FIG. 1. Effects of chronic oral administration of pCPA on ethanol intake of rats. Ethanol concentrations for each rat are as indicated. The points on the graphs show ethanol intake expressed as a percent of total fluid consumed during a 24-hr period. The first and last arrow on each graph indicates the 24-hr intake readings taken on the days following the first and last pCPA administrations, respectively.

The observation that pCPA increases ethanol preference in rats obtains support from the observations of other investigators. In their study of pCPA effects on intake of ethanol solutions prepared from 95% versus absolute alcohol, Cicero and Hill [2] observed an increase in ethanol preference at the 3% concentration of 95% ethanol. Similarly, Hill, who was unable to produce a pCPA-induced rejection of ethanol [6], found that pCPA increased intake of 3 and 5% ethanol concentrations.

Although biochemical correlative data were not obtained in the present experiment, it seems reasonable to assume that changes in alcohol intake may be related to the brain serotonergic system. The chemical agents used in this study were evaluated previously for their actions on levels of brain serotonin. Koe and Weissman [8] found that intraperitoneal administration of 316 mg/kg of pCPA reduced brain serotonin by 80% while Bloom and Giarman [1] observed that the same pCPA treatment reduced rat pineal 5-HT levels by better than 90%. On the other hand, whole brain 5-HT was increased 216% and pineal 5-HT, 400% following intravenous administration of 5-HTP to rats [13].

The possibility that ethanol may increase levels or turnover of brain 5-HT could account for the findings of this experiment. If ethanol produces an increase in brain 5-HT levels, it is conceivable that following pCPA administration and a concomitant reduction in brain 5-HT levels, rats will drink more alcohol in an attempt to restore levels of 5-HT to normal. Further support for this speculation derives from Frey *et al.* [3], who found that depletion of 5-HT with subtoxic 2 mg/kg doses pCPA did in fact increase drinking of a 5% ethanol solution when the drug was given twice a day for a period of 13 days.

If rats drink alcohol to raise or maintain levels of brain or pineal 5-HT, administration of 5-HTP should satisfy this requirement and reduce the animals' need to drink alcohol. Administration of 5-HTP reduced alcohol intake (Fig. 2), an effect which persisted after termination of the 5-HTP treatment.

If rats drink alcohol to inhibit 5-HT metabolism and thus maintain an optimal level of brain 5-HT, administration of 5-HTP might be expected to raise 5-HT levels [13], produce inhibition of 5-HT synthesis possible through a

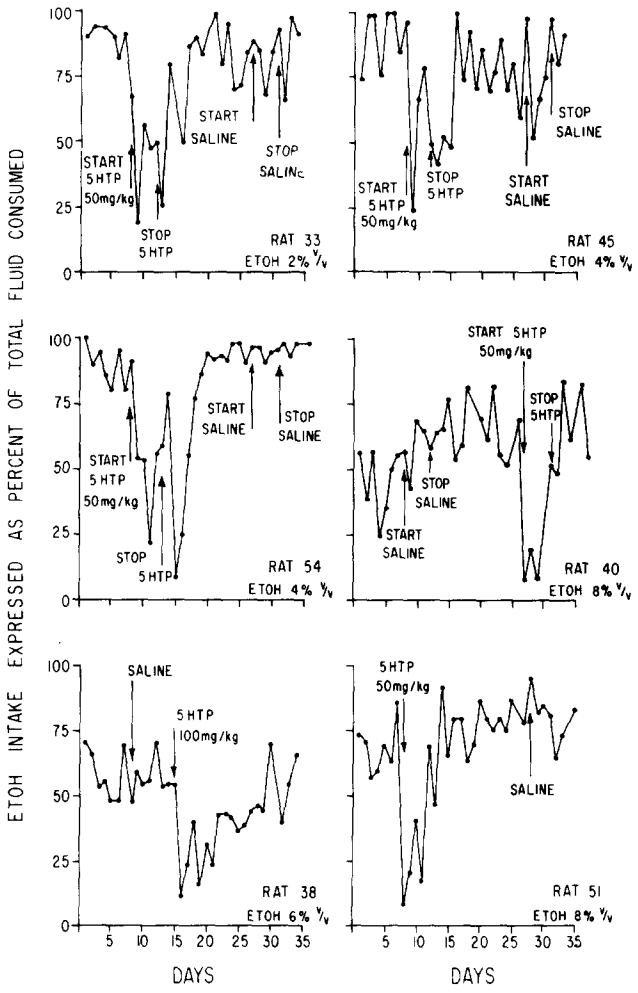

FIG. 2. Effects of chronic or acute administration of 5-HTP or saline on ethanol intake of rats. Ethanol concentrations for each rat are as indicated. The points on the graphs show ethanol intake expressed as a percent of total fluid consumed during a 24-hr period. The arrows show 24-hr readings obtained after the first, last or individual treatments.

feedback mechanism [14], and reduce the animals' need to drink alcohol. On the other hand, the increase in alcohol intake after pCPA treatment might be to satisfy a requirement for inhibition of 5-HT degradation, thereby conserving remaining 5-HT until new tryptophan hydroxylase is formed and pre-pCPA levels are restored.

Darkness-induced alcohol intake in laboratory rats [4] also might be due to an alteration of 5-HT turnover. The activity of hydroxyindole-O-methyl transferase, the pineal enzyme which converts n-acetylserotonin to melatonin, is highest at midnight and lowest at 6 p.m. [17]. Rats kept in continuous darkness can synthesize three to ten times as much melatonin as littermates kept in continuous light [16]. If the increased HIOMT activity produces an increased turnover of pineal 5-HT, the reported increase in alcohol drinking may have occurred in order to inhibit the increased 5-HT turnover.

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