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Effects of PCPA on the Consumption of Alcohol, Water and Other Solutions[1]

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WALTERS, J. K. *Effects of PCPA on the consumption of alcohol, water and other solutions.* PHARMAC. BIOCHEM. BEHAV. 6(4) 377–383, 1977. – The influence of para-chlorophenylalanine (PCPA) on the preference for alcohol, saccharin, glucose, and sodium chloride solutions and on water intake was studied in rats. Ten daily intragastric doses of 100 mg/kg PCPA were found to reduce alcohol preference both during and after PCPA treatment. Administration of daily 20 mg/kg IP doses of 5-hydroxytryptophan (5-HTP), plus a peripheral decarboxylase inhibitor, failed to reverse the PCPA-induced suppression in the post-PCPA test. Alcohol preference was not reduced below baseline following PCPA if this drug was administered between preference tests rather than coincident with alcohol drinking. Preferences for saccharin, glucose, and sodium chloride solutions were all affected by PCPA. Large increases in water intake were produced by 50, 100, or 200 mg/kg PCPA given orally for 10 days. These experiments suggest that alcohol preference, in studies where PCPA treatment and alcohol drinking occur concurrently, may be considerably influenced by learned aversions to alcohol. Also, PCPA may, under some circumstances, produce an increased consumption of water by rats.

Alcohol preference Water intake Serotonin Para-chlorophenylalanine 5-hydroxytryptophan

THE VOLUNTARY selection of alcohol solutions by rats has been found to be influenced by administration of the tryptophan hydroxylase inhibitor, DL-para-chlorophenylalanine (PCPA). Most studies have found that PCPA, which depletes the brain of serotonin (5-hydroxytryptamine; 5-HT), reduces the selection of alcohol solutions [7, 11, 12, 14, 15, 20, 21], although a few have reported PCPA to increase alcohol preference [8,9] or leave it unaffected [4,10]. Myers and his collaborators observed very long lasting reductions in alcohol preference which increased after drug administration ceased and could continue for up to 3 months [21]. However, the reduction in alcohol preference was not simply associated with lowered brain 5-HT levels, for brain 5-HT returned to normal long before alcohol selection did.

An alternative explanation for the PCPA-induced suppression of alcohol selection was proposed by Nachman, Lester and LeMagnen [17]. They showed that PCPA could induce learned aversions to saccharin solutions and proposed that the pairing of alcohol drinking with the noxious effects of PCPA might result in the establishment of a conditioned aversion to alcohol. Two recent studies lend some support for this conditioned aversion hypothesis. Holman, Hoyland, and Shillito [10] administered 316 mg/kg PCPA intraperitoneally to rats on an intermittent schedule during alcohol preference testing. This drug regimen produced a brain 5-HT depletion similar to the daily oral administration of 316 mg/kg PCPA used by Myers et al. No reduction in voluntary alcohol consumption was observed, however. Using a somewhat different design, Parker and Radow [20] gave daily 300 mg/kg IP injections of PCPA to rats not having access to alcohol. Testing occurred 16 days later when brain 5-HT should have been restored. Their rats maintained the same preference for alcohol solutions 16 days post-PCPA as they had during a pre-PCPA preference test.

In order to further investigate the contribution of conditioning in producing aversion to alcohol solutions after PCPA treatment, the following two experiments were conducted.

EXPERIMENT 1

To allow a direct comparison of this experiment with those of the Myers group, a design quite similar to theirs was employed. It consisted of a series of preference tests in which animals received a sequence of ascending concentrations of solution. Dissociation of alcohol drinking from PCPA treatment was accomplished by administering the drug between preference tests rather than concurrent with alcohol drinking. The effects on alcohol preference of 5-hydroxytryptophan (5-HTP), serotonin's immediate pre-

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cursor, were also tested. Nance and Kilby found that a single daily dose (50 mg/kg) of 5-HTP eliminated PCPA's influence on sucrose preference [18]. The same might be expected to occur with alcohol preference if brain serotonin level, rather than aversive conditioning, is truly a critical variable in determining the aversion to alcohol following PCPA. Finally, the influence of PCPA on preferences for saccharin, glucose and sodium chloride solutions was evaluated using the same multiple concentration, multiple preference test paradigm.

Method

Animals. One hundred-twelve male Holtzman albino rats were used. They were 120 days old when experimentation began and were housed individually in a room maintained at 22-25°C on a 14:10 light-dark cycle. All had Wayne Mouse Breeder Blox and tap water freely available.

Procedure. After a 2 week adaptation period, and before preference testing began, the animals were randomly divided into 14 groups of 8 rats each. All groups were tested in the absence of drugs to establish individual preference baselines. A second and then a third preference test followed the first; 4 days separated each of the 3 tests. Table 1 provides a summary of the experimental design. Eight groups of rats were used in testing preferences for alcohol; 4 received a drug treatment and 4 served as vehicle controls. The experimental groups were administered either PCPA or 5-HTP during the second preference test. To determine whether 5-HTP could counteract or reverse the effects of PCPA on alcohol preference, one group received PCPA during Test 2 and 5-HTP during Test 3. All animals who received 5-HTP were pretreated 45-60 minutes earlier with the peripheral decarboxylase inhibitor MK-486 to reduce the peripheral side effects of 5-HTP. An important exception to the standard 3 preference test procedure was made in order to test the hypothesis that the formation of a conditioned aversion might be responsible for PCPA's effects on alcohol preference. Two groups received only 2 tests; PCPA or CMC vehicle was administered to these animals between preference tests, but at the same time that the other PCPA groups were being treated.

The remaining 6 groups of rats were used to determine PCPA's effects on preferences for solutions other than alcohol. Two were tested with saccharin, 2 with glucose, and 2 with sodium chloride solutions; one group of each pair received PCPA and the other the CMC vehicle.

A preference test lasted for 8 days and consisted of offering each rat a choice between tap water and a different concentration of solution on each consecutive day. In order to induce consumption of the unpalatable concentrations, an ascending series was always used. Concentrations were the following: 2, 4, 6, 8, 10, 12, 16, and 20% alcohol; 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0, and 2.4% sodium saccharin; 15, 20, 25, 30, 35, 40, 45 and 50% glucose; 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, and 2.4% sodium chloride. All solutions were prepared fresh daily on a weight/weight basis with alcohol solutions being diluted from 95% alcohol. The 3 bottle, 24 hr preference method of Myers and Holman [13] was used to help eliminate bottle position and drinking spout preferences.

Drug preparation and administration. DL-Para-chlorophenylalanine (Charles Pfizer Co.) was prepared as a suspension in 0.5% carboxymethylcellulose (CMC) and

TABLE 1

SUMMARY OF THE EXPERIMENTAL DESIGN INCLUDING THE SOLUTION PRESENTED TO EACH GROUP AND THE TREATMENTS RECEIVED DURING PREFERENCE TESTS

Solution	Group	N	Test No. 1	Test No. 2	Test No. 3
Ethanol	drug	8	—	100 mg/kg pCPA	—
Ethanol	vehicle	8	—	CMC vehicle	—
Ethanol	drug	8	—	60 mg/kg MK-486 + 20 mg/kg 5-HTP	—
Ethanol	vehicle	8	—	60 mg/kg MK-486 + NaCl vehicle	—
Ethanol	drug	8	—	100 mg/kg PCPA	60 mg/kg MK-486 + 20 mg/kg 5 HTP
Ethanol	vehicle	8	—	100 mg/kg pCPA	60 mg/kg MK-486 + NaCl vehicle
Ethanol	drug	8	—	100 mg/kg pCPA **NO TEST**	— —
Ethanol	vehicle	8	—	CMC vehicle **NO TEST**	—
Saccharin	drug	8	—	100 mg/kg pCPA	—
Saccharin	vehicle	8	—	CMC vehicle	—
Glucose	drug	8	—	100 mg/kg pCPA	—
Glucose	vehicle	8	—	CMC vehicle	—
Sodium Chloride	drug	8	—	100 mg/kg pCPA	—
Sodium Chloride	vehicle	8	—	CMC vehicle	—

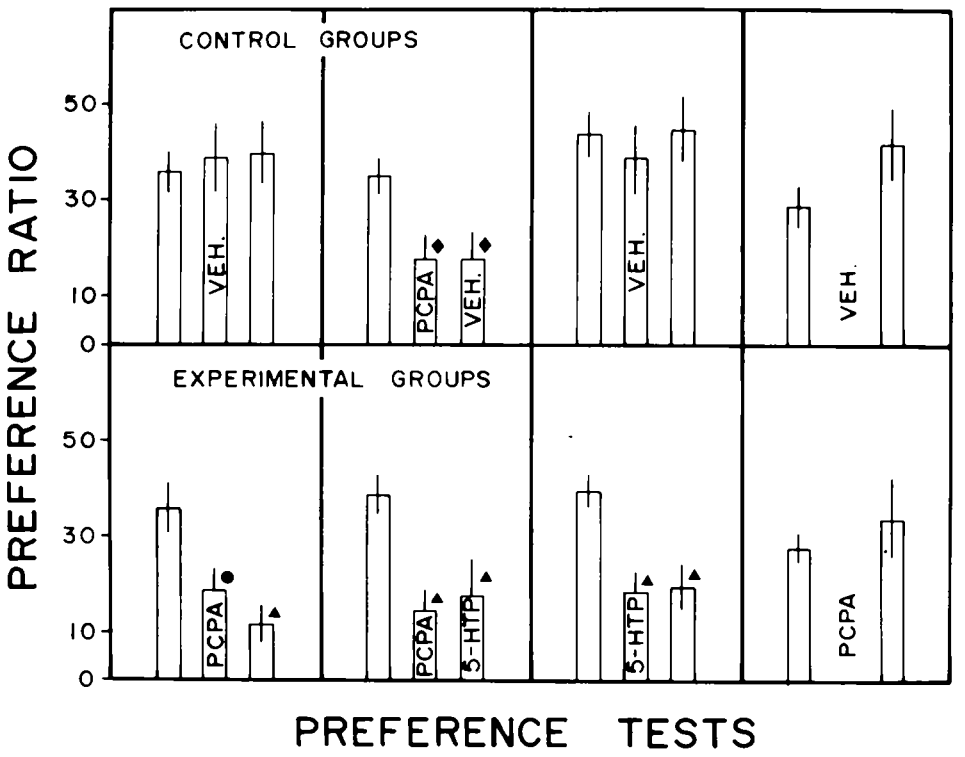

FIG. 1. Mean (+ S.E.) daily preference ratios of alcohol drinking groups for each preference sequence. On the bottom are experimental groups which received drug treatment and above each is its corresponding vehicle control group. See Table 1 for an explanation of drug treatments. Symbols represent probability levels in Duncan's tests of significant differences from baseline preference: $\blacklozenge p<0.05$; $\bullet p<0.01$; $\blacktriangle p<0.001$.

maintained under constant stirring prior to each administration. Control animals received the CMC vehicle alone. PCPA and CMC were given intragastrically in 5 ml volumes. DL-5-Hydroxytryptophan (Sigma Chemical Co.) was dissolved in 0.9% saline with gentle warming. The peripheral decarboxylase inhibitor L-2-hydrazino-2-methyl-B-3, 4 dihydroxyphenyl propionic acid (MK-486; Merck, Sharp, and Dohme Co.) was prepared as a suspension in 0.9% saline and maintained with constant stirring. Intraperitoneal (IP) injection volumes for 5-HTP animals and their saline controls were 3 ml; MK-486 or saline vehicle IP injections were 2 ml. Thus, all rats received a total of 5 ml of fluid on each day of drug treatment.

Drug treatment always started two days before a preference test began and continued throughout it. All drugs were prepared fresh daily and administered under very light ether anesthesia.

Results

The measures of self-selection behavior were mean proportion alcohol to total fluid intake per day (mean daily preference ratio) and mean g/kg consumed as absolute alcohol or solute per day. Both measures were calculated for each group for each preference sequence, as was mean g/kg total fluids consumed. Repeated measures analyses of variance were then conducted on each of these 3 dependent variables to determine if differences existed among the preference tests for a group. If differences were found, individual comparisons were made using Duncan's multiple range tests [3]. Probability levels are given in figures and tables.

Figure 1 shows the mean daily preference ratios of alcohol drinking groups for each preference test. The alcohol preferences of those groups which received either PCPA or MK-486 + 5-HTP were reduced from baseline during drug treatment. Their preferences remained suppressed in the post-drug period. Administration of MK-486 + 5-HTP during the third test was ineffective at reversing the PCPA-induced reduction in mean daily preference ratio. On the contrary, however, alcohol preference was not reduced below baseline in the post-drug period when PCPA was given between two preference tests.

Table 2 reveals a slightly different picture regarding g/kg absolute alcohol consumed by the same animals shown in Fig. 1. Groups receiving PCPA or MK-486 + 5-HTP during Test 2 did not significantly decrease their alcohol intake in g/kg until the post-drug preference test; there was a tendency toward reduced alcohol intake during drug treatment, however. Administration of MK-486 + 5-HTP during the third test did not prevent the PCPA-induced decline. The g/kg alcohol intake of animals receiving PCPA between tests did not decrease post-drug as it did for animals receiving PCPA during Test 2. However, neither did it increase significantly post-PCPA as it did for the controls receiving CMC vehicle between tests, although there appeared to be a similar tendency in both groups toward greater alcohol consumption in the post-drug period.

Total fluid intake, also shown in Table 2, increased significantly during the second preference test for every group which received either PCPA or MK-486 + 5-HTP concurrent with the test.

Results for groups drinking saccharin, glucose or sodium chloride solutions are given in Table 3. The saccharin and glucose groups showed significant decreases from baseline in mean daily preference ratio during PCPA treatment, while preference of the sodium chloride group decreased significantly during the post-PCPA test. No significant differences in preference were found among the 3 tests for any control group. Solute consumption in g/kg increased significantly above baseline during PCPA administration for the glucose and NaCl groups. It decreased significantly below baseline during the post-PCPA sequence, however, for the saccharin group. Vehicle controls drinking sodium chloride and saccharin solutions showed no changes in solute intake among the 3 tests. Glucose vehicle controls significantly decreased solute intake both during and after CMC treatment. The total fluid intakes of saccharin, glucose, and sodium chloride groups all increased significantly above baseline during PCPA treatment, while controls showed no increases during or after CMC treatment.

TABLE 2

MEAN (± SE) DAILY G/KG CONSUMED AS ETHYL ALCOHOL AND MEAN (± SE) DAILY G/KG TOTAL FLUIDS INGESTED DURING EACH PREFERENCE TEST FOR GROUPS DRINKING ETHANOL SOLUTIONS

Treatment	G/KG Alcohol	G/KG Total Fluids
Baseline	2.05 ± 0.42	100 ± 5.3
pCPA	1.51 ± 0.40	130 ± 11.4 b
Post-drug	0.73 ± 0.22 b	104 ± 5.2 w
Baseline	1.86 ± 0.29	96 ± 7.7
CMC vehicle	2.15 ± 0.44	79 ± 5.0 d
Post-drug	2.38 ± 0.45	94 ± 9.0 y
Baseline	1.91 ± 0.35	82 ± 4.2
pCPA	1.35 ± 0.44	150 ± 10.1 d
MK-486 + 5-HTP	1.01 ± 0.51 a	80 ± 4.3 z
Baseline	1.80 ± 0.21	93 ± 7.1
pCPA	1.39 ± 0.44	139 ± 14.2 c
MK-486 + NaCl vehicle	0.77 ± 0.24 a	85 ± 7.5 y
Baseline	1.75 ± 0.18	75 ± 3.2
MK-486 + 5-HTP	1.18 ± 0.23	119 ± 11.3 c
Post-drug	0.94 ± 0.34 a	75 ± 3.9 y
Baseline	2.08 ± 0.45	74 ± 4.1
MK-486 + NaCl vehicle	2.44 ± 0.50	92 ± 9.2 a
Post-drug	2.42 ± 0.46	78 ± 6.8 w
Pre-pCPA	1.27 ± 0.21	93 ± 5.1
Post-pCPA	1.75 ± 0.35	92 ± 4.5
Pre-CMC vehicle	1.51 ± 0.35	96 ± 6.5
Post-CMC vehicle	2.69 ± 0.57 a	98 ± 6.7

Letters represent levels of significance in statistical tests made among the preference tests for a given group.
Differences from baseline: a = .05, b = .01, c = .005, d = .001.
Differences from drug/veh: w = .05, x = .01 y = .005 z = .001.

TABLE 3

MEAN (± SE) DAILY PREFERENCE RATIO, G/KG SOLUTE CONSUMED AND TOTAL FLUIDS INGESTED FOR GROUPS DRINKING SACCHARIN, GLUCOSE, OR SODIUM CHLORIDE SOLUTIONS

Treatment	Pref. Ratio	G/KG	G/KG Total Fluids
		Saccharin	
Baseline	45 ± 3.4	0.34 ± 0.02	137 ± 9.6
pCPA	32 ± 2.7 a	0.30 ± 0.04	169 ± 13.0 d
Post-drug	36 ± 4.1	0.24 ± 0.03 a	121 ± 8.8 a, z
Baseline	39 ± 4.6	0.32 ± 0.06	136 ± 12.0
CMC vehicle	42 ± 3.7	0.31 ± 0.06	114 ± 12.6 c
Post-drug	38 ± 4.6	0.27 ± 0.05	117 ± 10.7 b
		Glucose	
Baseline	63 ± 4.6	22 ± 2.5	124 ± 10.1
pCPA	52 ± 5.4 a	28 ± 3.3 a	200 ± 17.5 d
Post-drug	63 ± 1.7 w	18 ± 0.8 y	104 ± 6.8 z
Baseline	78 ± 3.4	30 ± 1.9	133 ± 7.5
CMC vehicle	73 ± 3.3	25 ± 2.3 c	121 ± 9.0 a
Post-drug	76 ± 2.5	26 ± 2.1 c	120 ± 8.1 a
		Sodium Chloride	
Baseline	30 ± 3.5	0.39 ± 0.06	106 ± 8.2
pCPA	36 ± 6.2	0.76 ± 0.20 a	161 ± 16.0 c
Post-drug	15 ± 4.2 b, y	0.20 ± 0.08 y	95 ± 7.0 z
Baseline	27 ± 1.2	0.31 ± 0.05	105 ± 7.8
CMC vehicle	27 ± 2.9	0.31 ± 0.06	98 ± 8.9
Post-drug	22 ± 2.0	0.23 ± 0.04	103 ± 7.3

Letters represent levels of significance in statistical tests made among the preference tests for a given group.
Differences from baseline: a = .05, b = .01, c = .001, d = .001.
Differences from drug/veh: w = .05, x = .01, y = .005, z = .001.

Although the consumption data are not provided for individual concentrations of the 4 solutions offered, in general, PCPA reduced preferences proportionally for all concentrations. The only exception was seen with sodium chloride. Preferences for this solution were reduced at lower concentrations early in treatment, but elevated for higher (hypertonic) ones later in treatment.

EXPERIMENT 2

The elevated total fluid intakes evident for each PCPA group during the preference testing of Experiment 1 suggested that this drug may have a significant effect on water consumption. To date, only two studies have directly addressed this question, one found PCPA to increase water consumption [1], while the other found a decrease [19]. Experiment 2 was intended to determine the influence of 3 doses of PCPA (50, 100, and 200 mg/kg) on the water intake and body weight of rats.

Method

Animals. Twenty male Holtzman albino rats were used. Eight were 118 days old and 12 were 137 days at the start of experimentation. Housing and lighting conditions were the same as those in Experiment 1.

Procedure. At least two weeks of adaptation to the lab preceded the commencement of 24 hr measures of water intake (g) and body weight. Following 8 days of baseline

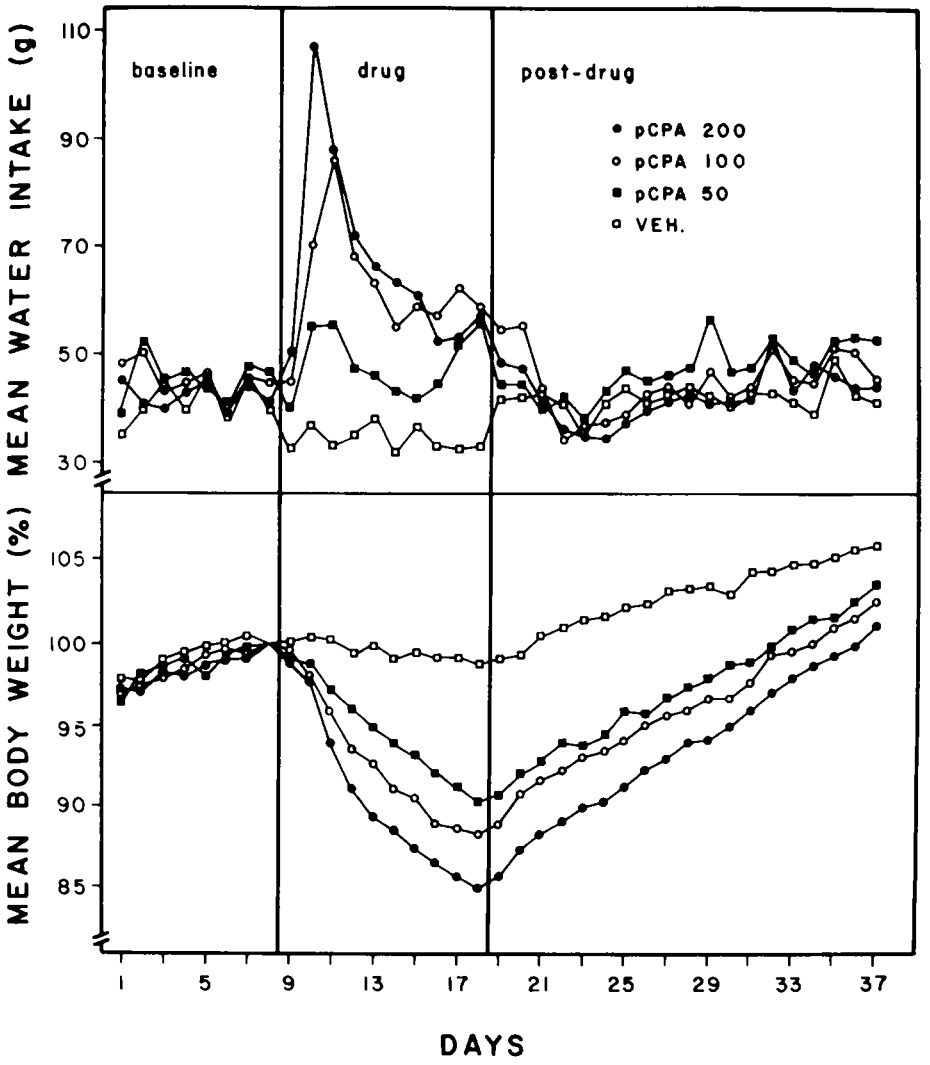

FIG. 2. Mean water intake in g (top) and mean body weight plotted as a percentage of each group's weight on the last day of baseline (bottom) as a function of days during baseline, drug and post-drug periods for animals receiving 50, 100, or 200 mg/kg PCPA or CMC vehicle alone during the 10-day drug period.

measures, animals were matched for water intake and divided into 4 groups of 5 rats which each included 3 older and 2 younger animals. Beginning on the eighth day, and continuing for 10 consecutive days, groups received either 50, 100, or 200 mg/kg PCPA or 0.5% CMC vehicle. Water intakes and body weights continued to be recorded for 19 days post-drug. Preparation and administration of PCPA were the same as that for Experiment 1.

Results

The top of Fig. 2 shows the mean water intake in grams during baseline, drug and post-drug periods. Significant differences were found among the groups during the 10 day drug period, $F(3,36) = 17.52$, $p<0.001$. All 3 PCPA groups drank significantly more during drug treatment than the vehicle controls (Duncan's test; $p<0.01$ for PCPA-50, $p<0.001$ for PCPA-100 and PCPA-200 groups). The PCPA-50 animals drank significantly less than the PCPA-100 ($p<0.01$) and PCPA-200 ($p<0.001$) groups which did not differ in mean water intake. No differences in mean water consumption were found among any of the groups during the first week or the last 12 days post-drug.

The bottom of Fig. 2 shows the mean body weight of each group plotted as a percentage of its weight on the last baseline day. This measure was used because the groups had not been matched for body weight and did differ significantly during baseline in body weight as measured in grams, $F(3,28) = 31.82$, $p<0.001$. In comparison to vehicle controls, the mean percent body weight of all 3 PCPA groups was significantly lower ($p<0.01$ for PCPA-50, $p<0.001$ for PCPA-100 and PCPA-200 groups). A significant difference was also found between the PCPA-50 and PCPA-200 groups ($p<0.05$). The PCPA animals continued to have lower mean percent body weights than the controls during both the first week, $F(3,24) = 65.54$, $p<0.001$, and the last 12 days post-drug, $F(3,44) = 23.28$, $p<0.001$.

GENERAL DISCUSSION

The results of Experiment 1 provide additional support for the hypothesis that learned aversions to alcohol, rather than brain serotonin depletion, may be the significant factor in determining alcohol self- selection in studies where PCPA treatment and alcohol drinking coincide. The basic finding of Myers *et al.* [12, 14, 15, 21] and others [7, 11, 20] that alcohol preference is reduced both during and after PCPA treatment was confirmed, despite the use of a lower dose (100 mg/kg vs 300 mg/kg) of PCPA. The interpretation of this result as being caused by depletion of brain 5-HT was not confirmed.

Although not actually measured in Experiment 1, brain serotonin level should have been on the rise, but still depleted, at 4 days post-PCPA when the post-drug preference sequence began. Nevertheless, animals which received PCPA concurrently with alcohol drinking reduced their alcohol consumption during the post-PCPA test, while those which received PCPA between preference tests did not reduce their alcohol selection at this time. These data corroborate those of Parker and Radow [20] who gave 300 mg/kg PCPA between preference tests. They found no suppression of alcohol selection 16 days later when brain serotonin should have been replete and at a time when Veale and Myers [21] were still finding decreased preference. Together, the results of Experiment 1 and those of Parker and Radow show that no simple association exists between alcohol preference and lowered brain serotonin.

Some evidence supporting the interpretation of PCPA's suppressing effect as resulting from aversive conditioning is revealed by the results with 5-HTP. One might expect the elevation of brain 5-HT with 5-HTP to eliminate or reverse PCPA's effect on alcohol preference if brain serotonin level is a critical variable. Nance and Kilby [18] successfully reversed the PCPA-induced elevation of sucrose consumption by administering 50 mg/kg 5-HTP to rats which had been treated with 100 mg/kg PCPA on the three previous days. On the other hand, if a learned aversion to alcohol is responsible for PCPA's effects, 5-HTP would not be expected to reverse a previously learned response. In Experiment 1, 20 mg/kg 5-HTP injections were totally ineffective at reversing the suppression in alcohol drinking caused by PCPA. In fact, 5-HTP itself produced a decreased preference for alcohol both during and after drug treatment in a fashion quite similar to PCPA and suggestive of aversive conditioning also. This agrees with the data of Myers, Evans, and Yaksh [16]. Unfortunately, 5-HTP may be taken up and converted to 5-HT in neurons other than serotonergic ones [5]. Care was taken in the present study to use a low dose of 5-HTP and pretreat the animals with a peripheral decarboxylase inhibitor (MK-486) which reduces the peripheral side effects and potentiates the cerebral dose of 5-HTP [22]. Additional doses of 5-HTP and/or multiple injections remain to be attempted.

In explaining their preference data in terms of aversive conditioning, Parker and Radow [20] have suggested that PCPA and alcohol may somehow interact to either make the post-ingestional effects of alcohol more aversive, or alternatively, to make the effects of PCPA more noxious. Thus, simultaneous exposure to both alcohol and PCPA produces a more robust aversive conditioning than normal. The data of Experiment 1 appear to support the Parker and Radow interpretation, but suggest that the interaction may not be specific to PCPA. An interaction of alcohol with other drugs, either peripherally or centrally, might explain why long-term reductions in preference after PCPA or 5-HTP have so far only been discovered for alcohol solutions.

The data concerning PCPA's effects on preferences for solutions other than alcohol are not clearcut. This is the only study to date, however, which allows a direct comparison of PCPA's influence on preferences for several solutions, because the same methodology was used in testing all solutions. Somewhat different pictures of the data emerge if one uses g/kg solute consumed rather than preference ratio as the dependent variable of choice. The two measures did not correlate well. This may have occurred because PCPA produced an increase in the total fluid consumption of the animals. Therefore, the proportion, not the absolute amount, of a substance consumed seems to be the better index of preference.

Unlike alcohol selection, the mean daily preference ratio for saccharin was decreased significantly only during PCPA treatment itself. This substantiates the results of Parker and Radow [20] who found saccharin preference to return to baseline levels on the sixth day of an 11 day post-PCPA test. Their finding may explain why mean daily saccharin preference in Experiment 1 had not quite returned to baseline during the post-drug sequence. Parker and Radow used a similar multiple test method, but their animals drank only 0.23% saccharin and received 300 mg/kg PCPA IP. The results for glucose preference parallel those for saccharin in that the only significant effect was a decrease during PCPA treatment. Although no other investigator has published results of PCPA's effects on glucose consumption, Nance and Kilby [18] did find 100 mg/kg PCPA (IP) to elevate the preference for several sucrose solutions (0.05 to 16%) during 12 hr tests. An explanation for this discrepancy is not obvious, although procedural differences such as the shorter preference test period or the use of only low, highly preferred sucrose concentrations may account for it.

Sodium chloride solution self-selection increased somewhat overall during PCPA treatment and then decreased significantly post-PCPA. Again, no other sodium chloride data are available for comparison. The increase during drug administration is interesting, for it is the only hint of a positive effect of PCPA on preference, and the post-PCPA decrease could then represent a rebound to counteract higher than normal body sodium levels. The effects of PCPA on sodium chloride preference definitely deserve further study.

Experiments 1 and 2 clearly demonstrated that daily intragastric PCPA treatment (100 mg/kg) can produce large increases in water intake alone and in total fluid consumption during preference testing. Holman, Hoyland and Shillito [10] and Kiianmaa [11] have also reported PCPA to increase total fluid intake during alcohol preference testing. Both used doses in the 300 mg/kg range, but one administered the drug IP on an intermittent schedule [10], while the other gave it daily by stomach tube [11]. Others have found the 300 mg/kg oral dose of PCPA to decrease total fluids consumed [15] or leave it unaffected [4,16]. Similar contradictions exist in those studies which have investigated PCPA's effects on water intake itself, apart from preference testing procedures. Brody [1,2] found that a single 300 mg/kg, but not 100 mg/kg, IP injection of PCPA could dramatically increase water consumption. Nance and Kilby [18] found no effect of 3 daily IP injections of 100 mg/kg PCPA. Finally, Panksepp and

Nance [19] reported treatment with 200 mg/kg PCPA on Day 1 and 100 mg/kg PCPA for 19 consecutive days to produce about a 28% decrease in water intake.

A simple procedural explanation for these discrepancies is not apparent. A variety of effects has been produced by PCPA at both high and low doses and with intragastric or IP administration. The rapid onset and decay over time of PCPA's effects at the 100 and 200 mg/kg doses in Experiment 2 suggests that falling, rather than steady state, brain serotonin levels may be the crucial factor. This hypothesis has been put forward by Davis and Sheard [6] to explain some effects of p-chloroamphetamine on the acoustic startle reflex in rats. On the other hand, since the quantity of water drunk increased soon after PCPA intubation began and returned to normal soon after it ended, it is possible that a peripheral side effect of PCPA is responsible for its facilitation of drinking.

In conclusion, these experiments suggest that the determination of alcohol preference in studies where PCPA or 5-HTP treatment and alcohol drinking occur concurrently is probably more influenced by learned aversions than brain serotonin level. Alcohol may interact with these drugs to make them more aversive than normal. In addition, PCPA may, under some circumstances produce large increases in water intake which could influence the interpretation of preference experiments.

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