EFFECTS OF PARA-CHLOROPHENYLALANINE AND 5-HYDROXYTRYPTOPHAN ON THE SELECTION OF ETHANOL AND OTHER SOLUTIONS BY RATS

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ABSTRACT

EFFECTS OF PARA-CHLOROPHENYLALANINE AND 5-HYDROXYTRYPTOPHAN ON THE SELECTION OF ETHANOL AND OTHER SOLUTIONS BY RATS

By

James Karl Walters

Within the past several years, experimental evidence has accumulated which suggests that an association may exist between the neurochemistry of brain serotonergic systems and the ethanol self-selection behavior of rats. This evidence is primarily of a pharmacological nature and most investigators have studied the effects on ethanol selection of manipulating whole-brain serotonin level with drugs. Para-chlorophenylalanine (pCPA), a serotonin depletor, and 5-hydroxytryptophan (5-HTP), a serotonin precursor, have been used for this purpose.

Questions have remained, however, as to whether these drugs are influencing only the selection of ethanol solutions and whether their effects are truly due to altered brain serotonin level. The present series of three experiments was therefore designed to further investigate the effects of pCPA and 5-HTP on the selection of ethanol and other solutions by rats.

In Experiment I, 50, 100 or 200 mg/kg pCPA or 25 mg/kg 5-HTP (plus a peripheral decarboxylase inhibitor) were intragastrically

administered to rats for 10 consecutive days to determine if this treatment influenced ad libitum water intake or body weight. Rats of Experiment II received 10 daily intragastric doses of either 100 mg/kg pCPA or 20 mg/kg 5-HTP (plus a peripheral decarboxylase inhibitor) during or between 8-day ethanol preference test sequences. Preference testing with saccharin, glucose or sodium chloride solutions was carried out in Experiment III while animals were intubated for 10 days with 100 mg/kg pCPA.

Results showed that pCPA, in most cases, and 5-HTP (plus peripheral decarboxylase inhibitor) both caused significant body weight decreases and water intake increases. The effects of pCPA on water intake were greatest early in treatment and also were positively related to dose. With regard to ethanol, both pCPA and 5-HTP produced significant reductions in selection, despite the fact that they should have had opposite effects on brain serotonin level. It was found that the one dose of 5-HTP employed failed to reverse the effects of pCPA on ethanol consumption and that pCPA had little influence on ethanol choice behavior when its administration did not coincide with ethanol drinking. The selection of saccharin solutions was suppressed during pCPA intubation, while the intake of glucose and sodium chloride solutions increased.

Taken together, these data may be interpreted to support the hypothesis that the effects of pCPA are not specific to the consumption of ethanol, and that pCPA and 5-HTP are most likely influencing ethanol self-selection through the formation of a conditioned taste aversion due to noxious drug effects.

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Special thanks go to my sister and brother-in-law, Jan and Bill, for showing me the light at the end of the tunnel at a time when it was looking very dim.

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INTRODUCTION

An enduring enigma for those concerned with determining the etiology of excessive alcohol intake in man is the contribution of central nervous system anatomy, physiology and biochemistry to this phenomenon. Within the past several years an association has apparently been demonstrated between the neurochemistry of brain serotonin systems and the consumption of alcohol by experimental animals (Myers & Veale, 1968; Veale & Myers, 1970; Geller, 1973; Ho, Tasi, Chen, Begleiter, & Kissin, 1974; Myers & Melchior, 1975). The import of this association for understanding human alochol abuse remains quite equivocal though, for no true animal model of alcoholism presently exists (Myers & Veale, 1970). Such knowledge may nonetheless provide clues to alcohol's interaction with the nervous system and may possibly help to elucidate some of the neurochemical processes which participate in determining alcohol consumption. Therefore, this proposed relationship deserves further close scrutiny.

Great interest was aroused in the brain's serotonergic neurochemical systems, among others, by the pioneering demonstration of Dahlstrom and Fuxe in 1964. Using fluorescent histochemical techniques, they showed that most, if not all, of the nerve cell bodies containing the putative neurotransmitter (Rech & Moore, 1971, p. 108)

serotonin (5-HT) are located within a number of nuclear groups in the brainstem. These raphe nuclei lie along the midline tegmentum throughout the medulla, pons and midbrain (Morgane & Stern, 1973). Axons from the serotonergic raphe neurons course both caudally and rostrally, ramifying to most areas of the central nervous system (Anden, Dahlstrom, Fuxe, Larsen, Olson, & Ungerstedt, 1966). Reviews of the anatomical, pharmacological, physiological and behavioral aspects of brain serotonin can be found in several recent books (Barchas & Usdin, 1973; Costa, Gessa, & Sandler, 1974a,b; Cooper, Bloom, & Roth, 1974).

A major step forward in the study of the functional significance of brain serotonergic systems was made in 1966 when Koe and Weissman reported that the drug para-chlorophenylalanine (pCPA) could selectively deplete 5-HT neurons of their serotonin. It apparently does so by inhibiting tryptophan hydroxylase activity and/or impairing tryptophan transport. Experimentally, pCPA has been used extensively despite the complication that it also depletes blood and peripheral tissue of 5-HT. As far as whole-brain serotonin levels are concerned, one result which "remains quite stable within and across laboratories is the amount of brain 5-HT depletion which results from pCPA" (Rechtscaffen, Lovell, Freedman, Whitehead, & Aldrich, 1973). Such depletion usually approximates 90% with only a slight 10 to 15% reduction in catecholamine levels early in treatment. Dosage and schedule of pCPA administration can, of course, make a difference (Holman, Hoyland, & Shillito, 1974). Extremely rapid reversal of both electrophysiological and behavioral changes resulting from pCPA is usually accomplished by giving small doses of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT (Weissman, 1973).

Unfortunately though, the ubiquitous nature of the decarboxylating enzyme which converts 5-HTP to 5-HT results in 5-HT being formed within neurons where it is not normally present (Moore, 1971).

In 1968 Myers and Veale first applied the pharmacological technique of depleting brain serotonin with pCPA to the study of alcohol preference in experimental animals. Their initial study revealed that the ethyl alcohol preference of rats was significantly reduced or abolished by pCPA treatment. Later studies by Myers and his collaborators tended to confirm and extend these original findings (Myers & Cicero, 1969; Veale & Myers, 1970; Myers & Tytell, 1972; Myers, Evans, & Yaksh, 1972; Myers & Martin, 1973). They showed that pCPA, given in daily oral doses of 300 mg /kg, reduced not only the preference but also the absolute amount of alcohol consumed, in g /kg /day, by animals selecting ethanol solutions for various reasons. These included: (1) an initial predisposition, (2) acclimation to ethanol, and (3) stress induced by electric shock delivered randomly during a conditioned shock avoidance task.

One unusual finding from these many studies was that an even more pronounced rejection of ethanol occurred in preference test sequences given up to two months after drug administration was discontinued. Surprisingly, Veale and Myers (1970) reported preliminary assays carried out in a parallel fashion which showed 5-HT content in discrete brain regions to recover to levels even greater than control following chronic pCPA administration. This seems to shed doubt on the hypothesis that the changes in alcohol consumption actually did reflect changes in central serotonin stores, since alcohol intake was depressed when serotonin levels were either depleted or elevated.

With regard to 5-HTP, Myers, Evans, and Yaksh (1972) found i.p. doses of 50 mg /kg injected every eight hours to also suppress ethanol intake. These effects persisted for over three months, despite the fact that 5-HT levels determined shortly after treatment ended revealed no significant differences in 5-HT content between the brains of control and experimental animals. Finally, in 1973 Myers and Martin reported that 5-HTP also lowered ethanol consumption when it was infused directly into the lateral cerebral ventricle. In these animals, oral pCPA administration during 5-HTP infusion reversed the lowered ethanol consumption. It also increased ethanol intake in a preference sequence given after infusion ended. In neither of these experiments did there seem to be a close correlation between preference behavior and brain serotonin levels.

These phenomena, particularly with regard to the effects of pCPA, have proven quite ephemeral to other investigative teams. For instance, Frey, Magnussen, and Nielsen (1970) found a less pronounced effect from pCPA treatment and equivocal results with p-chloroamphetamine which produced less serotonin depletion than pCPA at the doses they used. Cicero and Hill (1970) found a marked distinction between results obtained from pCPA treatment when ethanol solutions were prepared with 95% versus 100% alcohol. Interestingly, the typical pCPA effect was found with solutions made from 100% (absolute) alcohol. This drug little affected the consumption of solutions prepared with 95% alcohol, however. They suggested that the pCPA treated rat may be more sensitive to the taste or odor of a contaminant, benzene perhaps, which azeotropic distillation imparts upon absolute alcohol. Another very recent experiment by Holman, Hoyland, and

Shillito (1974) also questioned the hypothesis that the decreased ethanol consumption found by the Myers group was actually due to brain serotonin depletion. They gave pCPA to rats at intervals of three to four days and found it to be as effective as daily pCPA administration at depleting serotonin levels. Such intermittent treatment, however, did not produce a reduction in voluntary alcohol consumption. They speculate, as Nachman, Lester, and Le Magnen (1970) had done several years earlier, that a learned aversion or the route and time of pCPA administration may be responsible for the drug effects.

Results in direct contradiction to those of Myers and his collatorators have been obtained by both Geller (1973) and Hill (1971). Hill found pCPA to produce no rejection of ethanol, but rather an increased intake of 3% and 5% solutions. Geller's results were even stronger. According to him, pCPA nearly always increased alcohol intake when given in daily oral doses ranging from 75-300 mg /kg. Daily intraperitoneal doses of 50-100 mg /kg 5-HTP consistently reduced intake. The use of extended periods of daily drug administration (up to four weeks) and a debatable interpretation of results render Geller's study questionable.

The final studies concerning serotonergic involvement in ethanol preference have used the relatively new technique of lesioning central serotonin neurons by injecting 5,6-dihydroxytryptamine (5,6-DHT) intracisternally or intraventricularly. Two such studies have both shown 5,6-DHT to increase alcohol consumption within a few days after injection (Ho, Tasi, Chen, Begleiter, & Kissin, 1974; Myers & Melchior, 1975). Ho et al. found the effect to last from the fifth to the eleventh day post-treatment, while Myers and Melchior found

increased consumption to persist 60 days after drug administration ended.

Are any of these effects specific to the consumption of alcohol solutions? Unfortunately, few of the many studies implicating serotonin in ethanol self-selection also included preference testing for other solutions. Cicero and Hill, in 1970, reported preliminary findings of an increased saccharin intake due to pCPA treatment, but no detailed account of that study has apparently been published. Concerning sucrose consumption, Nance and Kilby (1973) showed pCPA to increase both preference and absolute amount consumed. They suggest that 5-HT neurons may participate in regulating the amount of carbohydrate ingested. Suppression of quinine intake by pCPA has been observed by Brody (1970) in a study of the "hyper-reactivity hypothesis" of serotonin depletion effects. Suppression was especially evident when the quinine solution was novel to the rats. As an aside, Brody reported increases in dextrose intake due to pCPA treatment but gave no details of the experiment.

Strikingly surprising to me in regard to most of these studies is the fact that little attention was paid to the effects which pCPA and 5-HTP might have on the health and water consumption of the experimental animals. Although little quantitative data are available concerning water intake, present results are conflicting. Brody (1969) found a single dose of 300 mg /kg pCPA to increase drinking; a second dose given to the same animals five days later again facilitated water consumption. Holman, Hoyland, and Shillito (1974) also reported increased total fluid intake during an ethanol preference test sequence when pCPA was administered; other investigators have not reported such

to be the case. In contrast to these findings, Panksepp and Nance (1974) found 20 days of pCPA treatment to reduce water intake by 28%. They also showed, as have others, that prolonged pCPA administration reduces food intake by 25% to 50% while lowering body weight 10% to 20%. The deleterious effects of pCPA on an animal's health might hamper data interpretation. In addition, drug-induced changes in water intake alone, if grams of alcohol consumed are not considered, could lead to erroneous conclusions.

Also obvious in this hodge-podge of studies is the lack of appreciation for variability due to differences in the species or strain of animals tested. As pointed out by Tilson and Rech (1974) and others (Miller, Cox, & Maickel, 1968; Rosecrans & Schechter, 1972), sex, strain or supplier differences in whole brain or regional distribution of 5-HT may sometimes account for contradictory results. Two experiments by Ahtee and Eriksson (1972, 1973) illustrate this crucial fact. They discovered whole-brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) contents to be 15% to 20% higher in Wistar rats genetically selected for high ethanol preference than in Wistar rats selected for low preference. Additionally, they demonstrated that the distribution of 5-HT in various brain regions was not the same in each strain.

As should be obvious from this brief overview, considerable evidence has accumulated which suggests that the consumption of ethanol, by experimental animals at least, may possibly be influenced by variations in the activity or anatomy of the brain's serotonergic neurochemical systems. The data are extremely contradictory, however, particularly in regard to the effects on ethanol intake of the peripherally administered drugs, pCPA and 5-HTP, which have been used

to manipulate brain serotonin content. Often there has been little correspondence between the consummatory behavior itself and actual brain serotonin levels in those instances where these drug treatments did prove effective.

Therefore, three interrelated experiments were carried out to further assess the effects on preference behavior of pharmacological manipulations, especially pCPA treatment, known to alter brain serotonin levels. In the first, three doses of pCPA and one of 5-HTP were administered to rats to determine whether the drugs themselves or the resulting brain serotonin depletion had an effect on body weight and ad libitum water consumption. The second was intended to determine whether decreasing brain serotonin with pCPA and increasing it with 5-HTP would affect ethanol self-selection in opposite directions, while giving both treatments to the same animals would produce a cancellation of effects. In addition, procedural variables were investigated to help provide insight into whether any changes in preference behavior resulting from pCPA treatment were actually due to brain serotonin manipulation or if they could have been due to drug administration itself. To assess this possibility, pCPA was given during the first of three preference test sequences and also between preference test sequences. Administration of pCPA at these times had not previously been attempted. Finally, the third experiment was aimed at discovering whether pCPA treatment would affect the selection of three other solutions (saccharin, glucose, and sodium chloride), or if its effects were specific to the consumption of ethanol.

EXPERIMENT I

The purpose of this experiment was to determine quantitatively the effects of 10-day periods of pCPA or 5-HTP treatment on the water consumption and body weight of rats. Primary interest was focused on the effects of pCPA, since this drug would be employed extensively in later preference testing experiments. No previous researchers have systematically varied the dosage of pCPA and made quantitative determinations of resulting changes in water intake or body weight. Three dose levels of pCPA and one of 5-HTP were employed. The peripheral decarboxylase inhibitor, L-α-hydrazino-α-methyl-β-3,4-dihydroxyphenyl propionic acid (HMD), was used in conjunction with 5-HTP to reduce its side effects.

To help shed some light on how pCPA might be influencing water intake and body weight, the intermediate dose level (100 mg/kg) was administered to rats housed in standard metaboloism cages. This allowed monitoring of such variables as food intake, urine volume and urine electrolytes.

Method

Subjects

Thirty-six male Sprague-Dawley albino rats supplied by Holtzman Co. of Madison, Wisconsin served as subjects. Thirty animals

were housed individually in a standard Wahman laboratory rack. Each had Wayne Mouse Breeder Blox and tap water freely available. Twelve of the 30 were 118 days old and 18 were 137 days old at the start of experimentation. The remaining six rats were 135 days old when experimentation began, and they were housed in standard metabolism cages supplied by Acme Metal Products Co. They had powdered Mouse Breeder Blox and tap water available ad libitum. All 36 animals were maintained in rooms kept at 22-25°C which were on 14:10 light-dark cycles.

Procedure

At least two weeks of adaptation to the laboratory preceded the commencement of all baseline readings. Following adaptation, 24-hour measures of water intake and body weight were begun for the 30 animals in standard cages; food intake and urine volume were additionally recorded for the six rats in metabolism cages and a urine sample saved each day. The 3-bottle method of Myers and Holman (1966) was used to dispense water to the animals in standard cages. Three 125 ml. bottles with stainless steel drinking spouts were attached to the front of each cage. Two were filled with tap water while the third always remained empty. Bottle positions were changed daily according to the random schedule provided by Myers and Holman. Animals in metabolism cages had just one water bottle attached to the rear of the cage.

Following eight days of baseline measures, the 30 rats in standard cages were divided into groups matched for mean daily water consumption during baseline. Each of the six groups of five animals contained two younger and three older rats. Beginning on Day 8 and continuing for 10 consecutive days, each of the groups received one of

the following drugs and dosages administered approximately three hours after taking daily water intake and body weight readings:

(1) 50 mg/kg pCPA
(2) 100 mg/kg pCPA
(3) 200 mg/kg pCPA
(4) vehicle
(5) 75 mg/kg HMD + 25 mg/kg 5-HTP
(6) vehicle + vehicle

Upon completion of 10 days of drug administration, water intakes and body weights continued to be measured for approximately three weeks.

The six rats in metabolism cages underwent four days of base-line readings. Beginning on Day 4 and continuing for 10 consecutive days, each animal received 100 mg/kg pCPA approximately three hours after water intake, food intake, urine volume and body weight were recorded. All measures continued to be recorded for four days post-drug. Urine samples from each animal were saved and frozen on all 18 days of treatment. Later determinations of urine specific grativty were made by refractometry; urine sodium and potassium concentrations were determined by flame photometry.

Drug Preparation and Administration

DL-Para-chlorophenylalanine (Charles Pfizer Co.) was prepared as a suspension in 0.5% carboxymethylcellulose (CMC) solution and maintained under constant stirring prior to each administration. It was prepared at a concentration of 20 mg/ml and then the proper dosage was diluted with additional 0.5% CMC vehicle if necessary so that each animal received a constant fluid volume of 5 ml. The pCPA vehicle group received 5 ml. of CMC alone. Both pCPA and vehicle were administered by stomach tube under very light ether anesthesia. Animals were placed in a covered glass jar (25 cm. high X 22 cm. diameter) which was half

filled with ether-saturated cob meal. Thirty to 60 seconds elapsed, in most instances, before they were removed and intubation began.

The peripheral decarboxylase inhibitor L-α-hydrazino-α-methyl-β-3,4-dihydroxyphenyl propionic acid (Merck, Sharp & Dohme Co.) was prepared as a suspension in 0.9% saline at a concentration of 20 mg /ml and maintained under constant stirring until administered. The proper dosage of HMD was diluted with 0.9% saline if necessary so that each animal received a constant fluid volume of 3 ml. The vehicle group received 3 ml. of 0.9% saline. Both HMD and saline were administered intraperitoneally under very light ether anesthesia.

The chief reason for using HMD was to eliminate some of the undesired peripheral side effects of the decarboxylated products of 5-HTP. HMD inhibits aromatic L-amino acid decarboxylase without crossing the blood brain barrier. By so doing, it allows 5-HTP to be decarboxylated mainly in the central nervous system (Moore, 1971; Swonger & Rech, 1972) and potentiates the central action of a dosage of 5-HTP by four to six times.

Approximately 45-60 minutes after injecting HMD or its vehicle, DL-5-hydroxytryptophan (Sigma Chemical Co.) was administered. It was dissolved in 0.9% saline with gentle warming and prepared as a 10 mg/ml solution. Dilution with additional 0.9% saline followed if necessary, so that each animal received the proper dosage in a 2 ml. fluid volume. The vehicle group received 2 ml. of 0.9% saline, making a total of 5 ml. Both 5-HTP and vehicle were administered under very light either anesthesia.

All drugs were prepared fresh daily, and every animal received a total of 5 ml. of fluid on each day of treatment.

Results and Discussion

Figure 1 shows the mean water intake in grams during baseline, drug and post-drug periods for animals in standard cages receiving pCPA or CMC vehicle. The groups did not differ significantly during baseline. An analysis of variance did reveal differences in water intake among the groups during the 10-day drug period ($F = 7.14$, df 3/16, $p < .005$). A Duncan's test showed that the vehicle group drank significantly less than the pCPA-100 group ($p < .05$) and the pCPA-200 group ($p < .005$). The pCPA-50 group also drank significantly less than the pCPA-200 animals ($p < .005$). In comparing other pairs of groups, no significant differences in water intake were found between the vehicle and pCPA-50 groups, pCPA-50 and pCPA-100 groups, or the pCPA-100 and pCPA-200 groups.

Plotted in Figure 2 are the mean water intakes for animals in standard cages receiving HMD + 5-HTP or saline vehicle injections. No differences in intake existed during baseline, but the HMD + 5-HTP group drank significantly more than the control group during the 10 days of drug treatment ($F = 23.7$, df 1/18, $p < .001$).

Mean body weight for animals in standard cages receiving pCPA or CMC vehicle is plotted as a percentage of each group's weight on the last day of baseline in Figure 3. There were no differences among the groups during baseline. During the drug period, however, an analysis of variance showed the groups to be significantly different ($F = 17.94$, df 3/16, $p < .001$). A Duncan's test found the vehicle group to be significantly different from the pCPA-50 ($p < .005$), the pCPA-100 ($p < .001$) and the pCPA-200 ($p < .001$) groups. The pCPA-50 group also differed in body weight from the pCPA-200 group ($p < .01$).

Figure 1. Mean water intake in grams as a function of days during baseline, drug and post-drug periods for animals in standard cages receiving 50, 100, or 200 mg/kg pCPA or CMC vehicle alone during the 10-day drug period.

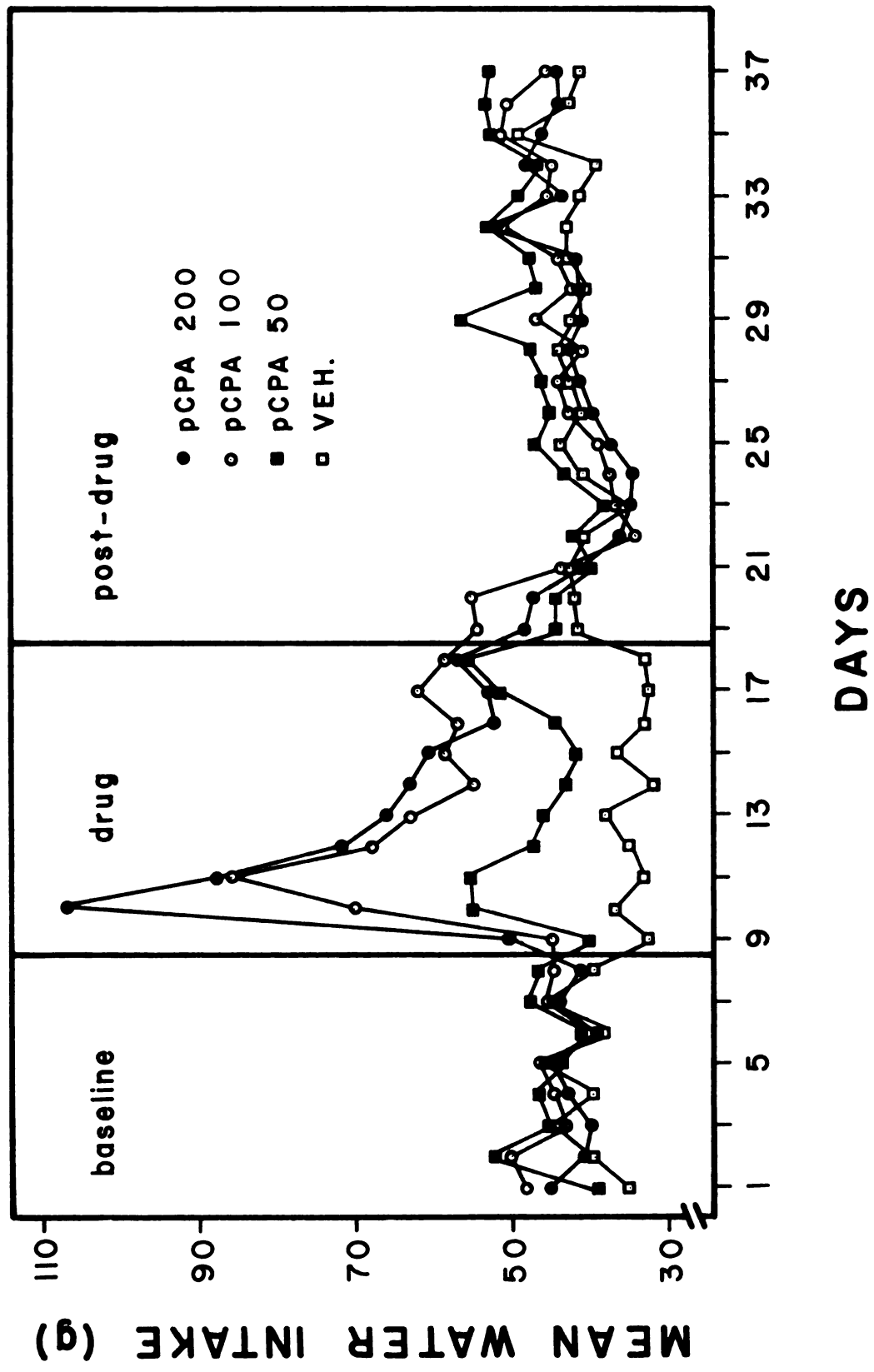

MEAN WATER INTAKE (g)
110
90
70
50
30
baseline
drug
post-drug
pCPA 200
pCPA 100
pCPA 50
VEH.
1
5
9
13
17
21
25
29
33
37
DAYS

Figure 2. Mean water intake in grams as a function of days during baseline, drug and post-drug periods for animals in standard cages receiving 75 mg/kg HMD + 25 mg/kg 5-HTP or two saline vehicle injections during the 10-day drug period.

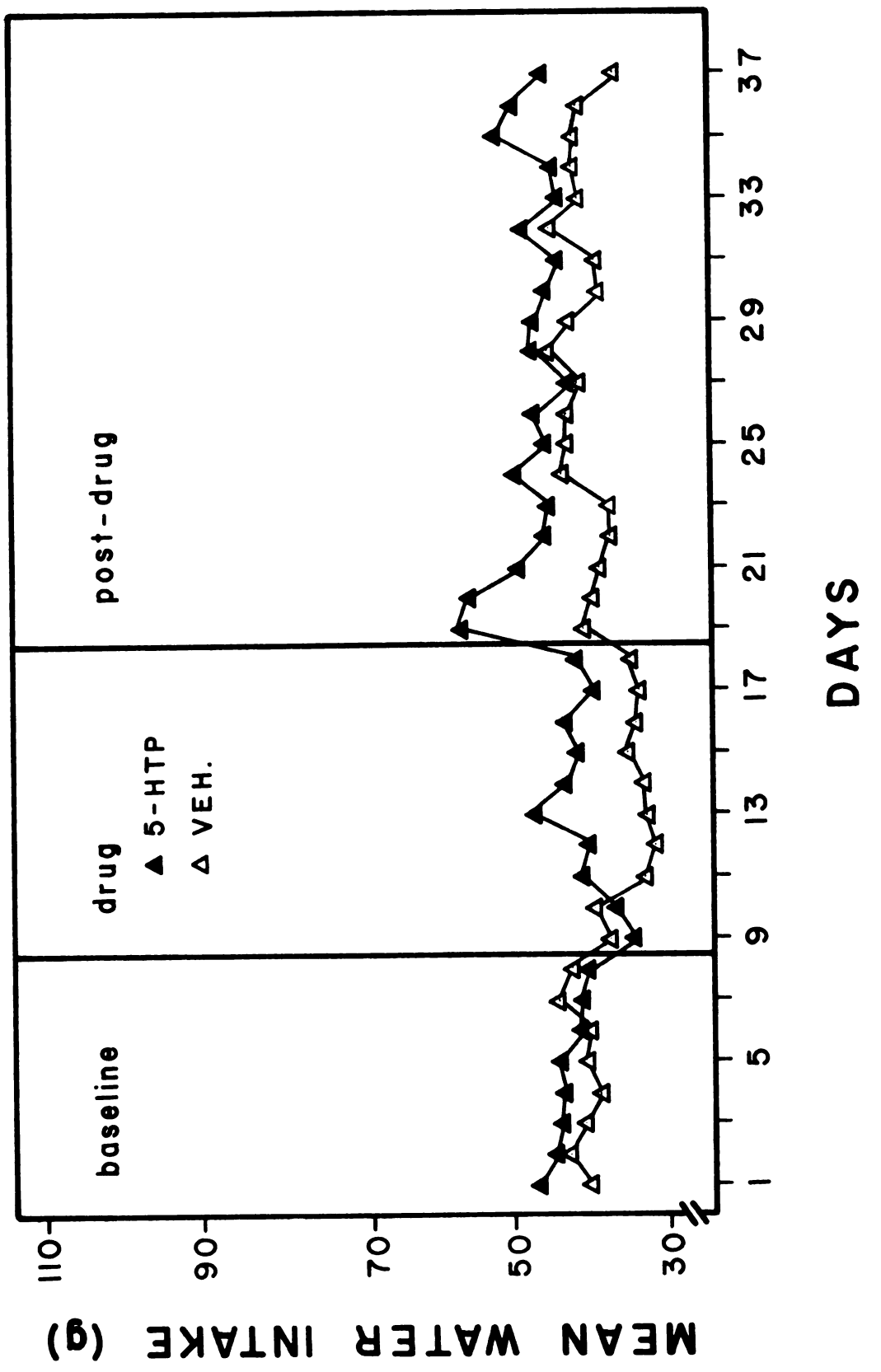

MEAN WATER INTAKE (g)
110
90
70
50
30
baseline
drug
▲ 5-HTP
△ VEH.
post-drug
1
5
9
13
17
21
25
29
33
37
DAYS

Figure 3. Mean percent body weight as a function of days during baseline, drug and post-drug periods for animals in standard cages receiving 50, 100, or 200 mg/kg pCPA or CMC vehicle alone during the 10-day drug period.

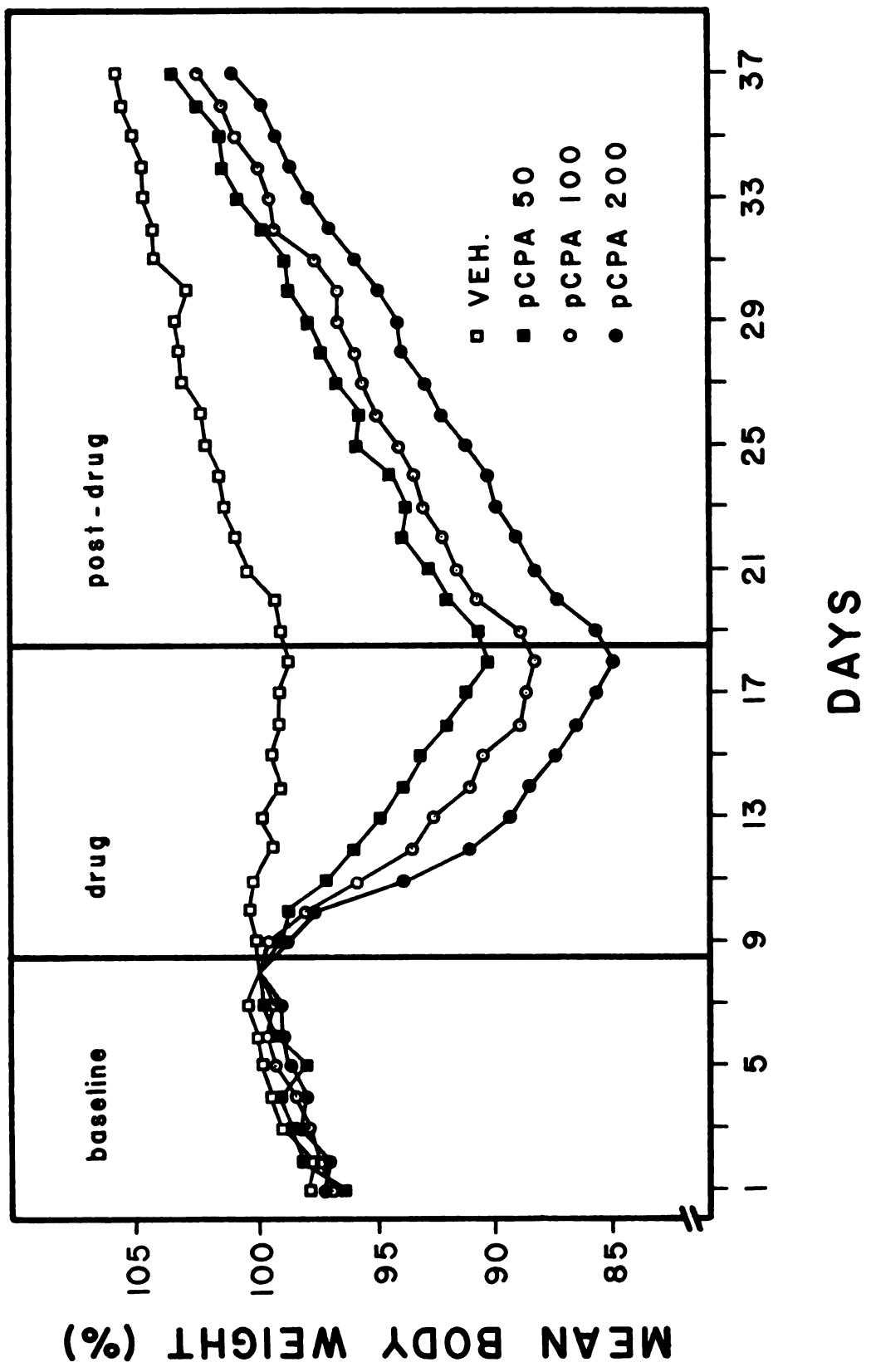

MEAN BODY WEIGHT (%)
105
100
95
90
85
baseline
drug
post-drug
1
5
9
13
17
21
25
29
33
37
DAYS
VEH.
pCPA 50
pCPA 100
pCPA 200

Figure 4 shows a similar plot of percent body weight for animals receiving HMD + 5-HTP or saline vehicle injections. The groups did not differ during baseline, but the HMD + 5-HTP group weighed significantly less than controls during the 10-day drug treatment period ($F = 11.3$, df 1/18, $p < .005$).

The water intake data for animals in standard cages were also analyzed in terms of g/kg water consumed as shown in Table 1. Analyses of variance revealed the pCPA-50, pCPA-100, pCPA-200, and pCPA vehicle groups to differ significantly on this measure during baseline ($F = 4.3$, df 3/28, $p < .025$), drug treatment ($F = 24.3$, df 3/36, $p < .001$) and the last nine days post-drug ($F = 8.2$, df 3/32, $p < .001$). During baseline the pCPA vehicle group was significantly different from the pCPA-50 ($p < .05$) and pCPA-100 ($p < .05$) groups. Also, the pCPA-50 animals differed significantly from the pCPA-200 animals ($p < .05$) and the pCPA-100 rats were reliably different from the pCPA-200 group ($p < .05$). While drugs were being administered, the pCPA vehicle group was significantly lower than the pCPA-50 ($p < .01$), pCPA-100 ($p < .001$) and pCPA-200 ($p < .001$) groups. The pCPA-50 rats were significantly below the pCPA-100 ($p < .01$) and pCPA-200 ($p < .001$) rats at this time also. For the last nine post-drug days the pCPA vehicle group was significantly different from the pCPA-50 ($p < .001$) and pCPA-100 ($p < .005$) groups; the pCPA-50 group was also reliably different from the pCPA-100 subjects ($p < .01$).

Analysis revealed that the HMD + 5-HTP group drank significantly fewer g/kg water than its vehicle control group during baseline ($p < .05$), drug treatment ($p < .001$), the first 10 days post-drug ($p < .001$) and the last nine post-drug days ($p < .001$).

Figure 4. Mean percent body weight as a function of days during baseline, drug and post-drug periods for animals in standard cages receiving 75 mg/kg HMD + 25 mg/kg 5-HTP or two saline vehicle injections during the 10-day drug period.

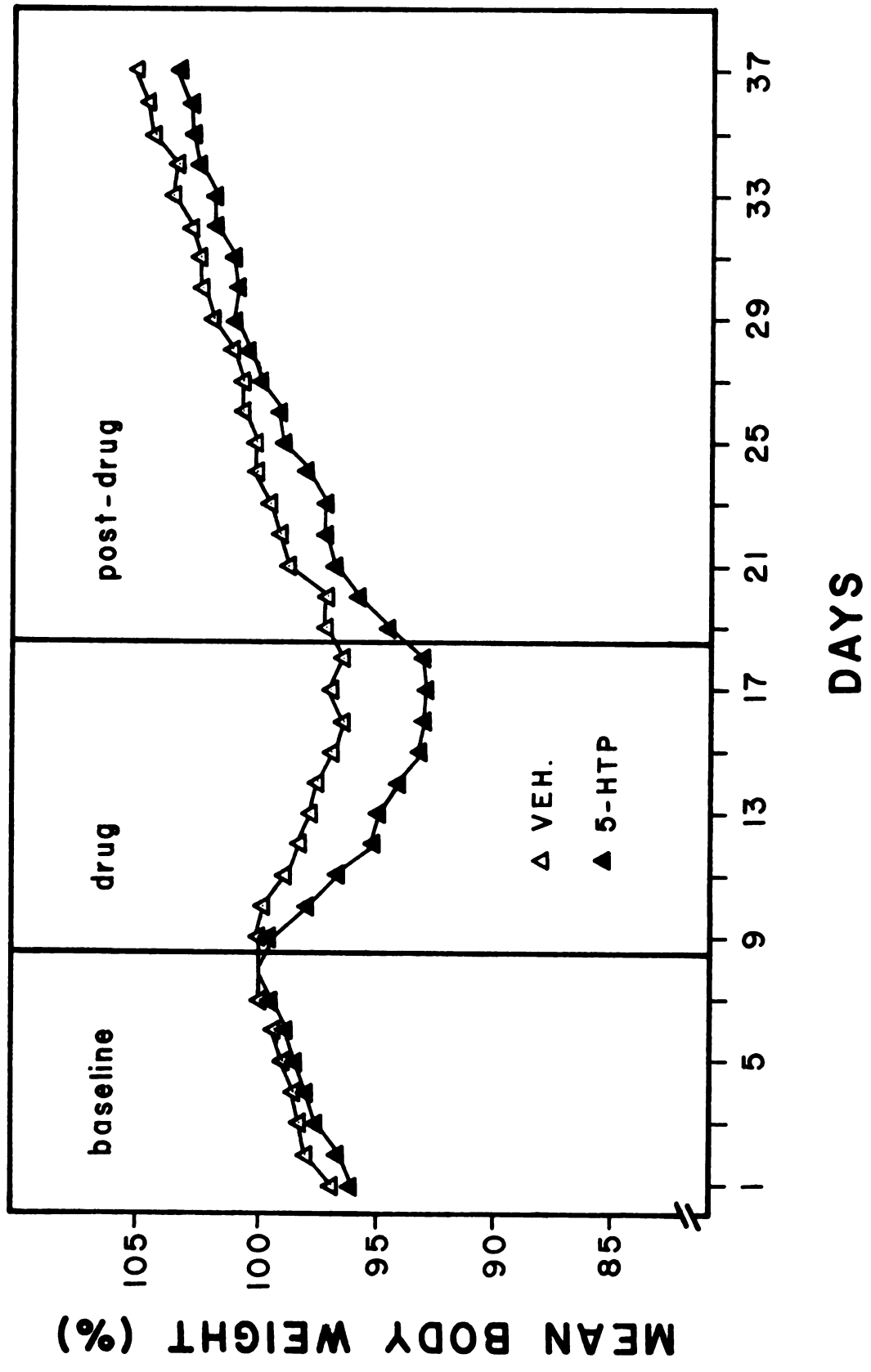

MEAN BODY WEIGHT (%)
105
100
95
90
85
baseline
drug
post-drug
Δ VEH.
▲ 5-HTP
1
5
9
13
17
21
25
29
33
37
DAYS

Table 1.--Mean water intake in g/kg during each day of baseline, drug treatment and post-drug period for rats of Experiment I housed in standard cages.

pCPA Vehicle Group

Baseline--72, 81, 87, 80, 90, 77, 86, 78

Drug Treatment--64, 72, 66, 71, 77, 65, 73, 67, 65, 67

First 10 Days Post-drug--83, 81, 80, 78, 75, 81, 84, 78, 80, 83

Last 9 Days Post-drug--81, 78, 81, 81, 79, 75, 94, 80, 78

pCPA-50 Group

Baseline--78, 103, 89, 90, 87, 80, 91, 89

Drug Treatment--78, 108, 110, 95, 94, 89, 88, 93, 111, 121

First 10 Days Post-drug--94, 93, 84, 87, 79, 89, 95, 91, 92, 94

Last 9 Days Post-drug--109, 89, 84, 103, 93, 86, 109, 100, 99

pCPA-100 Group

Baseline--98, 101, 87, 88, 92, 82, 87, 87

Drug Treatment--88, 141, 178, 143, 135, 120, 127, 127, 138, 130

First 10 Days Post-drug--120, 122, 95, 73, 78, 78, 80, 88, 91, 86

Last 9 Days Post-drug--92, 82, 83, 102, 89, 89, 102, 99, 87

pCPA-200 Group

Baseline--88, 80, 77, 83, 87, 77, 84, 78

Drug Treatment--96, 208, 178, 150, 140, 136, 126, 114, 118, 128

First 10 Days Post-drug--107, 102, 88, 77, 74, 72, 77, 82, 84, 85

Last 9 Days Post-drug--83, 82, 83, 100, 84, 91, 88, 82, 83

Table 1.--Continued.

5-HTP Group

Baseline--95, 90, 87, 87, 87, 82, 80, 78

Drug Treatment--66, 73, 82, 82, 96, 89, 87, 90, 84, 88

First 10 Days Post-drug--115, 112, 98, 92, 90, 97, 90, 92, 83, 93

Last 9 Days Post-drug--90, 86, 82, 91, 84, 83, 100, 96, 84

5-HTP Vehicle Group

Baseline--80, 85, 81, 77, 78, 78, 86, 76

Drug Treatment--72, 76, 65, 63, 66, 66, 70, 68, 68, 68

First 10 Days Post-drug--80, 82, 78, 76, 76, 83, 83, 77, 75, 86

Last 9 Days Post-drug--83, 82, 83, 100, 84, 91, 88, 82, 83

Table 2 provides the mean body metabolism data during baseline, pCPA treatment and post-drug period for the six animals which were housed in metabolism cages. It was expected that the data from these six animals might help to elucidate the causes for the pCPA-induced increases in water intake shown in Figure 1. As it turned out, the large transient increases in water consumption found previously to result from pCPA treatment did not occur among all six of these animals. Inspection of the individual drinking data revealed four quite different responses to 100 mg/kg daily oral doses of pCPA. Three animals actually showed slight decreases in water intake; one changed his intake little; one increased considerably and continued to drink excessively throughout the 10 days; and one other animal responded very similarly to the pCPA-100 group shown in Figure 1.

Therefore, the mean data presented in Table 1 do nothing to explain the pCPA-induced water consumption portrayed in Figure 1. It seems unwise to attempt to explain results which, for the most part, failed to be replicated by using data from a single animal who responded in the expected fashion. This unsuccessful attempt to replicate previous results was quite unexpected, since eight other animals housed earlier in metabolism cages and subjected to the same drug regimen all responded very much like the pCPA-100 group of Figure 1. Unfortunately, urine samples were not being saved at that time.

Only two known differences in procedure occurred during the unsuccessful attempt to replicate the water intake findings. They were: (a) the three-bottle method was not employed so animals had only one water bottle attached to the rear of the cage and (b) the pCPA

Table 2.--Daily body metabolism data ($\bar{X} \pm$ S.E.) during baseline, pCPA treatment and post-drug period for six animals.

Baseline				pCPA Treatment										Post-drug			
*BODY WEIGHT (%)																	
99	100	100	100	100	98	96	94	93	93	91	91	90	90	90	91	92	92
±1	±0	±0	±0	±0	±0	±1	±0	±2	±2	±2	±2	±2	±1	±1	±1	±1	±2
BODY WEIGHT (g)																	
482	484	483	484	480	475	465	458	452	448	442	440	436	434	436	439	442	444
±10	±9	±9	±9	±9	±9	±8	±8	±8	±8	±8	±7	±7	±7	±7	±6	±4	±4
FOOD INTAKE (g)																	
17	18	18	17	17	20	18	17	16	16	13	14	14	13	18	19	18	17
±1	±0	±1	±1	±2	±1	±1	±1	±2	±1	±1	±1	±1	±1	±0	±1	±1	±1
WATER INTAKE (g)																	
29	32	31	32	33	38	41	43	38	38	33	34	37	36	42	38	36	29
±2	±1	±2	±2	±4	±6	±6	±4	±6	±6	±4	±4	±6	±4	±9	±9	±7	±5
WATER INTAKE (g/kg)																	
59	65	65	65	69	80	88	94	84	86	74	77	85	82	96	88	82	66
±3	±3	±3	±3	±7	±14	±11	±8	±13	±14	±10	±8	±15	±10	±20	±21	±16	±12
URINE VOLUME (ml.)																	
18	18	20	19	30	34	36	35	33	32	30	28	34	30	26	23	23	17
±1	±1	±1	±2	±3	±4	±5	±4	±4	±5	±4	±3	±4	±4	±6	±6	±6	±3

Table 2.--Continued.

Baseline				pCPA Treatment										Post-drug			
#URINE SPECIFIC GRAVITY (refractive index)																	
423	424	412	417	402	408	409	404	413	410	411	411	401	402	437	456	462	476
±16	±12	±14	± 9	±9	±15	±11	±7	±13	±16	±13	±16	±12	±18	±23	±29	±28	±27
URINE SODIUM CONCENTRATION (mEq./L.)																	
164	170	146	148	130	124	104	84	100	100	94	90	86	90	142	164	178	220
±18	±14	±10	±16	±12	±14	±14	±12	±12	±16	±12	±16	±10	±12	±22	±28	±34	±36
URINE POTASSIUM CONCENTRATION (mEq./L.)																	
130	122	108	112	84	96	92	82	96	90	82	86	74	76	112	134	142	160
±12	±12	±8	±10	±8	±12	±12	±10	±12	±12	±12	±16	±10	±12	±20	±24	±28	±26

*Body weight is given as a percentage of the weight on the last day of baseline.

#Only the last three numbers of the refractive index are presented; full readings would take the form 1.3_ _ _.

used came from a different lot number than that administered to other animals. It seems unlikely that the number of water bottles would matter appreciably, but it is possible that the drug lot could have been a significant factor. It is unusual though that a whole range of responses to the same dose of pCPA was observed.

The extremely scant quantitative literature concerning pCPA's effects on water consumption are as contradictory as the present data. Brody (1969) found ad libitum water intake to be increased significantly by a single 300 mg/kg injection of pCPA. The effect was not secondary to urination and seemed partially independent of 5-HT levels, for a second dose five days later again increased water consumption. Brody never observed 100 mg/kg pCPA to facilitate ad libitum drinking. Panksepp and Nance (1974), on the other hand, found 20 daily intragastric doses of pCPA (200 mg/kg the first day; 100 mg/kg thereafter) to reduce water consumption by about 28%.

The preponderance of evidence from this experiment supports the notion that chronic pCPA treatment, in many instances, produces a dose dependent transient increase in water intake. That this increase might possibly be related to the pCPA-induced depletion of brain serotonin is supported by evidence from two raphe lesion studies. In each case raphe lesions produced a transient hyperdipsia beginning within a week of the lesion and lasting for three to five days (Lorens, Sorensen, & Yunger, 1971; Coscina, Grant, Balagura, & Grossman, 1972). Coscina et al. tested a group of raphe lesioned animals and found them to be no different than controls in excreting a water load; they did drink more than controls when nephrectomized. Therefore, it was concluded that the drinking was not secondary to urine output.

Contradicting the results of these two studies is that of Srebro and Lorens (1975). They found water intake to remain unchanged after ablation of the dorsal and median raphe nuclei.

It is quite possible, of course, that pCPA's effects on water intake are not related to its capacity for depleting brain serotonin. Instead they may be due to peripheral side effects of the drug. Alterations in kidney functioning, adrenal functioning or blood pressure could all lead to changes in water intake. Further research is needed along these lines.

The rise in water consumption resulting from HMD + 5-HTP treatment is consistent with the results of Joyce and Mrosovsky (1962). They found 5-HTP injections in doses ranging from 1.9 to 15 mg/rat to elevate water intake. It is difficult to attribute the present findings to 5-HTP alone, however, for HMD injections always preceded 5-HTP. One can only say that the HMD + 5-HTP combination reliably raises water consumption. Whether these are central or peripheral effects remains to be demonstrated.

With regard to body weight, it is obvious that pCPA administration had profound effects. Others have also found prolonged pCPA treatment to reduce body weight considerably (Myers & Veale, 1968; Panksepp & Nance, 1974). Table 1 indicates that the food intake of animals in metabolism cages did not begin to fall until the fifth day of treatment, a time when body weight was already reduced approximately 7%. Urine volume was elevated from the first day of pCPA treatment. This may mean that some of the initial body weight losses were due to body water losses. The data in Table 1 cannot validly be used to explain the weight losses of those animals represented in Figure 3,

unfortunately, because the drinking patterns were considerably different. Either decreases in food intake or increases in body water loss could have accounted for their rapid initial body weight decline.

Panksepp and Nance (1974) speculate that the reduced food intake and body weight of their chronically pCPA-treated rats was due to lowered brain serotonin levels. However, their animals, like those in the present study, began to gain weight almost immediately when drug administration ceased. This is in spite of the high probability that brain serotonin levels remained depressed for at least a few days and returned to normal only very slowly (Koe & Weissman, 1966).

To my knowledge, no one has investiaged the effects of prolonged HMD + 5-HTP treatment on body weight. The combination significantly reduces body weight as seen in Figure 4. The relationship of this reduction to food intake, urine output, brain serotonin levels, etc., remains to be elucidated.

EXPERIMENT II

The main thrust of this experiment was to make a precise specification of the effects on ethanol self-selection of pharmacological manipulations known to alter brain serotonin level. It was expected that this would help to determine whether resulting changes in ethanol selection were actually related to brain serotonin level or could be interpreted as reflecting drug effects and/or procedural variables.

Since it is thought that a valid measure of ethanol selection can only be obtained by sampling preference-aversion patterns over a wide range of concentrations (Gillespie & Lucas, 1958; Fuller, 1964), the multiple concentration method of preference testing with three repetitive ethanol test sequences was employed. The effects of pCPA on ethanol selection were determined when pCPA was administered (a) during the initial ethanol preference sequence, (b) during the second ethanol sequence, and (c) between two ethanol sequences so that drug treatment and preference testing did not coincide.

Ethanol choice behavior was also studied while 5-HTP (in conjunction with HMD) was being administered to raise brain serotonin levels. This drug combination was given to some animals during the second ethanol preference sequence and to others during sequence three after pCPA had been previously used to deplete serotonin levels.

Method

Subjects

Eighty adult male Sprague-Dawley albino rats of the Holtzman strain served as subjects. All were 120 days old at the start of experimentation. They were housed in individual cages with Wayne Mouse Breeder Blox and tap water freely available. The room was maintained on a 14:10 light-dark cycle and kept at 22-25°C.

Procedure

Upon arrival in the laboratory, animals were randomly divided into groups of eight animals each. Following at least two weeks of adaptation, ethanol preference testing procedures were begun. An ethanol preference test sequence required eight days to complete and consisted of offering each rat a choice between tap water and a different ethanol solution on each day for eight consecutive days. The ethanol solutions were always increased in concentration daily in the following ascending order: 2, 4, 6, 8, 10, 12, 16, and 20%. All ethanol solutions were prepared fresh daily from 95% ethyl alcohol and tap water using a weight/weight method (Pfaffman, Young, Dethier, Richert, & Stellar, 1954). The 3-bottle, 24 hour preference test procedure of Myers and Holman (1966) was employed. Three 125 ml. bottles with stainless steel drinking spouts were attached to the front of each cage. One contained tap water, another the ethanol solution and the third remained empty. Bottle positions were changed daily according to the random schedule provided by Myers and Holman. Drinking spouts were also rinsed and randomly changed every day. These

steps were taken to help prevent bottle position and drinking spout preferences from developing.

At approximately the same time each day all animals were weighed. Their fluid bottles were also weighed to determine ethanol and water consumptions. Care was taken to keep fluid spillage to a minimum when removing and replacing bottles. Spillage ranged from 0.00 to 0.30 g. when bottles were placed on cages and from 0.00 to 1.00 g. when bottles were removed. In removing bottles, more was spilled from those which contained less fluid.

Except where indicated below, all groups received three 8-day ethanol-water preference test sequences with four water-only days separating each. The 10 groups received the following drug treatments:

PREF. TEST #1	PREF. TEST #2	PREF. TEST #3
(1) 100 mg/kg pCPA	no drug	no drug
(2) vehicle	no drug	no drug
(3) no drug	100 mg/kg pCPA	no drug
(4) no drug	vehicle	no drug
(5) no drug	60 mg/kg HMD +20 mg/kg 5-HTP	no drug
(6) no drug	60 mg/kg HMD +vehicle	no drug
(7) no drug	100 mg/kg pCPA	60 md/kg HMD +20 mg/kg 5-HTP
(8) no drug	100 mg/kg pCPA	60 mg/kg HMD + vehicle

PREF. TEST #1	NO PREF. TEST	PREF. TEST #2
(9) no drug	100 mg/kg pCPA	no drug
(10) no drug	vehicle	no drug

Drug Preparation and Administration

All drugs were prepared and administered in the same manner as those of Experiment I, with one exception. Groups 1 and 2, unlike all others, received their gavage without ether anesthesia. This procedure proved to be too stressful, especially for pCPA animals who seemed not to adapt well, and was discontinued in favor of light ether anesthetization for later groups.

Each group received drug or vehicle treatment for 10 consecutive days, beginning two days prior to and continuing throughout an ethanol preference test sequence. Groups 9 and 10 received 16 water-only days between their first and second ethanol preference tests. Intubation of their pCPA or vehicle began two days after the first test ended and continued for 10 days. During this time the animals were not allowed access to ethanol. Following drug treatment there were, as for all other groups, four additional water-only days before the start of the next preference test sequence.

Results and Discussion

Two measures of self-selection behavior were employed in analyzing the intake data. The first was mean proportion ethanol (ETOH) to total fluids consumed. This is also called the preference ratio and was calculated in the following manner:

$$\frac{\text{mean g ETOH consumed}}{\text{mean g ETOH consumed + mean g water consumed}}$$

The second measure was mean g/kg consumed as absolute alcohol which was calculated as such:

$$\frac{\text{mean g ETOH consumed X g absolute alcohol per g solution}}{\text{mean body weight / 1000}}$$

Since each preference test consisted of 24-hour access periods to eight different ethanol concentrations, it was possible to plot each measure as a function of ethanol concentration for each preference test. This was done for all 10 groups described in the methods section, resulting in two figures for each experimental group and two for each control group.

For the purpose of statistical analysis, however, mean proportion ETOH consumed and mean g/kg alcohol consumed were collapsed across concentration for each animal for each preference sequence. It was then possible to do a repeated measures analysis of variance (Winer, 1962, p. 105) on the mean daily preference ratios and mean daily g/kg alcohol consumed for each group to determine if significant differences existed among the preference test sequences. If differences were found for either measure for any group, a Duncan's multiple range test (Bruning & Kintz, 1968, p. 115) was conducted to reveal which preference sequences differed from which others. All data were handled in this manner. Concentration effects were statistically analyzed only in specific instances.

Table 3 gives the mean daily preference ratios ($\pm$ S.E.), mean daily g/kg alcohol consumed ($\pm$ S.E.) and drug treatments for each group

Table 3.--Drug treatments, mean daily preference ratios (± S.E.), and mean daily g/kg alcohol consumed (± S.E.) for each group of Experiment II.

	Drug Treatment	Mean Daily Pref. Ratio	Mean Daily g/kg Alc. Consumed
Test #1	no drug	36 ± 5.6	2.05 ± 0.42
Test #2	pCPA	19 ± 4.7	1.51 ± 0.40
Test #3	no drug	12 ± 4.1	0.73 ± 0.22
Test #1	no drug	36 ± 4.7	1.86 ± 0.29
Test #2	CMC	39 ± 7.7	2.15 ± 0.44
Test #3	no drug	40 ± 7.0	2.38 ± 0.45
Test #1	no drug	39 ± 4.2	1.91 ± 0.35
Test #2	pCPA	15 ± 4.3	1.35 ± 0.44
Test #3	HMD + 5-HTP	18 ± 7.4	1.01 ± 0.51
Test #1	no drug	35 ± 4.0	1.80 ± 0.21
Test #2	pCPA	18 ± 5.6	1.39 ± 0.44
Test #3	HMD + NaCl	18 ± 5.6	0.77 ± 0.24
Test #1	no drug	40 ± 3.6	1.75 ± 0.18
Test #2	HMD + 5-HTP	19 ± 4.1	1.18 ± 0.23
Test #3	no drug	20 ± 5.0	0.94 ± 0.34
Test #1	no drug	44 ± 5.1	2.08 ± 0.45
Test #2	HMD + NaCl	39 ± 7.2	2.44 ± 0.50
Test #3	no drug	45 ± 7.1	2.42 ± 0.46

Table 3.--Continued.

	Drug Treatment	Mean Daily Pref. Ratio	Mean Daily g/kg Alc. Consumed
Test #1	pCPA	11 ± 4.0	0.62 ± 0.17
Test #2	no drug	21 ± 6.9	1.36 ± 0.53
Test #3	no drug	37 ± 7.6	2.80 ± 0.59
Test #1	CMC	19 ± 2.7	0.81 ± 0.12
Test #2	no drug	26 ± 5.6	1.65 ± 0.36
Test #3	no drug	31 ± 6.9	2.18 ± 0.45
Test #1	no drug	28 ± 2.7	1.27 ± 0.21
No Test	pCPA	----	----
Test #2	no drug	34 ± 8.4	1.75 ± 0.53
Test #1	no drug	29 ± 4.2	1.51 ± 0.35
No Test	CMC	----	----
Test #2	no drug	42 ± 7.6	2.69 ± 0.57

in Experiment II. It is upon these data which the statistical analyses are based.

Mean proportion ethanol consumed for rats receiving pCPA during the second preference test is shown in Figure 5. The same measure is plotted in Figure 6 for CMC vehicle control animals. Significant differences were found among the tests for the pCPA group ($F = 11.05$, df 2/14, $p < .005$). Preference was significantly below baseline during pCPA treatment ($p < .01$) and during the post-drug period ($p < .001$). As seen in Figure 5, the greatest depression in preference occurred mainly at the lower concentrations. There were no significant differences in preference among the three tests for CMC vehicle control animals, although Figure 6 shows a slight acclimation effect with preference elevated at the middle concentrations during the second and third preference sequences. Such was not the case for pCPA treated subjects. Mean g/kg alcohol consumed for the pCPA treated group is plotted in Figure 7, while similar data for CMC vehicle controls can be seen in Figure 8. Mean g/kg consumed was significantly different among the three tests for the pCPA group ($F = 6.11$, df 2/14, $p < .025$), with post-drug ingestion being significantly lower than baseline ($p < .01$). No significant differences in g/kg alcohol consumed were found among the three tests for the CMC vehicle group. Figure 8 does reveal an increased consumption at the middle concentrations during the second and third tests.

These results confirm the original findings of Myers and his collaborators (Myers & Veale, 1968; Myers & Cicero, 1969; Veale & Myers, 1970; Myers & Tytell, 1972; Myers & Martin, 1973). They too found pCPA treatment during the second preference test to exert its

Figure 5. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 6. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving CMC vehicle during the second of three preference test sequences.

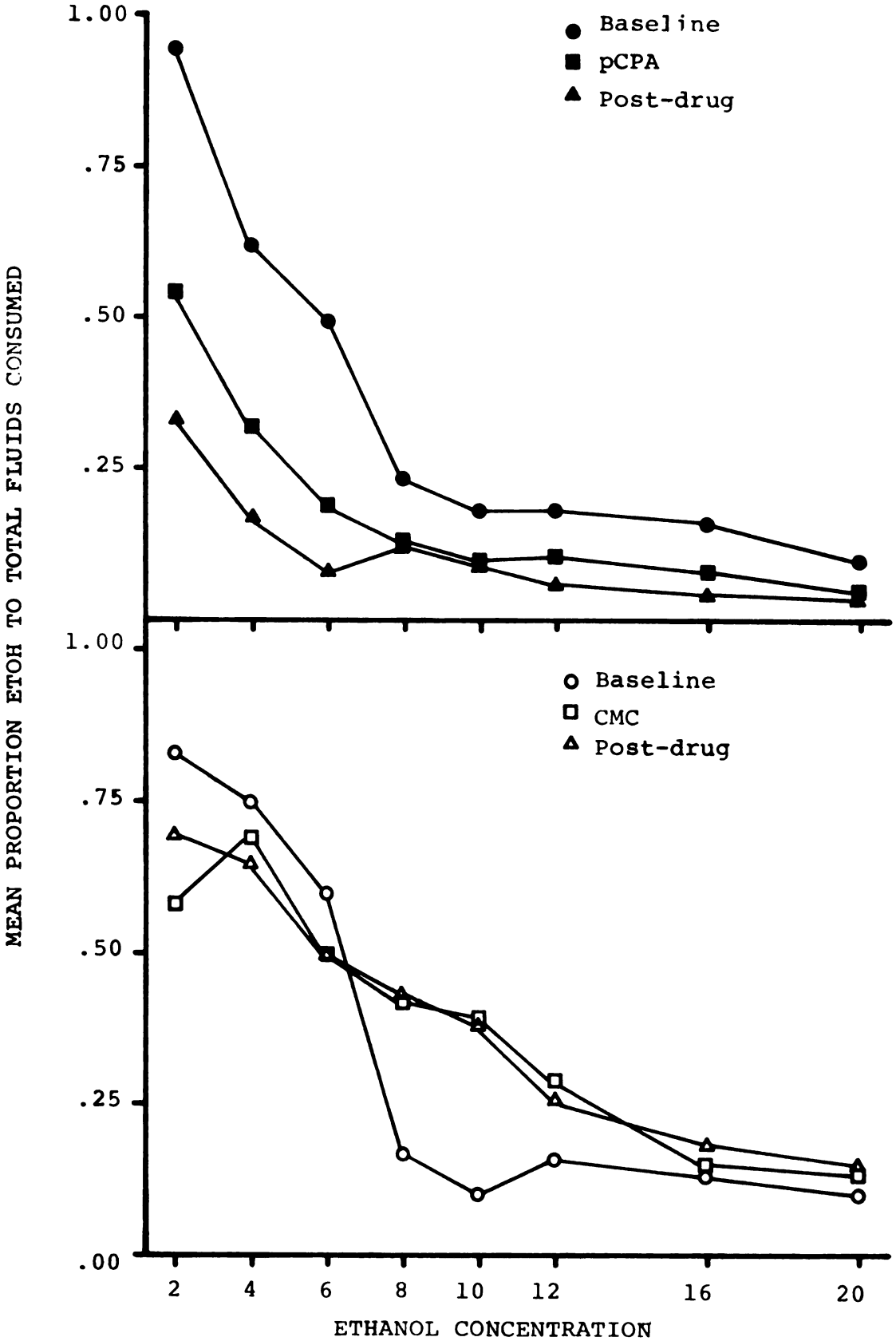
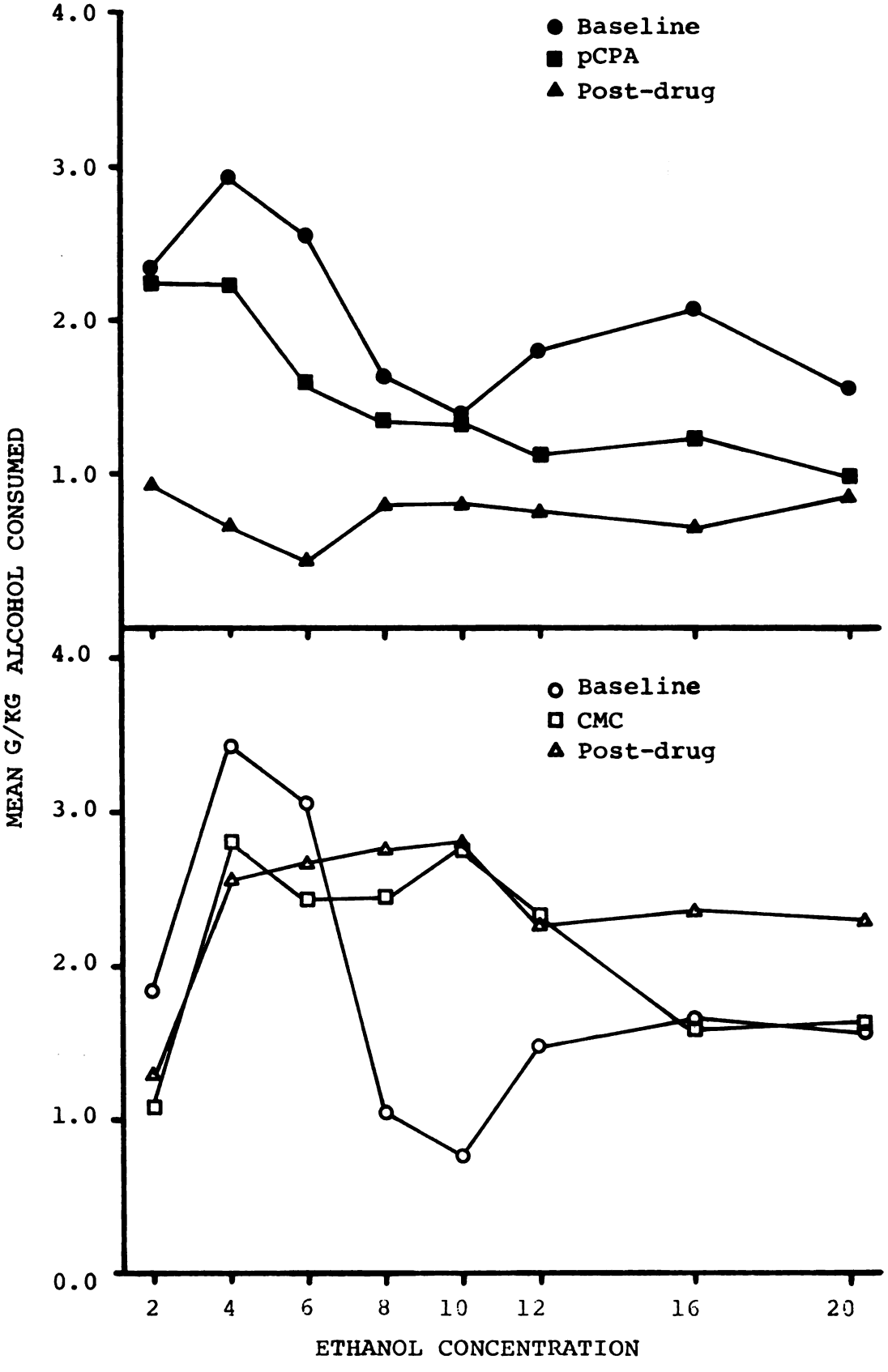

1.00
.75
.50
.25
1.00
.75
.50
.25
.00
Baseline
pCPA
Post-drug
Baseline
CMC
Post-drug
MEAN PROPORTION ETOH TO TOTAL FLUIDS CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

Figure 7. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 8. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving CMC vehicle during the second of three preference test sequences.


Baseline
pCPA
Post-drug
Baseline
CMC
Post-drug
MEAN G/KG ALCOHOL CONSUMED
4.0
3.0
2.0
1.0
0.0
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

primary influence on ethanol self-selection after drug administration had ended. As Figures 5 and 7 reveal, it was during the post-pCPA preference test when both proportion of ethanol and g/kg alcohol consumed were maximally depressed. Control animals, on the other hand, did not differ significantly from baseline in either preference of g/kg consumed during either of the two sequences which followed baseline testing. This indicates that it was in fact pCPA treatment producing the effects in experimental animals.

There seems to be no way to reconcile the present data with those of Cicero and Hill (1970) who failed to find a pCPA effect when solutions were diluted from 95% alcohol. All ethanol solutions used in Experiment II were made from 95% alcohol, and quite significant effects were observed. In any case, the Cicero and Hill results apparently stand alone in this research area. Most other investigators have prepared solutions from other than absolute alcohol and yet found pCPA to influence ethanol selection (Frey, Magnussen, & Nielsen, 1970; Myers, Evans, & Yaksh, 1972; Geller, 1973).

In the studies of Frey, Magnussen, and Nielsen (1970) and Geller (1973) animals were given a choice between tap water and only one concentration of ethanol. Geller employed extended periods of drug treatment with pCPA administered in daily intragastric doses ranging from 50 to 300 mg/kg. He found this to increase ethanol preference. Frey, Magnussen, and Nielsen, as did the Myers group, gave daily intragastric doses of 300 mg/kg pCPA and produced a decreased ethanol selection. It is unlikely that the conflicting results of these two experiments are due to drug dose, for Figures 5 and 7 show that 100 mg/kg pCPA will effectively suppress ethanol consumption.

Unfortunately, neither Geller nor Frey, Magnussen, and Nielsen computed g/kg absolute alcohol consumed for their animals so no comparisons can be made between their studies and the present one on that measure. In any case, the difference in preference methodologies also makes direct comparison difficult. The results shown in Figures 5 and 7 do, nonetheless, support the data of Frey, Nagmussen, and Nielsen, leaving Geller as still the only investigator producing elevated ethanol selection with pCPA.

The very recent experiment by Holman, Hoyland, and Shillito (1974) conflicts with the results of this study and with all of the previous studies mentioned. They gave pCPA intermittently in two different dose regiments: (1) 316 mg/kg followed four days later by 100 mg/kg or (2) 316 mg/kg three times at three day intervals. Neither treatment had any effect on ethanol selection. They used the multiple concentration method and began drug treatment two days before the second of three preference test sequences. Drugs were administered intraperitoneally. Assays for whole brain 5-HT showed their intermittent drug treatment to be as effective as eight daily intragastric doses of 316 mg/kg pCPA at reducing serotonin level. Their results definitely throw into question the hypothesis that the effects of pCPA on ethanol selection are related to brain serotonin. They suggest that a conditioned aversion may be operating in the experiments of the Myers group to cause the reduction in ethanol intake. Such an interpretation could apply to the results shown in Figures 5 and 7 also. Since 48 hours separated their initial injection from the start of ethanol preference testing, they suggest that a conditioned aversion may not have been produced in their own animals. The

conditioned aversion hypothesis, however, does nothing to explain Geller's finding of pCPA-induced ethanol selection increases. His study cannot be highly regarded though. A close inspection of his data for individual animals shows pCPA to sometimes reduce ethanol intake before increasing it and to sometimes suppress ethanol intake after treatment stopped. Geller totally ignores such discrepancies.

In general, there does not seem to be a close correlation between brain serotonin level and the changes in ethanol selection depicted in Figures 5 and 7. The standard procedure for producing maximal brain serotonin depletion involves administering 316 mg/kg pCPA for one day or giving 100 mg/kg pCPA on three consecutive days (Koe & Weissman, 1966). Therefore, the brains of pCPA-treated rats should have been maximally depleted of serotonin during most, if not all, of the second preference test, since 100 mg/kg daily intragastric doses of pCPA began two days prior to preference testing. During test two, however, the animals had not significantly decreased their intake of alcohol in g/kg. Only during the post-drug sequence were there reductions in both preference and g/kg alcohol consumed. Brain serotonin should still have been depleted at this time although most likely on the rise. No data have been published on the recovery of brain serotonin following extended periods of daily pCPA treatment, but it is known that 12 to 16 days are required for full recovery after a single 316 mg/kg injection (Koe, 1971). If brain serotonin plays a crucial role in determining an animal's ethanol selection, one would expect the effects of 5-HT depletion to be maximal at the time when depletion is maximal. Such was not the case in this experiment or in those conducted by Myers and coworkers.

In Figures 9 and 10 are plotted the mean proportion ethanol consumed for animals receiving pCPA on the second preference sequence followed by either HMD + 5-HTP or HMD + NaCl, respectively, on the third sequence. Both groups responded in precisely the same manner. Significant differences in preference were found among the three tests for each ($F = 20.21$, df 2/14, $p < .001$ for HMD + 5-HTP group; $F = 4.96$, df 2/14, $p < .025$ for HMD + NaCl group). Mean proportion of ethanol consumed was significantly decreased for both groups during pCPA administration ($p < .001$ for HMD + 5-HTP group; $p < .05$ for HMD + NaCl group) and also during the post-pCPA sequence ($p < .001$ for HMD + 5-HTP group; $p < .05$ for HMD + NaCl group) when an attempt was made to elevate brain serotonin in experimental animals. An examination of Figures 9 and 10 reveals that these animals responded very similarly to the animals receiving pCPA on the second sequence (Figure 5) and nothing on the third. The suppression of preference for all of these groups was most pronounced at lower concentrations; there is, of course, less possibility for decrease at the upper concentrations and spillage may be a more important factor. Mean g/kg alcohol consumed for animals receiving pCPA on the second sequence and either HMD + 5-HTP or HMD + NaCl on the third sequence is shown in Figures 11 and 12, respectively. Again, significant differences among the three tests were shown for both groups ($F = 3.77$, df 2/14, $p < .05$ for the HMD + 5-HTP group; $F = 3.91$, df 2/14, $p < .05$ for the HMD + NaCl group). Mean g/kg consumed was significantly lower than baseline only during the post-pCPA sequence for each group ($p < .05$ for each). Alcohol ingested during the pCPA sequence was not significantly different from baseline or post-pCPA sequences. These data are also strikingly

Figure 9. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving pCPA during the second and HMD + 5-HTP during the third of three preference test sequences.

Figure 10. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving pCPA during the second and HMD + NaCl during the third of three preference test sequences.

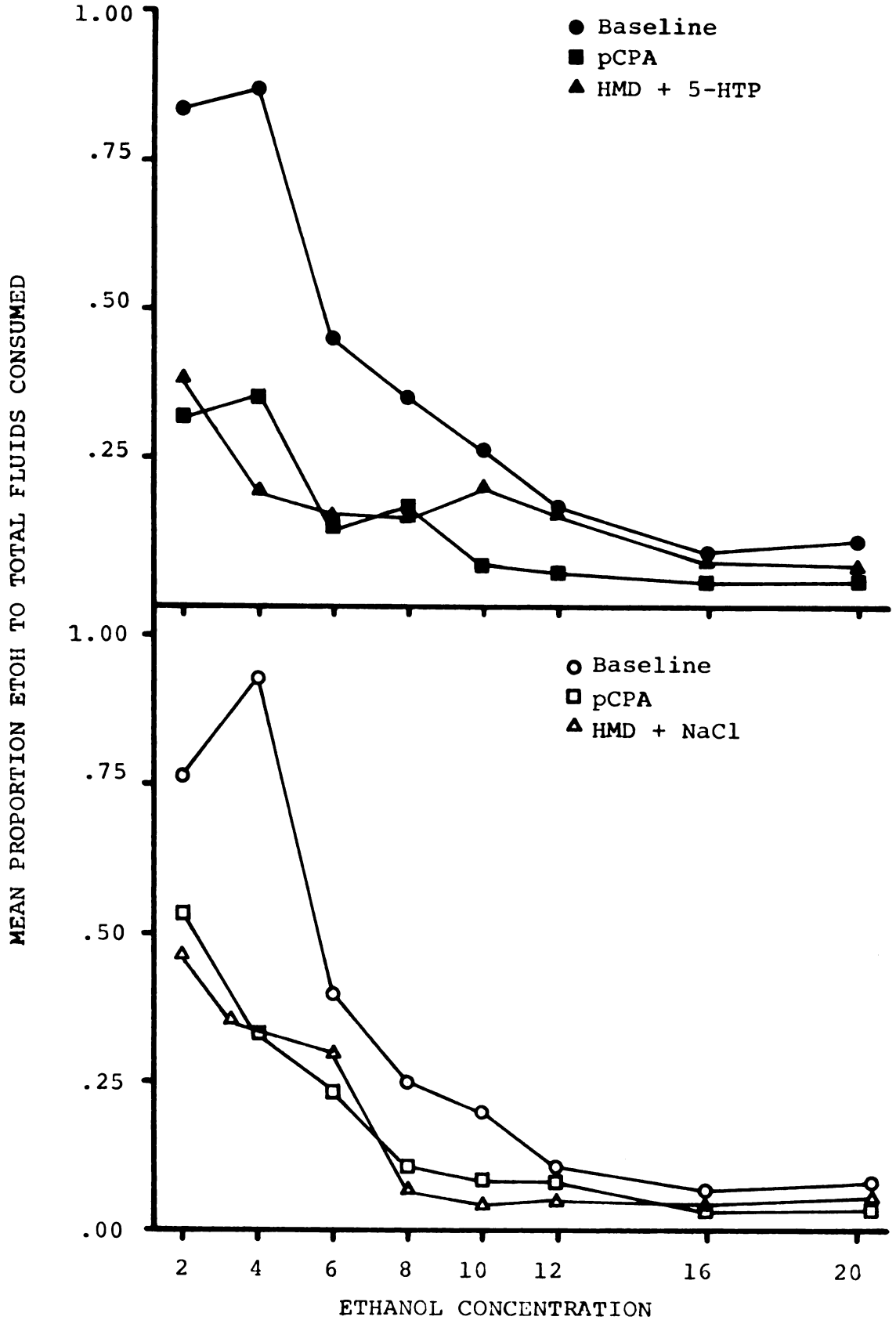
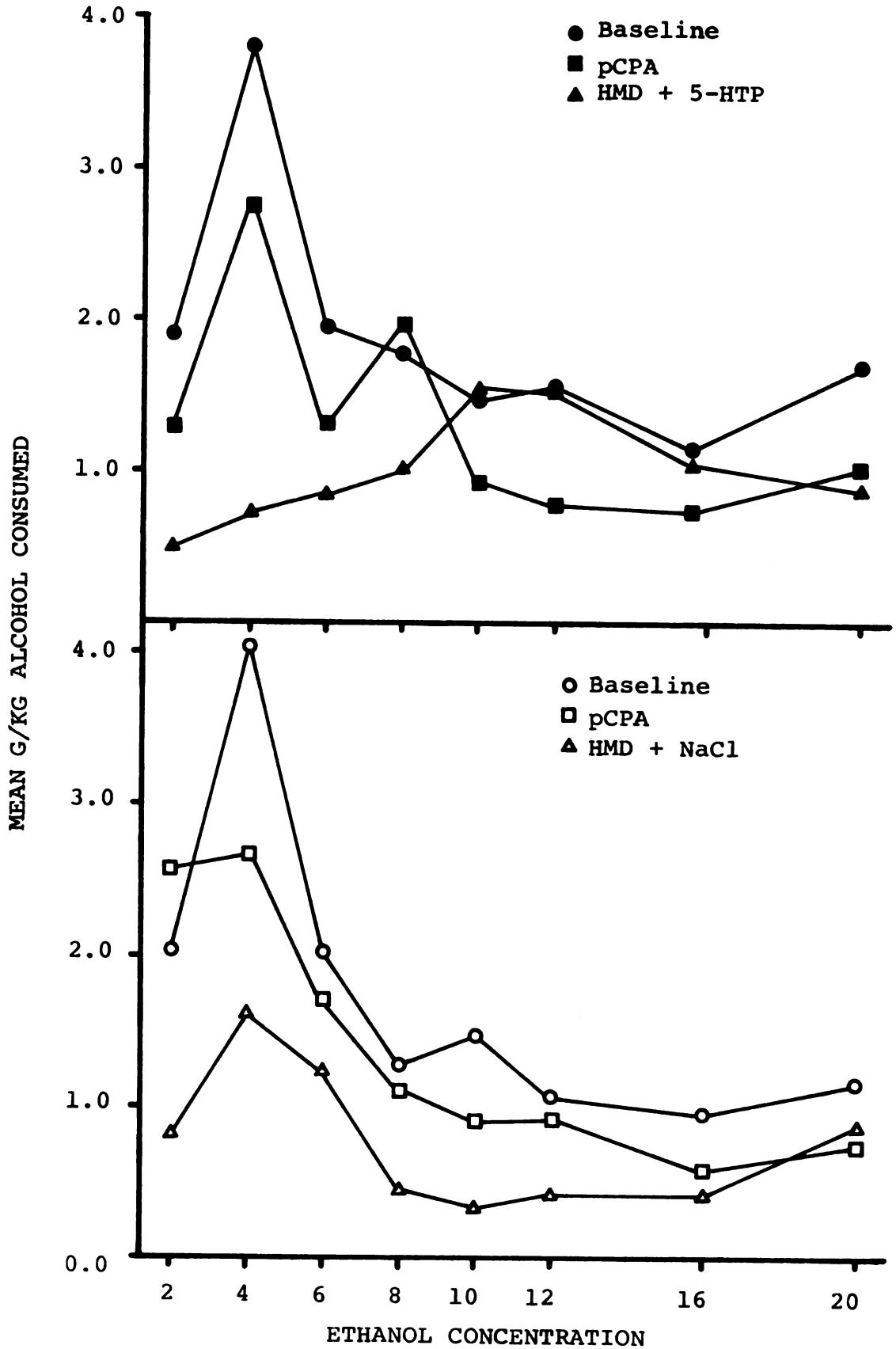

1.00
.75
.50
.25
1.00
.75
.50
.25
.00
Baseline
pCPA
HMD + 5-HTP
Baseline
pCPA
HMD + NaCl
MEAN PROPORTION ETOH TO TOTAL FLUIDS CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

Figure 11. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving pCPA during the second and HMD + 5-HTP during the third of three preference test sequences.

Figure 12. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving pCPA during the second and HMD + NaCl during the third of three preference test sequences.


4.0
3.0
2.0
1.0
Baseline
pCPA
HMD + 5-HTP
4.0
3.0
2.0
1.0
0.0
Baseline
pCPA
HMD + NaCl
MEAN G/KG ALCOHOL CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

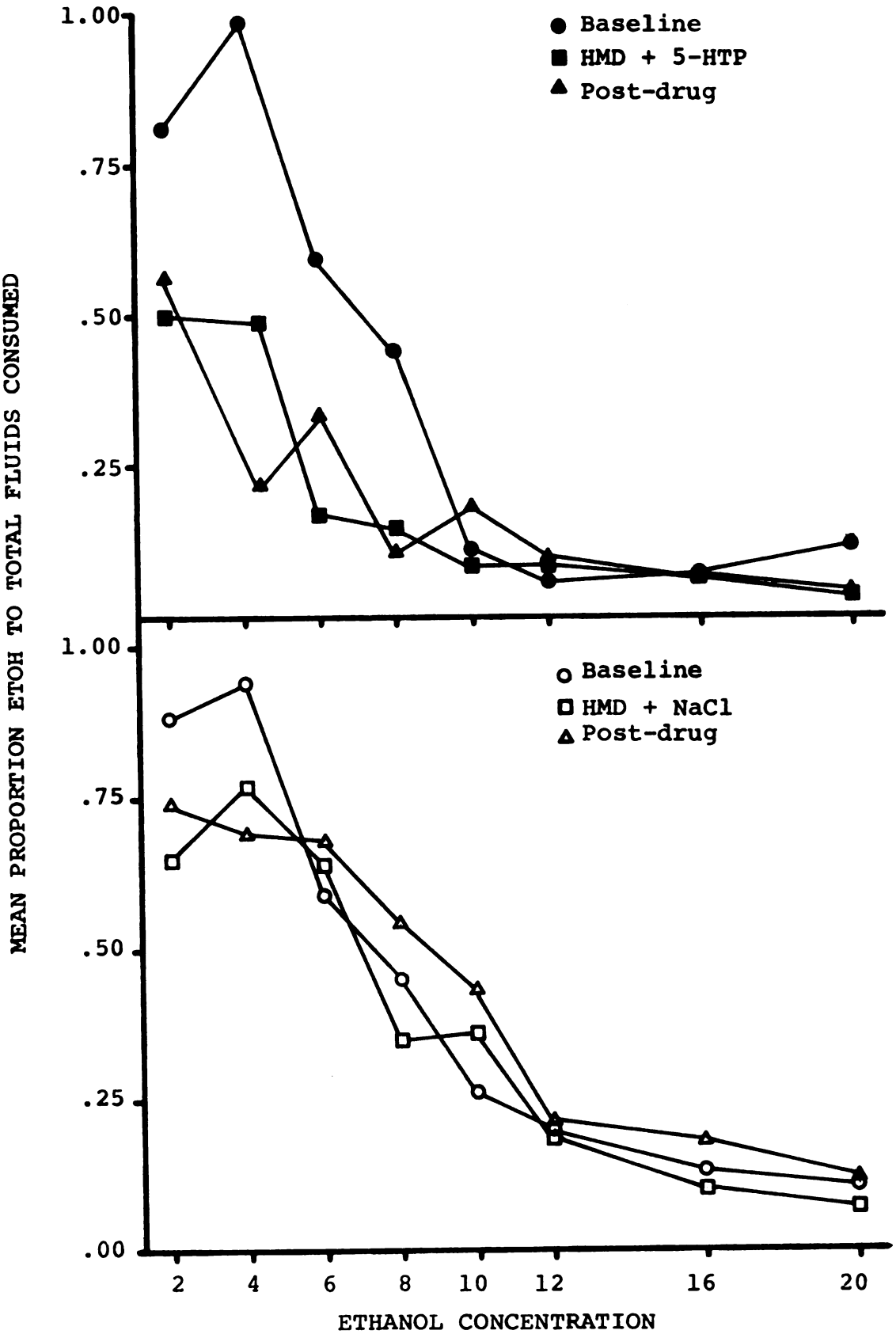
similar to those from animals receiving pCPA on the second sequence and no treatment whatsoever during the third (Figure 7).

The 5-HTP injections were totally ineffective at restoring to baseline levels either preference for ethanol or g/kg alcohol consumed after pCPA treatment. Although only one dose of HMD + 5-HTP was employed, it is quite likely that this treatment restored brain serotonin level to normal or above. Nance and Kilby (1973) were successful at repleting whole-brain serotonin and eliminating pCPA's effects on sucrose consumption with just 50 mg/kg 5-HTP and no peripheral decarboxylase inhibitor. They are the only previous investigators who have attempted to produce a cancellation of effects in self-selection studies by giving pCPA and 5-HTP to the same animals. The 20 mg/kg dose of 5-HTP used in this study was well within the range of doses (50-150 mg/kg) often administered to reverse the effects of pCPA by researchers studying other serotonergic mechanisms (Jouvet, 1973; Shopsin, Shenkman, Sanghvi & Hollander, 1974). With peripheral decarboxylation inhibited, quantities of 5-HTP reaching the brain should have been in the 80 to 120 mg/kg range due to the resulting potentiation of central nervous system dosage. All in all, the lack of efficacy of HMD + 5-HTP in pCPA-treated animals casts further doubt on the serotonin hypothesis of ethanol selection.

Plotted in Figure 13 is mean proportion ethanol consumed by rats receiving HMD + 5-HTP during preference test two, while Figure 14 shows the same measure for control rats who got HMD + NaCl during test two. Significant differences in preference among the tests were revealed for the HMD + 5-HTP group ($F = 8.57$, df 2/14, $p < .005$). Significantly decreased preference occurred during drug treatment

Figure 13. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving HMD + 5-HTP during the second of three preference test sequences.

Figure 14. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving HMD + NaCl during the second of three preference test sequences.

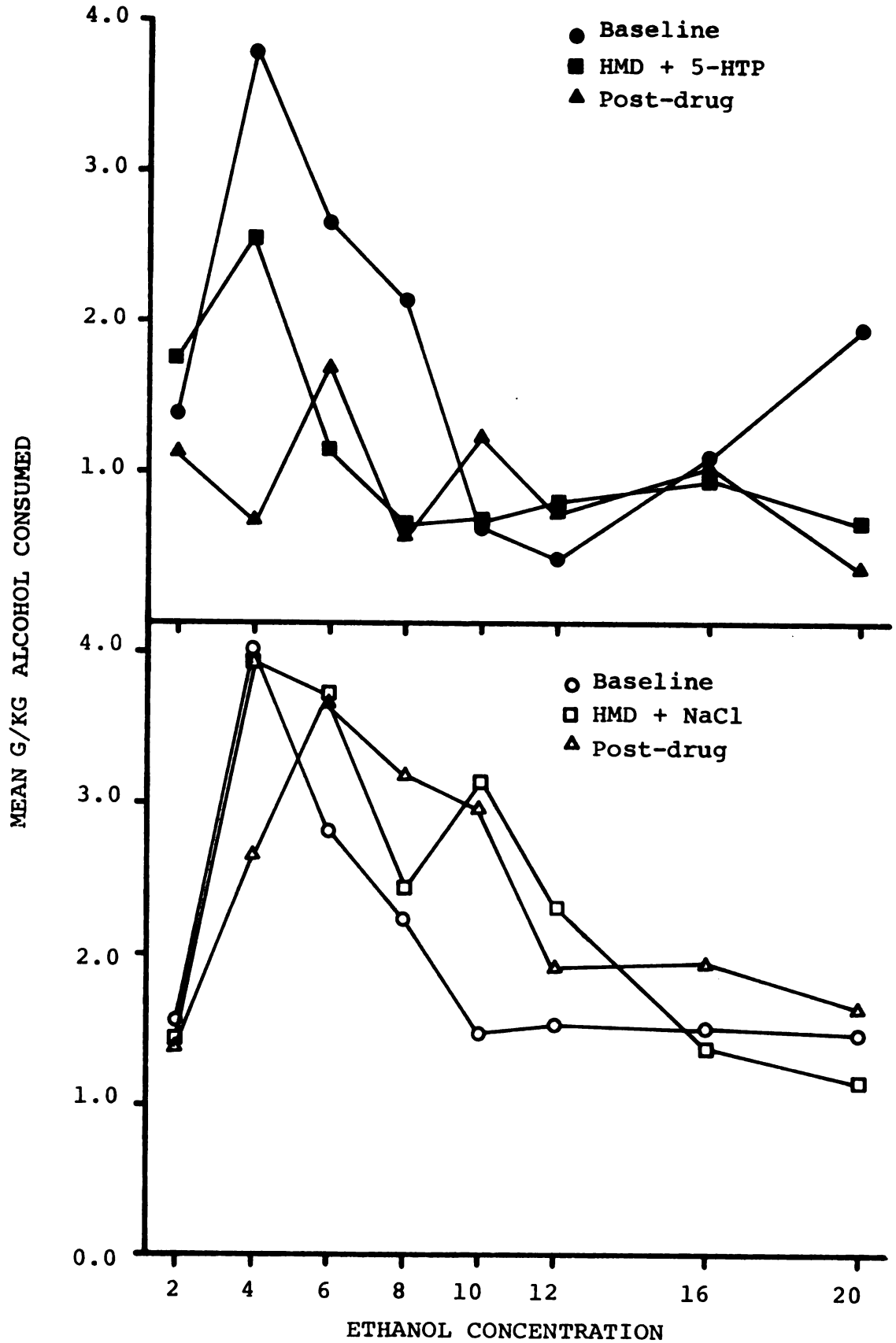

1.00
.75
.50
.25
Baseline
HMD + 5-HTP
Post-drug
1.00
.75
.50
.25
.00
Baseline
HMD + NaCl
Post-drug
MEAN PROPORTION ETOH TO TOTAL FLUIDS CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

($p < .001$) and during the post-drug period ($p < .001$). No significant differences were revealed for the HMD + NaCl control group. Comparison of Figure 13 with Figure 5 shows that animals receiving either HMD + 5-HTP to increase brain serotonin (Figure 13) or pCPA to decrease brain serotonin (Figure 5) responded in a very similar fashion. Preference was decreased for both groups during and after drug treatment, with the primary suppression occurring at lower concentrations. Figures 14 and 6 show the control groups for each of these treatments which also responded quite similarly. Figures 15 and 16 present mean g/kg alcohol consumed for HMD + 5-HTP and HMD + NaCl groups, respectively. No differences were found among the tests for the control group, while significant differences were revealed for the HMD + 5-HTP animals ($F = 4.23$, df 2/14, $p < .05$). Alcohol ingestion in g/kg was significantly lower than baseline only during the post-drug period ($p < .05$). Reduction of g/kg alcohol consumed by animals receiving HMD + 5-HTP was also somewhat similar to that of pCPA treated rats (Figures 7 and 15). Control groups for each of these drug treatments responded similarly (Figures 16 and 8).

These results substantiate a similar finding by Myers, Evans, and Yaksh (1972) who also showed 5-HTP to decrease ethanol selection with the primary effect coming after drug administration ceased. They gave three daily 100 mg/kg injections with no peripheral decarboxylase inhibitor, whereas only one daily 5-HTP injection in conjunction with HMD was used in this study. Geller (1973) also found 5-HTP to decrease ethanol preference when doses ranging from 50 to 150 mg/kg were given once daily. Again, his data interpretations cannot be

Figure 15. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving HMD + 5-HTP during the second of three preference test sequences.

Figure 16. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving HMD + NaCl during the second of three preference test sequences.


Baseline
HMD + 5-HTP
Post-drug
Baseline
HMD + NaCl
Post-drug
MEAN G/KG ALCOHOL CONSUMED
4.0
3.0
2.0
1.0
0.0
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

regarded highly because he ignores discrepancies and uses no statistical tests whatsoever.

It is necessary to interpret all 5-HTP results of this and other studies with caution due to the problems inherent in the technique. One is that 5-HT may be formed within neurons where it is not normally present because of the ubiquitous nature of the enzyme (aromatic L-amino acid decarboxylase) which converts 5-HTP to 5-HT (Moore, 1971, p. 111). Thus the use of 5-HTP may not precisely mimic the effects of endogenous 5-HT. Another problem is that increased serotonin levels might reduce the normal synthesis or release of 5-HT through feedback inhibition, leaving functional 5-HT levels unchanged. Nevertheless, it is surprising that the animals of this experiment responded in precisely the same manner to both pCPA and 5-HTP treatments which should have had opposite effects on brain serotonin content. The post-drug effect of 5-HTP is particularly unusual, since 5-HTP-induced increases in brain serotonin are not of long duration. All in all, little support for serotonin's reputed role in ethanol selection can be gleaned from these data.

Results for the groups receiving pCPA or CMC vehicle during the first preference test sequence are plotted in Figures 17-20. Figure 17 shows mean proportion ethanol consumed for the pCPA group. Significant differences among the tests were found ($f = 9.34$, df 2/12, $p < .005$) with preferences being significantly higher during the third sequence than during drug treatment ($p < .005$) or during the test immediately following drug treatment ($p < .05$). Mean proportion ethanol consumed during the sequence immediately following pCPA administration was not significantly different than that during drug

Figure 17. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving pCPA during the first of three preference test sequences.

Figure 18. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving CMC vehicle during the first of three preference test sequences.

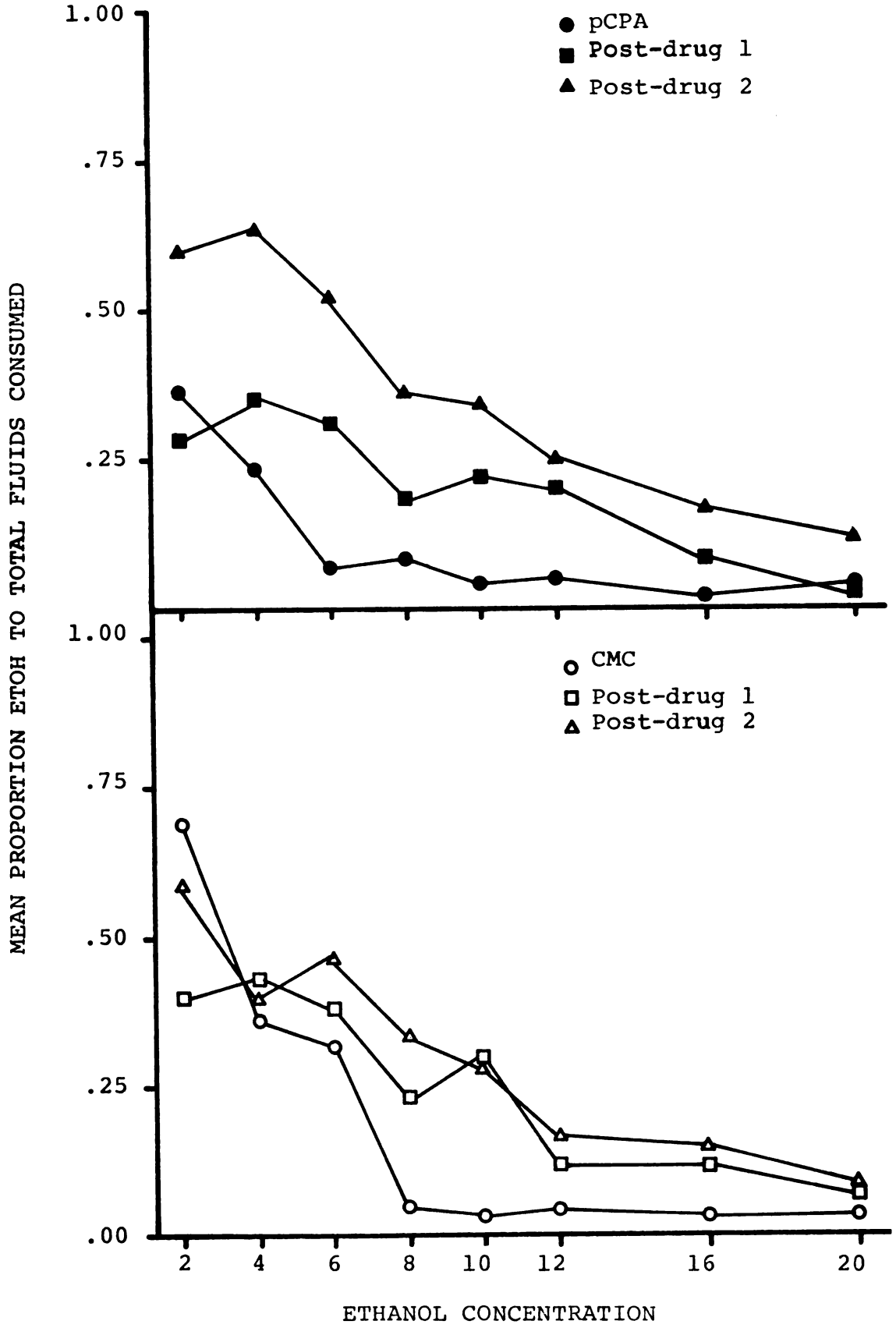
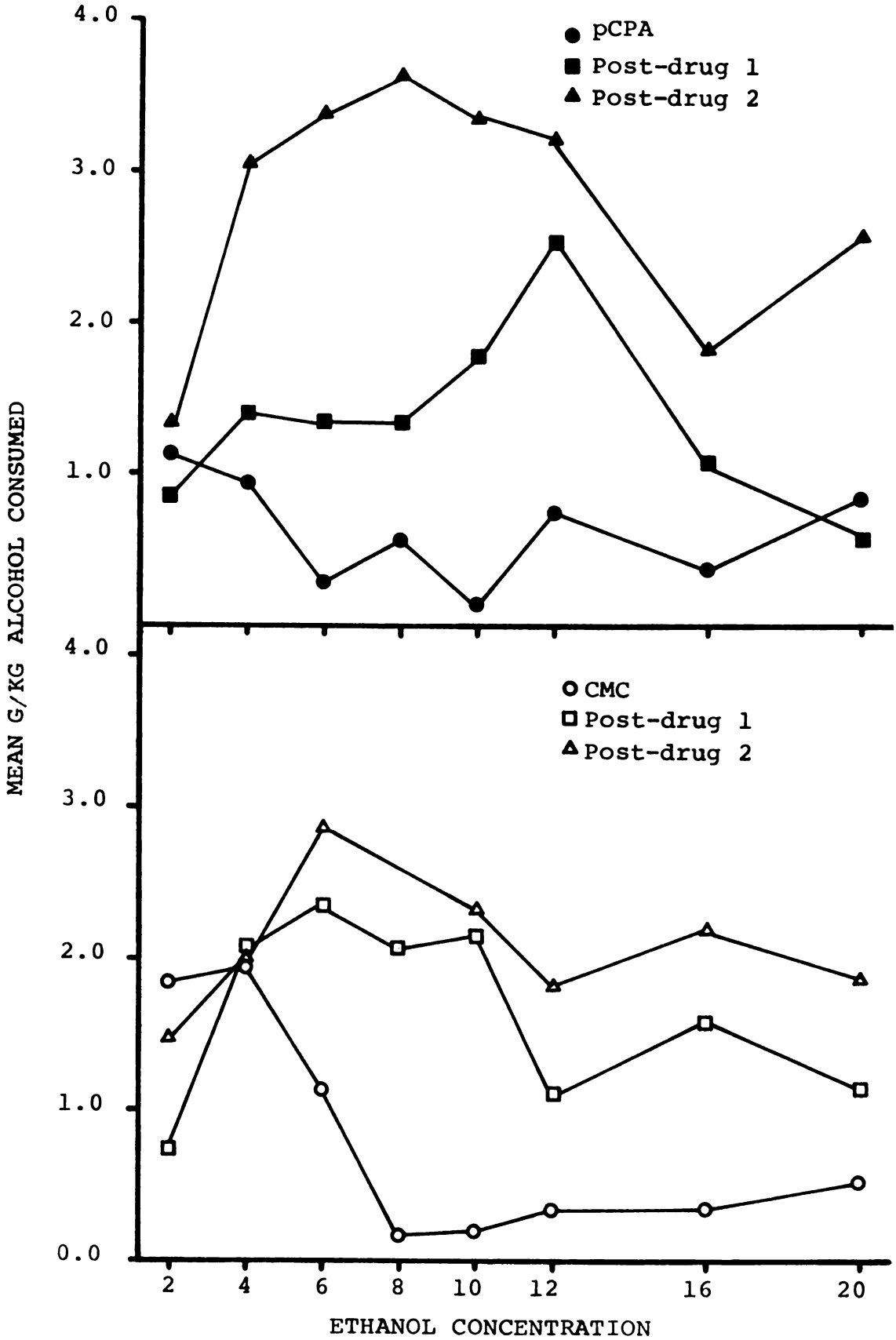

1.00
.75
.50
.25
pCPA
Post-drug 1
Post-drug 2
1.00
.75
.50
.25
.00
CMC
Post-drug 1
Post-drug 2
MEAN PROPORTION ETOH TO TOTAL FLUIDS CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

Figure 19. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving pCPA during the first of three preference test sequences.

Figure 20. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving CMC vehicle during the first of three preference test sequences.


4.0
3.0
2.0
1.0
pCPA
Post-drug 1
Post-drug 2
4.0
3.0
2.0
1.0
0.0
CMC
Post-drug 1
Post-drug 2
MEAN G/KG ALCOHOL CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

treatment. No significant differences among the tests on this measure were found for the CMC vehicle control animals (Figure 18), despite a trend toward higher preferences on later tests, especially at higher concentrations. Figures 19 and 20 provide data on mean g/kg alcohol consumed for pCPA and CMC vehicle groups, respectively. Differences among the tests were significant for each group ($F = 11.30$, df 2/12, $p < .005$ for the pCPA group; $F = 6.12$, df 2/14, $p < .025$ for the CMC vehicle group). Animals receiving pCPA consumed significantly greater g/kg alcohol on the third test than during either the drug sequence ($p < .001$) or the second sequence ($p < .01$), the latter two of which did not differ significantly. A significant increase above the first sequence in g/kg alcohol consumed was found for the CMC vehicle group during the test immediately following treatment ($p < .05$) and during the third sequence ($p < .01$), which did not differ from each other. Data from only seven animals were analyzed for the pCPA group, since one animal died on the day after pCPA treatment ended.

Interpretation of this information is difficult for two reasons. First, these are the only two groups which were intubated without light ether anesthetization, and the pCPA animals, in particular, did not adapt at all well to the procedure. What influence, if any, this had on their ethanol selection remains unclear. Second, since the treatments were given during the first preference sequence, there is no baseline behavior with which to compare a group's later performance. Direct comparison between experimental and control groups is dubious, since their initial ethanol preference functions could well have been different. Individual differences in ethanol preference are great among rats (Myers & Veale, 1970).

It does appear though that during drug treatment pCPA animals had a lower preference and consumed less alcohol than controls at the three lowest ethanol concentrations. At higher and thus later concentrations, both measures are quite low for each group, possibly indicating an effect of the intubation procedure. The tendency for mean increases in selection during the post-pCPA period (Figures 17 and 19) conflicts with the trend for further decreases following pCPA administration when animals got the drug on the second preference sequence (Figures 5 and 7). This again fails to support correlations between ethanol selection and brain serotonin.

The very long-lasting depression in ethanol selection caused by pCPA which Veale and Myers (1970) reported was not found when pCPA was given on the first preference test. This may indicate that procedural variables such as animal's prior exposure to ethanol may influence the effects produced by pCPA.

Finally, Figures 21 and 22 show the mean proportion of ethanol consumed by rats receiving either pCPA or CMC vehicle, respectively, between two preference sequences. No significant changes in preference occurred for either group, although both showed a slight acclimation effect with increased mean preference at the middle concentrations on the second test sequence. Mean g/kg alcohol consumed for the same groups is plotted in Figures 23 and 24. Treatment with pCPA did not result in significantly different amounts consumed during the two tests (Figure 23). On the other hand, animals receiving CMC vehicle between tests significantly increased their consumption of alcohol in g/kg on the second preference sequence ($F = 8.58$, df 1/7, $p < .025$).

Figure 21. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving pCPA between two preference test sequences.

Figure 22. Mean proportion ETOH to total fluids consumed as a function of ethanol concentration for animals receiving CMC vehicle between two preference test sequences.

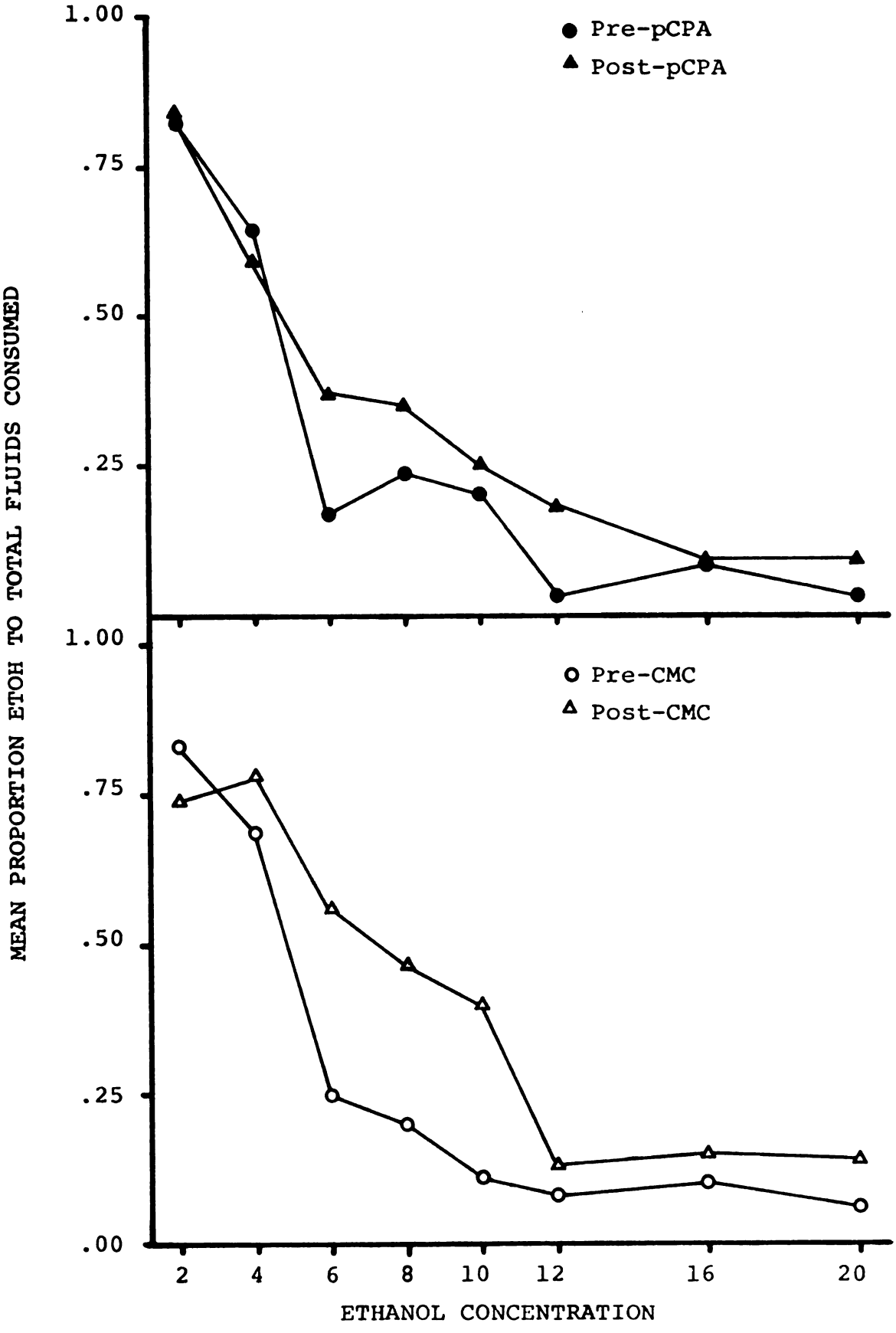
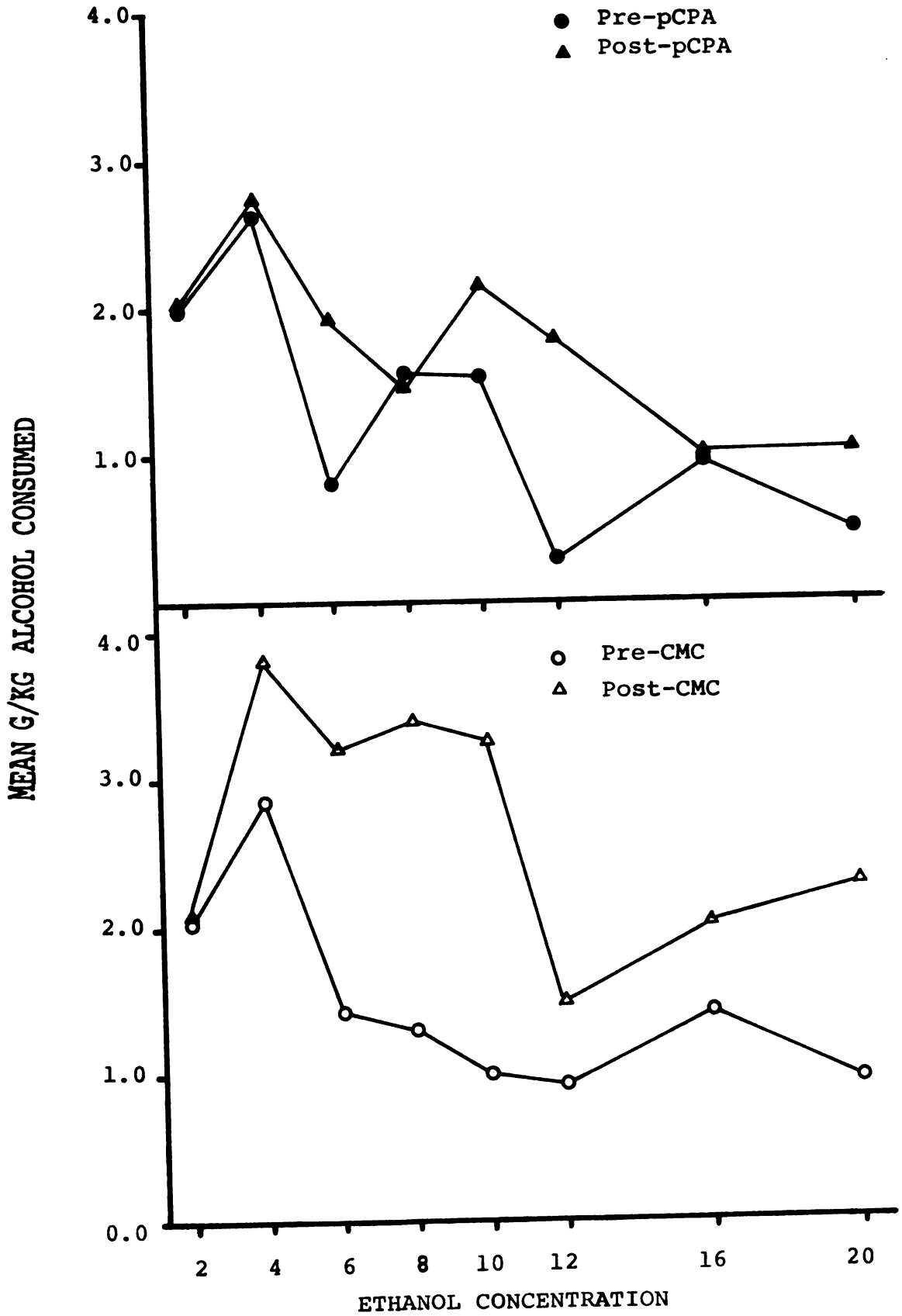

1.00
.75
.50
.25
1.00
.75
.50
.25
.00
Pre-pCPA
Post-pCPA
Pre-CMC
Post-CMC
MEAN PROPORTION ETOH TO TOTAL FLUIDS CONSUMED
2
4
6
8
10
12
16
20
ETHANOL CONCENTRATION

Figure 23. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving pCPA between two preference test sequences.

Figure 24. Mean g/kg alcohol consumed as a function of ethanol concentration for animals receiving CMC vehicle between two preference test sequences.


Pre-pCPA
Post-pCPA
Pre-CMC
Post-CMC
MEAN G/KG ALCOHOL CONSUMED
ETHANOL CONCENTRATION
4.0
3.0
2.0
1.0
0.0
2
4
6
8
10
12
16
20

These data reveal that post-pCPA ethanol selection is not decreased from baseline when there has been no association between ethanol preference testing and pCPA administration. The brains of pCPA-treated rats should still have been quite depleted of 5-HT, yet both preference and g/kg alcohol consumed showed only non-significant increases during the post-pCPA test. Even if brain serotonin level was on the rise in these animals, their brains should have been in a condition similar to those of animals shown in Figures 5 and 7 who decreased their selection of ethanol significantly in the post-pCPA period. It therefore seems rather questionable to use decreased brain serotonin level as an explanation for the greatly depressed post-pCPA ethanol selection seen when the drug is given during preference test two.

Control animals showed a greater acclimation to ethanol than pCPA-treated rats as seen by their significant increase in g/kg alcohol consumed during the post-CMC test. Thus although pCPA may not have decreased ethanol selection, it may have prevented an acclimation effect. This interpretation is weakened, however, by the fact that the preference of control animals did not also rise significantly.

EXPERIMENT III

This experiment was intended to determine whether the changes in ethanol self-selection resulting from the use of pCPA to deplete brain serotonin were specific to ethanol solutions. The effects of pCPA treatment were assessed when preference testing procedures similar to those of Experiment II were employed but the choice was between either a glucose, saccharin or sodium chloride solution and tap water.

Saccharin preference was tested because such solutions are preferred at lower concentrations and rejected at higher ones, as is ethanol. In addition, saccharin has no caloric value. Glucose preference was tested because glucose does have caloric value, as does ethanol, despite the fact that it is preferred at even very high concentrations. And finally, sodium chloride was employed because it also has both accepted and rejected concentration ranges, while having quite significant metabolic consequences for the maintenance of water balance, but no caloric value.

Method

Subjects

Forty-eight adult male albino rats supplied by Holtzman Co. were used. All were 120 days old at the start of experimentation.

Housing, feeding and lighting conditions were the same as those of Experiment II.

Procedure

The animals were randomly divided into six groups of eight rats each and allowed at least two weeks of adaptation to the laboratory before preference testing began. As in Experiment II, three 8-day preference test sequences were conducted. The 3-bottle, 24-hour preference method was again employed with body weights and fluid intakes recorded at approximately the same time each day. Two groups (one experimental and one control) received a choice between tap water and a glucose solution; two received a choice between tap water and a saccharin solution; and two received a choice between tap water and a sodium chloride solution. Concentrations of the solutions offered were increased on each day of a preference sequence as follows:

glucose--15, 20, 25, 30, 35, 40, 45, and 50%

saccharin--.2, .4, .6, .8, 1.2, 1.6, 2.0, and 2.4%

sodium chloride--.3, .6, .9, 1.2, 1.5, 1.8, 2.1, and 2.4%

Each group received the same test solution on all three preference sequences. Solutions were prepared fresh daily on a weight/weight basis.

Drug preparation and administration were the same as in Experiment I. Beginning two days before the second preference test, the 10 consecutive days of drug or vehicle treatment consisted of:

PREF. TEST #1	PREF. TEST #2	PREF. TEST #3
no drug	100 mg/kg pCPA	no drug
no drug	vehicle	no drug

Four water-only days separated each preference sequence.

Results and Discussion

The same measures of self-selection and the same statistical procedures were employed in Experiment III as had been used in Experiment II. Table 4 provides the drug treatments, mean daily preference ratios and mean daily g/kg alcohol consumed for groups in this experiment. Statistical analyses are based upon these data.

Saccharin

Figures 26 and 27 show the mean proportion saccharin solution consumed by animals receiving pCPA or CMC vehicle, respectively, during the second preference sequence. Significant differences were found among the tests for the pCPA group only ($F = 4.12$, df 2/14, $p < .05$). Treatment with pCPA significantly decreased preference from baseline only during the drug period itself ($p < .05$) and, as Figure 25 reveals, the effect was primarily at the lower concentrations. Mean g/kg saccharin consumed for the pCPA group can be seen in Figure 27, while the same measure is plotted for CMC vehicle controls in Figure 28. As with preference, only the pCPA treated group displayed significant differences among the three tests ($F = 4.20$, df 2/14, $p < .05$). Mean g/kg saccharin ingested during the post-pCPA period was significantly less than baseline ($p < .05$).

A similarity, although slightly obscure, can be seen between these saccharin solution results and those obtained with ethanol under the same test procedures (see Figures 5 and 7). Preference for each solution, but not g/kg consumed, was significantly decreased during pCPA treatment. Both measures of selection were significantly reduced

Table 4.--Drug treatments, mean daily preference ratios (± S.E.) and mean daily g/kg alcohol consumed (± S.E.) for each group of Experiment III.

	Drug Treatment	Mean Daily Pref. Ratio	Mean Daily g/kg Alc. Consumed
SACCHARIN			
Test #1	no drug	45 ± 3.4	0.34 ± 0.02
Test #2	pCPA	32 ± 2.7	0.30 ± 0.04
Test #3	no drug	36 ± 4.1	0.24 ± 0.03
Test #1	no drug	39 ± 4.6	0.32 ± 0.06
Test #2	CMC	42 ± 3.7	0.31 ± 0.06
Test #3	no drug	38 ± 4.6	0.27 ± 0.05
GLUCOSE			
Test #1	no drug	63 ± 4.6	22 ± 2.5
Test #2	pCPA	52 ± 5.4	28 ± 3.3
Test #3	no drug	63 ± 1.7	18 ± 0.8
Test #1	no drug	78 ± 3.4	30 ± 1.9
Test #2	CMC	73 ± 3.3	25 ± 2.3
Test #3	no drug	76 ± 2.5	26 ± 2.1
SODIUM CHLORIDE			
Test #1	no drug	30 ± 3.5	0.39 ± 0.06
Test #2	pCPA	36 ± 6.2	0.76 ± 0.20
Test #3	no drug	15 ± 4.2	0.20 ± 0.08
Test #1	no drug	27 ± 1.2	0.31 ± 0.05
Test #2	CMC	27 ± 2.9	0.31 ± 0.06
Test #3	no drug	22 ± 2.0	0.23 ± 0.04

Figure 25. Mean proportion saccharin solution to total fluids consumed as a function of saccharin concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 26. Mean proportion saccharin solution to total fluids consumed as a function of saccharin concentration for animals receiving CMC vehicle during the second of three preference test sequences.

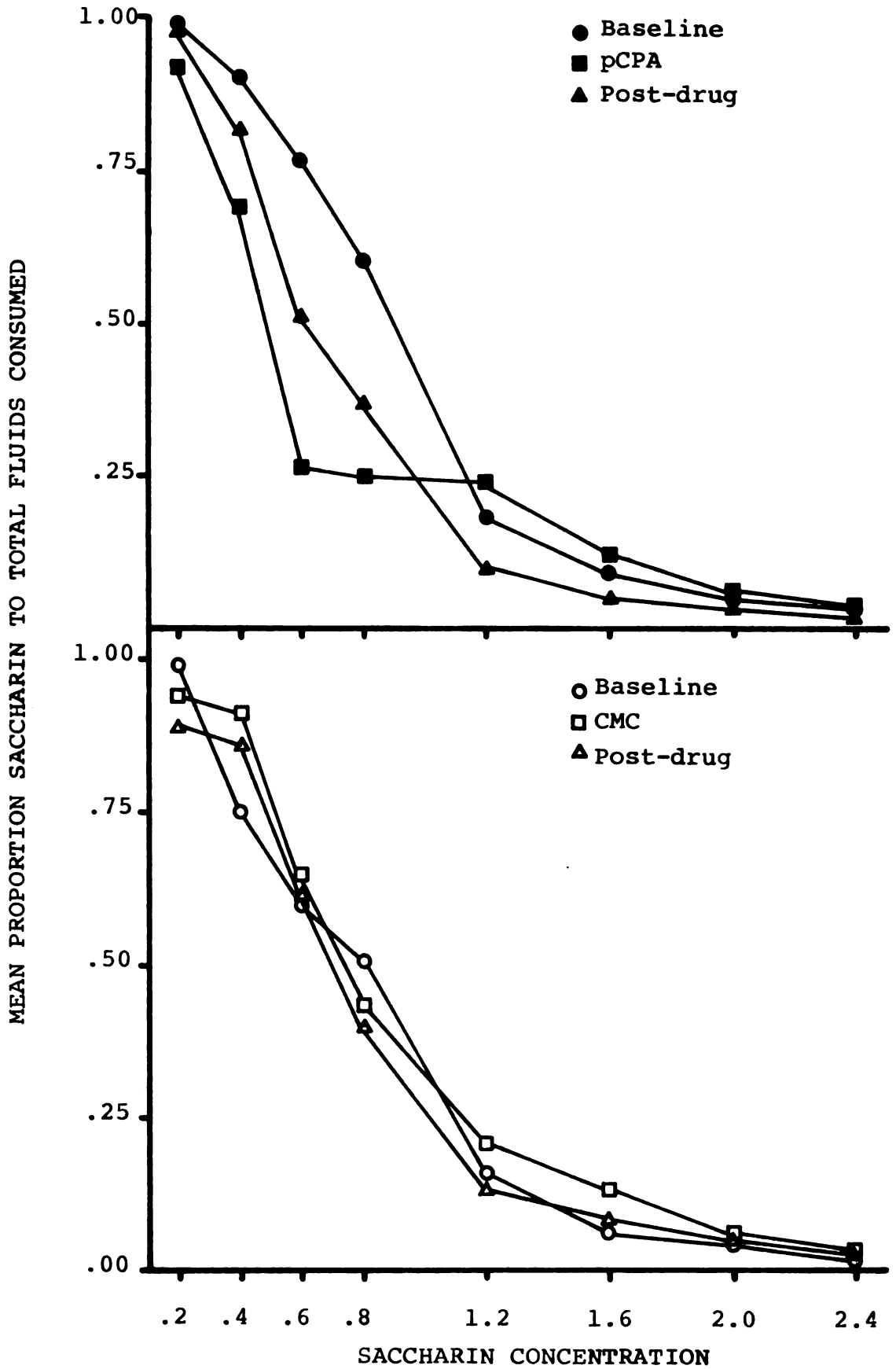
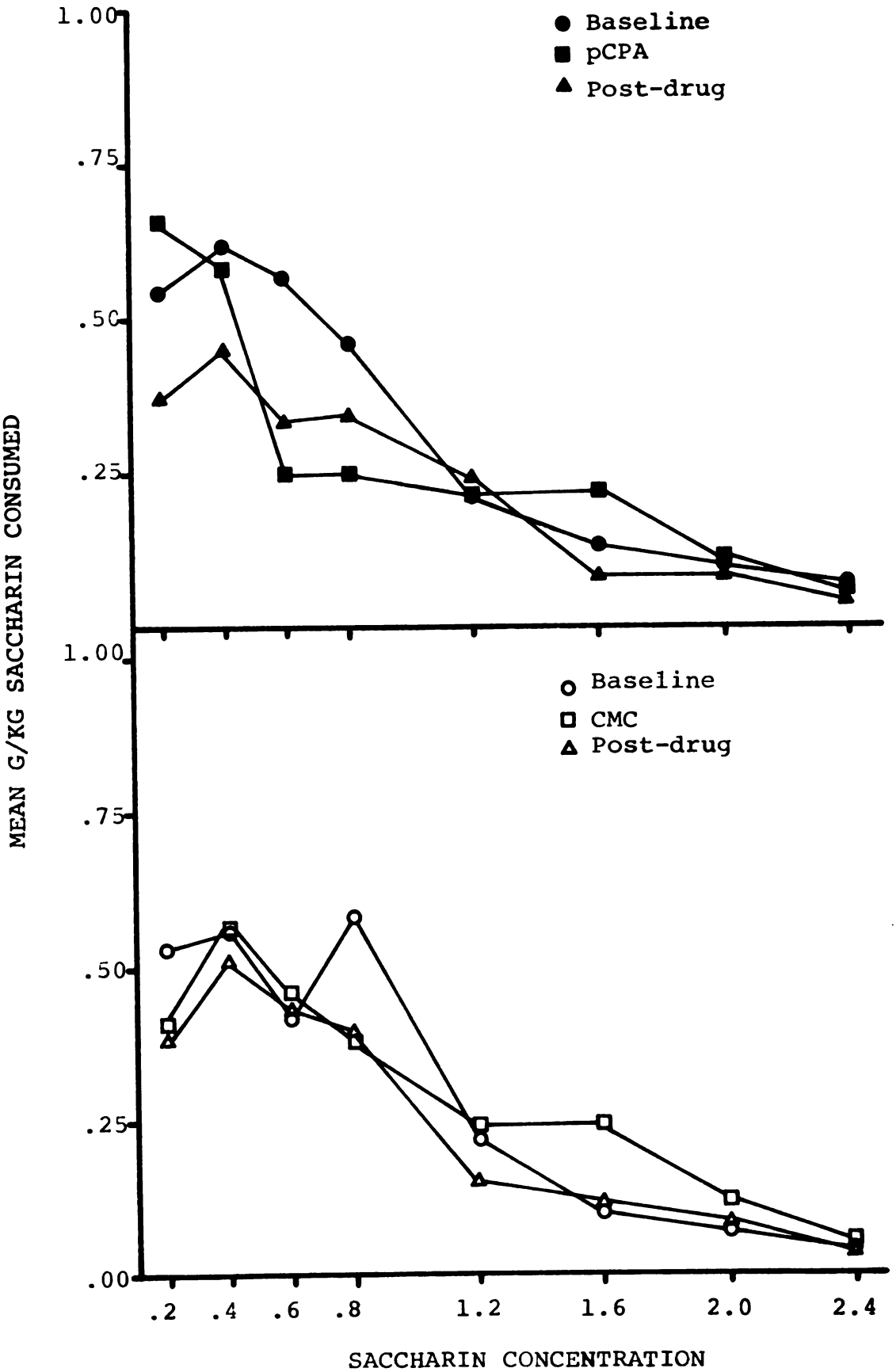

1.00
.75
.50
.25
Baseline
pCPA
Post-drug
1.00
.75
.50
.25
.00
Baseline
CMC
Post-drug
MEAN PROPORTION SACCHARIN TO TOTAL FLUIDS CONSUMED
.2
.4
.6
.8
1.2
1.6
2.0
2.4
SACCHARIN CONCENTRATION

Figure 27. Mean g/kg saccharin consumed as a function of saccharin concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 28. Mean g/kg saccharin consumed as a function of saccharin concentration for animals receiving CMC vehicle during the second of three preference test sequences.


1.00
.75
.50
.25
1.00
.75
.50
.25
.00
Baseline
pCPA
Post-drug
Baseline
CMC
Post-drug
MEAN G/KG SACCHARIN CONSUMED
.2
.4
.6
.8
1.2
1.6
2.0
2.4
SACCHARIN CONCENTRATION

from baseline during the post-pCPA test sequence for ethanol; only g/kg showed a significant post-pCPA drop when saccharin was tested. Mean post-drug preference for saccharin, although not different from baseline preference, was not significantly above the reduced preference during pCPA treatment. To summarize, the results for saccharin and ethanol were quite similar except that preference for saccharin was not as depressed post-drug. Control animals intubated with CMC and tested with either ethanol (Figures 6 and 8) or saccharin (Figures 26 and 28) showed no significant changes whatsoever during any of the three preference tests.

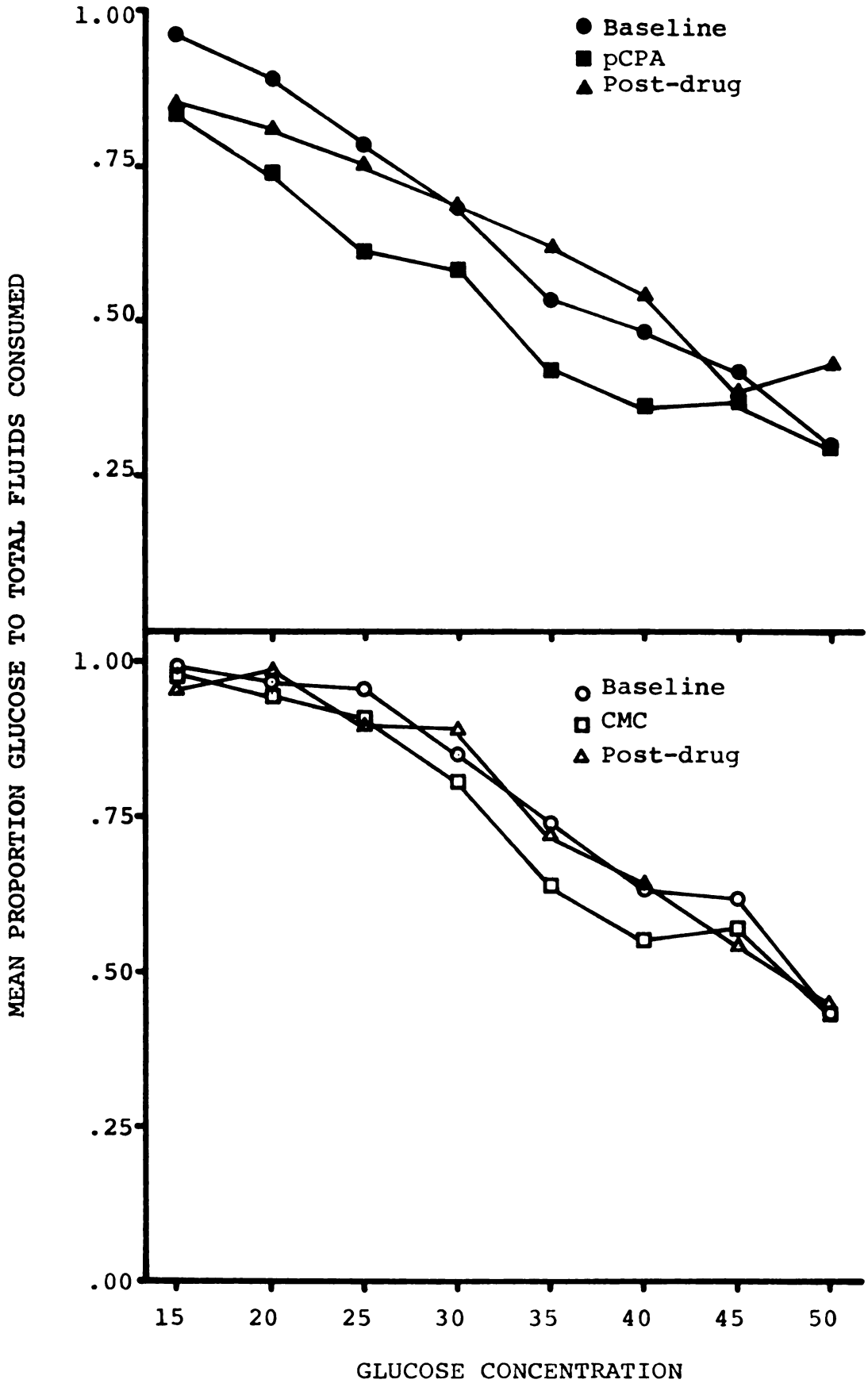
The findings for ethanol and saccharin do not correlate perfectly, but they do suggest that pCPA is similarly affecting the self-selection of each. It can be concluded then that pCPA's effects are definitely not specific to ethanol. Unfortunately, there are no other published studies with which to compare the saccharin results. A conflict is apparent with the statement made by Cicero and Hill in 1970. They mentioned unpublished observations of increased saccharin intake due to pCPA.

Glucose

Mean proportion glucose consumed during pCPA or CMC vehicle treatment is plotted in Figures 29 and 30, respectively. Analysis revealed significant differences among the three tests for the pCPA group ($F = 5.31$, df 2/14, $p < .025$), while no reliable differences were found for vehicle controls. During pCPA treatment, preference for glucose was significantly below that during the baseline and post-drug sequences ($p < .05$ each time). This effect was evident for nearly all

Figure 29. Mean proportion glucose solution to total fluids consumed as a function of glucose concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 30. Mean proportion glucose solution to total fluids consumed as a function of glucose concentration for animals receiving CMC vehicle during the second of three preference test sequences.

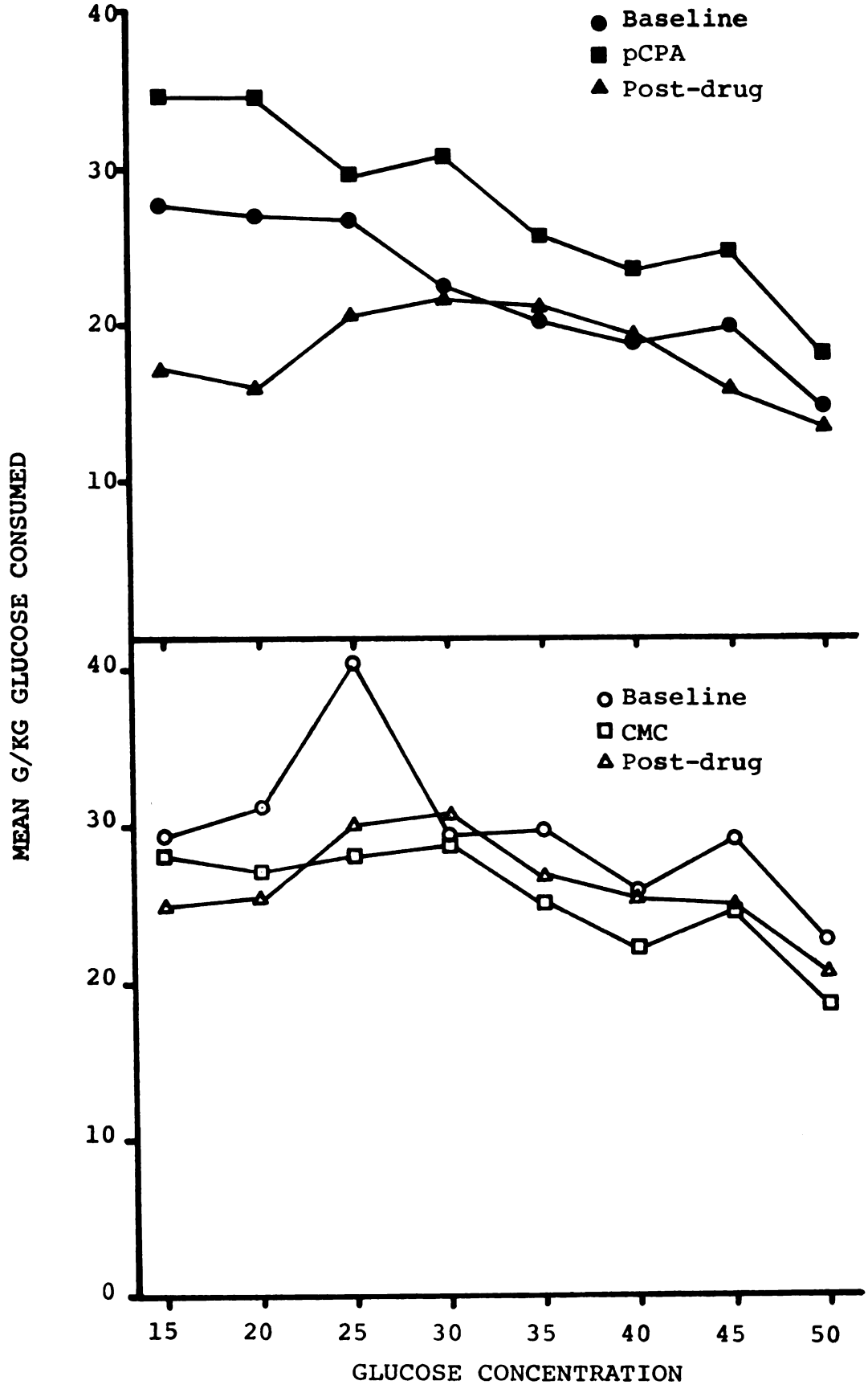

1.00
.75
.50
.25
Baseline
pCPA
Post-drug
1.00
.75
.50
.25
.00
Baseline
CMC
Post-drug
15
20
25
30
35
40
45
50
MEAN PROPORTION GLUCOSE TO TOTAL FLUIDS CONSUMED
GLUCOSE CONCENTRATION

concentrations. Figure 31 shows mean g/kg glucose consumed for the pCPA group. It presents quite a different picture than was seen with preference. Significant differences were found among the tests ($F = 8.08$, df 2/14, $p < .005$), but rather than being suppressed, g/kg glucose ingested was significantly higher during pCPA treatment than during baseline ($p < .05$) or the post-drug period ($p < .005$). Regarding the control animals receiving CMC (Figure 32), significant differences were also found in g/kg consumed among the three sequences ($F = 9.57$, df 2/14, $p < .005$). Their glucose ingestion, however, was significantly reduced from baseline during CMC treatment ($p < .005$) and remained depressed during test number three ($p < .005$).

The elevated consumption of glucose (in g/kg) during pCPA treatment is especially noteworthy when one considers that vehicle intubation actually reduced glucose intake. This finding agrees with an experiment by Nancy and Kilby (1973) which showed sucrose ingestion to be increased by daily 100 mg/kg i.p. injections of pCPA. Four or 12-hour preference tests were employed by them with random presentation of sucrose solutions ranging from 0.5 to 16%. They found preference to be elevated also, while such was not the case here. The discrepancy may be due in part to the longer 24-hour testing procedure employed in this study which allowed more time for post-ingestional factors influencing water intake to come into play. Brody (1970) also reported increases in glucose consumption in unpublished experiments. Nance and Kilby, however, are the only investigators who have reported a successful attempt to reverse pCPA's effects on self-selection by giving 5-HTP.

Figure 31. Mean g/kg glucose consumed as a function of glucose concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 32. Mean g/kg glucose consumed as a function of glucose concentration for animals receiving CMC vehicle during the second of three preference test sequences.


Baseline
pCPA
Post-drug
Baseline
CMC
Post-drug
MEAN G/KG GLUCOSE CONSUMED
GLUCOSE CONCENTRATION
0
10
20
30
40
15
20
25
30
35
40
45
50

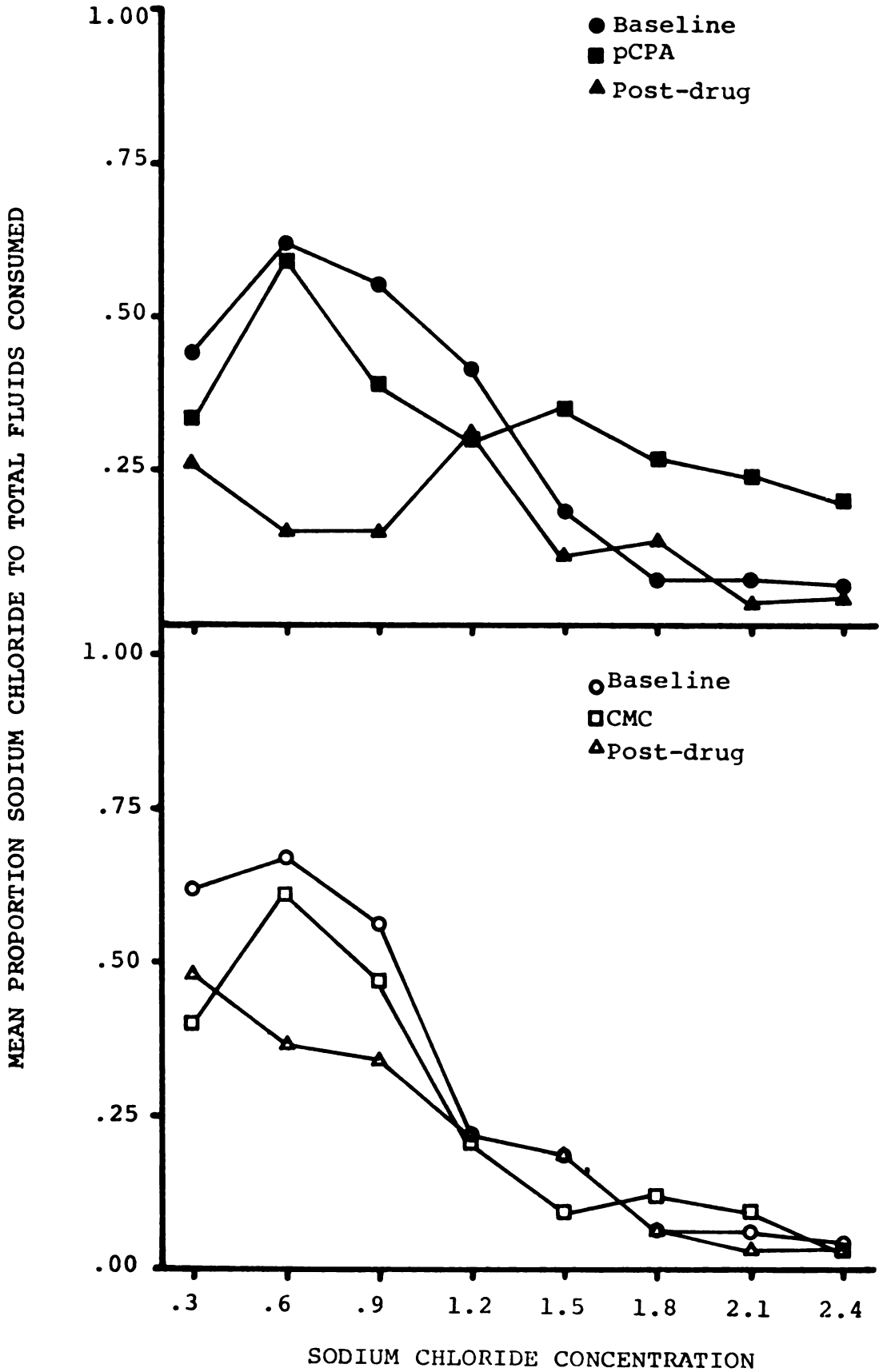
It may well be that 5-HT neurons are involved in the regulation of carbohydrate intake as Nance and Kilby (1973) suggest. Caloric value of the solution alone apparently does not determine whether pCPA will enhance or reduce its consumption. Both ethanol and glucose contain calories, but only glucose consumption was enhanced. It may be that glucose, an easily digestable source of calories, is less irritating to the digestive mucosa than either dry diet or ethanol. Sweetness of the solution, per se, cannot be the sole determinant of pCPA's effects either. Saccharin, at least at lower concentrations, is sweet, as is glucose. Yet saccharin intake was depressed while glucose consumption rose with pCPA treatment.

Sodium Chloride

Mean proportion sodium chloride solution consumed is shown in Figures 33 (pCPA group) and 34 (CMC controls). Analysis revealed significant differences in preference among the three sequences for the pCPA group ($F = 9.30$, df 2/14, $p < .005$) but no differences for controls. Treatment with pCPA caused sodium chloride preference to decrease significantly from baseline ($p < .01$) and drug sequence ($p < .005$) during the post-pCPA test. To statistically test for concentration effects during pCPA treatment, (Figure 33) a mean daily preference ratio was calculated for each animal for each test at concentrations $\leq$.9% NaCl and at concentrations $\geq$1.2% NaCl. Related measures t-tests (one-tailed) were then conducted. These revealed that for concentrations $\leq$.9% NaCl, post-drug preference was significantly different from both drug preference ($t = 5.66$, df 7, $p < .001$) and baseline preference ($t = 7.00$, df 7, $p < .001$). At concentrations $\geq$1.2% NaCl, drug preference was not significantly different than either baseline or post-drug preference.

Figure 33. Mean proportion NaCl solution to total fluids consumed as a function of NaCl concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 34. Mean proportion NaCl solution to total fluids consumed as a function of NaCl concentration for animals receiving CMC vehicle during the second of three preference test sequences.

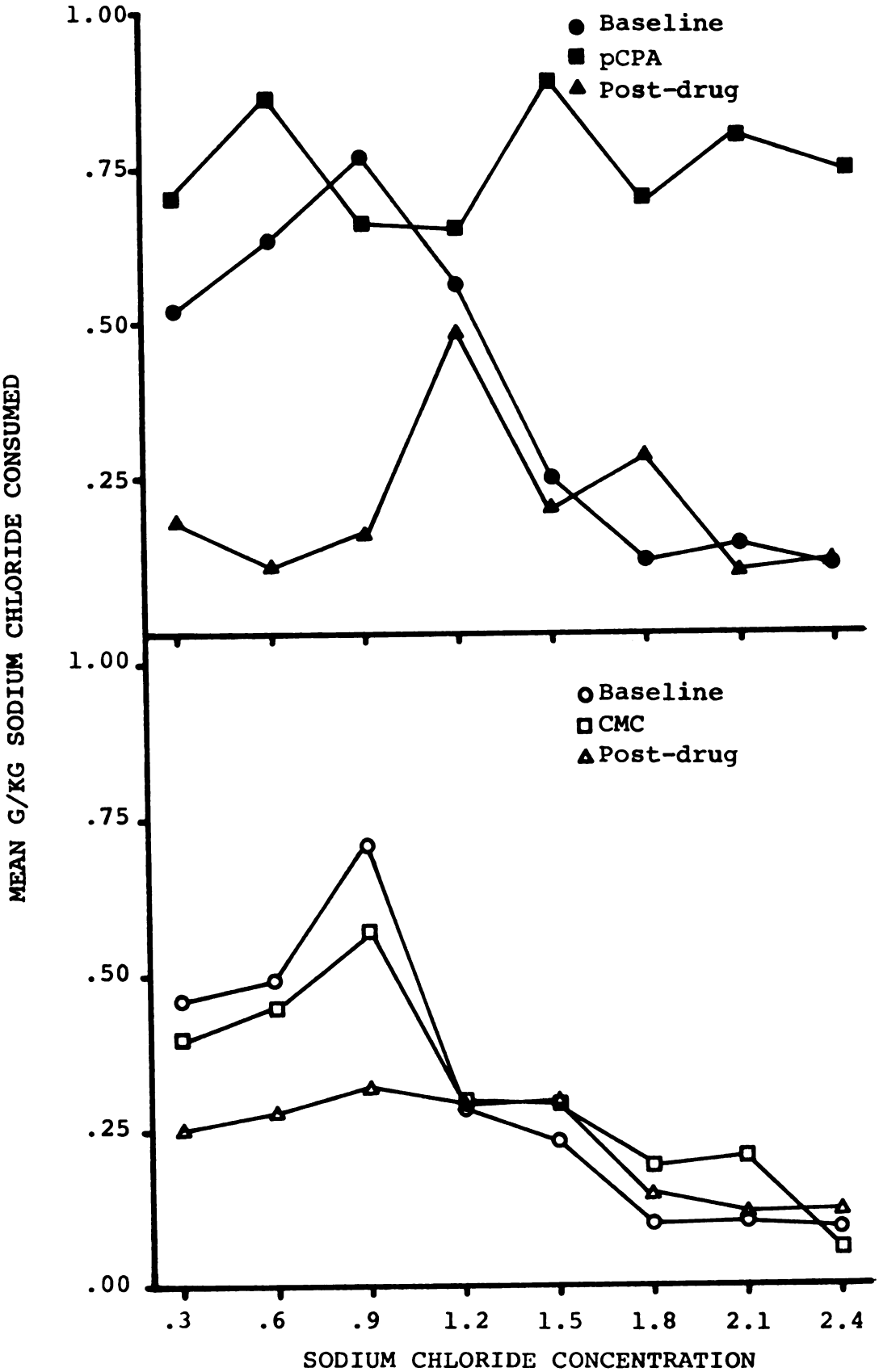

1.00
.75
.50
.25
1.00
.75
.50
.25
.00
Baseline
pCPA
Post-drug
Baseline
CMC
Post-drug
.3
.6
.9
1.2
1.5
1.8
2.1
2.4
MEAN PROPORTION SODIUM CHLORIDE TO TOTAL FLUIDS CONSUMED
SODIUM CHLORIDE CONCENTRATION

Figures 35 and 36 show mean g/kg NaCl consumed for the same two groups. Animals given pCPA (Figure 35) showed significant differences in NaCl consumption among the three tests ($F = 7.65$, df 2/14, $p < .01$). Mean g/kg consumed was significantly higher during pCPA treatment than it was during either baseline ($p < .05$) or the post-drug sequence ($p < .005$). There were no significant differences among the tests for the animals receiving CMC. Concentration effects during pCPA treatment (Figure 35) were again statistically analyzed using one-tailed t-test for related measures. Mean g/kg alcohol consumed were calculated for each animal for each test at concentrations $\leq .9\%$ NaCl and $\geq 1.2\%$ NaCl. For the lower concentrations post-drug intake was significantly below baseline ($t = 9.0$, df 7, $p < .001$) and drug treatment intakes ($t = 4.9$, df 7, $p < .005$). Alcohol intake in g/kg during drug treatment concentrations $\geq 1.2\%$ NaCl was significantly below baseline ($t = 2.17$, df 7, $p < .05$) and post-drug intakes ($t = 2.30$, df 7, $p < .05$).

These results provide the only example of an increased consumption of the little preferred concentrations of a solution near the end of pCPA treatment. Unfortunately, duration of pCPA administration is confounded with concentration. This makes it impossible to determine from these data whether animals increased their intake of NaCl at high concentrations only or whether they would also have increased at lower concentrations if they had come later in treatment. Preliminary data from three additional animals given a choice between 1.8% NaCl and tap water while receiving daily doses of pCPA indicated that the duration of drug administration may be the important factor. Further experiments are needed to make a precise determination. Random presentation of a range of NaCl solutions during pCPA treatment

Figure 35. Mean g/kg NaCl consumed as a function of NaCl concentration for animals receiving pCPA during the second of three preference test sequences.

Figure 36. Mean g/kg NaCl consumed as a function of NaCl concentration for animals receiving CMC vehicle during the second of three preference test sequences.


1.00
.75
.50
.25
Baseline
pCPA
Post-drug
1.00
.75
.50
.25
.00
Baseline
CMC
Post-drug
MEAN G/KG SODIUM CHLORIDE CONSUMED
.3
.6
.9
1.2
1.5
1.8
2.1
2.4
SODIUM CHLORIDE CONCENTRATION

would be enlightening. There are no data in the literature concerning pCPA's effects on NaCl consumption with which to compare these results.

If an increased need for sodium chloride does result from brain serotonin depletion, this may indicate that serotonergic neurons may modulate the gustatory, olfactory or other areas within the brain which are involed in sodium appetite. On the other hand, if the enhanced NaCl intake merely reflects drug toxicity, it may mean that aldosterone secretion is inhibited or kidney reabsorption of sodium is disturbed. However, the metabolism data of Experiment I did not reveal elevated urine sodium concentrations toward the end of drug treatment. It would be interesting to give rats a shorter preference test (one, four, or 12 hours) with a choice between tap water and a normally non-preferred NaCl solution after one 316 mg/kg dose of pCPA. This and other research along these lines is definitely called for.

Why there should have been a significantly reduced NaCl intake at the start of preference test three remains unclear. One possibility is that a sequestering of sodium occurred during late drug treatment. Such a sodium accumulation could then have been reduced by an avoidance of the NaCl solution in the post-drug test. Another possibility is that the reduction represents a transitory aversive conditioning factor. A review of the data for each case where a drug was administered on the second of three preference sequences reveals that, regardless of the solution being offered, either preference and/or g/kg alcohol consumed was suppressed at the start of the post-drug test.

GENERAL DISCUSSION

The primary thrust of these experiments was to further investigate the hypothesized relationship between brain serotonin and ethanol self-selection through the use of pharmacological techniques. The results of several previous experiments were confirmed concerning the effects of pCPA (Myers & Veale, 1968; Myers & Cicero, 1969; Veale & Myers, 1970; Frey, Magnussen, & Nielsen, 1970; Myers & Tytell, 1972; Myers & Martin, 1973) and 5-HTP (Myers, Evans, & Yaksh, 1972; Geller, 1973) on ethanol self-selection. Both drugs suppressed ethanol intake, each having a more pronounced effect after drug treatment had ended. The pCPA results of Geller (1973) indicating that pCPA enhances ethanol selection were definitely not confirmed.

Several lines of evidence converge to support the position taken by Nachman, Lester, and Le Magnen (1970) and Holman, Hoyland, and Shillito (1974) that the reduced ethanol consumption may well represent a conditioned aversion resulting from the pairing of noxious drug effects with the distinct taste of ethanol. The evidence is as follows: (1) chronic administration of both pCPA and HMD + 5-HTP caused a reduction in body weight indicating noxious drug effects, (2) pCPA and HMD + 5-HTP treatments each reduced the selection of ethanol, although they should have had opposite effects on brain

serotonin, (3) HMD + 5-HTP (at the one dose employed) failed to reverse the effects of pCPA and restore ethanol selection to baseline levels, (4) pCPA had little effect on ethanol selection when its administration did not coincide with ethanol drinking, and (5) pCPA similarly affected the selection of ethanol and saccharin solutions, indicating that its effects are not specific to ethanol.

It is difficult to explain why pCPA affected the consumption of saccharin in a manner rather similar to ethanol, while the intakes of glucose and sodium chloride solutions were affected in quite different ways. The reason may be that glucose, being easily digestable, and sodium chloride, having consequences for water balance, helped the animals to counteract some aspects of drug toxicity. The animals may also have been deconditioned to these substances since they undoubtedly had previous experience with both sodium chloride and carbohydrates. Ethanol, on the other hand, being irritating to the gastrointestinal tract, and saccharin, having no caloric value were probably more likely candidates for conditioned aversion formation. In addition, these substances were novel to the animals. This is an explanation based primarily on post-ingestional factors, rather than on changes in the sensitivity of the chemical senses, taste and small. Sensitivity changes were not assessed in the present experiments.

Stronger conclusions concerning brain serotonin's role in fluid self-selection might result from the use of these pharmacological agents if much shorter (i.e., one or four hour) preference tests were used and at least a day intervened between drug administration and preference testing. Also, random or counterbalanced presentation of test concentrations would help to eliminate the confounding of two

variables, namely, duration of drug treatment and concentration. These methods should much more reliably determine the significance of gustatory and/or olfactory influences, in particular.

Undoubtedly, the greatest liability limiting the interpretation of the present fluid selection experiments, and many other experiments in this area, is the lack of closely corresponding brain 5-HT assay data. In those instances where assays have been carried out by others, usually only one or a few 5-HT determinations have been made, often coming at the end of drug treatment. No data are available which provide a function for the recovery of brain serotonin level after chronic pCPA or 5-HTP adminiatration. This is quite disturbing since many of the effects of these drugs on fluid selection occur in the post-drug period. Veale and Myers (1970) allude to preliminary assays revealing an overshoot of brain 5-HT level after chronic pCPA administration. No details of these assays have appeared in print.

Holman, Hoyland, and Shillito (1974), who used a preference testing methodology very similar to that of Experiments II and III, did made several 5-HT determinations following pCPA treatment. They discovered that whole-brain 5-HT was reduced during drug administration (sequence two) but not different from control at the end of post-drug sequence three. Their dose regimen (316 mg/kg pCPA two days before sequence two and 100 mg/kg pCPA four days later) was not really chronic, however, and the drug was given intraperitoneally. Despite reduced brain serotonin, the selection of ethanol was not affected by pCPA treatment.

The pharmacological manipulations used in these experiments each have their own inherent limitations. When using pCPA one can

never reduce brain serotonin level to zero, and the significance of remaining 5-HT stores cannot be determined. Peripheral 5-HT is depleted by pCPA also. With 5-HTP there is no true physiological distribution within the brain and its effects are relatively short-lived. These complications restrict the interpretations which can be drawn from any study using these two drugs to alter brain serotonin level. In addition, manipulation of whole-brain serotonin provides absolutely no insight whatsoever into precisely where in the brain the crucial serotonergic terminals might be located. The use of pCPA and HMD + 5-HTP in a relatively long-term preference situation poses other problems due to their influences on water intake as shown in Experiment I. In addition, over a period of days both must be relatively noxious (and probably toxic) to the animals, since each causes a significant reduction in body weight.

Despite the methodological limitations, the present experiments have several major procedural advantages over all previous investigations of serotonin's involvement in self-selection behavior. First, the possibility of attributing conflicting or unexpected results to strain or supplier differences (Tilson & Rech, 1974) has been eliminated. Second, standardized procedures were employed throughout the experiments and all animals were of very similar ages and body weights. Third, this is the only study in which the effects of pCPA on a variety of test solutions has been determined under such exacting experimental conditions.

That there may yet be some relationship between brain serotonin level and ethanol consumption has been indicated by the recent investigations of Ho, Tasi, Chen, Begleiter, and Kissin (1974) and

Myers and Melchior (1975). Each group injected 5,6-dihydroxytryptamine, either intracisternally or intraventricularly, and found it to enhance ethanol selection. Unfortunately, 5,6-DHT may not be as specific a toxin for 5-HT neurons as some would hope (Bjorklund, Nobin, & Stenevi, 1973; Longo, Scotti de Carolis, Liuzzi, & Massotti, 1974; Nygren, Fuxe, Jonsson, & Olson, 1974). Two methods of altering central serotonergic processes which no one has utilized in the study of ethanol choice behavior include lesioning and electrical stimulation of the dorsal and/or median raphe nuclei. Application of these techniques should prove enlightening, despite their drawbacks.

Obviously, only further experimentation can determine beyond a reasonable doubt whether brain serotonergic systems play any role in determining ethanol self-selection. Perhaps even this will not clarify the situation until more effective pharmacological techniques for manipulating these systems can be developed. The present experiments, nevertheless, cast serious doubt on the hypothesis that the consumption of ethanol is dependent upon the level of serotonin within the brain. In any case, given the vast complexity of the central nervous sytem, it is undoubtedly naive for one to suspect that a particular behavior, such as ethanol selection, is totally determined by the level of a single chemical wihtin the brain. Serotonergic neurons may possibly be involved in modulating some aspect of brain function which participates in the control of consummatory behavior, but it is unlikely that this is specific to ethyl alcohol.

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APPENDICES

APPENDIX A

DRUGS, SUPPLIES, AND EQUIPMENT

APPENDIX A

DRUGS, SUPPLIES, AND EQUIPMENT

Drugs

1. DL-p-chlorophenylalanine (courtesy of Pfizer Inc.)
2. L-α-hydrazino-α-methyl-β-3,4-dihydroxyphenyl propionic acid (courtesy Merck, Sharp & Dohme Co.)
3. DL-5-hydroxytryptophan (Sigma Chemical Co.)

Supplies

1. Carboxymethylcellulose (Sigma Chemical Co.)
2. Sodium saccharin (Sigma Chemical Co.)
3. Sodium chloride (M.S.U. General Stores)
4. Glucose (M.S.U. General Stores)
5. Stainless steel drinking spouts (M.S.U. Center for Laboratory Animal Resources)
6. 125 ml. glass bottles (M.S.U. General Stores)
7. #0 rubber stoppers (M.S.U. General Stores)
8. 5cc plastic syringes (VWR Scientific)

Equipment

1. Metabolism cages with bases (Acme Metal Products Co.)
2. Dial-o-gram balance (Ohaus)
3. Autogram balance (Ohaus)
4. Magnetic stirrer (Corning Glass Works)

5. Refractometer (American Optical)

6. Flame photometer model 143 (American Instrumentation Laboratories)

APPENDIX B

SUMMARY DATA--EXPERIMENT I

APPENDIX B

SUMMARY DATA--EXPERIMENT I

Mean Water Intakes for Rats in Standard Cages

Day	pCPA Vehicle	pCPA-50	pCPA-100	pCPA-200	5-HTP Vehicle	5-HTP-25
1	35	39	48	45	40	47
2	40	52	50	41	43	45
3	43	45	43	40	41	44
4	40	46	44	43	39	44
5	45	44	46	45	40	44
6	38	41	41	40	40	42
7	43	47	44	44	44	41
8	39	46	44	41	39	40
9	32	40	44	50	37	34
10	36	55	70	107	39	37
11	33	55	86	88	33	41
12	35	47	68	72	32	40
13	38	46	63	66	33	47
14	32	43	55	63	33	43
15	36	42	58	58	35	42
16	33	44	57	52	34	43
17	32	52	62	53	34	40
18	33	56	58	57	34	42
19	41	44	54	48	40	56
20	40	44	56	47	41	55
21	40	40	44	41	40	49
22	39	42	34	36	39	46

Day	pCPA Vehicle	pCPA-50	pCPA-100	pCPA-200	5-HTP Vehicle	5-HTP-25
23	38	38	37	35	39	45
24	41	43	37	34	43	49
25	43	47	38	37	43	46
26	40	45	42	40	40	47
27	41	46	44	41	39	43
28	43	47	42	42	45	48
29	42	55	45	41	42	47
30	40	45	40	41	38	45
31	42	43	41	42	38	43
32	42	53	51	51	44	48
33	41	48	45	43	41	44
34	39	45	45	47	42	44
35	49	57	52	46	42	53
36	42	53	51	43	41	51
37	41	53	45	44	36	45

Mean Body Weights for Rats in Standard Cages

Day	pCPA Vehicle	pCPA-50	pCPA-100	pCPA-200	5-HTP Vehicle	5-HTP-25
1	486	497	490	509	498	495
2	487	505	494	512	503	498
3	491	507	495	517	505	503
4	494	509	497	517	506	505
5	497	507	502	519	509	508
6	496	512	501	522	510	508
7	499	515	506	524	514	513
8	498	515	506	526	514	515
9	498	513	501	521	514	514
10	499	508	495	515	513	504
11	498	500	483	494	509	499
12	495	494	474	479	505	490
13	496	489	468	470	503	488
14	494	483	460	464	501	484
15	494	480	457	460	499	481
16	493	473	449	454	497	479
17	493	469	448	451	499	478
18	491	464	445	446	498	478
19	493	467	449	450	502	486
20	496	473	459	459	502	493
21	502	478	464	465	509	497
22	502	482	466	468	510	500
23	505	482	471	471	511	501
24	506	485	472	474	515	504
25	509	493	475	479	515	510
26	510	492	480	485	518	511
27	512	497	483	489	518	519
28	515	501	485	492	522	518
29	516	504	488	495	525	520
30	514	507	488	498	527	520
31	519	510	493	504	528	522

Day	pCPA Vehicle	pCPA-50	pCPA-100	pCPA-200	5-HTP Vehicle	5-HTP-25
32	519	515	501	511	529	525
33	521	518	504	514	533	526
34	522	522	505	519	532	528
35	524	524	510	521	536	530
36	526	529	514	525	539	533
37	528	533	518	533	540	535

APPENDIX C

SUMMARY DATA--EXPERIMENT II

APPENDIX C

SUMMARY DATA--EXPERIMENT II

Mean Daily Preference Ratios for Each Animal

pCPA (Test #2)

Test #1	50	31	49	20	30	63	27	19	($\bar{X}$ = 36 ± 5.6)
Test #2	10	6	29	6	27	36	33	6	($\bar{X}$ = 19 ± 4.7)
Test #3	28	2	1	15	4	28	19	3	($\bar{X}$ = 12 ± 4.1)

pCPA Vehicle (Test #2)

Test #1	17	60	35	29	40	26	32	46	($\bar{X}$ = 36 ± 4.7)
Test #2	2	68	36	49	58	45	43	14	($\bar{X}$ = 39 ± 7.7)
Test #3	0	67	42	50	54	30	39	39	($\bar{X}$ = 40 ± 7.0)

pCPA (Test #2) + 5-HTP (Test #3)

Test #1	37	24	34	22	47	57	47	43	($\bar{X}$ = 39 ± 4.2)
Test #2	13	1	14	0	19	36	9	26	($\bar{X}$ = 15 ± 4.3)
Test #3	5	0	11	0	2	55	29	39	($\bar{X}$ = 18 ± 7.4)

pCPA (Test #2) + Vehicle (Test #3)

Test #1	28	33	41	33	26	25	36	60	($\bar{X}$ = 35 ± 4.0)
Test #2	4	50	17	28	21	6	1	16	($\bar{X}$ = 18 ± 5.6)
Test #3	27	40	1	7	11	20	0	39	($\bar{X}$ = 18 ± 5.6)

5-HTP (Test #2)

Test #1	23	52	39	27	41	46	39	50	($\bar{X}$ = 40 ± 3.6)
Test #2	13	11	3	38	24	28	27	11	($\bar{X}$ = 19 ± 4.1)
Test #3	8	20	1	27	40	28	6	34	($\bar{X}$ = 20 ± 5.0)

5-HTP Vehicle (Test #2)

Test #1	46	30	51	51	33	46	71	26	($\bar{X}$ = 44 ± 5.1)
Test #2	54	36	24	62	29	57	49	2	($\bar{X}$ = 39 ± 7.2)
Test #3	54	52	54	50	30	62	56	1	($\bar{X}$ = 45 ± 7.1)

pCPA (Test #1)

Test #1	10	3	19	2	2	31	11	($\bar{X}$ = 37 ± 7.6)
Test #2	18	41	50	2	3	14	17	($\bar{X}$ = 21 ± 6.9)
Test #3	33	64	56	23	7	49	30	($\bar{X}$ = 37 ± 7.6)

pCPA Vehicle (Test #1)

Test #1	17	19	18	23	29	3	18	25	($\bar{X}$ = 19 ± 2.7)
Test #2	24	8	27	43	53	29	11	12	($\bar{X}$ = 26 ± 5.6)
Test #3	44	2	5	37	55	39	21	46	($\bar{X}$ = 31 ± 6.9)

pCPA (Between Tests)

Test #1	21	32	20	28	42	31	19	27	($\bar{X}$ = 28 ± 2.7)
Test #2	16	41	2	48	78	41	34	15	($\bar{X}$ = 34 ± 8.4)

pCPA Vehicle (Between Tests)

Test #1	21	27	46	44	37	26	19	13	($\bar{X}$ = 29 ± 4.2)
Test #2	63	54	58	58	50	29	3	22	($\bar{X}$ = 42 ± 7.6)

Mean Daily g/kg Alcohol Consumed by Each Animal

pCPA (Test #2)

Test #1	2.18	3.86	1.45	0.82	2.58	1.06	3.56	0.88	($\bar{X}$=2.05±0.42)
Test #2	2.34	3.13	2.26	0.59	0.37	0.35	2.45	0.61	($\bar{X}$=1.51±0.40)
Test #3	0.19	1.58	1.22	0.19	1.55	0.18	0.19	0.71	($\bar{X}$=0.73±0.22)

pCPA Vehicle (Test #2)

Test #1	0.63	3.61	1.52	1.92	1.95	2.05	1.64	1.58	($\bar{X}$=1.86±0.29)
Test #2	0.21	3.16	1.56	2.66	3.41	3.32	2.22	0.65	($\bar{X}$=2.15±0.44)
Test #3	0.00	3.72	2.14	3.80	3.25	2.25	2.51	1.38	($\bar{X}$=2.38±0.45)

pCPA (Test #2) + 5-HTP (Test #3)

Test #1	1.21	0.78	1.56	0.75	3.36	3.01	2.24	2.36	($\bar{X}$=1.91±0.35)
Test #2	1.06	0.09	0.83	0.00	1.49	3.57	1.03	2.71	($\bar{X}$=1.35±0.44)
Test #3	0.09	0.03	0.11	0.01	0.08	3.85	1.99	1.93	($\bar{X}$=1.01±0.51)

pCPA (Test #2) + Vehicle (Test #3)

Test #1	1.46	1.76	1.60	2.02	1.64	1.08	1.70	3.12	($\bar{X}$=1.80±0.21)
Test #2	0.22	3.77	1.52	2.39	1.66	0.26	0.12	1.21	($\bar{X}$=1.39±0.44)
Test #3	0.94	1.94	0.02	0.32	1.09	0.58	0.00	1.26	($\bar{X}$=0.77±0.24)

5-HTP (Test #2)

Test #1	0.70	2.00	1.50	2.23	2.28	1.70	1.58	2.02	($\bar{X}$=1.75±0.18)
Test #2	0.53	0.69	0.35	1.59	1.60	1.60	2.19	0.86	($\bar{X}$=1.18±0.23)
Test #3	0.27	0.57	0.02	1.05	3.01	1.21	0.07	1.30	($\bar{X}$=0.94±0.34)

5-HTP Vehicle (Test #2)

Test #1	2.42	0.79	2.10	2.13	1.39	2.16	4.80	0.86	($\bar{X}$=2.08±0.45)
Test #2	3.48	1.07	2.31	2.79	1.87	4.38	3.51	0.10	($\bar{X}$=2.44±0.50)
Test #3	4.36	2.01	2.52	2.42	1.32	3.63	2.96	0.14	($\bar{X}$=2.42±0.46)

pCPA (Test #1)

Test #1	1.04	0.21	0.91	0.11	0.14	0.89	1.03		($\bar{X}$=0.62±0.17)
Test #2	0.79	3.38	3.33	0.08	0.08	0.84	1.02		($\bar{X}$=1.36±0.53)
Test #3	1.73	4.88	4.77	1.29	1.03	2.91	2.96		($\bar{X}$=2.80±0.59)

pCPA Vehicle (Test #1)

Test #1	0.73	1.16	0.63	0.65	0.94	1.23	0.98	0.16	($\bar{X}$=0.81±0.12)
Test #2	1.32	1.26	1.23	0.15	1.13	2.69	3.40	2.02	($\bar{X}$=1.65±0.36)
Test #3	2.23	3.52	2.73	0.09	0.33	2.74	2.80	2.99	($\bar{X}$=2.18±0.45)

pCPA (Between Tests)

Test #1	1.01	1.12	0.67	1.73	2.49	1.29	0.78	1.04	($\bar{X}$=1.27±0.21)
Test #2	0.80	1.76	0.16	3.16	4.61	1.22	1.92	0.37	($\bar{X}$=1.75±0.53)

pCPA Vehicle (Between Tests)

Test #1	0.80	1.51	2.89	2.97	1.87	0.85	0.61	0.57	($\bar{X}$=1.51±0.35)
Test #2	3.98	3.92	3.94	4.24	2.86	1.60	0.24	0.75	($\bar{X}$=2.69±0.57)

APPENDIX D

SUMMARY DATA--EXPERIMENT III

APPENDIX D

SUMMARY DATA--EXPERIMENT III

Mean Daily Preference Ratios for Each Animal

Saccharin (pCPA)

Test #1	52	38	51	47	51	33	33	58	($\bar{X}$ = 45 ± 3.4)
Test #2	26	44	30	24	40	29	26	39	($\bar{X}$ = 32 ± 2.7)
Test #3	47	18	35	26	29	35	46	52	($\bar{X}$ = 36 ± 4.1)

Saccharin (CMC)

Test #1	28	51	50	45	43	30	15	49	($\bar{X}$ = 39 ± 4.6)
Test #2	43	37	60	50	30	32	34	48	($\bar{X}$ = 42 ± 3.7)
Test #3	23	28	54	49	18	42	41	46	($\bar{X}$ = 38 ± 4.6)

Glucose (pCPA)

Test #1	40	79	51	64	60	65	77	69	($\bar{X}$ = 63 ± 4.6)
Test #2	24	68	50	69	50	51	64	40	($\bar{X}$ = 52 ± 5.4)
Test #3	60	68	58	73	61	60	64	63	($\bar{X}$ = 63 ± 1.8)

Glucose (CMC)

Test #1	68	76	80	80	84	87	87	59	($\bar{X}$ = 78 ± 3.4)
Test #2	70	66	78	74	77	83	81	54	($\bar{X}$ = 73 ± 3.3)
Test #3	73	71	80	75	80	83	82	62	($\bar{X}$ = 76 ± 2.5)

Sodium Chloride (pCPA)

Test #1	30	24	29	37	15	35	48	24	($\bar{X}$ = 30 ± 3.5)
Test #2	24	65	12	37	25	47	52	26	($\bar{X}$ = 36 ± 6.2)
Test #3	10	16	6	12	7	6	42	18	($\bar{X}$ = 15 ± 4.2)

Sodium Chloride (CMC)

Test #1	24	32	30	30	25	25	26	27	($\bar{X}$ = 27 ± 1.2)
Test #2	30	28	23	24	39	21	14	36	($\bar{X}$ = 27 ± 2.9)
Test #3	26	18	14	22	25	18	23	32	($\bar{X}$ = 22 ± 2.0)

Mean Daily g/kg Alcohol Consumed by Each Animal

Saccharin (pCPA)

Test #1	0.42	0.25	0.40	0.30	0.41	0.31	0.26	0.36	($\bar{X}$=0.34±0.02)
Test #2	0.23	0.27	0.28	0.13	0.51	0.31	0.28	0.35	($\bar{X}$=0.30±0.04)
Test #3	0.30	0.08	0.25	0.13	0.21	0.26	0.33	0.33	($\bar{X}$=0.24±0.03)

Saccharin (pCPA)

Test #1	0.21	0.38	0.44	0.38	0.29	0.14	0.09	0.59	($\bar{X}$=0.32±0.06)
Test #2	0.30	0.20	0.61	0.40	0.13	0.15	0.20	0.49	($\bar{X}$=0.31±0.06)
Test #3	0.14	0.11	0.43	0.38	0.09	0.24	0.26	0.48	($\bar{X}$=0.27±0.05)

Glucose (pCPA)

Test #1	9.9	26.0	16.0	16.6	28.3	24.2	25.5	29.9	($\bar{X}$=22.0±2.5)
Test #2	11.2	30.0	23.2	26.1	41.4	36.9	30.0	22.8	($\bar{X}$=27.7±3.3)
Test #3	15.9	18.1	18.0	15.8	21.9	20.3	17.7	17.2	($\bar{X}$=18.1±0.7)

Glucose (CMC)

Test #1	23.6	32.6	27.2	29.7	39.0	34.8	23.1	28.6	($\bar{X}$=29.8±1.9)
Test #2	20.6	27.2	23.8	24.2	39.4	26.6	22.8	17.8	($\bar{X}$=25.2±2.3)
Test #3	23.3	30.1	25.4	23.0	37.9	28.0	22.4	18.4	($\bar{X}$=26.1±2.1)

Sodium Chloride (pCPA)

Test #1	0.32	0.28	0.39	0.46	0.15	0.47	0.71	0.33	($\bar{X}$=0.39±0.06)
Test #2	0.34	1.73	0.16	0.59	0.38	0.71	1.50	0.64	($\bar{X}$=0.76±0.20)
Test #3	0.10	0.21	0.07	0.12	0.08	0.05	0.69	0.30	($\bar{X}$=0.20±0.08)

Sodium Chloride (CMC)

Test #1	0.23	0.59	0.30	0.42	0.31	0.16	0.21	0.24	($\bar{X}$=0.31±0.05)
Test #2	0.28	0.27	0.18	0.43	0.39	0.17	0.12	0.62	($\bar{X}$=0.31±0.06)
Test #3	0.22	0.16	0.12	0.29	0.20	0.15	0.22	0.46	($\bar{X}$=0.23±0.04)