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The Effect of para-Chlorophenylalanine on the Intake of Ethanol and Saccharin Solutions[1]

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STEIN, J. M., M. J. WAYNER AND H. A. TILSON. *The effect of para-chlorophenylalanine on the intake of ethanol and saccharin solutions.* PHARMAC. BIOCHEM. BEHAV. 6(1) 117–122, 1977. – The effects of para-chlorophenylalanine (PCPA) on the ingestive behavior of rats offered a choice between ethanol (3%) and water, saccharin (0.125%) and water, or water alone were examined. Following a baseline period three saline or PCPA injections, 80 mg/kg, were administered. Decreases in both ethanol and saccharin intakes were observed. Increases in water intake occurred in the ethanol group without a change in total fluid intake. Increases in water intake did not occur in the saccharin group and these animals displayed decreases in total fluid intake. Water intake in the nonchoice group was unaffected. There were no changes in food intake associated with any of these effects. The data demonstrate that decreases in intakes following PCPA are not specific to ethanol solutions.

Para-chlorophenylalanine Serotonin Ethyl alcohol Drinking Alcoholism

THE POSSIBLE role of various putative neurotransmitters in the selection and preference of ethanol has been studied extensively. Catecholamines (CA) have been implicated in behavioral studies which examined ethanol ingestion following monoamine depletion [13, 14, 20]. In neurochemical studies, central CA turnover rates but not brain concentrations seem to be altered by acute or chronic ethanol administration [1, 11, 25]. Recent data have also implicated acetylcholine both behaviorally and neurochemically [8, 9, 26]. Evidence concerning the role of serotonin (5-HT) has remained unclear.

Ethanol administered either acutely or chronically is reported to alter the catabolism of the 5-HT metabolite, 5-hydroxyindoleacetaldehyde in the periphery [2,3]. This shift however is not accompanied by similar changes in the central nervous system [2] or in either brain 5-HT concentrations or turnover rates [4, 12, 17, 26]. In behavioral studies, attempts to increase 5-HT or 5-hydroxytryptophan have produced decreases in ethanol preference [6, 7, 18]. Consistent with these data are reported increases in ethanol preference following 5,6-dihydroxy-tryptamine [8,20]. Other studies have presented contradictory evidence concerning 5-HT involvement. Increases in dietary tryptophan produced strain dependent increases in ethanol consumption in rats [21]. More importantly, electrolytic lesions of the dorsal and median raphe nuclei produced no discernable changes in the selection of a 10% ethanol solution [13].

Para-chlorophenylalanine (PCPA), a drug whose effects include tryptophan hydroxylase inhibition, has been utilized to lower 5-HT levels with subsequent examination of ethanol ingestion. While the results of several studies have demonstrated decreased ethanol intakes following PCPA [18, 22, 27] other studies have seen no change in preference [7,10] and at least one study has reported decreases followed by increases in preference [5]. These contradictions might be attributable to several factors. First, PCPA has been shown to be capable of eliciting taste aversion comparable to lithium chloride when associated with novel solutions [23]. Recent studies [24,28] have demonstrated nonspecific decreased intake of a variety of sapid solutions following PCPA. Second, studies using PCPA have utilized chronically administered high doses. The effects of such injection regimens have been reported to include inhibition of tryptophan hydroxylase and tyrosine hydroxylase and thereby reduce both 5-HT and CA levels [15,16]. Finally, repeated PCPA administrations can increase total fluid intake in rats [13,28].

The purpose of the present study was to examine the effect of PCPA, 80 mg/kg administered intraperitoneally on three consecutive days, on ethanol consumption. Similar regimens have been reported to initially decrease both CA

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and 5-HT levels [15]. This is followed by a period when only 5-HT levels remain depleted. In this manner CA and 5-HT depletion effects might be separable. Saccharin preference was also examined to determine if changes seen after PCPA were specific for ethanol solutions.

METHOD

Animals

Forty-four female hooded rats, 4 months old and 230–285 g in weight, were selected from our colony and placed in individual cages, 10 x 11 x 12 in. A 12 hr light-dark cycle began at 0600 hr and was followed by a 12 hr dark phase. The room temperature was maintained at 70 ± 2° F.

Solutions and Drugs

Ethanol solutions (ETOH), 3% v/v, were prepared from 95% ethyl alcohol in distilled water. Saccharin solutions (SAC), 0.125%, were prepared from purified, sodium saccharin (Fisher Scientific). Para-chlorophenylalanine injection solutions, 30.9 mg/cc, were prepared from dl-para-chlorophenylalanine, methyl ester hydrochloride (Regis) dissolved in 0.9% NaCl and triple distilled water. PCPA was administered in a dose of 80 mg of the base per kilogram of body weight. All saline injections were of 0.9% NaCl and were administered in volumes equivalent to the PCPA injections. All injections were administered intraperitoneally between 1600 and 1800 hr.

Procedure

Animals were divided into 3 groups with 14, 16 and 14 rats comprising Groups 1, 2, and 3 respectively. Group 1 had water available from a single ball bearing spout protruding through the front grid cage door and attached to an inverted plastic graduated cylinder. Groups 2 and 3 had water available from 2 of 3 spouts and tubes. The third tube remained empty. Locations of tubes were changed daily in a predetermined order to eliminate perferences due to position [19]. Ad lib food and water intakes and body weights were measured daily for 10 days between 1200 and 1500 hr.

During the next 12 days of the experiment, Days 1–12, Group 1 continued to have water available in a nonchoice situation. Group 2 was exposed to ETOH and water, and Group 3 was exposed to SAC and water. On Days 13, 14 and 15 all animals received an injection of saline. Following these injections, each group was further subdivided and injected with either PCPA or saline. In Group 1, the HOHPCPA animals received PCPA and the HOHSAL animals received saline on Days 16, 17 and 18. In Group 2, ETOHPCPA animals received PCPA and ETOHSAL animals received saline on Days 16, 17 and 18. Identically in Group 3, SACPCPA animals received PCPA and SACSAL animals received saline on these days. Water, ETOH and SAC intakes, food intakes, and body weights were recorded for the next 30 days.

RESULTS

Food and water intake data for all groups were analyzed by means of 2 x 13 ANOVAs with repeated measures [29]. In addition, 2 x 13 ANOVAs with repeated measures for solution intakes and total fluid intakes were performed for Groups 2 and 3. The factors were groups, PCPA or saline, and days on the solution. Days represented the mean intake of all animals during Days 7–12, the mean intake of all animals during Days 13–15, the individual mean intakes on Days 16–25, and the mean intake of all animals during Days 26–31. Post hoc Dunnett tests [29] were performed for all significant days by group interactions. The control mean constituted the mean intake of all animals on Days 7–12.

In Group 1, analysis of water intakes indicated no significant differences. These effects are shown in Fig. 1 where water intakes in the HOHPCPA group are repre-

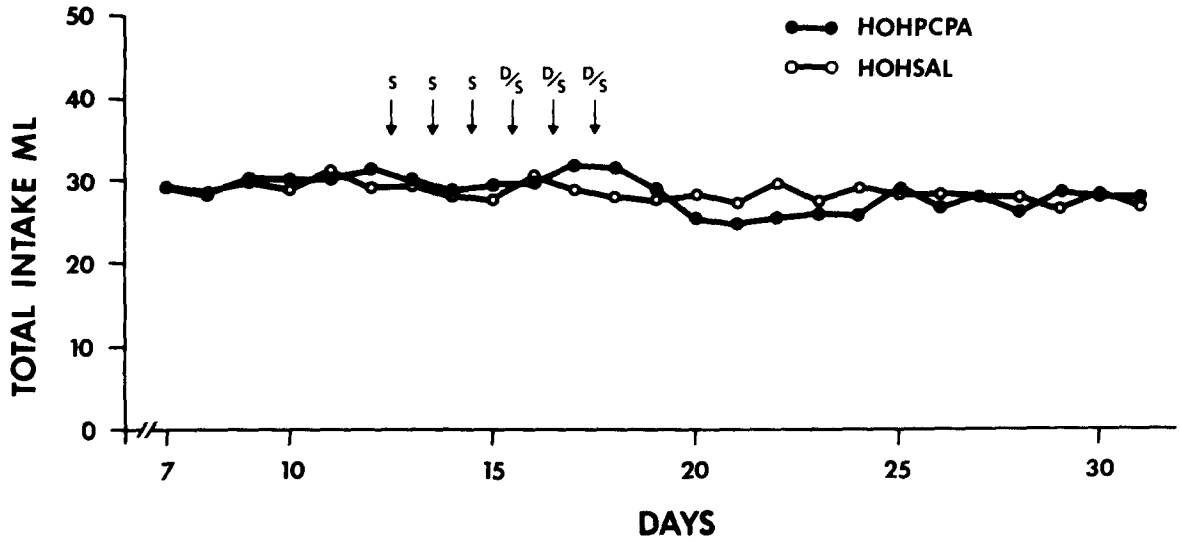

FIG. 1. Water intakes in ml of Group 1 presented as daily group means. Water intakes of the HOHPCPA animals are represented as closed circles connected by solid lines. Water intakes of the HOHSAL animals are represented as open circles connected by solid lines. Vertical arrows with an S indicate when all animals received saline injections. Vertical arrows with a D/S indicate when the HOHPCPA animals received PCPA, 80 mg/kg, and when the HOHSAL animals received saline injections.

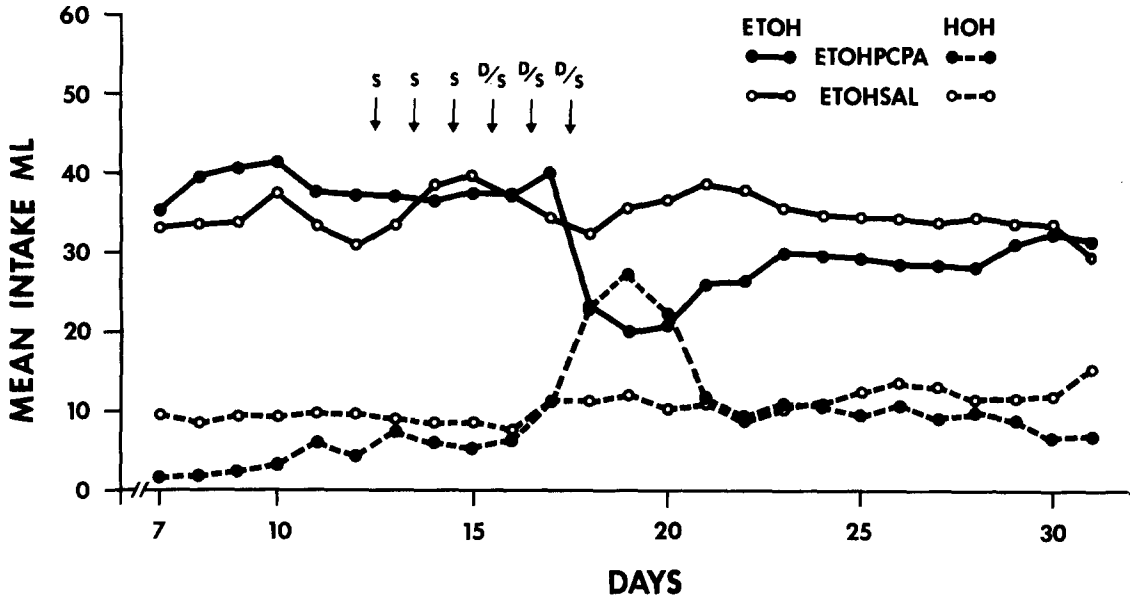

FIG. 2. ETOH and water intakes in ml of Group 2 presented as daily group means. ETOH intakes of the ETOHPCPA animals are represented as solid circles connected by solid lines. ETOH intakes of the ETOHSAL animals are represented as open circles connected by solid lines. Water intakes of the ETOHPCPA animals are represented as closed circles connected by broken lines. Water intakes of the ETOHSAL animals are represented as open circles connected by broken lines. Vertical arrows with an S indicate when all animals received saline injections. Vertical arrows with a D/S indicate when the ETOHPCPA animals received PCPA, 80 mg/kg, and when the ETOHSAL animals received saline injections.

sented by closed circles with solid lines and water intakes in the HOHSAL group are represented by open circles with solid lines. Analysis of food intakes indicated no significant differences.

In Group 2, analysis of solution intakes indicated significant differences between days, $F(12,168) = 3.53$, $p<0.01$. A days by group interaction was also significant, $F(12,168) = 3.90$, $p<0.01$. Further analysis with Dunnett tests revealed significant decreases in ETOH intakes on Days 18–22 in the ETOHPCPA group, $p<0.02$. No significant decreases in ETOH intakes were found within the ETOHSAL group. These effects are shown in Fig. 2 where ETOH intakes in the ETOHPCPA group are represented by closed circles with solid lines and ETOH intakes in the ETOHSAL group are represented by open circles with solid lines. Analysis of water intakes indicated significant differences between days, $F(12,168) = 3.31$, $p<0.01$. A days by group interaction was significant, $F(12,168) = 2.25$, $p<0.02$. Further analysis with Dunnett tests revealed significant increases in water intakes in the ETOHPCPA group on Days 18, 19 and 20, $p<0.01$. No significant differences were found within the ETOHSAL group. These effects are shown in Fig. 2 where water intakes for the ETOHPCPA group are represented by closed circles with broken lines and water intakes for the ETOHSAL group are represented by open circles with broken lines.

Analysis for total fluid intakes indicated significant differences between days, $F(12,168) = 2.10$, $p<0.02$. A days by group interaction was significant, $F(12,168) = 2.29$, $p<0.02$. Further analysis with Dunnett tests revealed no significant differences within either group. These effects are shown in Fig. 3 where total fluid intakes for the ETOHPCPA group are represened by closed circles with solid lines and total fluid intakes for the ETOHSAL group are represented by open circles with solid lines.

Analysis of food intakes indicated significant differences between days for both groups, $F(12,168) = 2.77$, $p<0.01$. The days by group interaction was not significant.

In summary, in Group 2, decreases were seen in the intake of ETOH following PCPA with simultaneous increases in water intakes. Increases in water intakes only occurred when the decrease observed in ETOH intake fell below the fluid intake levels seen in Group 1. These changes were not associated with either a change in food intakes or a change in total fluid intakes.

In Group 3, analysis for solution intakes indicated significant differences between days, $F(12,144) = 4.59$, $p<0.01$. A days by group interaction was significant, $F(12,144) = 8.36$, $p<0.01$. Further analysis with Dunnett tests revealed significant decreases in SAC intakes in the SACPCPA group on Days 20–24, $p<0.01$. No significant differences were found within the SACSAL group. These effects are shown in Fig. 4 where SAC intakes for the SACPCPA group are represented by closed circles with solid lines and SAC intakes for the SACSAL group are represented by open circles with solid lines. Analysis for water intakes indicated no significant differences. These effects are shown in Fig. 4 where water intakes for the SACPCPA group are represented by closed circles with broken lines and water intakes for the SACSAL group are represented by open circles with broken lines.

Analysis for total fluid intakes indicated significant differences between days, $F(12,144) = 4.43$, $p<0.01$. A days by group interaction was significant, $F(12,144) = 8.28$, $p<0.01$. Further analysis with Dunnett tests revealed significant decreases in total fluid intakes within the SACPCPA group on Days 20–24, $p<0.01$. No significant differences were found within the SACSAL group. These effects are shown in Fig. 5 where total fluid intakes for the SACPCPA group are represented by closed circles with solid

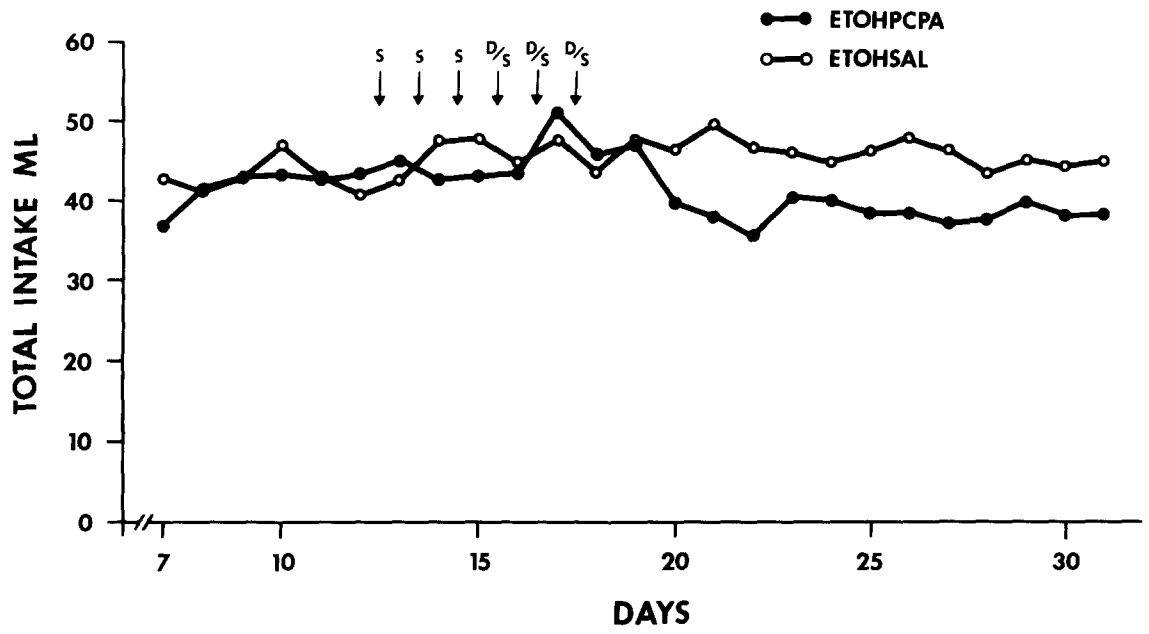

FIG. 3. Total fluid intakes in ml of Group 2 presented as daily group means. Total fluid intakes of the ETOHPCPA animals are represented as solid circles connected by solid lines. Total fluid intakes of the ETOHSAL animals are represented as open circles connected by solid lines. Vertical arrows with an S indicate when all animals received saline injections. Vertical arrows with a D/S indicate when the ETOHPCPA animals received PCPA, 80 mg/kg, and when the ETOHSAL animals received saline injections.

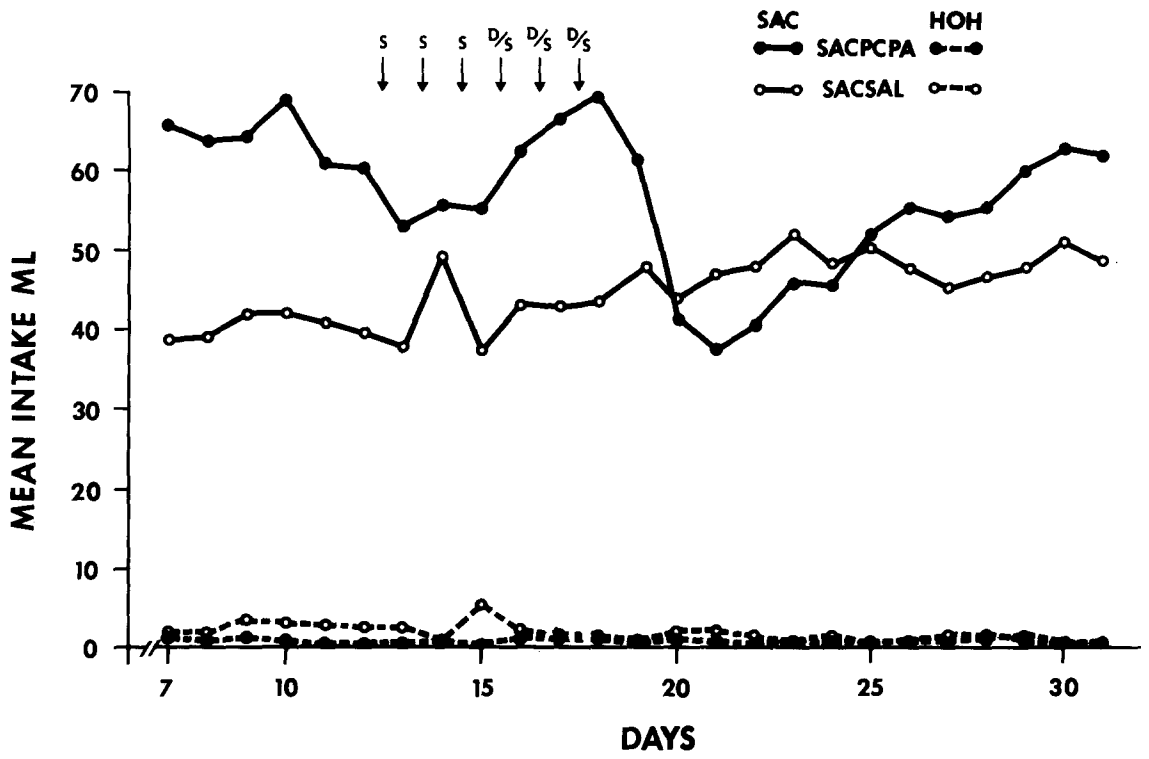

FIG. 4. SAC and water intakes in ml of Group 3 presented as daily group means. SAC intakes of the SACPCPA animals are represented as solid circles connected by solid lines. SAC intakes of the SACSAL animals are represented as open circles connected by solid lines. Water intakes of the SACPCPA animals are represented as closed circles connected by broken lines. Water intakes of the SACSAL animals are represented as open circles connected by broken lines. Vertical arrows with an S indicate when all animals received saline injections. Vertical arrows with a D/S indicate when the SACPCPA animals received PCPA, 80 mg/kg, and when the SACHOH animals received saline injections.

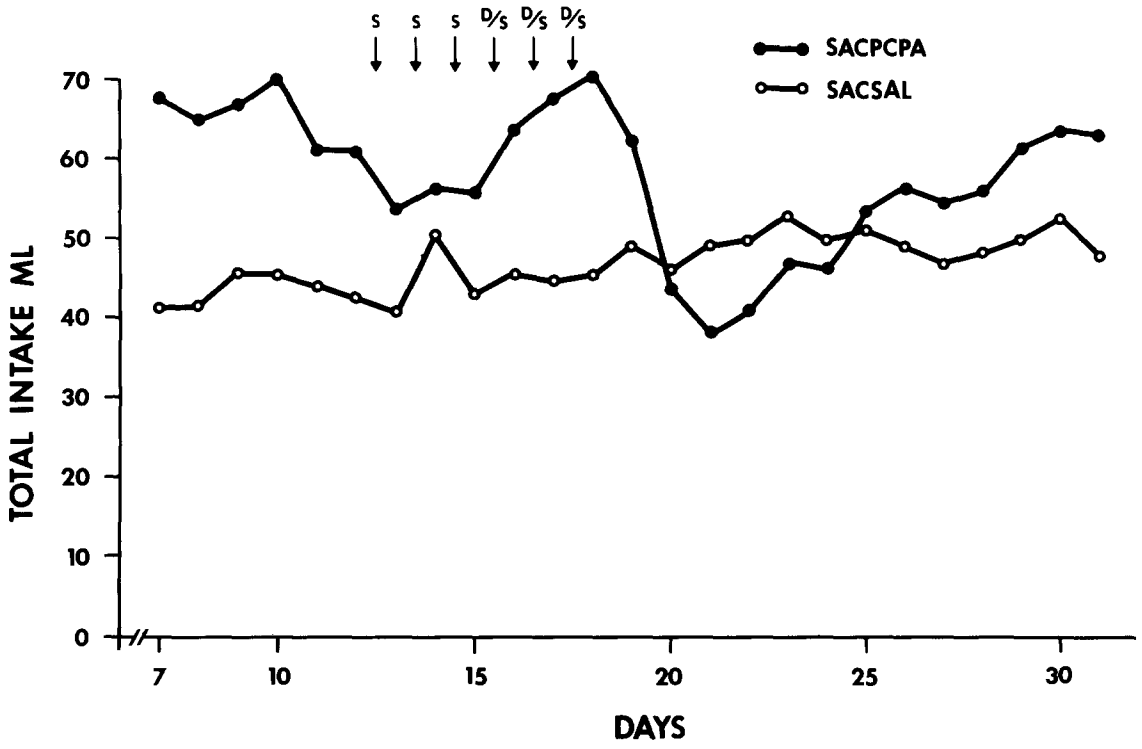

FIG. 5. Total fluid intakes in ml of Group 3 presented as daily group means. Total fluid intakes of the SACPCPA animals are represented as solid circles connected by solid lines. Total fluid intakes of the SACSAL animals are represented as open circles connected by solid lines. Vertical arrows with an S indicate when all animals received saline injections. Vertical arrows with a D/S indicate when the SACPCPA animals received PCPA, 80 mg/kg, and when the SACSAL animals received saline injections.

lines and total fluid intakes for the SACSAL group are represented by open circles with solid lines. Analysis of food intakes indicated no significant differences.

In summary, in Group 3, decreases were seen in the intakes of SAC following PCPA with no simultaneous increase in water intake. Decreases observed in SAC and in total fluid intakes never decreased below the fluid intake levels seen in Group 1. These changes were not associated with changes in food intakes.

DISCUSSION

These results indicate that changes in solution consumption following PCPA can occur with an ethanol solution or a nonethanol sweet solution. Decreases in ethanol intake first occurred immediately following the last drug injection. Decreases in saccharin intake first occurred 3 days after the last injection. Intakes of both solutions returned to baseline levels within 5 days. Increases in water intake occurred in Group 2 but not in either Group 1 or 3. Such changes were only observed when the physiological total fluid intake requirements of the rats, based upon the data of Group 1, would not have otherwise been attained when ethanol intake decreased. In Group 3, no decreases in total fluid intake below the Group 1 level occurred. In none of the groups were significant changes in food intake associated with changing fluid intake patterns.

In conclusion, the decreases in ethanol intake following PCPA appear to be related to some form of mild taste aversion and nonspecific for ethanol solutions. These decreases do not appear to be associated with changes in food, water or total fluid intake. All these effects are relatively transient and might be correlated with decreases in CA and 5-HT concentrations. During the period when only 5-HT levels remain depleted, no behavioral effects occurred. Recent unpublished data in this laboratory on the effect of p-chloroamphetamine on ethanol preference seems to support these observations. Finally, these PCPA effects were seen to occur following a lower dose administered less frequently than in previous studies.

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