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Synergistic Action of p-Chloroamphetamine and Fluoxetine on Food and Water Consumption Patterns in the Rat[1]

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STEIN, J. M., M. J. WAYNER, K. M. KANTAK AND R. L. ADLER-STEIN. *Synergistic action of p-chloroamphetamine and fluoxetine on food and water consumption patterns in the rat.* PHARMAC. BIOCHEM. BEHAV. 9(5) 677–685, 1978.—The distribution of eating, drinking and body weight changes during the 24 hr day were examined following brain 5-HT depletion with p-chloroamphetamine (PCA). Following a baseline period, measurements of food and water intakes and body weights were recorded 1, 2, 3, 6, 12, 20 and 24 hr following PCA, 5.0 mg/kg, or saline. Other animals were pretreated with fluoxetine, 10.0 mg/kg, prior to either PCA or saline in an attempt to block the PCA effects. The results indicate acute hypophagia, hypodipsia, and body weight losses. These decreases were not influenced by the time of day when PCA was administered. Pretreatment with fluoxetine enhanced rather than blocked these effects. No long term changes in ingestive behavior were seen. These results are discussed with respect to the possible role of 5-HT in the control of ingestive behavior.

p-Chloroamphetamine Eating Fluoxetine Drinking Body weight Light/dark rhythms

DETERMINATION of the possible role of brain serotonin (5-HT) in ingestive behavior has been of interest for several years. Systemic or intraperitoneal administration of the serotonin precursor, 5-hydroxytryptophan (5-HTP), decreases food intake and increases water intake [24,37]. 5-Hydroxytryptophan administered in this manner is chiefly metabolized to 5-HT in both the central nervous system and periphery. Intraperitoneal, subcutaneous or systemic 5-HT administration, procedures which primarily elevate only peripheral 5-HT concentrations, decrease food intake [5, 19, 25]. Therefore, changes in consummatory behavior following 5-HTP administration might be attributable to peripheral 5-HT modulation which can include changes in gastrointestinal secretion and motility, plasma insulin, glucose and renin-angiotensin concentrations, intralumenal intestinal secretion, body temperature, and cardiovascular physiology [3, 10, 21, 29]. Infusion of 5-HT directly into specific brain nuclei or into the lateral cerebroventricle has failed to produce consistent changes in consummatory behavior [4, 19, 25, 35, 37]. In addition a lack of effect has been reported [36].

Discrete brain lesions and the administration of relatively specific neuropharmacological agents have been utilized to chronically deplete brain 5-HT. Intracranial administration of 5,6- or 5,7-dihydroxytryptamine (DHT), peripheral administration of p-chloroamphetamine (PCA) or fenfluramine, and lesions of the median or dorsal raphe nuclei produce long term decreases in brain 5-HT concentration [1, 15, 22, 26]. Short term decreases in food intakes and body weights are reported to occur following each of these procedures [2, 6, 16, 26, 27, 38, 39] while other reports have indicated no effects [28, 31, 33]. In contrast, hyperphagia and chronically increased body weight growth have been demonstrated following intraventricular p-chlorophenylalanine (PCPA), or 5,7-DHT plus desmethylimipramine pretreatment [7,32]. In light of these conflicting reports it can be suggested that brain 5-HT depletion might alter the pattern, rate or distribution of eating and drinking with or without affecting 24 hr total intakes. Serotonergic depletion following intraventricular PCPA produces the greatest increase in food intake during the light portion of the light-dark cycle [7] and intraventricular 5,6-DHT changes meal size and dietary selection without affecting 24 hr intake [9].

PCA is a halogenated amphetamine analogue whose long term effects include chronic reduction of brain 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan hydroxylase concentrations [15,34]. During the first 24 hr following administration increases in norepinephrine (NE), dopamine (DA) and 5-HT release occur [40] and 5-HT reuptake, synthesis and turnover is inhibited. The ability of PCA to lower chronically brain 5-HT concentrations can be blocked or reversed by treatment with fluoxetine (Lilly 110140) up to 24 hr prior to or following PCA administration [12,14]. Fluoxetine is a specific 5-HT reuptake blocker which lowers 5-HIAA concentrations and decreases 5-HT synthesis without affecting brain 5-HT, NE or DA concentrations [13]. In the present investigation, the pattern of food and water in-

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takes and body weight changes during the 24 hr day were examined following PCA, fluoxetine, PCA plus fluoxetine or saline administration. In addition the effect of PCA on ingestive behavior was examined at two different injection times.

EXPERIMENT 1

METHOD

Animals

Twenty eight female hooded rats, at least 4 months old and 240–330 g in weight, were selected from our colony and placed into individual cages, 25×28×30 cm. A 12 hr light-dark cycle began at 0700 hr and was followed by a 12 hr dark phase. The room temperature was maintained at 20 ± 1°C.

Drugs

PCA injection solutions, 5.0 mg/cc calculated from the base, were prepared from d,l-para-chloroamphetamine hydrochloride (Sigma Chemical). Fluoxetine (Lilly 110140; Eli Lilly) was prepared as a 10 mg/cc injection solution. Both PCA and fluoxetine were dissolved in 0.9% NaCl in triple distilled water. Saline injections were of 0.9% NaCl. All injections were administered intraperitoneally in volumes of 1 cc per kilogram of body weight.

Procedure

For a period of at least 10 days, Days 1–10, animals were adapted to their home cages. Ad lib food and water intakes and body weights were measured daily between 1400 and 1600 hr. On Days 11, 13, 15 and 17 all animals received saline injections at 1800 hr. Food and water intakes and body weights were recorded 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection on Days 15 and 17. On Day 19, animals were divided into four groups of 7 rats each comprising the Saline, PCA, Saline+Fluoxetine, and PCA+Fluoxetine groups. At 0600 hr, animals in the Saline+Fluoxetine and PCA+Fluoxetine Groups received 10.0 mg/kg of fluoxetine. At 1800 hr, animals in the Saline and Saline+Fluoxetine Groups received saline and animals in the PCA and PCA+Fluoxetine Groups received 5.0 mg/kg of PCA. Food and water intakes and body weights were recorded 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection on Day 19 beginning at 1800 hr and 25, 26, 27, 30, 36, 44 and 48 hr postinjection on Day 20 beginning at 1800 hr. On Days 21, 23 and 26 all animals received saline injections at 1800 hr. Food and water intakes and body weights were recorded, 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection on Day 26.

RESULTS

Body Weight

Mean body weights on Days 15 and 17 and net body weight growth during the entire experiment were analyzed by means of one way analyses of variances [41]. The 4 levels of the groups factor were the Saline, PCA, Saline+Fluoxetine and PCA+Fluoxetine Groups. There were no significant differences between groups in terms of mean body weights on Days 15 and 17 or between body weight growth during the experiment. Cumulative body weight changes recorded during each test day were analyzed by means of a 4×4×7 analysis of variance with repeated measures. The factors for the analysis were groups, days and hours postinjection. Cumulative body weight changes refer to the net change in body weight during the hours postinjection and starting at 0 hr postinjection. The 4 levels of the groups factor were the Saline, PCA, Saline+Fluoxetine and PCA+Fluoxetine Groups. The 4 levels of the days factor were the baseline test day, the drug day, the postdrug day and the retest day. The baseline test day refers to the mean of the 24 hr post saline injection periods on Days 15 and 17. The drug day, the postdrug day and the retest day refer to the 24 hr post injection periods on Days 19, 20 and 26, respectively. The 7 levels of the hours factor were 1, 2, 3, 6, 12, 20 and 24 hr postinjection.

Analysis of cumulative body weight changes indicated significant differences between groups, $F(3,24)=23.42$, $p<0.01$; days, $F(3,72)=91.17$, $p<0.01$; and hours, $F(6,144)=48.47$, $p<0.01$. The interaction of all factors was significant: groups by days, $F(9,72)=35.09$, $p<0.01$; days by hours, $F(18,432)=27.53$, $p<0.01$; groups by hours, $F(18,144)=3.66$, $p<0.01$; and groups by days by hours, $F(54,432)=6.44$, $p<0.01$. Fig. 1 illustrates the mean cumulative body weights for each group on the drug day, begining at 1800 hr on Day 19. Figure 2 illustrates the mean cumulative body weight for each group on the postdrug day beginning at 1800 hr on Day 20.

Within group and between group differences were compared using Tukey A tests. There were no significant differences in cumulative body weight between groups on the baseline test day. Cumulative body weight in the PCA Group was significantly lower on the drug day compared to Saline Group, Saline+Fluoxetine Group, and its own baseline day 1, 2, 3, 6, 12, 20 and 24 hr postinjection, $p<0.01$. On the postdrug day the cumulative body weight in the PCA group was significantly lower compared to the Saline Group, Saline+Fluoxetine Group, and its own baseline day 25, 26, 27, 30 and 36 hr postinjection, $p<0.01$. There were no significant differences on the retest day. In the Saline+Fluoxetine Group, significantly lower cumulative body weight on the drug day was found 2, 3, 6 and 12 hr postinjection compared to its own baseline day and 6 hr postinjection compared to the Saline Group, $p<0.01$. Body weight returned to normal on the postdrug day. Cumulative

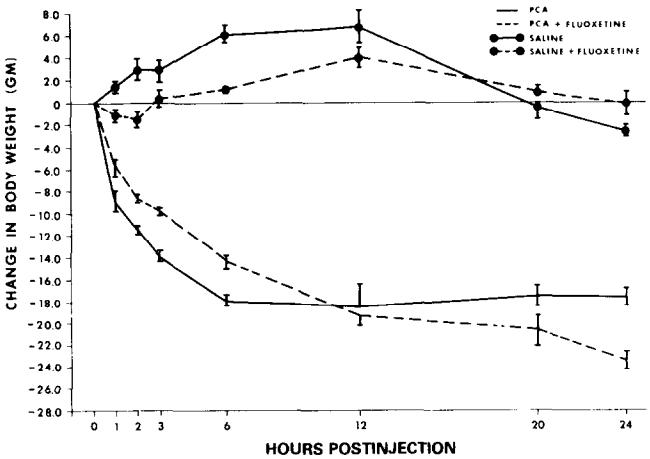

FIG. 1. Mean cumulative body weight changes 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection in the PCA, PCA+Fluoxetine, Saline, and Saline+Fluoxetine Groups. All body weight changes were calculated with respect to individual body weights at 0 hr postinjection (1800 hr on Day 19). n=7 animals/group.

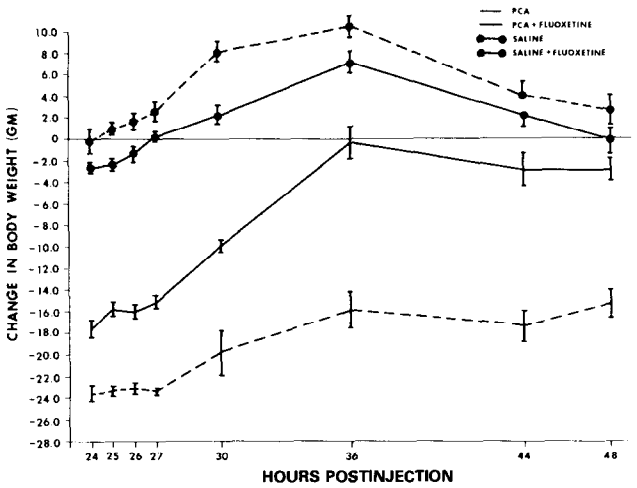

FIG. 2. Mean cumulative body weight changes 24, 25, 26, 27, 30, 36, 44 and 48 hr postinjection in the PCA, PCA+Fluoxetine, Saline and Saline+Fluoxetine Groups. All body weight changes were calculated with respect to the individual body weights at 0 hr postinjection (1800 hr on Day 19). n=7 animals/group.

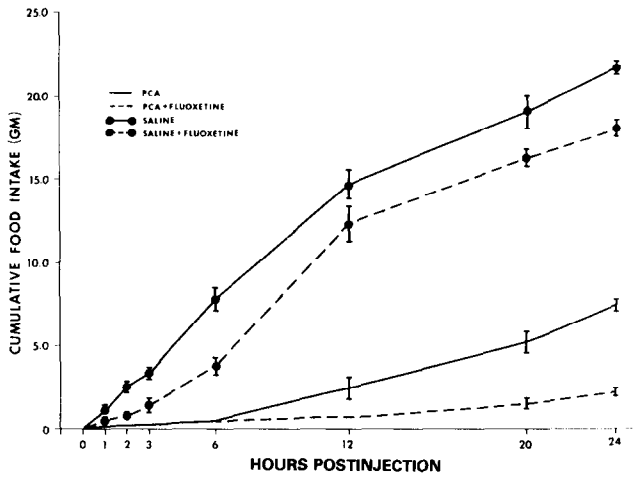

FIG. 3. Mean cumulative food intakes in the PCA, PCA+Fluoxetine, Saline and Saline+Fluoxetine Groups 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection. All food intakes were calculated with respect to 0 hr postinjection (1800 hr on Day 19). n=7 animals/group.

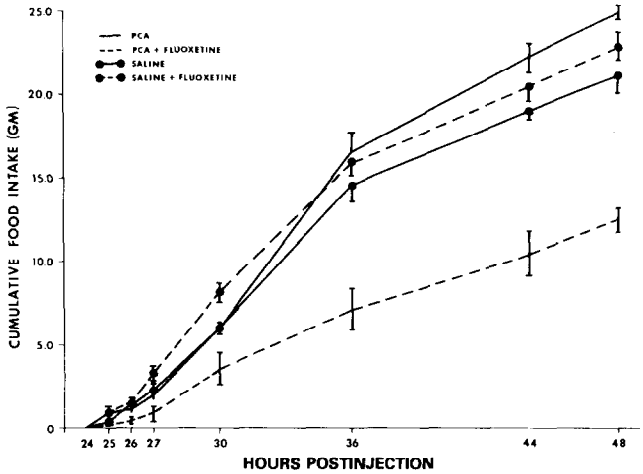

FIG. 4. Mean cumulative food intakes in the PCA, PCA+Fluoxetine, Saline and Saline+Fluoxetine Groups 24, 25, 26, 27, 30, 36, 44 and 48 hr postinjection. All food intakes were calculated with respect to 24 hr postinjection (1800 hr on Day 20). n=7 animals/group.

body weight in the PCA+Fluoxetine Group was significantly lower on the drug day compared to Saline Group, Saline+Fluoxetine Group, and its own baseline day 1, 2, 3, 6, 12, 20 and 24 hr postinjection, $p<0.01$. In addition the PCA+Fluoxetine Group had significantly lower cumulative body weight than the PCA Group 24 hr postinjection, $p<0.01$. On the postdrug day the cumulative body weight in the PCA+Fluoxetine Group was significantly lower compared to the Saline Group, Saline+Fluoxetine Group, PCA Group, and its own baseline day 25, 26, 27, 30, 36, 44 and 48 hr postinjection, $p<0.01$. There were no significant differences between the PCA+Fluoxetine Group and all other Groups on the retest day.

In summary, PCA produced rapid and large decreases in body weights. Body weight remained significantly lower for 36 hr postinjection. Fluoxetine produced only small decreases during the first 6 hr postinjection and had no significant effect on the normal daily body weight patterns after this period. Animals administered PCA following fluoxetine pretreatment exhibited the longest lasting decreases in body weight. During the 0–20 hr postinjection period cumulative body weight was almost identical in the PCA and PCA+Fluoxetine Groups. However, body weight in the PCA+Fluoxetine Group remained depressed throughout the first postdrug day while body weight in the PCA Group rapidly recovered. No long term changes in the normal daily body weight patterns were measured in any group.

Food Intake

Cumulative food intake recorded during each test day was analyzed by means of a 4×4×7 analysis of variance with repeated measures. The factors for the analysis were groups, days and hours postinjection. The 4 levels of the groups factor were the Saline, PCA, Saline+Fluoxetine and PCA+Fluoxetine Groups. The 4 levels of the days factor were the baseline test day, the drug day, the postdrug day and the retest day. The baseline test day refers to the mean of the 24 hr post saline injection periods on Days 15 and 17. The drug day, the postdrug day and the retest day refer to the 24 hr postinjection periods on Days 19, 20 and 26, respectively. The 7 levels of the hours factor were 1, 2, 3, 6, 12, 20 and 24 hr postinjection.

Analysis of cumulative food intakes indicated significant differences between groups, $F(3,24)=11.76$, $p<0.01$; days, $F(3,72)=76.38$, $p<0.01$; and hours, $F(24,144)=1090.55$, $p<0.01$. The interaction of all factors was significant: groups by days, $F(9,72)=16.11$, $p<0.01$; days by hours, $F(18,432)=37.71$, $p<0.01$; groups by hours, $F(18,144)=6.37$, $p<0.01$; and groups by days by hours, $F(54,432)=11.09$, $p<0.01$. Figure 3 illustrates the mean cumulative food intake for each group on the drug day. Figure 4 illustrates the mean cumulative food intake for each group on the postdrug day.

Within group and between group differences were compared using Tukey A tests. There were no significant differences in cumulative food intake between groups on the baseline test day. Cumulative food intake in the PCA Group

was significantly lower on the drug day compared to the Saline Group and its own baseline day 6, 12, 20 and 24 hr postinjection; and compared to the Saline+Fluoxetine Group 12, 20 and 24 hr postinjection, $p<0.01$. On the postdrug day, food intake was significantly increased 48 hr postinjection compared to the Saline Group, $p<0.01$. Cumulative food intakes on the retest day were normal. In the Saline+Fluoxetine Group significantly lower cumulative food intake on the drug day was found 6 and 24 hr postinjection compared to the Saline Group and its own baseline day, $p<0.01$. Normal intakes were found on the postdrug day and retest day. Cumulative food intake in the PCA+Fluoxetine Group was significantly lower on the drug day compared to the Saline Group, Saline+Fluoxetine Group, and its own baseline day 6, 12, 20 and 24 hr postinjection, $p<0.01$. On the postdrug day the cumulative food intake in the PCA+Fluoxetine Group was significantly lower compared to the Saline Group, Saline+Fluoxetine Group, PCA Group, and its own baseline day 36, 44 and 48 hr postinjection, $p<0.01$. There were no significant differences between the PCA+Fluoxetine Group and all other Groups on the retest day.

In summary, PCA produced large decreases in food intakes during the first 24 hr postinjection. Fluoxetine treatment also produced decreases during this period but these decreases were of a smaller magnitude. Animals administered PCA following fluoxetine pretreatment exhibited the longest lasting decreases in food intakes. During the 0–24 hr postinjection period cumulative food intake was almost identically depressed in the PCA and PCA+Fluoxetine Groups. However, food intakes in the PCA+Fluoxetine Group remained depressed throughout the postdrug day while food intakes in the PCA Group returned to baseline levels. No long term changes in the normal daily food intake patterns were measured in any group.

Water Intake

Cumulative water intake recorded during each test day was analyzed by means of a 4×4×7 analysis of variance with repeated measures. The factors for the analysis were groups, days and hours postinjection. The 4 levels of the groups factor were the Saline, PCA, Saline+Fluoxetine and PCA+Fluoxetine Groups. The 4 levels of the days factor were the baseline test day, the drug day, the postdrug day and the retest day. The baseline test day refers to the mean of the 24 hr post saline injection periods on Days 15 and 17. The drug day, the postdrug day and the retest day refer to the 24 hr postinjection periods on Days 19, 20 and 26, respectively. The 7 levels of the hours factor were 1, 2, 3, 6, 12, 20 and 24 hr postinjection.

Analysis of cumulative water intake indicated significant differences between days, $F(3,72)=52.42$, $p<0.01$; and hours, $F(6,144)=696.80$, $p<0.01$. There were no significant differences between groups. There were significant interactions of groups by days, $F(9,72)=9.48$, $p<0.01$; days by hours, $F(18,432)=32.03$, $p<0.01$; and groups by days by hours, $F(54,432)=6.82$, $p<0.01$. The groups by hours interaction was not significant. Figure 5 illustrates the mean cumulative water intake for each group on the drug day. Figure 6 illustrates the mean cumulative water intake for each group on the postdrug day.

Within group and between group differences were compared using Tukey A tests. There were no significant differences in cumulative water intake between groups on the baseline test day. Cumulative water intake in the PCA Group was significantly lower on the drug day compared to the Saline Group, Saline+Fluoxetine Group, and its own baseline day 6, 12, 20 and 24 hr postinjection, $p<0.01$. In contrast on the postdrug day cumulative water intake in the PCA Group was significantly increased compared to the Saline Group and PCA+Fluoxetine Group 36, 44 and 48 hr postinjection; and compared to the Saline+Fluoxetine Group and its own baseline 44 and 48 hr postinjection, $p<0.01$. No changes were found on the retest day. In the Saline+Fluoxetine Group significantly lower cumulative water intake on the drug day was found 6 and 12 hr postinjection compared to the Saline Group and its own baseline day, $p<0.01$. Water intakes returned to normal on the postdrug day. Cumulative water intake in the PCA+Fluoxetine Group was significantly lower on the drug day compared to the Saline Group, Saline+Fluoxetine Group, and its own baseline day 6, 12, 20 and 24 hr postinjection; and compared to the PCA Group 20 and 24 hr

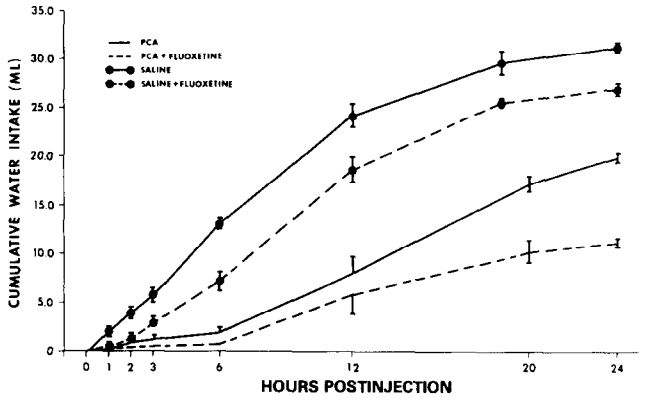

FIG. 5. Mean cumulative water intakes in the PCA, PCA+Fluoxetine, Saline and Saline+Fluoxetine Groups 0, 1, 2, 3, 6, 12, 20 and 24 hr postinjection. All water intakes were calculated with respect to 0 hr postinjection (1800 hr on Day 19). n=7 animals/group.

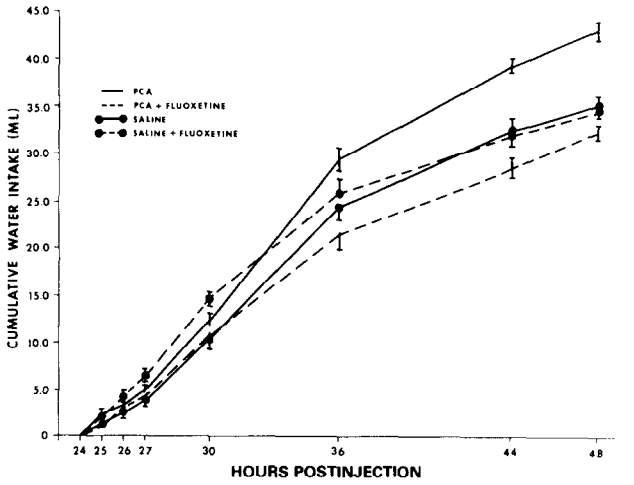

FIG. 6. Mean cumulative water intakes in the PCA, PCA+Fluoxetine, Saline and Saline+Fluoxetine Groups 24, 25, 26, 27, 30, 36, 44 and 48 hr postinjection. All water intakes were calculated with respect to 24 hr postinjection (1800 hr on Day 20). n=7 animals/group.

postinjection, $p<0.01$. On the postdrug day the cumulative water intake in the PCA+Fluoxetine Group was significantly lower compared to its own baseline day 36, 44 and 48 hr postinjection, $p<0.01$. There were no differences on the retest day.

In summary, PCA produced large decreases in water intakes during the first 24 hr postinjection. Fluoxetine treatment produced smaller decreases during this same period. Animals administered PCA following fluoxetine pretreatment exhibited the longest lasting decreases in water intake. During the 0–12 hr postinjection period cumulative water intake was almost identically depressed in the PCA and PCA+Fluoxetine Groups. However, water intakes in the PCA+Fluoxetine Group remained depressed during part of the postdrug day while water intake in the PCA Group exhibited a significant increase. No long-term changes in the normal daily water intake patterns were measured in any group.

DISCUSSION

PCA produced large decreases in food and water intakes and body weights shortly following its administration. These deficits endured for at least 24 hr and ingestive behavior and body weights slowly recovered during the 24–48 hr postinjection period. The initial losses in body weights were not entirely due to the hypophagia and hypodipsia. Animals deprived of food and water for 24 hr lose weight at a slower rate compared to PCA injected rats (unpublished observations). Increases in urination and/or defecation are, therefore, implicated. Fluoxetine produced small decreases in food and water intakes and body weights. In contrast, animals pretreated with fluoxetine 12 hr prior to PCA initially had decreases in food and water intakes and body weight deficits almost identical to that of the PCA Group. During the 24–48 hr postinjection period, the PCA+Fluoxetine Group continued to be hypophagic, hydodipsic and remained at lower body weight levels. In the PCA+Fluoxetine Group, complete recovery did not occur until 72 hr postinjection.

EXPERIMENT 2

METHOD

Animals

Fourteen female hooded rats, at least 4 months old and 212–310 g in weight, were selected from our colony and housed under identical experimental conditions as described in Experiment 1.

Drugs

PCA injection solutions, 5.0 mg/cc calculated from the base, were prepared identically as described in Experiment 1.

Procedure

For a period of at least 10 days, Days 1–10, animals were adapted to their home cages. Ad lib food and water intakes and body weights were measured daily between 1400 and 1600 hr. On Days 11, 13, 15 and 17 all animals received saline injections at 1200 hr. Food and water intakes and body weights were recorded 0, 1, 2, 3, 6, 12, 18 and 24 hr postinjection on Days 15 and 17. On Day 19, animals were divided into two groups of 7 rats each comprising the Saline and PCA Groups. At 1200 hr, animals in the PCA Group received 5.0 mg/kg of PCA and animals in the Saline Group received saline. Food and water intakes and body weights were recorded 0, 1, 2, 3, 6, 12, 18 and 24 hr postinjection on Day 19 beginning at 1200 hr and 25, 26, 27, 30, 36, 42 and 48 hr postinjection on Day 20 beginning at 1200 hr.

RESULTS

Data collected from the Saline and PCA Groups in Experiment 1 were analyzed together with data collected in the Saline and PCA Groups in Experiment 2 in order that the effects of PCA administered at 1800 or 1200 hr could be compared. The Saline and PCA Groups from Experiment 1 are referred to as the Night-Saline and Night-PCA Groups, respectively. The Saline and PCA Groups from Experiment 2 are referred to as the Day-Saline and Day-PCA Groups, respectively.

Cumulative body weight changes and cumulative food and water intakes recorded during each test period were analyzed by means of 4×3×6 analyses of variances with repeated measures. The factors were groups, days and hours. The 4 levels of the groups factor were the Night-Saline, Night-PCA, Day-Saline and Day-PCA Groups. The 3 levels of the days factor were the baseline test day, the drug day, and the postdrug day. The baseline test days in the Night-Saline and Night-PCA Groups refer to the mean of the two 24 hr postinjection periods beginning at 1800 on Days 15 and 17. The baseline test days in the Day-Saline and Day-PCA Groups refer to the mean of the two 24 hr postinjection periods beginning at 1200 hr on Days 15 and 17. The drug day and the postdrug day refer to the 24 hr postinjection periods beginning at 1800 and 1200 hr on Days 19 and 20. The 6 levels of the hours factor were the 1, 2, 3, 6, 12 and 24 hr postinjection periods. The data recorded 20 hr postinjection in the Night-PCA and Night-Saline Groups and the data recorded 18 hr postinjection in the Day-PCA and Day-Saline Groups were not analyzed. Post hoc Tukey A tests were used to compare the data within and between groups. Because of normal differences in the pattern of eating, drinking and body weight changes at different times of day, direct comparisons between groups were confined to comparing the Night-PCA Group with the Night-Saline Group and the Day-PCA Group with the Day-Saline Group. The only exception was a comparison of food intakes and water intakes in all 4 groups 24 and 48 hr postinjection. Finally, planned contrast analyses were performed comparing the net differences in terms of body weights and food and water intakes in the Night-PCA minus the Night-Saline compared to the Day-PCA minus the Day-Saline.

Body Weight

There were no significant differences between groups in terms of mean body weights on Days 15 and 17. Analysis of cumulative body weight changes indicated significant differences between groups, $F(3,24)=16.41$, $p<0.01$; days, $F(2,48)=36.96$, $p<0.01$; and hours, $F(5,120)=20.19$, $p<0.01$. The interaction of all factors was significant: groups by days, $F(6,48)=22.74$, $p<0.01$; days by hours, $F(10,240)=24.19$, $p<0.01$; groups by hours, $F(15,120)=11.52$, $p<0.01$; and groups by days by hours, $F(30,240)=7.10$, $p<0.01$. Figure 7 illustrates the mean cumulative body weight changes for each group on the drug day beginning at 1800 hr on Day 19 in the Night Groups and at 1200 hr on Day 19 in the Day Groups.

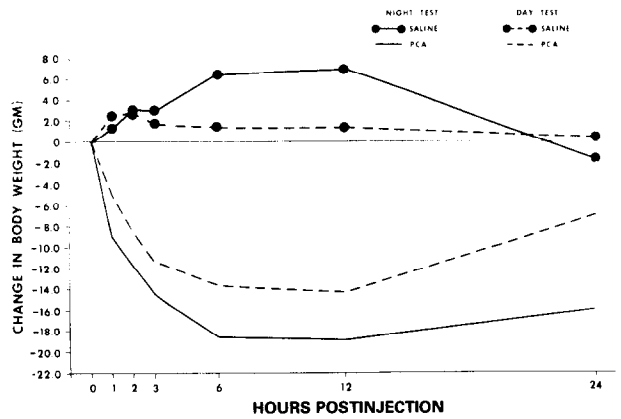

FIG. 7. Mean cumulative body weight changes 0, 1, 2, 3, 6, 12 and 24 hr postinjection in the Night-PCA, Night-Saline, Day-PCA and Day-Saline Groups. All body weight changes were calculated with respect to the individual body weights at 0 hr postinjection (1800 or 1200 hr on Day 19). n=7 animals/group.

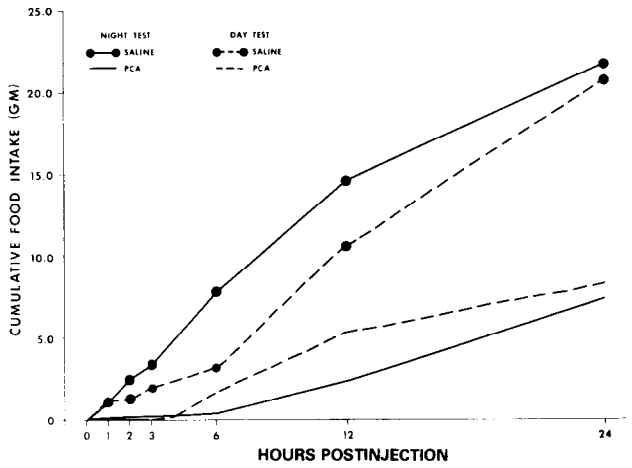

FIG. 8. Mean cumulative food intakes in the Night-PCA, Night-Saline, Day-PCA, and Day-Saline Groups 0, 1, 2, 3, 6, 12 and 24 hr postinjection. All food intakes were calculated with respect to 0 hr postinjection (1800 or 1200 hr on Day 19). n=7 animals/group.

Cumulative body weight in the PCA Groups was significantly lower on the drug day compared to their respective Saline Groups and their own baseline days 1, 2, 3, 6, 12 and 24 hr postinjection, $p<0.01$. On the postdrug day cumulative body weight in the Night-PCA Group was significantly lower compared to the Night-Saline Group and its own baseline day 25, 26, 27, 30 and 36 hr postinjection, $p<0.01$. In the Day-PCA Group on the postdrug day, cumulative body weight was significantly lower 25, 26, 27, 30 and 36 hr postinjection compared to the Day-Saline Group and 25 and 26 hr postinjection compared to its own baseline day, $p<0.01$. Analyses with planned contrasts revealed significant differences in cumulative body weight change in the Night-PCA Group minus the Night-Saline Group compared to the Day-PCA Group minus the Day-Saline Group. There were greater body weight deficits in the Night-PCA Group than in the Day-PCA Group: 3 hr, $p<0.05$; 6, 12, 24, 25, 26, 27 and 30 hr postinjection, $p<0.01$.

In summary, PCA produced rapid and large decreases in body weight when given 6 hr following the beginning of the light phase or 1 hr prior to the beginning of the dark phase. The initial decreases, 1 and 2 hr postinjection, were unaffected by the time of day when the drug was administered. Body weights in both drug groups remained lowered throughout the first 24 hr postinjection period. Body weights were more severely affected in the group injected at night with PCA. On the postdrug day the animals injected with PCA at 1200 hr recovered their body weights more rapidly than animals injected with PCA at 1800 hr.

Food Intake

Analysis of cumulative food intakes indicated significant differences between groups, $F(3,24)=11.07$, $p<0.01$; days $F(2,48)=80.41$, $p<0.01$; and hours, $F(5,120)=1183.35$, $p<0.01$. The interaction of all factors was significant: groups by days, $F(6,48)=33.94$, $p<0.01$; days by hours, $F(10,240)=64.55$, $p<0.01$; groups by hours, $F(15,120)=19.10$, $p<0.01$; and groups by days by hours, $F(30,240)=19.11$, $p<0.01$. Figure 8 illustrates the mean cumulative food intake for each group on the drug day beginning at 1800 hr on Day 19 in the Night Groups and at 1200 hr on Day 19 in the Day Groups.

Cumulative food intake on the drug day in the Night-PCA Group was significantly lower 3, 6, 12 and 24 hr postinjection compared to the Night-Saline Group and its own baseline day, $p<0.01$. In the Day-PCA Group, however, cumulative food intake was significantly lower on the drug day 6, 12 and 24 hr postinjection compared to the Day-Saline Group and its own baseline day, $p<0.01$. On the postdrug day there were no differences between the Night Groups or between the Day Groups. In addition there were no significant differences in the 24 hr total food intake on the drug day and postdrug in the Night-PCA Group compared to the Day-PCA Group. However, analysis with planned contrasts revealed significant differences in cumulative food intake in the Night-PCA Group minus the Night-Saline Group compared to the Day-PCA Group minus the Day-Saline Group. There were greater food intake deficits in the Night-PCA Group than in the Day-PCA Group 6 and 12 hr postinjection on the drug day, $p<0.01$.

In summary, PCA produced decreases in food intakes when given either 6 hr following the beginning of the light phase or 1 hr prior to the beginning of the dark phase. Although 24 hr total intake was not different, cumulative food intake in the Night-PCA was more affected by PCA than food intakes in the Day-PCA Group during the first 24 hr postinjection. On the postdrug day food intakes returned to normal.

Water Intake

Analysis of cumulative water intakes indicated significant differences between groups, $F(3,24)=7.88$, $p<0.01$; days, $F(2,48)=27.35$, $p<0.01$; and hours, $F(5,120)=1122.61$, $p<0.01$. The interaction of all factors was significant: groups by days, $F(6,48)=12.05$, $p<0.01$; days by hours, $F(10,240)=18.72$, $p<0.01$; groups by hours, $F(15,120)=8.72$, $p<0.01$; and groups by days by hours, $F(30,240)=6.56$, $p<0.01$. Figure 9 illustrates the mean cumulative water intake for each group on the drug day.

Cumulative water intake in the Night-PCA Group was significantly lower on the drug day compared to the Night-Saline Group and its own baseline day 3, 6, 12 and 24 hr postinjection, $p<0.01$. In the Day-PCA Group cumulative

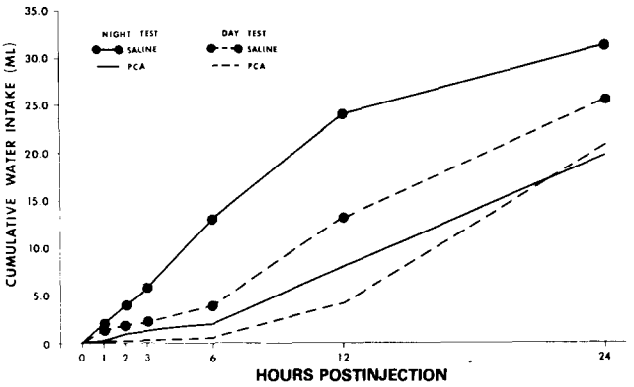

FIG. 9. Mean cumulative water intakes in the Night-PCA, Night-Saline, Day-PCA and Day-Saline Groups 0, 1, 2, 3, 6, 12 and 24 hr postinjection. All water intakes were calculated with respect to 0 hr postinjection (1800 or 1200 hr on Day 19). n=7 animals/group.

water intake on the drug day was significantly lower compared to the Day-Saline Group and its own baseline day 12 and 24 hr postinjection, $p<0.01$. On the postdrug day cumulative water intake in the Night-PCA Group was significantly elevated compared to the Night-Saline Group and its own baseline day 48 hr postinjection, $p<0.01$. Cumulative water intake in the Day-PCA Group on the postdrug day was significantly elevated compared to the Day-Saline Group and its own baseline day 36 and 48 hr postinjection, $p<0.01$. In addition there were no significant differences in the 24 hr total water intake on the drug day and postdrug day in the Night-PCA Group compared to the Day-PCA Group. However, analysis with planned contrasts revealed significant differences in cumulative water intake in the Night-PCA Group minus the Night-Saline Group compared to the Day-PCA Group minus the Day-Saline Group. There were greater water intake deficits in the Night-PCA Group than in the Day-PCA Group 6, 12 and 24 hr postinjection on the drug day, $p<0.01$.

In summary, PCA produced decreases in water intakes when administered either 6 hr following the beginning of the light phase or 1 hr prior to the beginning of the dark phase. Although 24 hr total intake was not different, cumulative water intake in the Night-PCA Group was more affected by PCA than water intake in the Day-PCA Group. On the postdrug day, animals injected with PCA at either 1200 or 1800 hr on the previous day showed significant increases in water intake.

DISCUSSION

PCA produced large deficits in body weights and food and water intakes whether administered 6 hr following the beginning of the light phase or 1 hr prior to the beginning of the dark phase. The initial rapid losses in body weights appeared to be independent of the time of day during which animals normally eat and drink. These deficits in body weight might have been caused by increased urination and defecation. Although 24 hr food and water intakes were not different when the Night-PCA and Day-PCA Groups were compared, a hypodipsic and hypophagic effect was evident in both groups. In contrast to total 24 hr intake data, there were some differences in the patterns of food and water deficits measured at various times during the day–night cycle. Therefore an interaction of drug injection time and deficits in ingestive behavior is suggested. The long duration of the PCA actions at this dose, however, tends to limit comparing differences between drug groups directly.

GENERAL DISCUSSION

These results indicate that PCA produces marked acute decreases in food and water intakes and body weights in the rat. These effects are not greatly influenced by the time of day when the drug is administered. The initial hypophagia, hypodipsia and body weight losses are in agreement with several earlier reports [15, 30, 38, 39]. If these effects can be attributed to decreases in brain 5-HT concentrations remains unclear since changes in brain 5-HT, NE and DA occur shortly following PCA administration [15, 34, 40]. Fluoxetine pretreatment, a procedure known to block 5-HT depletion induced by PCA, failed to block the decreases in ingestive behavior and body weights seen during the first 24 hr postinjection. During the 24–48 hr postinjection period, fluoxetine actually prolonged the hypophagic and hypodipsic effects of PCA. The uptake of PCA into 5-HT neurons is apparently inhibited by fluoxetine. This exclusion prevents PCA from producing its neurotoxic effects. Simultaneously, however, greater concentrations of PCA and extraneuronal 5-HT are available to influence catecholaminergic systems. The prolonged effects seen in the PCA+Fluoxetine Group might be attributable to catecholaminergic influences on ingestive behavior. Similar mechanisms of action might account for the synergistic hypophagic effects seen following treatment of rats with 5-HTP following fluoxetine [20]. Mediation by catecholaminergic systems is also suggested by chlorimipramine's antagonism of fenfluramine but not amphetamine or p-chloromethamphetamine induced hypophagia [8, 18, 23]. In addition pretreatment with PCPA [11] or lesions to the midbrain raphe nuclei [17] do not antagonize the hypophagic effects of PCA. However, a catecholaminergic mechanism of action can be questioned since it was shown that PCA induced hypophagia could not be blocked by NE and DA depletion following treatment with 6-hydroxydopamine and pargyline [17]. It appears likely that the initial hypophagic and hypodipsic effects of PCA are produced by catecholaminergic influences on non-serotonergic systems. Whether an influence on some other mechanism is responsible for these effects remains unclear.

Decreases in body weights in the PCA Group during the first 3 hr postinjection were significantly greater than the weight losses which occur in food and water deprived animals. Increased urination and/or defecation was suspected and confirmed by observations made during this initial period. PCA animals were saturated with urine, an artifact which probably tended to mask some of the initial weight losses. In addition recently collected data revealed a dose dependent increase in defecation and urination following PCA (Stein, *et al.*, manuscript in preparation). Increased urination could be responsible for the rebound in water intakes observed during the 24–48 hr period both in the present study and previously [38,39]. If these effects are centrally or peripherally mediated remains uncertain and is presently being investigated.

Within 4 days following PCA administration, body weights and 24 hr total food and water intakes return to baseline levels [39]. It was suggested that the distribution of eating and drinking might be changed following 5-HT depletion with PCA. However, the results of the present experiments do not support this hypothesis. Eight days following PCA, the distribution of body weight changes and food and

water intakes during the 24-hr day were not significantly different from baseline test day measurements. Chronic changes in the rates of eating and drinking or dietary selection remain possibilities. Finally, these results are in contrast with the long term effects of 5-HT depletion following 5,7-DHT plus desmethylimipramine [32] and are probably related to different mechanisms of action, brain regions affected, and specificity of their 5-HT depleting effects.

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REFERENCES

1. Baumgarten, H., L. Lachemayer, A. Bjorklund, A. Nobin and E. Rosengren. Long-term recovery of serotonin concentrations in the rat CNS following 5,6-dihydroxytryptamine. *Life Sci.* **12:** 357–364, 1973.
2. Blundell, J. E. and M. B. Leshem. Central action of anorexic agents: Effects of amphetamine and fenfluramine in rats with lateral hypothalamic lesions. *Eur. J. Pharmac.* **28:** 81–88, 1974.
3. Bogdanski, D. F., H. Weissbach and S. Udenfriend. Pharmacological studies with the serotonin precursor, 5-hydroxytryptophan. *J. Pharmac. exp. Ther.* **122:** 182–194, 1958.
4. Booth, D. A. Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J. Pharmac. exp. Ther.* **160:** 336–348, 1968.
5. Bray, G. A. and D. A. York. Studies on food intake of genetically obese rats. *Am. J. Physiol.* **223:** 176–179, 1972.
6. Breese, G. R., B. R. Cooper, L. D. Grant and R. D. Smith. Biochemical and behavioral alterations following 5,6-dihydroxytryptamine administration into brain. *Neuropharmac.* **13:** 177–187, 1974.
7. Breisch, S. T., F. P. Zemlan and B. G. Hoebel. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. *Science* **192:** 382–384, 1976.
8. Clineschmidt, B. V., J. V. McGuffin and A. B. Werner. Role of monoamines in the anorexigenic actions of fenfluramine, amphetamine and p-chloromethamphetamine. *Eur. J. Pharmac.* **27:** 313–323, 1974.
9. Diaz, J., G. Ellison and D. Masuoka. Opposed behavioral syndromes in rats with partial and more complete central serotonergic lesions made with 5,6-dihydroxytryptamine. *Psychopharmacologia* **37:** 67–79, 1974.
10. Donowitz, M., A. N. Charney and J. M. Heffernan. Effect of serotonin treatment on intestinal transport in the rabbit. *Am. J. Physiol.* **232:** E85–94, 1977.
11. Frey, H.-H. and R. Schulz. On the central mediation of anorexigenic drug effects. *Biochem. Pharmac.* **22:** 3041–3049, 1973.
12. Fuller, R. W., K. W. Perry and B. B. Molloy. Effect of an uptake inhibitor on serotonin metabolism in rat brain: Studies with 3-(p-trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine (Lilly 110140). *Life Sci.* **15:** 1161–1171, 1974.
13. Fuller, R. W., K. W. Perry and B. B. Molloy. Effect of 3-(p-trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. *J. Pharmac. exp. Ther.* **193:** 796–803, 1975.
14. Fuller, R. W., K. W. Perry and B. B. Molloy. Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. *Eur. J. Pharmac.* **33:** 119–124, 1975.
15. Fuller, R. W. and H. D. Snoddy. Long-term effects of 4-chloroamphetamine on brain 5-hydroxyindole metabolism in rats. *Neuropharmac.* **13:** 85–90, 1974.
16. Fuxe, K., L.-O. Farnebo, B. Hamburger and S.-O. Ogren. On the in vivo and in vitro actions of fenfluramine and its derivatives on central monoamine neurons, especially 5-hydroxytryptamine, and their relation to the anorectic activity of fenfluramine. *Postgrad. Med. J.* **51:** Suppl. 1, 35–45, 1975.
17. Garattini, S., A. Bizzi, G. de Gaetano, A. Jori and R. Samanin. Recent advances in the pharmacology of anorectic agents. In: *Recent Advances in Obesity Research I*, edited by A. Howard. New York: Newman Publishing Co., 1975, pp. 354–367.
18. Ghezzi, D., R. Samanin, S. Bernasconi, G. Tognoni, M. Gerna and S. Garattini. Effects of thymoleptics on fenfluramine-induced depletion of brain serotonin in rats. *Eur. J. Pharmac.* **24:** 205–210, 1973.
19. Goldman, H. W., D. Lehr and E. Friedman. Antagonistic effects of alpha and beta-adrenergically coded hypothalamic neurones on consumatory behavior in the rat. *Nature, Lond.* **231:** 453–455, 1971.
20. Goudie, A. J., E. W. Thornton and T. J. Wheeler. Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan anorexia. Evidence for serotonergic inhibition of feeding. *J. Pharm. Pharmac.* **28:** 318–320, 1976.
21. Hano, J., J. Bugajski, L. Danek and C. Wantuch. The influence of serotonin on insulin-stimulated gastric secretion, blood glucose and serum electrolyte levels in the unanesthetized rat. *Arch. int. Pharmacodyn. Thér.* **216:** 28–39, 1975.
22. Harvey, J. A. and S. E. McMaster. Fenfluramine: Cumulative neurotoxicity after chronic treatment with low dosages in the rat. *Communs Psychopharmac.* **1:** 3–17, 1977.
23. Jespersen, S. and J. Scheel-Kruger. Evidence for a difference in mechanism of action between fenfluramine- and amphetamine-induced anorexia. *J. Pharm. Pharmac.* **25:** 49–54, 1973.
24. Joyce, D. and N. Mrosovsky. Eating, drinking and activity in rats following 5-hydroxytryptophan (5-HTP) administration. *Psychopharmacologia* **5:** 417–423, 1964.
25. Kruk, Z. L. Dopamine and 5-hydroxytryptamine inhibit feeding in rats. *Nature New Biol.* **246:** 52–53, 1973.
26. Lorens, S. A., J. P. Sorenson and L. M. Yunger. Behavioral and neurochemical effects of lesions in the raphe system of the rat. *J. comp. physiol. Psychol.* **77:** 48–52, 1971.
27. Melchior, C. L. and R. D. Myers. Genetic differences in ethanol drinking of the rat following injection of 6-OHDA, 5,6-DHT or 5,7-DHT into the cerebral ventricles. *Pharmac. Biochem. Behav.* **5:** 63–72, 1976.
28. Messing, R. B., L. Phebus, L. A. Fisher and L. D. Lytle. Effects of p-chloroamphetamine on locomotor activity and brain 5-hydroxyindoles. *Neuropharmacology* **15:** 157–163, 1976.
29. Meyer, D. K., M. Adele and G. Hertting. Influence of serotonin on water intake and renin-angiotensin system in the rat. *Arch. int. Pharmacodyn.* **212:** 130–140, 1974.
30. Nielsen, C. K., M. P. Magnussen, E. Kampmann and H.-H. Frey. Pharmacological properties of racemic and optically active p-chloroamphetamine. *Arch. int. Pharmacodyn.* **170:** 428–443, 1967.
31. Richardson, J. S., N. Cowan, R. Hartman and D. M. Jacobowitz. On the behavioral and neurochemical actions of 6-hydroxydopa and 5,6-dihydroxytryptamine in rats. *Res. Commun. Chem. Path. Pharmac.* **8:** 29–45, 1974.
32. Saller, C. F. and E. M. Stricker. Hyperphagia and increased growth in rats after intraventricular injection of 5,7-dihydroxytryptamine. *Science* **192:** 385–387, 1976.
33. Samanin, R., D. Ghezzi, L. Valzelli and S. Garattini. The effects of selective lesioning of brain serotonin or catecholamine containing neurones on the anorectic activity of fenfluramine and amphetamine. *Eur. J. Pharmac.* **19:** 318–322, 1972.
34. Sanders-Bush, E., J. A. Bushing and F. Sulser. Long-term effects of p-chloroamphetamine and related drugs on central serotonergic mechanisms. *J. Pharmac. exp. Ther.* **192:** 33–41, 1975.

35. Sharpe, L. G. and R. D. Myers. Feeding and drinking following stimulation of the diencephalon of the monkey with amines and other substances. *Expl Brain Res.* **8:** 295–310, 1969.
36. Slangen, J. L. and N. E. Miller. Pharmacological tests for the function of hypothalamic norepinephrine in eating behavior. *Physiol. Behav.* **4:** 543–552, 1969.
37. Singer, G., I. Sanghvi and S. Gershon. Exploration of certain behavioral patterns induced by psychoactive agents in the rat. *Communs behav. Biol.* **6:** 307–314, 1972.
38. Stein, J. M., M. J. Wayner, R. C. Cook and H. A. Tilson. The effect of para-chloroamphetamine on the intake of ethanol, saccharin, food and water. In: *Currents in Alcoholism*, Vol. 3, edited by F. A. Seixas. New York: Grune and Stratton, 1978, pp. 173–190.
39. Stein, J. M., M. J. Wayner, K. M. Kantak and R. C. Cook. The short and long term effects of para-chloroamphetamine on ingestive behavior. *Pharmac. Biochem. Behav.*, **9:** 115–122, 1978.
40. Steranka, L. and E. Sanders-Bush. Temporal effects of p-chloroamphetamine on catecholamine synthesis. *Eur. J. Pharmac.* **45:** 83–86, 1977.
41. Winer, B. J. *Statstical Principles in Experimental Design*. New York: McGraw-Hill, 1971.