Pharmacology Biochemistry and Behavior, Vol. 1, pp. 255–258. ANKHO International Inc., 1973. Printed in the U.S.A.

Effects of *dl*-Para-Chlorophenylalanine on Sucrose Preference and Intake: Reversal by 5-Hydroxytryptophan and 6-Methoxy 1,2,3,4,-Tetrahydro-β-Carboline

DWIGHT M. NANCE

Department of Anatomy, School of Medicine, University of California, Los Angeles, California 90024

AND

M. MARLYNE KILBEY

Department of Biophysics, University of Houston, Houston, Texas 77004

(Received 19 July 1972)

NANCE, D. M. AND M. M. KILBEY. *Effects of dl-para-chlorophenylalanine on sucrose preference and intake: reversal by 5-hydroxytryptophan and 6-methoxy-1,2,3,4-tetrahydro-β-carboline.* PHARMAC. BIOCHEM. BEHAV. 1(3) 255–258, 1973.–In two experiments involving 12- or 4-hr two-bottle preference tests, rats depleted of brain serotonin by *dl*-para-chlorophenylalanine (pCPA) injections consumed larger amounts of sucrose and had a higher preference for sucrose solutions over distilled water than vehicle-injected control animals. Injection of 5-hydroxy-tryptophan (5-HTP) or 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) was found to reverse the effects of pCPA on brain serotonin as well as reverse the increased sucrose preference produced by pCPA.

Taste preference *dl*-Para-chlorophenylalanine 5-Hydroxytryptophan Serotonin 6-Methoxy-1,2,3,4-tetrahydro-β-carboline Carbohydrate regulation

---

SEPTAL lesions decrease serotonin (5-HT) [6] as well as increase preference for sucrose [1]. Recently, Fernstrom and Wurtman [3] have suggested a direct relationship between brain levels of 5-HT and carbohydrate intake. They found an increase in brain 5-HT content following the ingestion of carbohydrate or the injection of insulin. However, Gagliardino *et al.* [4] found that serotonin stimulated insulin release. Thus, the relation between 5-HT and carbohydrate regulation appears to be quite complex.

If 5-HT is involved in carbohydrate regulation, chronic shifts in brain levels would be expected to alter regulatory behavior related to carbohydrate intake. Depletion of brain 5-HT by *dl*-para-chlorophenylalanine (pCPA) has been shown to alter the taste preference for a variety of substances such as alcohol [8], quinine [2] and 5-hydroxy-tryptophan [9]. The present experiments tested: (1) whether rats depleted of brain 5-HT by injections of pCPA show altered sucrose preference; and, (2) whether the behavioral effects of pCPA could be reversed by reestablishing normal brain levels of 5-HT.

EXPERIMENT 1

Method and Procedure

Animals were 20 270-g male Sprague-Dawley rats. Animals were individually caged and maintained on a 12-hr light (7:00 a.m.–7:00 p.m.) and 12-hr dark schedule. Purina Rat Chow and distilled water were available throughout the experiment. Animals were weighed daily at 9:00 p.m.

During the first phase of the experiment, food and water consumption were measured at 9:00 a.m. and 9:00 p.m. for three days. Then, on succeeding days, each animal received either a 100 mg/kg injection of pCPA (12 animals) or an equivalent volume of the injection vehicle (8 animals). pCPA was suspended in a 0.2% agar solution and the injection volume was 10 ml/kg. The injections were given for nine consecutive days. On the first three days, only food and water intake were measured; on the remaining six days, each animal was also subjected to 12-hr (9:00 a.m.–9:00 p.m.) sucrose preference testing. Six concentrations of sucrose (0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 percent) were

presented in a different random order for each animal. Sucrose solutions were paired with distilled water and the positions of the bottles were counterbalanced across days. A 12-hr distilled water maintenance period separated each 12-hr test. Immediately following the last 12-hr preference test, animals were guillotined and the brains removed and frozen. Within a week a fluorimetric assay [13,14] for whole brain 5-HT was completed on the brain tissue.

Results

Analysis of the whole brain 5-HT data by Kruskal-Wallace nonparametric analysis of variance [10] indicated a significant, $\bar{X} = 86.1 \pm 3.1\%$, depletion ($H = 20.0, p = 0.01$) for the rats injected with pCPA. Analysis of variance with repeated measures for unequal groups [12] was performed on all other data. Main effects and simple main effects were tested with an F-test.

Analysis of the body weight data indicated a significant Treatment X Days interaction ($F_{8,144} = 9.21, p = 0.05$). As shown in Fig. 1, pCPA-treated rats fail to show weight gains as exhibited by the agar control animals. Therefore, chow and sucrose intakes were also analyzed in terms of kcal/g body weight.

Analysis of water intake for the baseline and pCPA injection phases, as well as the preference testing maintenance period, indicated no significant difference between groups. Also, chow intake did not differ between groups during the baseline period. However, a significant Treatment X Days interaction ($F_{2,36} = 28.00, p = 0.01$) was found for chow intake during the injection phase, indicating that across days, drug-treated animals decreased their chow intake.

Table 1 shows the mean intake of sucrose and chow (kcal/g body wt) during the preference test and maintenance periods. The pCPA-treated animals continued to consume significantly less chow throughout the six days of preference testing. Analysis of sucrose consumed indicated that pCPA-treated animals consumed significantly more sucrose than controls (Table 1). Yet in terms of total caloric intake (sucrose + chow), there was no statistically significant difference between the groups during the preference-testing period (Table 1). Thus, pCPA-treated animals consume a larger proportion of their daily calories as sucrose than controls..

The greater caloric intake of sucrose by the pCPA animals is reflected in both the mean percent preference and, of course, in terms of

$$\frac{\text{Sucrose Intake}}{\text{Sucrose} + H_2O \text{ Intake}} \times 100$$

absolute intake (ml) of sucrose (Table 2). The pCPA group showed significantly greater preference for sucrose at all but the 8 and 16 percent concentrations (Table 2). Analysis of the simple main effects for the absolute intake of the six concentrations showed that pCPA animals drank significantly more sucrose than control subjects at every concentration, although the difference tended to be less pronounced at the 0.5 and 16.0% concentrations (Table 2).

Discussion

Results of Experiment 1 indicate that chronic depletion

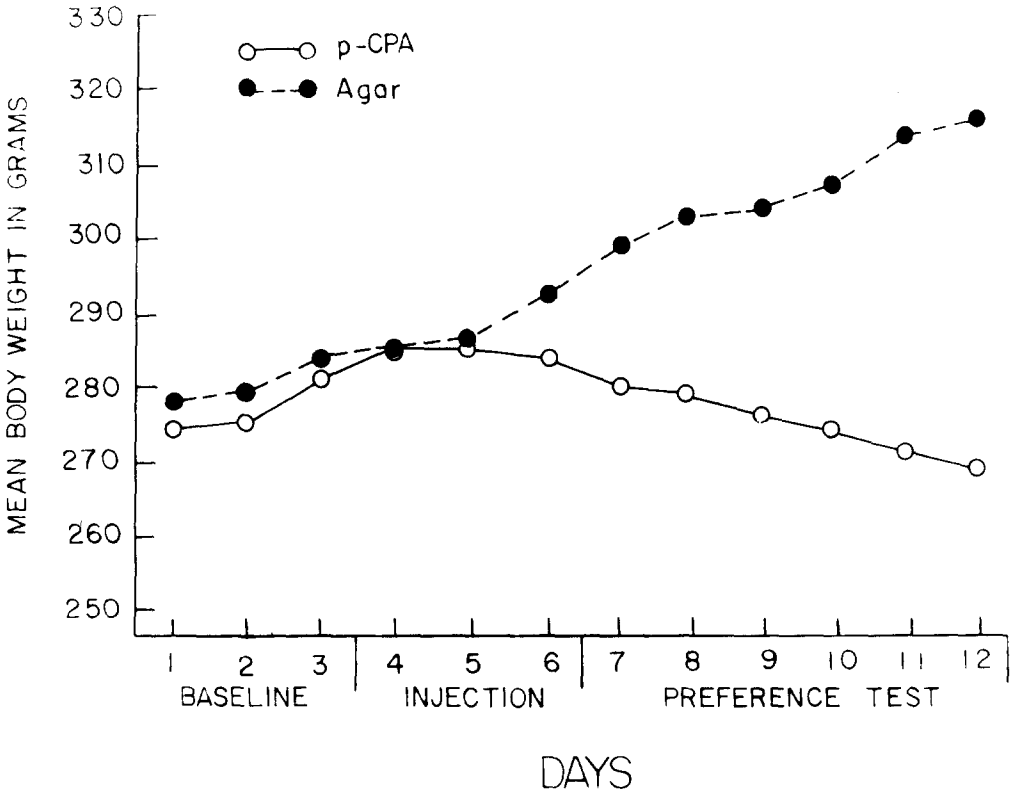

FIG. 1. Mean body weight of pCPA and agar-treated rats during baseline, injection, and preference-testing periods of Experiment 1.

TABLE 1

MEAN INTAKE OF FOOD AND SUCROSE (kcal/g BODY WEIGHT ± SE DURING PREFERENCE TESTING PERIODS OF EXPERIMENT 1)

Variable	Period	Duration	*p*-CPA	Agar	F Ratio
Food	test + maintenance periods	6 days/24 hr ea.	0.1314 ± 0.0069	0.1864 ± 0.0064	13.03+
Sucrose	test period	6 days/12 hr ea.	0.0603 ± 0.0062	0.0235 ± 0.0001	24.38+
Total calories	test + maintenance periods	6 days/24 hr ea.	0.1917 ± 0.0060	0.2099 ± 0.0074	1.74

+ $F(1,18)$ 8.28, $p = 0.01$

TABLE 2

MEAN PERCENT PREFERENCE ± SD AND MEAN ABSOLUTE INTAKE (ml) ± SD OF SIX CONCENTRATIONS OF SUCROSE DURING SIX 12-HR TWO-BOTTLE PREFERENCE TESTS

Group	Percent Preference			Absolute Intake‡	
	p-CPA	Agar	F Ratio	*p*-CPA	Agar
Concentration:					
0.5	70 ± 26	52 ± 24	7.86*	25 ± 22	06 ± 02
1.0	82 ± 14	62 ± 31	8.07*	55 ± 47	13 ± 08
2.0	93 ± 07	69 ± 23	12.60*	112 ± 66	29 ± 26
4.0	95 ± 10	82 ± 31	3.99†	136 ± 58	37 ± 31
8.0	95 ± 03	93 ± 32	n.s.	103 ± 34	40 ± 11
16.0	93 ± 04	87 ± 04	n.s.	55 ± 14	36 ± 09

*$p = 0.01$, 1,109 *df*
†$p = 0.05$, 1,109 *df*
‡Significantly different at all concentrations $F_{1,109} = 6.90$, $p = 0.01$

of whole brain 5-HT increases sucrose preference and intake. The pCPA-treated animals consume less chow and larger quantities of carbohydrate than normal animals, an effect which was found at all sucrose concentrations tested. In spite of similar total caloric intakes between groups during the preference testing period, the pCPA-treated group failed to gain weight as the control animals did (Fig. 1).

EXPERIMENT 2

Experiment 2 repeated the results of Experiment 1 during a briefer (4-hr) preference test and tested whether the pCPA effect on sucrose preference could be reversed by reestablishing normal brain levels of 5-HT. Both 5-hydroxytryptophan (5-HTP) [11] and 6-methoxy 1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) have been shown to elevate brain levels of 5-HT [7] and to reverse the biochemical and behavioral effects of pCPA [5] (Ho, personal communication). 6-MeO-THBC presumably elevates 5-HT by peripheral activation of 5-HTP decarboxylase [7].

TABLE 3

TREATMENT SCHEDULE FOR EXPERIMENT 2

Group	Days 1, 2, 3	Day 4
(1) Control	0.2% Agar	Saline
(2) *p*-CPA	100 mg/kg *p*-CPA	Saline
(3) *p*-CPA + 5-HTP	100 mg/kg *p*-CPA	50 mg/kg 5-HTP
(4) *p*-CPA + 6-MeO-THBC	100 mg/kg *p*-CPA	50 mg/kg 6-MTH

Method and procedure

Forty 370-g male Sprague-Dawley rats were used. Light–dark cycles, time of injection, volume, time of test, as well as time of chow and water intake measurements were the same as in Experiment 1. Animals were divided into four groups of ten animals each and treated according to the schedule in Table 3.

On day four, injections of saline, 5-HTP or 6-MeO-THBC in saline solutions, were given and water bottles were removed. One-half hour postinjection, 1/2 of Group 1, 1/2 of Group 2, and all of Group 3 were given a 4-hr, two-bottle preference test pairing a 2.0% sucrose solution with distilled water. The 4-hr preference test was begun for the remaining animals in Groups 1 and 2 and Group 4 two hours postinjection. Animals were sacrificed immediately

TABLE 4

MEAN PERCENT PREFERENCE ± SE, ABSOLUTE SUCROSE INTAKE ± SE, AND MEAN WHOLE BRAIN 5-HT ± SE OF RATS TREATED ACCORDING TO TABLE 3

Group	Mean % Preference 2.0% Sucrose ± S.E.	Sucrose Intake (ml) ± S.E.	Mean Whole Brain 5-HT μg/g ± S.E.
(1) Control	70.5 ± 2.10	12.7 ± 2.06	0.299 ± 0.013
(2) *p*-CPA	83.0 ± 2.31*	34.7 ± 4.93†	0.063 ± 0.033†
(3) *p*-CPA + 5-HTP	66.1 ± 2.53	24.5 ± 6.26	0.296 ± 0.027
(4) *p*-CPA + 6-MeO-THBC	68.3 ± 1.98	11.5 ± 3.92	0.270 ± 0.025

Group 2 vs Groups 1, 3, 4. *$p = 0.05$, †$p = 0.01$

following the conclusion of the preference test. As in Experiment 1, brains were removed and frozen for determination of whole brain 5-HT.

Results

Comparison of Groups 1 and 2 for whole brain serotonin data using a nonparametric analysis of variance [11] yielded an H = 12.88, $p = 0.01$, indicating a significant depletion of brain 5-HT in the pCPA-treated Group 2 animals (Table 4). Comparison of the 5-HTP and 6-MeO-THBC treated Groups 3 and 4 yielded an H = 0.26, $p = 0.9$, indicating that treatment with both drugs reestablished normal 5-HT levels in animals pretreated with pCPA. Analysis of the percent sucrose preference data indicated significantly greater sucrose preference (H = 8.28, $p = 0.05$) for the pCPA, Group 2 animals in comparison to the other groups (Table 4). Analysis of variance of the absolute sucrose intake indicated that Group 2 also ingested significantly more ($F_{3,36} = 6.00$, $p = 0.01$) sucrose than the other groups.

Discussion

Results of Experiment 2 indicate the increased sucrose intake and preference seen during a 12-hr preference test (Experiment 1) can also be demonstrated in a shorter 4-hr period. This phenomenon appears to be related to 5-HT depeltion by pCPA, as it is reversed when normal brain 5-HT levels are reestablished by administration of 5-HTP or 6-MeO-THBC. Thus, as suggested by Fernstrom and Wurtman [3], 5-HT may be involved in integrating information about the metabolic state of rats in relation to the control of homeostasis and behavior. An additional observation regarding the metabolic effects of pCPA is that during total food deprivation, pCPA-treated rats lose weight more rapidly than vehicle-injected control animals (Nance and Panksepp, unpublished observation).

An alternative explanation of the experiments reported here which cannot yet be ruled out is that the observed effects were due to depletion of gastric 5-HT, leading to disorders which favor ingestion of daily food from a more easily digested substance such as sucrose solutions.

REFERENCES

1. Beatty, W. W. and J. S. Schwartzbaum. Consummatory behavior for sucrose following septal lesions in the rat. *J. comp. physiol. Psychol.* **65**: 93–102, 1968.
2. Brody, J. F. Behavioral effect of serotonin depletion and of *p*-chlorophenylalanine (a serotonin depletor). *Psychopharmacologia (Berl.)* **17**: 14–33, 1970.
3. Fernstrom, J. D. and R. J. Wurtman. Brain serotonin content: increase following ingestion of carbohydrate diet. *Science* **174**: 1023–1025, 1971.
4. Gagliardino, J. J., L. M. Zieher, F. C. Iturriza, R. E. Hernández and R. R. Rodriguez. Insulin release and glucose changes induced by serotonin. *Horm. Metab. Res.* **3**: 145–150, 1971.
5. Geller, I. and K. Blum. The effects of 5-HTP on parachlorophenylalanine (pCPA) attentuation of "conflict" behavior. *Eur. J. Pharmac.* **9**: 319–324, 1970.
6. Heller, A., J. A. Harvey and R. Y. Moore. A demonstration of a fall in brain serotonin following central nervous system lesions in the rat. *Biochem. Pharmac.* **11**: 859–866, 1962.
7. McIsaac, W. M., D. Taylor, K. E. Walker and B. T. Ho. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline – a serotonin elevator. *J. Neurochem.* **19**: 1203–1206, 1972.
8. Myers, R. D. and T. J. Cicero. Effects of serotonin depletion on volitional alcohol intake of rats during a condition of psychological stress. *Psychopharmacologia (Berl.)* **15**: 373–381, 1969.
9. Nance, D. M. and M. M. Kilbey. Self-selection of 5-hydroxytryptophan in *dl*-para-chlorophenylalanine treated rats. *Physiol. Behav.* **8**: 545–547, 1972.
10. Siegel, S. *Nonparametric Statistics.* New York: McGraw-Hill, 1956.
11. Tenen, S. S. The effects of *p*chlorophenylalanine, a serotonin depletor on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia (Berl.)* **10**: 204–219, 1967.
12. Winer, B. *Statistical Principles of Experimental Design.* New York: McGraw-Hill, 1962.
13. Wise, C. D. An improved and simplified method for the fluorometric determination of brain serotonin. *Analyt. Biochem.* **18**: 94–101, 1967.
14. Wise, C. D. The fluorometric determination of brain serotonin. *Analyt. Biochem.* **20**: 369–371, 1967.