Pharmacology Biochemistry & Behavior, Vol 10, pp 951–955 Printed in the U.S A

Hyperphagia Following Intraventricular p-chlorophenylalanine-, Leucine- or Tryptophan-Methyl Esters: Lack of Correlation with Whole Brain Serotonin Levels

R. G. MACKENZIE*, B. G. HOEBEL, R. P. DUCRET AND M. E. TRULSON

Program in Neuroscience, Department of Psychology, Princeton University, Princeton NJ 08540
**Veterans Administration Hospital, 3495 Bailey Avenue, Buffalo NY 14215*

(Received 16 March 1979)

MACKENZIE, R G., B. G. HOEBEL, R. P. DUCRET AND M. E. TRULSON *Hyperphagia following intraventricular p-chlorophenylalanine-, leucine- or tryptophan-methyl esters: Lack of correlation with whole brain serotonin levels.* PHARMAC. BIOCHEM. BEHAV. 10(6) 951–955, 1979—The methyl ester hydrochlorides of DL-p-chlorophenylalanine (PCPA), L-leucine and L-tryptophan were intraventricularly administered to rats. All compounds produced increased food intake compared to saline administration. PCPA and leucine administration significantly decreased serotonin levels by 15–18%, while no serotonin depletion occurred following tryptophan injections. The data suggest that intraventricular injections of large quantities of neutral amino acid methyl esters may cause hyperphagia in rats through non-serotonergic effects on brain function.

p-Chlorophenylalanine Leucine Tryptophan Serotonin Hyperphagia

RESULTS from numerous experiments suggest that feeding in rats might be reciprocally related to activity at serotonin-mediated synapses in the central nervous system. Hyperphagia in rats has been reported following large depletions of brain serotonin (5-hydroxytryptamine, 5HT) by intraventricular administration of the 5HT synthesis inhibitor p-chlorophenylalanine (p-CPA) [3,11] or the 5HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) [18]. Conversely, adminstration of the 5HT precursor, 5-hydroxytryptophan, is followed by anorexia [2] which is potentiated by the 5HT uptake inhibitor fluoxetine [9]. 5-Hydroxytryptophan also can reverse the p-CPA- induced hyperphagia [11]. The anorectic effect of fenfluramine has been attributed to its serotonin-releasing capacity because (a) the anorectic potency of fenfluramine is reduced when administered to rats pre-treated with the uptake inhibitor chlorimipramine [14] and (b) electrolytic lesions of the median raphe nucleus or intraventricular injections of the neurotoxin, 5,6-dihydroxytryptamine, which deplete forebrain 5HT, are reported to greatly attenuate fenfluramine-induced anorexia [5,20]. In addition, when serotonin receptors are directly stimulated by quipazine, anorexia ensues which is unaffected by lesions of the median raphe [19].

In short, there exists an impressive body of evidence suggestive of an important role for brain 5HT pathways in food intake regulation. Recently, however, the results, or the interpretation of the results of some of these experiments, have been questioned. For example, several experimenters have noted that depletion of forebrain 5HT by intracerebral administration of 5,7-DHT is not followed by hyperphagia [6, 11, 17, 21]. Also, the specificity of the 5HT system as the target of action of 5-hydroxytryptophan-induced anorexia has been questioned because tryptophan loading, which produces large increments in brain 5HT only in serotonergic neurons, does not produce anorexia [23]. Furthermore, attempts to replicate the median raphe lesion-induced attenuation of fenfluramine anorexia have failed despite large lesion-induced depletions of forebrain 5HT [4,22], and depletions of brain 5HT by intracerebral administration of 5,6-DHT [22] or 5,7-DHT [11,12] are now reported to either not affect or potentiate the anorectic efficacy of fenfluramine.

The present experiments are designed to test if p-CPA-induced hyperphagia, in fact, is mediated via alterations of brain 5HT. To test this, the effect of intraventricularly administered p-CPA on food intake was compared to the effects of saline administration and other neutral amino acids which might not be expected to reduce brain 5HT concentrations.

METHOD

Sherman female rats (Camm Research, Inc., Wayne NJ) weighing 275–300 g were individually caged in a room with a 9 a.m.–9 p.m. light-dark schedule. The rats ate powdered rat chow (Ralston Purina Co., St. Louis MO) from specialized feeders designed to prevent spillage. Following a one-week period of acclimatization to this environment, 24 hr food

TABLE 1

EFFECTS OF AMINO ACID METHYL ESTER HCl ADMINISTRATION ON 24 HOUR FOOD INTAKE
MEAN 24 HOUR FOOD INTAKE ± SEM (g) ON FIVE DAYS PRIOR THROUGH 3 DAYS POST TREATMENT

Drug	n	-5	-4	-3	-2	-1
Experiment 1*						
Saline	7	20.0 ± 1.2	22 4 ± 0.6	21 5 ± 0.7	20 5 ± 0.4	20 2 ± 0 4
p-CPA-ME	7	20.1 ± 0.4	21.2 ± 0.9	20.8 ± 0.3	20 6 ± 0.4	19 8 ± 0.4
Leucine-ME	7	19.2 ± 0.6	20.7 ± 0.6	20.7 ± 0.4	21 0 ± 0 7	20 0 ± 0 8
Experiment 2†						
Saline	8	22 4 ± 1.1	22 4 ± 0 4	20 1 ± 0.7	20.9 ± 0 3	21.2 ± 0.6
p-CPA-ME	8	22 0 ± 0 5	21.3 ± 0 5	22.0 ± 0.4	22 1 ± 0 6	21.8 ± 0.6
Tryptophan-ME	8	21.4 ± 0.5	21.6 ± 0.5	22.1 ± 0.6	21 5 ± 0.5	21.7 ± 0.7

n=number of rats.
*p-CPA differs from saline, $p < 0.05$.
†p-CPA differs from saline, $p < 0\ 05$ and tryptophan differs from saline, $p < 0.01$
ME=methyl ester

intake was measured for 5 consecutive days to verify that daily food intake was stable. On the sixth day, the rats were lightly anesthetized with sodium pentobarbital (35 mg/kg, IP), placed in a stereotaxic instrument and injected intraventricularly with vehicle or one of the test substances. Ether-soaked cotton was placed beneath the nose of the rat as needed during the injection period. Immediately following surgery, the rats were returned to the home cages and food intake measures were continued for 3 more days. On the third post-injection day, the rats were killed by decapitation and the brains were rapidly removed, dissected into right and left halves minus the cerebellum and immediately immersed in liquid N_2. The rats were killed on the third post-injection day because this is the time at which p-CPA injected rats exhibited maximal hyperphagia in previous studies [3,11], and brain 5HT levels are still maximally depleted following p-CPA administration [3,15]. The right half-brains were assayed for 5HT, dopamine and norepinephrine using the fluorometric method developed by Jacobowitz and Richardson [13]. Recoveries for all monoamine assays reported in this paper were: 5HT=97.4 ± 2.3%, DA= 95.0 ± 3.4% and NE=96.1 ± 2.8%. The left half-brains were assayed for tryptophan using modification by Bloxam and Warren [1] of the method of Denckla and Dewey [8]. The catecholamines were measured in addition to serotonin in order to monitor the specificity of the drug treatments on neurotransmitter levels. Tryptophan was measured to indicate whether the intraventricular administration of such a large dose of neutral amino acids would interfere with the transport of endogenous amino acids from the blood to the brain. The entire procedure described above was performed for each of the two experiments in the present report.

Intraventricular Injections

With the rat positioned in the stereotaxic instrument, small burr holes were made in the skull. The injectates were back-loaded into 10 μl Hamilton syringes (Hamilton Co., Reno NV) mounted in a syringe pump (Sage, White Plains NY) and connected by PE tubing to cannulae made of 29 ga stainless steel tubing. The tips of the cannulae were stereotaxically lowered into the lateral ventricles and 5 μl of injectate was delivered into each ventricle at a rate of 0.5 μl/minute. The cannulae were left in the ventricles for an additional 5 minutes to allow for diffusion of the injectate into the ventricular space prior to withdrawal. Delivery of the injectate into the ventricular space by this method was verified in a separate group of rats by observing staining throughout the ventricular system when dye was injected. The injection procedure was successful in every case.

Test Compounds

Separate solutions of DL-p-chlorophenylalanine methyl ester HCl (p-CPA), L-leucine methyl ester HCl, and L-tryptophan methyl ester HCl (all obtained from Sigma Chemical Co., St. Louis MO) were made by dissolving the compounds in 0.9% saline and adjusting the pH to 7.0 by the addition of 0.1 N NaOH, such that the final concentrations were 300 mg/ml. Each rat received 3 mg of one of the compounds in 10 μl, or 10 μl of 0.9% saline. Solutions of the test compounds were prepared immediately prior to injection. In the first experiment, rats received p-CPA, leucine or saline. In the second experiment, rats received p-CPA, tryptophan or saline. The methyl esters of the amino acids were used because of their high solubility.

Statistical Analysis

Food intake was analyzed by two-way analysis of variance between treatments and across days (−1 to 3) appropriate for a two-factor experiment with repeated measures on one factor. Differences between means within a single factor were tested by the Newman-Keuls procedure [24].

TABLE 1
(continued)

Drug	0	1 24 to 48 hrs post- injection	2 48 to 72 hrs post- injection	3 72 to 96 hrs post- injection
Experiment 1*				
Saline	13.5 ± 1.2	17.0 ± 1 6	18.1 ± 1.1	20.9 ± 1.1
p-CPA-ME	17.6 ± 2.2	22.3 ± 2.9	25.6 ± 2.4	28.5 ± 2.6
Leucine-ME	15.9 ± 1.4	21.1 ± 1.1	24.0 ± 2.1	26.4 ± 2.2
Experiment 2†				
Saline	12.2 ± 0.7	19.0 ± 0.8	21.2 ± 0.8	21.3 ± 0.6
p-CPA-ME	17.4 ± 2.1	24.0 ± 3.1	27.0 ± 2.8	28.9 ± 2.1
Tryptophan-ME	16.0 ± 1.4	23.4 ± 1.6	29.7 ± 2.0	29.9 ± 1.6

TABLE 2
EFFECTS OF AMINO ACID METHYL ESTER HCl ADMINISTRATION ON BRAIN MONOAMINES AND TRYPTOPHAN

Drug	n	Brain Concentration ± SEM (μg/g Wet Weight) 5-HT	NE	DA	TRY
Experiment 1					
Saline	7	0.49 ± 0.01	0.28 ± 0.01	0.51 ± 0.03	3.69 ± 0.16
p-CPA-ME	7	0.40 ± 0.01*	0.24 ± 0.01‡	0.44 ± 0.03	3.49 ± 0 12
Leucine-ME	7	0.41 ± 0.01†	0.27 ± 0.02	0.46 ± 0.02	3.62 ± 0 17
Experiment 2					
Saline	8	0.48 ± 0.01	0.23 ± 0.01	0.39 ± 0.02	3.95 ± 0.14
p-CPA-ME	8	0.41 ± 0.01§	0.22 ± 0.01	0 40 ± 0.02	4.00 ± 0.14
Tryptophan-ME	8	0.45 ± 0.02	0.21 ± 0.01	0.37 ± 0.03	3.89 ± 0.15

n=number of rats.
*p-CPA differs from saline, $p < 0.01$
†Leucine differs from saline, $p < 0.05$.
‡p-CPA differs from leucine and saline, $p < 0.05$.
§p-CPA iffers from tryptophan and saline, $p < 0.01$.
ME=methyl ester

The neurochemical data were analyzed by a one-way analysis of variance and differences between individual means were tested by the Newman-Keuls method [24].

RESULTS

Statistical analysis of the feeding data from Experiment 1 (Table 1) indicates that food intake is affected by the drug treatments ($F(2,18)=3.6, p<0.05$). There also is a significant effect across days ($F(4,72)=32.3, p<0.01$), and a significant treatment × days interaction ($F(8,72)=2.4, p<0.05$). A Newman-Keuls test for differences between means within the treatment factor indicates that the food intake in the p-CPA treated rats differed significantly from the saline-treated animals. There also is a marked tendency for the leucine-treated group to overeat, although this difference was not statistically significant.

Results from the neurochemical assays for Experiment 1 (Table 2) indicate the p-CPA treatment reduced brain 5HT concentrations relative to saline treated rats by 18.4% ($p<0.01$) and norepinephrine concentrations by 14.3% ($p<0.05$). The amount of 5HT depletion caused by p-CPA methyl ester may vary from one drug shipment to another. We originally obtained 75% depletion [3,11], but in this study obtained only 15–18% depletion. Leucine administration also lowered brain 5HT concentrations (−16.3%, $p<0.05$). 5HT concentrations did not differ between the p-CPA and leucine-treated groups. No effects by any treatments were noted on brain dopamine or tryptophan concentrations.

Analysis of the feeding data from Experiment 2 (Table 1) again indicates a drug treatment effect ($F(2,21)=6.3, p<0.01$), a day effect ($F(4,84)=46.8, p<0.01$), and a treatment×day interaction ($F(8,84)=2.6, p<0.05$). Within the drug treatment factor both the tryptophan-treated and p-

CPA-treated animals ate more than the saline-treated group ($p<0.01$, $p<0.05$ respectively).

The neurochemical measures from Experiment 2 (Table 2) demonstrate that brain 5HT concentrations are lower in the p-CPA-treated animals relative to both the tryptophan and saline-treated groups ($p<0.01$), which do not differ statistically from each other. No other treatment effects were found on the remaining measures.

DISCUSSION

The induction of hyperphagia in rats administered p-CPA methyl ester HCl by the intraventricular route has been interpreted as supportive evidence for a role of 5HT systems in food intake regulation [3,11]. Although the present study confirms that this manipulation can reliably produce hyperphagia, the results question previous interpretations regarding 5HT mediation of the effect.

In both Experiments 1 and 2, p-CPA treatment was followed by hyperphagia, but the concentration of 5HT in the brain was reduced by only 18 and 15%, respectively. This minimal 5HT depletion contrasts sharply with 75% reductions reported in former p-CPA studies [3,11], although the hyperphagia observed in the present report appears to be comparable to that obtained previously [3,11]. In this regard, there are numerous experimental reports in which brain 5HT concentrations have been reduced over 50% by electrolytic lesions or 5,7-DHT administration without producing hyperphagia [4, 11, 16, 20, 21,22]. Indeed, in the one study where 5,7-DHT treatment is linked to hyperphagia [18], the authors indicate that telencephalic 5HT must be reduced by at least 60%, and preferably by 80%, to obtain the effect. Therefore, the hyperphagia induced by administration of p-CPA in the present study may have been due to some non-serotonergic effect on brain function which occurs when a large dose of a neutral amino acid methyl ester HCl is injected into the ventricles of rats.

This interpretation is supported by the present results where leucine injections produced as much 5HT depletion as p-CPA injections but less hyperphagia (Experiment 1), and where tryptophan injections produced as much hyperphagia as p-CPA injections but no 5HT depletion (Experiment 2). At present we do not know what the basis for the hyperphagia might be following intraventricular injections of a neutral amino acid methyl ester. Norepinephrine depletion can result in hyperphagia [10], but the levels of this neurotransmitter were only slightly decreased in Experiment 1, and were unchanged in Experiment 2. Therefore, norepinephrine does not appear to be involved in this phenomenon.

A recent study by Coscina *et al.* [7] reported that intraventricular administration of PCPA- or phenylalanine-methyl ester HCl (2–4 mg) produced no significant changes in forebrain NE, 5HT or DA, but resulted in hyperphagia comparable to that observed in the present study. These results support the hypothesis that neutral amino acid methyl ester-induced hyperphagia is not mediated by 5HT or NE systems. In addition, Coscina *et al.* [7] demonstrated that the hyperphagia is not due to hyperosmotic stress produced by infusions of large quantities of the drug into the brain, nor to methanol-induced toxicity produced by in vivo hydrolysis of the neutral amino acid methyl ester.

We conclude that large 5HT depletions, which would be detected by whole brain assay, are not necessary for neutral amino acid methyl ester-induced hyperphagia. Therefore, the effect is either produced by small 5HT depletions, which would not be detected by whole brain assay, or is not 5HT-mediated. The fact that tryptophan loading (Experiment 2) would not be expected to decrease brain 5HT points to the latter interpretation.

ACKNOWLEDGEMENT

This research was supported by P H.S. Grant DA01672

REFERENCES

1. Bloxam, D. L. and W. H Warren Error in the determination of tryptophan by the method of Denckla and Dewey· A revised procedure. *Analyt. Biochem* **60:** 621–625, 1974
2. Blundell, J. E. and M B. Leshem The effect of 5-hydroxytryptophan on food intake and on the anorexic action of amphetamine and fenfluramine. *J Pharm. Pharmac.* **27:** 31–37, 1975.
3. Breisch, S. T , F. P. Zemlan and B. G. Hoebel. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. *Science* **192:** 382–385, 1976.
4. Carey, R. J. Effects of selective forebrain depletions of norepinephrine and serotonin on the activity and food intake effects of amphetamine and fenfluramine. *Pharmac. Biochem. Behav* **5:** 519–523, 1976.
5 Clineschmidt, B. V. 5-6-Dihydroxytryptamine suppression of the anorexigmic action of fenfluramine. *Eur J Pharmac* **24:** 405–409, 1973.
6. Coscina, D. V Effects of central 5,7-dihydroxytryptamine on the medial hypothalamic syndrome in rats. *Ann. N Y. Acad Sci* **308:** 627–644, 1978.
7 Coscina, D. V , J Daniel and J. J Warsh Potential non-serotonergic basis of hyperphagia elicited by intraventricular p-chlorophenylalanine. *Pharmac. Biochem Behav.* **9:** 791–797, 1978.
8. Denckla, W. E. and K K Dewey. Determination of tryptophan content in tissues. *J Lab clin Med.* **69:** 160–169, 1967
9 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 induced anorexia Evidence for serotonergic inhibition of feeding *J Pharm Pharmac.* **28:** 318–320, 1976.
10. Hoebel, B. G. The psychopharmacology of feeding. In: *Handbook of Psychopharmacology*, edited by L. L Iversen, S. D Iversen and S H. Snyder New York· Plenum, 1977, pp 55–129.
11. Hoebel, B G , F P Zemlan, M. E. Trulson, R. G. MacKenzie, R P. DuCret and C. R Norelli. Differential effects of p-chlorophenylalanine and 5,7-hydroxytryptamine on feeding in rats. *Ann N Y Acad. Sci* **308:** 590–594, 1978.
12. Hollister, A. S., G. N. Ervin, B R Cooper and G R. Breese. The roles of monoamine neural systems in the anorexia induced by (+)-amphetamine and related compounds *Neuropharmacology* **14:** 715–723, 1975
13 Jacobowitz, D. M and J. S Richardson Method for the rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. *Pharmac Biochem Behav.* **8:** 515–519, 1978.

14 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.
15. Koe, B. K. and A. Weissman. p-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac exp Ther.* **154:** 499–516, 1966.
16. Kostowski, W , E. Giacalone, S. Garattini and L Valzelli. Studies on behavioral and biochemical changes in rats after lesion of midbrain raphe *Eur. J Pharmac* **4:** 371–376, 1968.
17. Myers, R. D. Hypothalamic actions of 5-hydroxytryptamine neurotoxins: Feeding, drinking, and body temperature. *Ann N Y. Acad Sci* **308:** 556–575, 1978.
18. Saller, C R. and E. M. Stricker Hyperphagia and increased growth in rats after intraventricular injection of 5,7-dihydroxytryptamine. *Science* **192:** 385–387, 1976.
19. Samanin, R., C. Bendotti, F. Miranda and S. Garattini. Decrease of food intake by quipazine in the rat: relation to serotonergic receptor stimulation *J Pharm. Pharmac* **29:** 53–54, 1977.
20. Samanin, R., D. Ghezzi, L. Valzelli and S. Garattini. The effects of selective lesioning of brain serotonin or catecholamine-containing neurons on the anorectic activity of fenfluramine and amphetamine. *Eur. J Pharmac.* **19:** 318–322, 1972.
21. Srebro, B. and S. A. Lorens. Behavioral effects of selective midbrain raphe lesions in the rat. *Brain Res.* **89:** 303–325, 1975.
22. Sugrue, M. F., I. Goodlet and I. McIndewar. Failure of depletion of rat brain 5-hydroxytryptamine to alter fenfluramine-induced anorexia. *J. Pharm. Pharmac.* **27:** 950–953, 1975.
23. Weinberger, S. B., S. Knapp and A. J. Mandell. Failure of tryptophan load-induced increases in brain serotonin to alter food intake in the rat. *Life Sci* **22:** 1595–1602, 1978.
24. Winer, B. J. *Statistical Principles in Experimental Design,* Edition 2, New York: McGraw Hill, 1971.