Effects of para-chlorophenylalanine on food intake in rats*

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Food intakes, water intakes, and body weights of rats were monitored for up to 30 days during daily intragastric administration of 100 mg/kg p-chlorophenylalanine. During treatment, food intake was reduced to 50%-75% of control levels, water intake was decreased to about 28% of control levels, and body weights stabilized at about 80%-90% of control levels. Body weight remained low as long as treatment continued, and animals defended the low body weights against food deprivation and weight gain induced by insulin injections. It is unlikely that the decrease in body weight during PCPA treatment is due to malaise, since anorexia could be prevented by depriving animals of food during the first 2 days of drug treatment.

METHOD

Experiment 1

Forty-two male Long-Evans hooded rats, approximately 120 days old at the start of experimentation, were used. Animals were individually caged and were allowed free access to powdered Purina Laboratory Chow from spill-proof containers and to water from 100-ml graduated drinking tubes. Lighting was on a diurnal cycle (lights on at 8:00 a.m.; lights off at 8:00 p.m. Animals were divided into six groups matched for body weight. Three groups served as volumetric vehicle controls. From each set of animals, one group (N = 6) was tested under food deprivation, one (N = 9) was to be tested during long-term insulin treatment, and one (N = 6) served as a baseline control. Throughout the experiment, daily food intakes were measured to 0.1 g, water intakes to 1.0 ml, and body weights to 1.0 g.

After 8 days of baseline measurements, PCPA treatment was initiated. On the initial day, all animals in drug groups were administered intragastric loads of 200 mg/kg dl-PCPA (Pfizer) suspended in 0.9% saline at concentrations of 50 mg/cc. Loading was done with the aid of a 16-ga stainless steel feeding tube (Popper & Sons, Inc.). On all subsequent days, the daily dose of PCPA was reduced to 100 mg/kg, and treatment was continued for 19 additional days. The drug-free groups received volumetric loads of the saline carrier each day. The intragastric route of drug administration was used in preference to the more widely employed intraperitoneal route to minimize tissue irritation, which is common when PCPA is administered for prolonged periods into the peritoneal cavity.

Ten days after initiation of PCPA treatment, deprivation groups were placed on a limited feeding schedule consisting of 0.5 h of food each day for 6 consecutive days. Simultaneously, the insulin groups were started on a daily Protamine zinc insulin (Lilly) regimen, which was to consist of 4 U, 6 U, 8 U, 8 U, 8 U, 8 U of insulin on 6 successive days. The daily dose of insulin was administered subcutaneously at the nape of the neck in two equal injections separated by 12 h.

Experiment 2

Six male Sprague-Dawley albino rats, 90 days old at the start of experimentation, were employed. Three animals were administered PCPA for 30 days as described in Experiment 1. Three animals served as vehicle controls. To obtain pilot data for a prospective study, all animals received intraperitoneal injections of 500 mg/kg 2-deoxy-D-glucose 5 and 10 days after the start of PCPA treatment. Beginning 12 days after the initiation of drug treatment, all animals were limited to 1 h of food per day for 4 consecutive days. Beginning on the 25th day

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Fig. 1. Mean body weights for Experiment 1. Flags for first 8 days indicate the range of group means.

of treatment, all animals were administered 4, 8, and 8 units of NPH insulin (Lilly) on 3 successive days. Injections were administered as in Experiment 1. Upon termination of insulin treatment, ad lib food consumption was measured for 3 days. On the last day of PCPA treatment, all animals were sacrificed, and brains were removed and frozen for brain serotonin analysis. Spectrophotofluorometric analysis of serotonin (Maickel, Lox, Saillant, & Miller, 1968) was performed on three parts of each brain: (1) telencephalon, (2) the diencephalon from septum to the superior colliculus, and (3) the brainstem caudal to the superior colliculus.

Experiment 3

Twelve male Sprague-Dawley albino rats, weighing 277-320 g, were employed. Animals were tested for 4 days. On the first day, daily ad lib food intake and body weights of all animals were measured. At the beginning of the second day, all animals were placed on food deprivation, and six of the animals were injected intragastrically with 200 mg/kg PCPA; 100 mg/kg was administered on the next 2 days. The remaining animals received

equivolumetric loads of the saline carrier. Food deprivation continued for 2 days, and 24-h refeeding intake was measured on the fourth day.

RESULTS

Experiment 1

Mean body weights of control and deprivation groups for the duration of the experiment are presented in Fig. 1. Average daily food and water intakes for these animals during the 6 days prior to the start of PCPA treatment (Days 3-8, Fig. 1), for the 6 days prior to the start of deprivation (Days 13-18), for the 6 days of deprivation (Days 19-24), and for the first 3 days of refeeding (Days 25-27) are presented in Table 1. The PCPA treatment produced an unambiguous depression in

Table 1
Food (Grams) and Water (Milliliters) Intakes for Experiment I

Groups		N	Pre-PCPA (Days 3-8)	Ad Lib (Days 13-18)	Deprivation (Days 19-24)	Postdeprivation (Days 25-27)
Food Intake						
Saline	Control	6	21.9 ± 0.9	18.9 ± 1.9	21.1 ± 1.7	20.6 ± 1.3
	Deprived	6	23.6 ± 1.2	19.6 ± 1.8	5.0 ± 1.4	25.0 ± 0.9
PCPA	Control	6	23.0 ± 1.0	9.6 ± 2.3*	10.0 ± 2.8*	14.1 ± 0.6**
	Deprived	6	23.0 ± 1.5	10.1 ± 2.3*	3.0 ± 1.3***	16.8 ± 2.0**
Water Intake						
Saline	Control	6	33 ± 2	29 ± 2	32 ± 2	34 ± 1
	Deprived	6	35 ± 2	30 ± 2	21 ± 4	43 ± 6
PCPA	Control	6	34 ± 2	21 ± 4**	18 ± 3**	27 ± 2**
	Deprived	6	32 ± 2	22 ± 3**	10 ± 2*	30 ± 6**

Note—Days refer to Fig. 1; values are means ± SD.

* $p < .001$

** $p < .01$

*** $p < .05$, drug vs appropriate no-drug group, t test

food intake to about 50% of pretreatment levels and a concurrent decline in body weight. Water intake was decreased to a smaller extent (approximately 28%). The body weight of PCPA-treated animals stabilized at about 80% of control weight and showed no tendency to recover for the duration of PCPA treatment. When drug treatment was terminated, animals overate and gradually regained the lost weight.

Data for insulin-treated animals will not be presented in full, because before the projected period of insulin treatment could be completed, seven of nine PCPA animals had died. No fatalities occurred in the control group. Five animals died within the first 3 days of treatment, and two died on the fourth day. Accordingly, treatment of the two surviving animals was terminated. Of the five animals which died during the first 3 days, none exhibited overeating in response to insulin. In fact, all ate less during insulin than prior to treatment. The two animals which died later, as well as the two surviving animals, exhibited overeating to insulin. For instance, on the last 2 days of insulin treatment (8 U/day), the two surviving animals ate an average of 40.5 g per day as compared to preinsulin intake of 17.4 g per day (mean of 2 days prior to insulin). On the second and third days following termination of insulin treatment, the intakes of these animals dropped to an average of 7.5 g per day. These values are comparable to the data of control animals, all of which exhibited hyperphagia during insulin and hypophagia after cessation of treatment.

Experiment 2

Mean food intakes and body weights are depicted in

Fig. 2. Mean food intakes and body weights for Experiment 2.

Fig. 2. These data essentially replicate the previous experiment, but there was one difference. In the previous experiment PCPA animals lost weight, while in this experiment they failed to gain weight. The difference may be due to the use of younger and lighter animals in this experiment. It may also be noted that on the days animals received 500 mg/kg 2-deoxy-D-glucose, PCPA-treated animals tended to eat more while control animals ate less.

Food intake during insulin treatment is depicted in Fig. 3. Insulin increased food consumption and body weight of both groups. The increased weight was lost by a compensatory decrease in food intake when insulin treatment was terminated.

Biochemical analyses indicated that brain serotonin levels of PCPA-treated animals were less than 23% of control levels (Table 2).

Experiment 3

The mean ($\pm$ SD) food intake of control animals on the day prior to deprivation was 21.7($\pm$ 2.9) g and on the day following the 48-h fast, 24.0($\pm$ 3.0) g. The corresponding values for the PCPA-treated group were 23.0($\pm$ 2.8) and 25.0($\pm$ 3.2) g. By comparison, feeding in nondeprived animals at comparable points in time were 59% (Experiment 1) and 62% (Experiment 2) of predrug levels.

DISCUSSION

Chronic PCPA treatment reduces food intake, water intake, and body weight of rats. Since food intake is

Fig. 3. Mean food intakes for 3 days prior to insulin treatment, for 3 days during insulin (4, 8, and 8 units/day), and for 3 postinsulin days. Mean body weights for the last day of each period are indicated.

reduced more than water intake, it is likely that the main effect is to reduce hunger rather than thirst. In line with such a conclusion, our attempts to reverse PCPA-induced anorexia by forced loading of water have been without success. Likewise, humans treated with PCPA report a loss of appetite (VanWoert, Ambani, & Levine, 1972).

Within the course of the present experiment, there was no tolerance to the weight-reducing effect of PCPA. The animals remained at 80%-90% of control levels for the duration of treatment. They also defended this lower body weight in response to imposed regulatory challenges. After food deprivation, they overate and recovered lost weight. After weight gain induced by insulin treatment, they exhibited compensatory reduction of food intake and a concomitant fall of body weight. In demonstrating this last point, we were confronted by a noteworthy peculiarity of PCPA-treated animals. They were deficient in the behavioral or physiological resources needed to resist insulin coma. A significant proportion of PCPA-treated animals died during the treatment. These deaths cannot be attributed merely to their low body weights, for the same insulin regimen has been successfully employed in normal animals lighter than the ones which died (personal observation). Since a common complaint voiced by humans administered PCPA is tiredness and weakness (Cremata & Koe, 1966; Sjoerdsma, 1970; Van Woert et al, 1972), it is possible that our rats experienced

Table 2
Brain Serotonin Concentrations (ug/g) Following 30 Days of Treatment With PCPA as Described in Experiment II

Treatment	Amine Concentration in		
	Telencephalon	Diencephalon	Brain Stem
Control	0.536 ± .010	1.026 ± .044	0.932 ± .105
PCPA	0.126 ± .057*	0.199 ± .081*	0.218 ± .064*

Note—Values are means ± SD, $N = 3$ for both treatments. * $p < .001$, t test for independent groups.

similar symptoms and that the further imposition of a tendency toward hypoglycemia may have produced lassitude beyond the animals' ability to cope.

Whether the observed anorexia should be attributed to reduction of brain serotonin or to other effects of PCPA cannot yet be decided. At least four alternative possibilities to a central action should be considered: (1) PCPA reduces serotonin not only in the brain, but also within the gastrointestinal tract and other tissues (Koe & Weissman, 1966). (2) PCPA also inhibits phenylalanine hydroxylase, leading to abnormally high blood phenylalanine levels (Lipton, Gordon, Guroff, & Udenfriend, 1967). (3) Metabolites of PCPA may suppress feeding. In line with such a possibility, Stark and Fuller (1972) have presented evidence that such products of PCPA metabolism as 3-chlorotyrosine and 3-chlorotyramine can inhibit self-stimulation behavior in rats. (4) The PCPA effect on feeding may be due to illness. Although our experimental animals appeared perfectly healthy, PCPA treatment has been reported to produce side effects such as nausea and abdominal pain in human patients (Cremata & Koe, 1966; Van Woert et al, 1972). However, our data argues against such causality since food-deprived animals receiving PCPA did not exhibit anorexia (Experiment 3).

Finally, we would like to discuss the possibility that PCPA reduces feeding as a direct consequence of reduced brain serotonin. There are some compelling lines of argument which suggest this possibility. For instance, the feeding behavior of animals treated with PCPA and animals with small hypothalamic lesions just lateral to the fornix columns (Powley & Kessey, 1970) are similar. Both preparations maintain and defend body weight below control levels. Furthermore, the location of weight-reducing perifornical lesions (Powley & Kessey, 1970) is within the trajectory of a serotonergic fiber bundle. Histofluorescence mapping has demonstrated a serotonergic fascicle concentrated just lateral to the fornix bundle (Fuxe, Hökfelt, & Ungerstedt, 1970), and lesions in this general area are known to markedly reduce forebrain serotonin levels (Harvey & Lints, 1971; Sheard & Freedman, 1966/67). Attenuation of activity within this system may be the common feature of the anorexia which results from midlateral hypothalamic lesions and PCPA treatment. Additional inferential support for this general line of reasoning can be culled from biochemical changes in brain serotonin activity which occur concurrently with satiation. The synthesis rate of brain serotonin is about 30% lower in fed rats

than in fasted rats (Perez-Cruet, Tagliamonte, Tagliamonte, & Gessa, 1972). Moreover, this period of decreased synthesis is accompanied by decreased neuronal utilization of serotonin, as indicated by the low 5-hydroxyindoleacetic acid levels (Perez-Cruet et al, 1972).

Behavioral results obtained from animals with serotonin-depleting lesions of the rostral raphe nuclei are only partially congruent with the above reasoning. Since ascending serotonergic systems in the brain are derived exclusively from midline raphe nuclei of the brainstem (Fuxe et al, 1970), it might be expected that destruction of those cells would also lead to a chronic reduction of body weight. In fact, the lesions do produce a period of weight reduction, but gradually the animals recover normal body weights and exhibit normal daily food intakes (Coscina, Grant, Balagura, & Grossman, 1972; Lorens, Sorensen, & Yunger, 1971).

In summary, chronic treatment of rats with PCPA produces a very systematic reduction in their feeding behavior. These animals also appear to actively defend their lower body weights. Although the reduction in feeding behavior may be due to effects other than reduction of brain serotonin, much of our present knowledge about the brain serotonergic systems is consistent with the notion that PCPA may be reducing appetite by attenuating activity in a serotonergic neural system which courses through the midlateral hypothalamus. Accordingly, it will be informative to determine whether PCPA-induced anorexia can be modified with lesions of the perifornical hypothalamus or raphe nuclei.

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