Decreased gastrointestinal motility in rats after parenteral injection of *p*-chlorophenylalanine

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Administration of 5,7-dihydroxytryptamine into the cerebral ventricles of rats, following treatment with the noradrenaline uptake blocker desmethylimipramine, produces large and apparently specific depletions of 5-hydroxytryptamine (5-HT) in the brain (Björklund, Baumgarten & Rensch, 1975; Gerson & Baldessarini, 1975). Recently we reported that rats given this treatment often showed a marked depression of gastrointestinal motility that was associated with considerable accumulation of food and wastes in the lower intestines (Saller & Stricker, 1978). We now report that such dysfunctions also occur in rats given parenteral injections of *p*-chlorophenylalanine (PCPA), the 5-HT synthesis inhibitor most generally employed to reduce brain 5-HT concentrations.

Male Sprague-Dawley rats (n = 87), 450–550 g, were housed and tested singly in wire mesh cages in a room maintained at 23°, with fluorescent lighting from 06·00 to 18·00 h. All rats had continuous access to Purina Lab Chow pellets (placed on the cage floor), and tap water (presented in bottles with metal drinking nozzles), except as noted.

56 rats received either one or two injections of *p*-chlorophenylalanine methyl ester (doses expressed as mg free base kg^{-1}). The drug was freshly prepared in 0·9% w/v sodium chloride, adjusted to pH 6·5 with NaOH, and administered either intraperitoneally or subcutaneously in a volume of 2 ml kg^{-1}. Animals given two injections received the second 24 h after the first. (The various doses chosen represent the treatments that seem to be most popular for reducing brain 5-HT concentrations.)

We used two procedures to assess the effects of PCPA on gastrointestinal motility, as in our previous study (Saller & Stricker, 1978). In one, motility was estimated simply by measuring the amount of food that was present in the gastrointestinal tract after 48 h of food deprivation. 40 rats were given one or two injections of PCPA (120–360 mg kg^{-1}); food was removed immediately before the first injection, and animals were decapitated 48 h later. The stomach and entire length of intestine was excised, their ends were clamped, and they were placed in preweighed glass beakers. Wet tissue weights were recorded before heating them at 100–110° to dryness, after which they were reweighed. Wet and dry weights of comparable tissues from 12 control rats that had been given only the vehicle solution, 5 untreated rats that were not fasted, and 6 rats that had been fasted for four days (whose gastrointestinal tracts were devoid of food and wastes) were obtained for purposes of comparison.

Gastrointestinal motility also was measured by determining the passage of the non-absorbable marker polyethylene[1,2-^{14}C]glycol (PEG) by a modification of previous methods (Bridges, Dent & Johnson, 1976). 16 rats were given one subcutaneous injection of PCPA (300 mg kg^{-1}) and were decapitated 5, 48, or 96 h later. Food and water remained available during this time, and intakes were monitored, until 1 h before the animals received an intragastric intubation of water containing PEG (0·5 μCi in 1 ml). Three h later they were killed and the stomachs, six equal segments of small intestine, and the colon were removed. The tissues were immersed in 10 ml of a solution containing 2M NaOH, methanol, and Triton-X-405 (60:30:10) for 12 h, following which 10 ml of water was added and the tissues were homogenized. The homogenate was adjusted to pH 7·0 with concentrated nitric acid, and an 0·5 ml aliquot was analysed for ^{14}C-PEG by liquid scintillation spectrometry. Counting efficiency was monitored using ^{14}C-PEG as an internal standard. A relative index of gastrointestinal motility in PCPA-treated rats was computed by assigning the numbers 0–7 to the 8 successive sections of gastrointestinal tracts (i.e., stomach, 6 segments of small intestine, and colon), multiplying those numbers by the percentage of total recovered radioactivity that was found in each section, and dividing the sum of these products by the mean value obtained from comparable computations using tissue from 8 control animals killed 96 h after vehicle injection.

To assess the effects of PCPA on brain 5-HT, when animals were killed each brain was rapidly removed from the skull, frozen on dry ice, stored at —70° for not more than 1 week, and later analysed fluorometrically for 5-HT using minor modifications of methods that have been described elsewhere (Atack & Lindqvist, 1973). Trace amounts of radioactive 5-HT were added to the samples to permit determinations of recovery.

The results of the first procedure are summarized in Table 1. In control animals almost 75% of the dry material within the intestines had disappeared within 2 days of fasting. Two intraperitoneal injections of 120 mg kg^{-1} PCPA reduced whole brain 5-HT by 65% but did not significantly affect wet or dry weights of the gastrointestinal tract. In contrast, after each of the four higher doses of PCPA (i.p.), brain 5-HT was decreased by 80–95% and only 25–35% of the dry intragastric material was emptied. In addition, the elevated wet weights of the tract documented the

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Table 1. *The effect of* p-*chlorophenylalanine (PCPA) on gastrointestinal tract weights and brain 5-HT concentrations in the rat.*

Treatment	n	Dose (mg kg^{-1})	Wet weight (g)	Dry weight (g)	Brain 5-HT (μg g^{-1})
Controls—not fasted	5	—	38·5 ±5·9*	10·0 ±1·1*	—
Controls—fasted 2 days	12	—	17·5 ±1·6	5·2 ±0·4	0·84 ±0·01
Controls—fasted 4 days	6	—	10·7 ±1·2*	3·4 ±0·6	—
PCPA (i.p.)	6	2 × 120	18·8 ±1·7	5·8 ±0·4	0·29 ±0·02**
PCPA (i.p.)	5	1 × 300	29·4 ±2·4**	8·0 ±0·9*	0·13 ±0·04**
PCPA (i.p.)	6	1 × 360	35·5 ±1·3**	8·2 ±0·6**	0·10 ±0·03**
PCPA (i.p.)	6	2 × 240	37·4 ±2·0**	7·8 ±0·8*	0·13 ±0·05**
PCPA (i.p.)	6	2 × 360	44·1 ±2·2**	8·1 ±0·8*	0·05 ±0·02**
PCPA (s.c.)	7	2 × 120	17·9 ±1·3	5·6 ±0·5	0·27 ±0·03**
PCPA (s.c.)	4	2 × 360	25·7 ±1·4*	8·4 ±0·6**	0·10 ±0·02**

Results represent the mean values ±s.e. Rats given one or two injections of PCPA were fasted for 2 days, and their results were compared with data from controls that were fasted for 2 days. Significant differences (two-tailed *t*-tests): $*P<0.01$; $**P<0.001$.

considerable accumulation of fluid within the intestinal lumen that had been apparent at autopsy. The largest dose of PCPA (2 × 360 mg kg^{-1}), when administered subcutaneously, produced the same depletion of brain 5-HT and decrease in gastric emptying as that produced by intraperitoneal injection but without the increase in intestinal wet weight (relative to dry weight).

A decrease in gastrointestinal motility produced by administering a large dose of PCPA subcutaneously also was apparent when motility was measured by passage of PEG and food was freely available. As indicated in Table 2, 5 h after the drug treatment motility was depressed by 30%, and comparable reductions were evident 2 and 4 days later. Whole brain 5-HT concentrations also were reduced throughout this period. The daily food intakes of the rats given PCPA were substantially lower than those of the control animals (mean ± s.e. = 5·4 ± 2·0 g and 25·7 ± 1·1 g, respectively; $P< 0.001$), and their body weights decreased progressively.

Table 2. *Gastrointestinal motility and brain 5-HT in rats at various times after* p-*chlorophenylalanine (PCPA).*

Treatment	n	Motility	Brain 5-HT
Saline	8	100·0 ± 1·0	100 ± 1·5
PCPA–5 h	6	69·7 ± 4·7	48 ± 2·7
PCPA–48 h	5	60·7 ± 1·1	9 ± 1·2
PCPA–96 h	5	65·9 ± 13·0	15 ± 2·5

Results represent mean values ±s.e., expressed as a percentage of controls. All differences between rats given PCPA (300 mg kg^{-1}, s.c.) and controls were statistically significant ($P<0.001$, two-tailed *t*-test).

Thus, with both procedures we observed that relatively large doses of PCPA reduced gastrointestinal motility in rats. These doses of PCPA also produced significant decreases in brain 5-HT concentrations. Although the present experiments do not provide evidence by which the alterations in gastrointestinal function might be linked directly to disruptions in central serotoninergic function, specific 5-HT depletions in brain following intraventricular injections of 5,7-dihydroxytryptamine have been shown to decrease gastrointestinal motility in rats (Saller & Stricker, 1978). In light of the numerous reports that central serotoninergic neurons inhibit peripheral sympathetic activity (e.g., Quik & Sourkes, 1977), it seems reasonable to suggest that the present findings might have resulted from an increase in sympathoadrenal function. Of course, it remains possible that PCPA may have disrupted peristalsis by some additional or alternative action, such as by decreasing 5-HT concentration in the gastrointestinal tract (cf. Bülbring & Crema, 1959).

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