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Effect of 2-(*p*-chlorophenyl)cyclopropylamine on 5-hydroxyindole concentration and monoamine oxidase activity in rat brain

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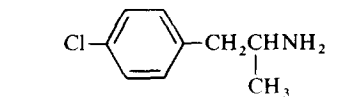
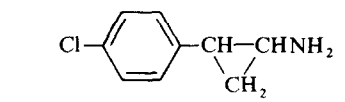
p-Chloroamphetamine (PCA) causes a rapid and long-lasting depletion of serotonin in rat brain [1]. PCA is thought to affect serotonin neurons acutely by (a) inhibition of tryptophan hydroxylation, (b) release of vesicular bound serotonin, (c) inhibition of serotonin reuptake from the synaptic cleft, and (d) inhibition of serotonin oxidation by monoamine oxidase, and to produce neurotoxic degeneration of some serotonin neurons resulting in a chronic decrease in tryptophan hydroxylase activity, serotonin and 5-hydroxyindoleacetic acid (5-HIAA) concentration, and serotonin uptake capacity (variables associated specifically with serotonin neurons).

Among the analogs of PCA that have been studied [1], *N*-cyclopropyl-PCA is characterized by being a potent irreversible inhibitor of monoamine oxidase [2]. Its relatively greater inhibition of monoamine oxidase than that by PCA results in serotonin concentration not being decreased initially (during the first 24 hr), whereas at 1 week, when the monoamine oxidase-inhibiting effects have diminished, serotonin concentration is decreased [2]. 5-HIAA concentration decreases rapidly and remains decreased at 1 week after *N*-cyclopropyl-PCA injection [2]. Thus, *N*-cyclopropyl-PCA (a) inhibits monoamine oxidase and (b) has PCA-like serotonin-depleting activity. These two actions have been dissociated first by giving harmaline, which protects against the irreversible inactivation of type A monoamine oxidase by *N*-cyclopropyl-PCA and exposes its ability to deplete serotonin earlier [3], and second by giving fluoxetine, which inhibits *N*-cyclopropyl-PCA uptake into serotonin neurons and thus prevents its depletion of serotonin but permits monoamine oxidase inhibition to occur [4].

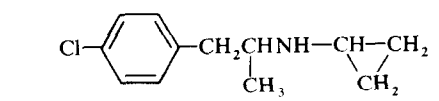
We are describing here studies on a PCA analog similar in structure to *N*-cyclopropyl-PCA but with the cyclopropyl group interposed between the *p*-chlorophenyl group and the amino group. The structure of this compound, 2-(*p*-chlorophenyl) cyclopropylamine (PCCA), is shown in Fig. 1 compared to PCA and *N*-cyclopropyl-PCA.



p-Chloroamphetamine (PCA)



2-(*p*-Chlorophenyl) cyclopropylamine (PCCA)



N-cyclopropyl-PCA

Fig. 1. Chemical structures.

In these experiments, 130–200 g male Wistar rats from Harlan Industries, Cumberland, IN, were used. The rats were housed in hanging wire cages in a light-controlled room (12 hr light/12 hr dark) and had free access to food and water. PCCA hydrochloride was synthesized at the Smith Kline & French Laboratories. Chromatographic and spectral data indicated that it consists of 77.6% *trans* and 22.4% *cis* isomers. Harmaline hydrochloride was purchased from the Sigma Chemical Co., St. Louis, MO. PCCA was injected at a dose of 0.05 mmole/kg, the same as that used previously for *N*-cyclopropyl-PCA [2]. Both drugs were injected i.p. in aqueous solutions. Rats were decapitated after treatment, and brains were rapidly excised, split longitudinally along with the midline, and frozen on dry ice. Tissue samples were stored at −15° prior to analysis. Serotonin and 5-hydroxyindoleacetic acid concentrations were determined spectrofluorometrically after extraction and reaction with *o*-phthalaldehyde by the method of Miller *et al.* [5]. Monoamine oxidase activity was assayed with 100 μM [^{14}C]serotonin or 80 μM [^{14}C]phenylethylamine (from New England Nuclear, Boston, MA) as substrate [6].

The effects of PCCA on 5-hydroxyindole concentrations in rat brain at various times are shown in Table 1. Within 6 hr there was a large increase in serotonin concentration and a marked fall in 5-HIAA concentration. At 24 hr the serotonin concentration was still significantly increased and the 5-HIAA concentration was still decreased. By 1 week, the concentration of both 5-hydroxyindoles had returned to normal. These results contrast with those obtained earlier with *N*-cyclopropyl-PCA [2] in two ways. First, the initial increase in serotonin concentration was greater with PCCA than with *N*-cyclopropyl-PCA. Second, PCCA had no effect at 1 week, whereas *N*-cyclopropyl-PCA had produced depletion both of serotonin and of 5-HIAA at 1 week.

Monoamine oxidase activity is also shown in Table 1. Virtually complete inhibition of monoamine oxidase was observed at 6 hr and at 24 hr. At 1 week, significant inhibition remained. At this time point, some selectivity was apparent in that serotonin oxidation was inhibited to a significantly lesser extent than was phenylethylamine oxidation. The 35 per cent reduction in serotonin-oxidizing capacity was not associated with any change in serotonin or 5-HIAA concentration at 1 week. These results indicate that PCCA produces a much greater inhibition of monoamine oxidase compared to an equimolar dose of *N*-cyclopropyl-PCA [2].

The small amount of monoamine oxidase inhibition remaining at 1 week might have masked a decline in serotonin concentration if PCCA had long-term serotonin-depleting effects. This possibility is unlikely, since the 5-HIAA concentration had returned to normal at 1 week, whereas if monoamine oxidase inhibition had been sufficient to prevent a decline in serotonin concentration it should have resulted in a continued decrease in the 5-HIAA concentration. Nonetheless, in a separate experiment with the same 10.2 mg/kg dose of PCCA, 5-hydroxyindole concentrations were measured at 2 weeks or 3 weeks to ensure that a long-term decline in serotonin concentration was not

Table 1. Effect of 2-(*p*-chlorophenyl)cyclopropylamine on 5-hydroxyindole concentration and monoamine oxidase activity in rat brain*

Time after injection	Brain 5-hydroxyindoles (μg/g) Serotonin	5-HIAA	MAO activity (nmoles · min^{-1} · g^{-1}) Serotonin	Phenylethylamine
0	0.66 ± 0.02	0.60 ± 0.02	107.8 ± 0.9	86.9 ± 0.8
6 hr	1.14 ± 0.04†	0.17 ± 0.01†	0.8 ± 0.1†	0.8 ± 0.1†
1 day	1.34 ± 0.06†	0.40 ± 0.01†	3.3 ± 0.4†	1.9 ± 0.1†
1 week	0.70 ± 0.02	0.55 ± 0.02	69.9 ± 0.6†	37.4 ± 0.3†

* 2-(*p*-Chlorophenyl)cyclopropylamine hydrochloride was injected i.p. at a dose of 10.2 mg/kg (0.05 mmole/kg). Mean values ± standard errors for five rats per group are shown.
† Significantly different from control group ($P < 0.01$).

missed. In this experiment, serotonin concentration was 0.67 ± 0.02 μg/g in control rats, 0.67 ± 0.03 μg/g 2 weeks after PCCA, and 0.65 ± 0.01 μg/g 3 weeks after PCCA. The concentration of 5-HIAA was 0.55 ± 0.01 μg/g in control rats, 0.55 ± 0.01 μg/g 2 weeks after PCCA, and 0.56 ± 0.01 μg/g 3 weeks after PCCA. None of these differences was statistically significant.

In a separate study (Table 2), PCCA and harmaline were co-administered, as reported earlier with *N*-cyclopropyl PCA [3]. Monoamine oxidase measured at 24 hr revealed, again, essentially complete inhibition by PCCA, and the co-administration of harmaline had no effect on the inhibition of either serotonin or phenylethylamine oxidation. These results also contrast with those obtained earlier with *N*-cyclopropyl-PCA, in which case harmaline co-administration had protected against the inactivation of monoamine oxidase and had antagonized the inactivation of serotonin oxidation to a greater extent than the inactivation of phenylethylamine oxidation [3]. 5-Hydroxyindole measurements in these rats (Table 2) indicated that the increase in serotonin and the decrease in 5-HIAA produced by PCCA were unchanged by harmaline co-administration.

The inability of harmaline to antagonize the inhibition of monoamine oxidase by PCCA in this experiment, in contrast to the antagonism that had been seen with *N*-cyclopropyl-PCA at an equimolar dose [3], seemed probably due to the greater degree of monoamine oxidase inhibition by PCCA than by *N*-cyclopropyl-PCA. To test that possibility, we injected two lower doses of PCCA along with harmaline. A dose of 5 mg/kg of PCCA produced nearly complete (≥ 95 per cent) inhibition of serotonin and phenylethylamine oxidation, and co-treatment with harmaline had little effect. A 1 mg/kg dose of PCCA produced 82 ± 1 per cent inhibition of serotonin oxidation in control rats but only 39 ± 2 per cent inhibition of serotonin oxidation in harmaline-treated rats. This difference was statistically significant ($P < 0.001$). Phenylethylamine oxidation was inhibited by 90 ± 1 per cent in control rats and 82 ± 0.4 per cent in harmaline-treated rats; this difference was also statistically significant ($P < 0.001$) but was slight compared to the difference when serotonin was substrate. Thus, harmaline can antagonize the inhibition of monoamine oxidase by PCCA just as it does the inhibition by *N*-cyclopropyl-PCA (more antagonism with serotonin as substrate than with phenylethylamine as substrate), but harmaline can competitively antagonize inhibition by PCCA only at a low dose (1 mg/kg) approximately equal in effectiveness to the 8 mg/kg dose of *N*-cyclopropyl-PCA [3]. Serotonin concentration (0.64 ± 0.02 μg/g) in rats treated with the 1 mg/kg dose of PCCA in combination with harmaline was not significantly different from control (0.60 ± 0.02 μg/g). Thus, for harmaline to be able to antagonize the monoamine oxidase inhibiting action of PCCA, the dose of PCCA has to be lowered below that required for serotonin depletion even by the most potent depletors in the chloroamphetamine series, leaving no possibility of unmasking a latent serotonin-depleting potential in PCCA by antagonism of its monoamine oxidase inhibition.

These studies indicate that PCCA manifests none of the serotonin-depleting activity of PCA. In contrast to PCA, PCCA causes pronounced and long-lasting (hence presumably irreversible) inhibition of monoamine oxidase, the inhibition being even greater than that produced by *N*-cyclopropyl-PCA at an equimolar dose. PCCA is thus a PCA analog for which only monoamine oxidase inhibition is apparent in its *in vivo* actions on serotonin neurons.

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Table 2. Effect of 2-(*p*-chlorophenyl)cyclopropylamine (PCCA) on 5-hydroxyindole concentration and monoamine oxidase activity in brains of control and harmaline-treated rats*

Rats	Dose of PCCA (mg/kg)	MAO activity with [^{14}C]Serotonin as substrate (nmoles · min^{-1} · g^{-1})	[^{14}C]Phenylethylamine as substrate (nmoles · min^{-1} · g^{-1})	Serotonin concn (μg/g)	5-HIAA concn (μg/g)
Control	0	135.6 ± 0.7	87.7 ± 1.3	0.64 ± 0.02	0.53 ± 0.01
	10.2	5.7 ± 1.2†	2.6 ± 0.5†	1.16 ± 0.06†	0.38 ± 0.01†
Harmaline treated	0	129.8 ± 2.2	82.6 ± 1.8	0.59 ± 0.02	0.49 ± 0.02
	10.2	2.6 ± 0.5†	1.8 ± 0.3†	1.36 ± 0.04†	0.37 ± 0.02†

* 2-(*p*-Chlorophenyl)cyclopropylamine hydrochloride and harmaline hydrochloride (20 mg/kg) were injected i.p. 24 hr before rats were killed. Mean values ± standard errors for five rats per group are shown.
† Significant effect of PCCA ($P < 0.01$).

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Alpha$_1$-adrenergic activation of phosphatidylinositol labeling in isolated brown fat cells*

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In the early 1960's, Smith and Hock [1] suggested that the primary physiological function of brown adipose tissue is that of heat production. It is now considered that brown adipose tissue is the primary thermogenic effector organ in arousing hibernators, most cold-exposed newborns and cold-exposed adults of many non-hibernating species [2]. Brown adipose tissue is richly innervated, and catecholamines seem to play a major physiological role in modulating its activity.

Electrophysiological studies have demonstrated depolarization of brown adipocytes in response to endogenous (nerve stimulation) or exogenous catecholamines [3–5]. Recently, Fink and Williams [6] have shown that both alpha and beta adrenoceptors mediate depolarization in brown adipocytes.

Alpha adrenoceptors have been subdivided in two subtypes—alpha$_1$ and alpha$_2$ [7, 8]. Fain and Garciá-Sáinz [9] have suggested that the subdivision of the alpha adrenoceptors is both strucural (affinity for agonists and antagonists) and functional (underlying mechanism of action). Activation of alpha$_1$ adrenoceptors mediates those effects of catecholamines that involve phosphatidylinositol turnover and the entry or mobilization of calcium, whereas activation of alpha$_2$ adrenoceptors mediates those effects of adrenergic amines resulting from inhibition of adenylate cyclase [9]. The present experiments were designed to determine whether an alpha-adrenergic stimulation of phosphatidylinositol turnover can be seen in brown fat cells.

Epinephrine, isoproterenol, and propranolol were obtained from the Sigma Chemical Co. (St. Louis, MO) yohimbine from ICN Nutritional Biochemicals (Cleveland, OH) crude collagenase (*Clostridium histolyticum*) from the Worthington Biochemical Corp. (Freehold, NJ) (Lot No. CLS 48A281), bovine serum albumin (Fraction V) from the Armour Pharmaceutical Co. (Kankakee, IL) (Lot No. S 11709) and [^{32}P]P$_i$ as orthophosphoric acid (carrier-free) from the New England Nuclear Corp. (Boston, MA). Prazosin and phentolamine were provided by Pfizer Inc. (Groton, CT) and the CIBA Pharmaceutical Co. (Summit, NJ) respectively.

Brown fat cells were isolated from the dorsal interscapular brown adipose tissue of Sprague–Dawley rats (Charles River CD strain) according to the method of Fain *et al.* [10] with minor modifications. In brief, brown adipose tissue from ten to fifteen rats was removed and carefully trimmed of adhering skeletal muscle or white adipose tissue; the tissue was digested in buffer containing 0.75 mg collagenase/ml. After 20 min, the non-digested tissue was filtered onto a layer of nylon chiffon, and the remaining pieces of brown adipose tissue were cut into small pieces and incubated for 40 min in buffer containing 1.5 mg collagenase/ml. This procedure is similar to that of Pettersson and Vallin [11] and allowed us to get at least 80% multilocular fat cells.

The incorporation of [^{32}P]P$_i$ into phospholipids was studied as described previously for white fat cells [12] with some modifications. In brief, brown fat cells (about 10^5 cells) were incubated in 1 ml of buffer containing 6% albumin and 10 μCi/ml of [^{32}P]P$_i$ for 60 min in a water bath shaker at 37°. Lipids were extracted with chloroform–methanol (2:1), and phospholipids separated by one-dimensional thin-layer chromatography as described previously [12]. The phosphorus content of each phospholipid was determined by the microprocedure of Bartlett [13]. Krebs-Ringer Tris buffer of the following composition was used in all the experiments: 120 mM NaCl, 1.4 mM $CaCl_2$, 5.2 mM KCl, 1.4 mM $MgSO_4$ and 5 mM Tris. The buffer was prepared daily and adjusted to pH 7.4 at 37° with NaOH after addition of the albumin powder.

Cyclic AMP accumulation was measured at 10 min in the presence of adenosine deaminase (0.5 μg/ml) and theophylline (100 μM) by a modification of the method of Gilman [14].

Incubation of brown cells in buffer containing radioactive phosphate resulted in significant incorporation of label into phospholipids. The specific activity of major phospholipids was as follows: cardiolipin + phosphatidylglycerol 135 ± 45 cpm/μg phosphate; phosphatidylethanolamine, 150 ± 15 cpm/μg phosphate; phosphatidylcholine, 865 ± 150 cpm/μg phosphate; phosphatidylinositol, 1195 ± 305 cpm/μg phosphate; and phosphatidic acid + phosphatidylserine, 819 ± 150 cpm/μg phosphate. The values are the means ± S.E.M. of seven experiments.

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