Modification by harmaline of the effects of *N*-cyclopropyl-*p*-chloroamphetamine on 5-hydroxyindole concentration in rat brain

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Among numerous analogues of *p*-chloroamphetamine (PCA) whose effects on brain 5-hydroxytryptamine metabolism we have studied previously (Fuller, 1978), *N*-cyclopropyl-PCA has been of particular interest because of its effectiveness as an inhibitor of monoamine oxidase. PCA itself apparently has multiple actions on 5-hydroxytryptamine (5-HT)neurons in brain, including inhibition of tryptophan hydroxylase, release of 5-HT from storage sites, inhibition of monoamine oxidase, and inhibition of 5-HT reuptake (Fuller & Molloy 1974). Although PCA and most of its analogues are weak reversible inhibitors of monoamine oxidase, *N*-cyclopropyl-PCA is a potent irreversible inhibitor of that enzyme (Fuller & Molloy 1973; Fuller & Perry 1977). Thus in addition to the PCA-like depleting effect of *N*-cyclopropyl-PCA on brain 5-hydroxytryptamine, the *N*-cyclopropyl compound—through inhibition of monoamine oxidase—tends to elevate 5-HT at early times. After *N*-cyclopropyl-PCA injection, 5-HT concentration in brain initially is not decreased or in fact is slightly increased, whereas 5-hydroxyindoleacetic acid (5-HIAA) concentration falls rapidly (Fuller & Molloy 1973). At longer times, when the monoamine oxidase inhibition is subsiding (days 2–14 after a single injection of *N*-cyclopropyl-PCA), 5-HT as well as 5-HIAA concentration is lowered in rat brain (Fuller & Molloy 1973). Thus *N*-cyclopropyl-PCA differs from PCA primarily in that the monoamine oxidase-inhibiting component of its action is stronger and therefore prevents 5-HT concentration from being lowered initially. The two predominant effects of *N*-cyclopropyl-PCA on 5-HT-containing neurons in rat brain appear to be (1) inhibition of monoamine oxidase and (2) a PCA-like depletion of 5-hydroxyindoles requiring active uptake of the drug into 5-HT-containing neurons. Earlier we were able to prevent the second component of action by giving fluoxetine, an inhibitor of active uptake into 5-HT-containing neurons (Fuller & Perry 1977). In fluoxetine-treated rats, 5-HT concentration was markedly increased after *N*-cyclopropyl-PCA injection as the first component of its action, monoamine oxidase inhibition, predominated. Now we are reporting that action (1) of *N*-cyclopropyl-PCA can be prevented while permitting action (2) to occur.

Harmaline, a short-acting reversible inhibitor of monoamine oxidase, was co-administered with *N*-cyclopropyl-PCA to protect against the irreversible inactivation of the enzyme by the latter compound. As expected, antagonizing the action of *N*-cyclopropyl-PCA that tends to increase 5-HT (monoamine oxidase inhibition) resulted in lowering of 5-HT concentration. The study was based on our recent observations that harmaline, by occupying active sites on type A monoamine oxidase, selectively prevents the irreversible inactivation of that enzyme form by agents that additionally and preferentially inhibit type B monoamine oxidase (Fuller & Hemrick 1978; Fuller et al 1978).

Groups of 5 male albino rats from the Wistar strain (Harlan Industries, Cumberland, Indiana) were given intraperitoneal injections of harmaline hydrochloride (Sigma), 20 mg kg^{-1}, along with *N*-cyclopropyl-PCA maleate (Lilly), 8·2 mg kg^{-1}. Rats were decapitated 24 h later, and monoamine oxidase activity in whole brain homogenates was assayed radiometrically with either ^{14}C-5-HT or [^{14}C]phenethylamine as substrate for type A and type B forms of the enzyme, respectively (Fuller & Roush 1972). 5-HT and 5-HIAA concentrations in whole brain were determined spectrofluorometrically (Miller et al 1970).

Table 1 shows the ability of *N*-cyclopropyl-PCA to inhibit the oxidation of 5-HT and phenethylamine in rat brain 24 h after its intraperitoneal injection. This compound is a relatively non-selective inhibitor, causing 75% inhibition of 5-HT oxidation and 80% inhibition of phenethylamine oxidation. In rats treated with harmaline alone, no significant inhibition of either form of monoamine oxidase occurred at this time. However, harmaline largely prevented the inhibition of 5-HT oxidation by *N*-cyclopropyl-PCA. 5-HT oxidation by brain homogenates from rats treated with the drug combination were depressed only 20% below the control level; although this difference from control was statistically significant ($P < 0{\cdot}001$), it was much less than had occurred in rats treated with *N*-cyclopropyl-PCA

Table 1. Monoamine oxidase activity and 5-hydroxyindole concentration in rat brain 24 h after treatment with *N*-cyclopropyl-PCA and/or harmaline.

Treatment group	Monoamine oxidase activity nmol^{-1} min^{-1} 5-HT as substrate	PE as substrate	5-Hydroxyindole concn μg g^{-1} 5-HT	5-HIAA
Control	97 ± 3	77 ± 2	0·71 ± 0·01	0·73 ± 0·02
N-Cyclopropyl-PCA	24 ± 1 (−75%)	15 ± 1 (−80%)	0·76 ± 0·03	0·36 ± 0·006 (−51%)
Harmaline	96 ± 1	74 ± 2	0·69 ± 0·006	0·62 ± 0·02 (−15%)
N-Cyclopropyl-PCA + harmaline	77 ± 2 (−20%)	25 ± 1 (−68%)	0·51 ± 0·02 (−28%)	0·39 ± 0·01 (−47%)

Percentage changes are shown in parentheses for all values that differed significantly ($P > 0{\cdot}05$) from the control group. All values represent mean ± standard error for 5 rats per group.
Statistical comparisons between groups were made by Student's *t*-test.
PE = phenethylamine.

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alone. In contrast, the inhibition of phenethylamine oxidation by *N*-cyclopropyl-PCA was only slightly influenced by harmaline (68% inhibition compared to 80% inhibition in rats receiving *N*-cyclopropyl-PCA alone).

The concentrations of 5-HT and of 5-HIAA are also shown in Table 1. *N*-Cyclopropyl-PCA alone markedly reduced 5-HIAA concentration without affecting 5-HT concentration significantly at this time, in agreement with earlier data (Fuller & Perry 1977). Although harmaline is an inhibitor of type A monoamine oxidase, it is short-acting so that no inhibition of type A monoamine oxidase remained at 24 h; 5-HT concentration therefore was not changed in the group of rats that received harmaline alone, though a slight but statistically significant decrease in 5-HIAA persisted. In rats that received harmaline along with *N*-cyclopropyl-PCA, 5-HT was significantly depressed, and 5-HIAA concentration was lowered to about the same extent as in rats treated with *N*-cyclopropyl-PCA alone.

Considering these data in light of our previous studies on *N*-cyclopropyl-PCA, we make the following interpretations. At times up to 24 h after *N*-cyclopropyl-PCA injection into rats, the concentration of 5-HT in brain was not decreased because inhibition of monoamine oxidase prevented the expression of the depleting effect of the drug. The concentration of 5-HIAA was lowered since both monamine oxidase inhibition and the PCA-like inhibition of 5-hydroxyindole formation by *N*-cyclopropyl-PCA would lower that metabolite. Prevention of type A monoamine oxidase inhibition through co-administration of harmaline, a short-acting reversible inhibitor that acts preferentially on type A MAO, revealed the 5-hydroxyindole-depleting effect of *N*-cyclopropyl-PCA. Both 5-HT and 5-HIAA concentrations were lowered in rats receiving this drug combination. Earlier we had shown that the PCA-like depleting effect of *N*-cyclopropyl-PCA could be prevented by inhibition of its uptake into 5-HT-containing neurons (Fuller & Perry 1977), resulting in expression of monoamine oxidase inhibition (an increase in 5-HT concentration). Thus the two major components of the action of *N*-cyclopropyl-PCA on 5-HT-containing neurons in brain can be dissociated pharmacologically.

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Metoclopramide reverses inhibitions of electrically-induced contractions of guinea-pig isolated ileum by anti-inflammatory drugs

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Metoclopramide stimulates peristaltic movements of the stomach, duodenum and small intestine leading to gastric emptying in man (Justin-Besançon et al 1964; Duret & Arguello 1969; Howells et al 1971) and animals (Guerrin et al 1967; Jacoby & Brodie 1967; Johnson 1971) but its mode of action is not well understood. Besides a central and anti-emetic action, the drug seems to have a peripheral action (Jacoby & Brodie 1967; Duret & Arguello 1969; Johnson 1971). It stimulates various preparations of the gastrointestinal tract (Reuse 1973) including the guinea-pig isolated ileum (Fontaine & Reuse 1973). It is also able to increase the response of the guinea-pig ileum to the electrical transmural stimulation (Fontaine & Reuse 1972) and to reverse the inhibition of these electrically-induced contractions by procaine, morphine and atropine (Fontaine & Reuse 1973).

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Non-steroidal anti-inflammatory drugs (NSAID) and steroidal anti-inflammatory drugs (SAID) inhibit electrically-induced contractions of guinea-pig isolated ileum and this is reversed by prostaglandins (PG), nicotine and caerulein, a polypeptide with gastrointestinal stimulating properties (Famaey et al 1975; Fontaine 1976). Thus the inhibition and its reversal seems to be non-specific. We therefore examined the effects of the drug on these inhibitions.

Segments of ileum (4 cm) at least 10 cm from the caecum were suspended under an initial load of 1 g in Krebs-Henseleit solution maintained at 37° C and gassed with a mixture of 5% CO_2 in oxygen. Isometric contractions (registered by a force transducer) were elicited by coaxial electrical stimulation (pulse width 0·5 ms, pulse strength 5–25 V, frequency 0·1 Hz; Paton 1955).

At concentrations known to induce approximately 50% inhibitions of these contractions one of 11 NSAID