The role of metabolic *N*-dealkylation in the action of *p*-chloromethamphetamine and related drugs on brain 5-hydroxytryptamine

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p-Chloromethamphetamine (PCMA) was the first ring-halogenated amphetamine reported to lower brain 5-hydroxytryptamine (5-HT) in rats (Pletscher, Burkard & others, 1963). We found *p*-chloroamphetamine (PCA) to be the most active of a series of chlorinated amphetamines in depleting rat brain 5-HT, though our findings (Fuller, Hines & Mills, 1965) and those of others (Miller, Cox & others, 1970) have indicated there is little difference between PCA and PCMA in this action. This is possibly because PCMA is extensively metabolized in rats to PCA. Several *N*-alkyl derivatives of PCA are converted metabolically to PCA by the rat and this may be adequate to account for their depletion of brain 5-HT.

Male albino rats (Wistar, Harlan Industries, Cumberland, Indiana), 150 g, were given injections of substituted amphetamines intraperitoneally and decapitated. Whole brains were removed, frozen rapidly and stored at —15°. 5-HT concentrations were determined fluorometrically by condensation with *o*-phthalaldehyde (Miller & others, 1970; Fuller, Perry & others, 1974), and PCA concentrations were determined spectrofluorometrically by condensation with fluorescamine (Fuller & others, 1974) which is specific for primary amines; none of the *N*-alkyl derivatives examined interfered.

When PCA was injected, PCA concentration in brain was highest at 2 h and declined slowly (Fig. 1). When PCMA was injected, a substantial concentration of PCA was already present at 2 h, and this increased at 4 and 6 h until it resembled that present after PCA (Fig. 1). The extensive metabolism of PCMA by *N*-demethylation suggested that the concentration of PCA in brain might be adequate to account for the lowering of brain 5-HT after PCMA injection.

We compared other *N*-alkyl derivatives of PCA, measuring both 5-HT and PCA concentrations in brain (Table 1). The highest concentration of PCA and the greatest depletion of 5-HT were obtained when PCA itself was injected. The *N*-methyl and *N*-ethyl derivatives depleted 5-HT almost as much and produced substantial though lower concentrations of PCA in brain. The *N*-isopropyl and *NN*-dimethyl derivatives had much less effect on brain 5-HT, and the concentration of PCA in brain was still lower.

The fact that the *N*-methyl and *N*-ethyl derivatives lowered 5-HT equally despite PCA concentrations differing approximately two-fold suggests that the actions of these *N*-alkyl derivatives may not be due entirely to the formation of PCA. We have attempted to follow the time course of drug effects and establish a relation between PCA concentration in brain and degree of 5-HT depletion after injection of these drugs, but the data have not clearly revealed whether conversion to PCA is the only mechanism by which the *N*-alkyl derivatives work. Most data have indicated that PCA could account substantially though probably not entirely for the actions of the *N*-alkyl derivatives on brain 5-HT. We thought a definitive answer could be obtained if the metabolic conversion could be blocked. However, we have not so far been able to find inhibitors of microsomal enzymes that will prevent the dealkylation of the PCA derivatives to an extent adequate to prevent the effects on brain 5-HT. For example, we found that SKF 525A (β-diethylaminoethyl-diphenylpropylacetate) injected intraperitoneally at 10 mg kg^{-1} 1 h before PCMA did not prevent its conversion to

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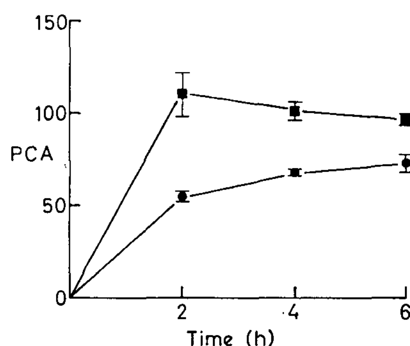

FIG. 1. PCA concentration (n mol g^{-1}) in rat brain after the injection of PCA (■) itself or PCMA (●). Mean values ± s.e. for 5 rats per group are shown.

Table 1. *Brain 5-hydroxytryptamine and PCA concentrations after treatment of rats with* N-*alkyl*-p-*chloroamphetamines.*

Drug	Brain 5-HT $\mu g\ g^{-1}$	PCA in brain nmol g^{-1}
None	0·64 ± 0·04	—
PCA	0·36 ± 0·02*	104 ± 6
N-Methyl PCA	0·38 ± 0·01*	73 ± 3
N-Ethyl PCA	0·38 ± 0·01*	34 ± 2
N-Isopropyl PCA	0·53 ± 0·02*	23 ± 2
NN-Dimethyl PCA	0·50 ± 0·03*	14 ± 1

* Significantly different from control, $P < 0·05$. All drugs were injected intraperitoneally at 0·1 mmol kg^{-1} 4 h before the rats were killed. Mean values ± s.e. for 5 rats per group are shown.

PCA. Brain concentrations of PCA at 4 h after PCMA injection (0·1 mmol kg^{-1}) were 70 ± 9 in control rats and 70 ± 3 nmol g^{-1} in rats pretreated with SKF 525A. Another microsomal enzyme inhibitor, DPEA (2,4-dichloro-6-phenylphenoxyethylamine), was similarly ineffective in lowering PCA concentration after PCMA injection. Brain concentrations of PCA were 58 ± 10 nmol g^{-1} 4 h after PCMA injection (0·1 mmol kg^{-1}) in control rats and 69 ± 11 nmol g^{-1} in rats treated with DPEA (25 mg kg^{-1}, i.p.). After the injection of PCA itself (0·1 mmol kg^{-1}), PCA concentration in brain was 81 ± 10 nmol g^{-1} in control rats and 93 ± 8 nmol g^{-1} in DPEA-treated rats in this experiment. Perhaps intraventricular injection of the *N*-alkyl derivatives of PCA would bypass *N*-dealkylating enzymes and permit determination of the direct actions of these compounds on brain 5-HT neurons. Since we may not conclude at present that metabolic *N*-dealkylation is a requirement for various *N*-alkyl derivatives of PCA to lower brain 5-HT, we simply would like to call attention to the fact that such *N*-alkylation does occur extensively in rats.

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The effects of clomipramine and desmethylclomipramine on the *in vitro* uptake of radiolabelled 5-HT and noradrenaline into rat brain cortical slices

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A number of workers have previously shown that in the tricyclic series of antidepressants, the secondary amine members, e.g. desipramine, are more potent inhibitors of noradrenaline uptake than are their tertiary amine counterparts, e.g. imipramine (Maxwell, 1969, 1971; Carlsson, Corrodi & others, 1969a). In contrast, the tertiary amines are more potent blockers of 5-hydroxytryptamine (5-HT) uptake (Carlsson, Corrodi & others, 1969b; Todrick & Tait, 1969). Of the existing tricyclic antidepressants, clomipramine, which is a tertiary amine, has been shown to be the most potent 5-HT uptake inhibitor, whilst its ability to interfere with noradrenaline uptake is much less (Waldmeier, Greengrass & others, 1976). We have shown that the secondary amine derivative of clomipramine, desmethylclomipramine is a major metabolite of clomipramine in man, where it has been detected in high concentrations in the plasma of depressed patients receiving treatment with the drug (Jones & Luscombe, 1977). The ability of desmethylclomipramine to interfere with the uptake of either noradrenaline or 5-HT has not previously been demonstrated, and the purpose of this communication is to describe the results of our *in vitro* studies with clomipramine and desmethylclomipramine on noradrenaline and 5-HT uptake into rat brain cortical slices.

The procedure used to determine the effects of various agents on the *in vitro* uptake of [^{3}H]noradrenaline (^{3}H-NA) and [^{14}C]5-hydroxytryptamine (^{14}C-5-HT) was that described by Sugden (1974). Briefly, male Wistar rats, 180 to 220 g, were killed by cervical dislocation, and the brains were removed over ice-cold Krebs solution. Cerebral cortical slices were obtained and 10 mg samples were incubated in 4·5 ml of Krebs solution at 27° for 15 min before the addition of tricyclic drug and radiolabelled amine in a total additional volume of 0·5 ml. Samples were incubated for a further 20 min and vacuum-filtered. Filter papers containing the tissue slices were solubilized to release their radioactivity by placing them in scintillation vials containing 4 ml of an ethanol–methanol (3:1 v/v) mixture for 45 min. Scintillation fluid, 10 ml (from a mixture containing 4 g PPO, 300 ml of 2-ethoxyethanol, 700 ml of toluene and 10 ml of formic acid), was added to each vial and radioactivity quantitatively determined by liquid scintillation spectrometry, using an ICN Tracerlab scintillation counter. The results are expressed as a % inhibition of uptake (by comparison with non-drug controls), using the equation (Sugden, 1974): % inhibition of amine uptake =

$$\frac{[\text{mean cpm control}] - [\text{mean cpm test}]}{[\text{mean cpm control}] - [\text{mean cpm background}]} \times 100$$

^{3}H-NA and ^{14}C-5-HT were obtained from the Radiochemical Centre, Amersham, and were used at

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