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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incubation concentrations of $0{\cdot}5 \times 10^{-8}$ M and $1{\cdot}0 \times 10^{-7}$ M respectively. Imipramine, desipramine, clomipramine and desmethylclomipramine, kindly donated by Ciba-Geigy Ltd., were each used as their hydrochloride salt.

Each of the tricyclic agents tested demonstrated a concentration-related inhibition of both noradrenaline and 5-HT uptake into rat cerebral cortex slices (see Fig. 1A, B). With the molar concentrations of antidepressant which inhibited amine uptake by 50% (IC50 values) for comparison, the rank order of potency as inhibitors of 5-HT uptake was: clomipramine > imipramine > desmethylclomipramine > desipramine. The rank order of potency as inhibitors of noradrenaline uptake was: desipramine > imipramine > desmethylclomipramine > clomipramine. In agreement with the existing data on other tricyclic antidepressants, the conversion of the parent tertiary amine clomipramine to its primary metabolite and secondary amine desmethylclomipramine, produces a substantial swing away from 5-HT uptake inhibition to a greater inhibition of noradrenaline; thus, whereas the IC50's of clomipramine for noradrenaline and 5-HT uptake inhibition were respectively $2{\cdot}5 \times 10^{-6}$ M and $5{\cdot}5 \times 10^{-7}$ M (ratio of activity against 5-HT and noradrenaline uptake being 4·5:1), for desmethylclomipramine the IC50's were $6{\cdot}5 \times 10^{-7}$ M and 7×10^{-6} M (ratio 1:9). It is not intended to attach any significance to the actual molar concentrations of the IC50's, since these will vary according to the operator, strain of animal, relative concentrations of the two amines in the incubations and other variations of technique. There is however a marked shift towards noradrenaline uptake inhibition in the secondary amine desmethylclomipramine. Thus, as a blocker of 5-HT uptake, it is some 12 times less potent than clomipramine but it is about 3 times more potent in blocking noradrenaline uptake. Similar swings have been observed with desipramine and other secondary amines.

Thus as with other tricyclic agents, desmethylclomipramine is a more potent inhibitor of noradrenaline uptake than is the parent clomipramine. Furthermore studies *in vivo* have shown desmethylclomipramine to possess 'antidepressant' activity, in that it induced a significant reversal of the depressive syndrome induced in mice by tetrabenazine. Recent clinical studies have shown that under steady state conditions, plasma

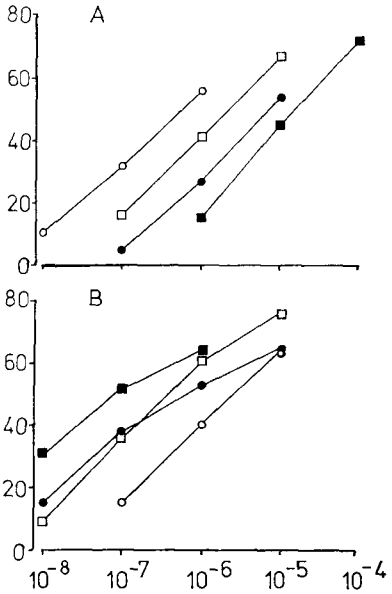

FIG. 1. Percentage inhibition of uptake of, (A) ^{14}C-5-HT and (B) ^{3}H-NA, compared to molar concentrations of tricyclic agents. Each point is the mean of at least three separate determinations. ○—○ clomipramine; ●—● desmethylclomipramine; □—□ imipramine; and ■—■ desmethylimipramine. Ordinate—Percentage inhibition of uptake. Abscissa—Conc. (M).

concentrations of desmethylclomipramine may exceed those of clomipramine in depressed patients receiving the parent compound (Jones & Luscombe, 1977). Consequently it has to be accepted that the metabolite desmethylclomipramine may be making a significant contribution to the overall antidepressant effect of clomipramine. The present observations also suggest that until the relative plasma concentrations of clomipramine, desmethylclomipramine and other metabolites have been determined, for the appropriate species, dose and duration of administration, then care must be exercised when regarding clomipramine as a pharmacological tool with relatively specific 5-HT uptake blocking activity.

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