Long-lasting reduction of brain 5-hydroxytryptamine concentration by 3-chloroamphetamine and 4-chloroamphetamine in iprindole-treated rats

Previously we have shown that 3-chloroamphetamine has little effect on brain 5-hydroxytryptamine concentrations in rats because it is rapidly metabolized by ring hydroxylation, but if that route of metabolism is blocked, then 3-chloroamphetamine lowers brain 5-hydroxytryptamine concentrations as effectively as does 4-chloroamphetamine (Fuller, Schaffer & others, 1972). Those results were obtained at short times after the chloroamphetamines, and the duration of action of the drugs was not examined. More recently, it has been recognized that 4-chloroamphetamine depresses 5-hydroxytryptamine concentrations for several months after a single dose of the drug (Sanders-Bush, Bushing & Sulser, 1972). This prolonged lowering of brain 5-hydroxytryptamine concentrations (and of tryptophan hydroxylase activity and of 5-hydroxytryptamine uptake) may result from a neurotoxic action of 4-chloroamphetamine or a metabolite formed from it, since the duration resembles that of 5,6-dihydroxytryptamine or of 6-hydroxydopamine, agents that are toxic to 5-hydroxytryptamine and catecholamine neurons, respectively. We therefore wanted to know whether 3-chloroamphetamine shared with 4-chloroamphetamine the ability to cause long-lasting depletion of 5-hydroxyindole concentrations when their short-term effects were made essentially equal by blocking the rapid metabolism of the 3-chloro compound.

Iprindole was chosen as the inhibitor of ring hydroxylation to avoid any effects on the neuronal membrane pump that might occur with a hydroxylation inhibitor like desipramine (Freeman & Sulser, 1972). We also determined the possible influence of iprindole on the long-term depletion of 5-hydroxytryptamine by 4-chloroamphetamine, since conceivably a ring-hydroxylated metabolite could be responsible for the long-term effect of that drug. Reasons for considering such a possibility are (*a*) the long-lasting depletion of 5-hydroxytryptamine does not occur with the β,β-difluoro derivative of 4-chloroamphetamine, which would not be expected to be ring hydroxylated (Fuller, Snoddy & Molloy, 1973) and (*b*) the long-lasting effect of 4-chloroamphetamine does not occur in mice, a species that is said not to ring hydroxylate amphetamines to nearly the extent that the rat does (Dring, Smith & Williams, 1970).

Male albino rats of the Wistar strain, weighing about 150 g, received intraperitoneally saline or iprindole (10 mg kg^{-1}) 1 h before 3-chloroamphetamine or 4-chloro-

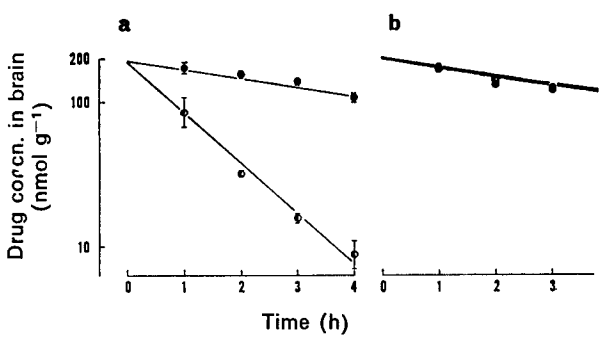


FIG. 1. Rate of disappearance of 3-chloroamphetamine (half-circles) and of 4-chloroamphetamine (solid circles) from whole brain of rats pretreated with (a) saline or with (b) iprindole.

The half lives found were: 3-chloroamphetamine (a) 0·9 h, (b) 4·4 h; 4-chloroamphetamine (a) 5·0 h, (b) 4·4 h.

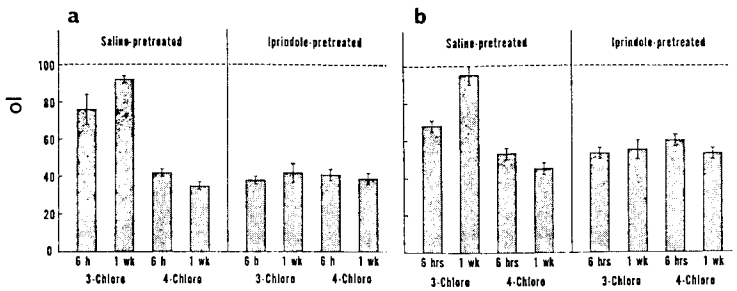


FIG. 2. (a) 5-Hydroxytryptamine concentrations in whole brain after the administration of chloroamphetamines to saline-pretreated or iprindole-pretreated rats. Control concentration of 5-hydroxytryptamine indicated by the dashed line was 0·66 ± 0·01 μg g^{-1}.
(b) 5-Hydroxyindoleacetic acid concentrations in whole brain after the administration of chloroamphetamines to saline-pretreated or iprindole-pretreated rats. Control concentration of 5-hydroxyindoleacetic acid indicated by the dashed line was 0·40 ± 0·03 μg g^{-1}.

amphetamine (both at 0·1 mmol kg^{-1} i.p.). Rats in groups of 5 were killed at 6 h or at 1 week after the chloroamphetamines. Brain concentrations of 5-hydroxytryptamine and of 5-hydroxyindoleacetic acid were measured spectrofluorometrically by condensation with *o*-phthalaldehyde (Miller, Cox & others, 1970). Concentrations of chloroamphetamines were measured colorimetrically by reaction with methyl orange (Axelrod, 1954; Dubnick, Leeson & others, 1963; Fuller & others, 1973). Mean values ± standard errors for 5 rats per group were calculated, and comparisons between groups were made by Student's *t*-test.

Fig. 1 shows that the half-life of 3-chloroamphetamine in control rats was about 1 h, whereas in iprindole-treated rats, the half-life was more than 4 h. The latter value was similar to that for 4-chloroamphetamine in control or in iprindole-treated rats. Iprindole had little or no effect on the half-life of 4-chloroamphetamine, though that finding does not mean that a small but pharmacologically significant amount of ring hydroxylation of 4-chloroamphetamine may not have occurred in control rats.

Fig. 2a shows 5-hydroxytryptamine concentrations in whole brain. Whereas 3-chloroamphetamine had only a slight effect on the 5-hydroxytryptamine in control rats (and only at 6 h, not at one week), in iprindole-treated rats 3-chloroamphetamine caused as much lowering of 5-hydroxytryptamine concentrations both at 6 h and at 1 week as did 4-chloroamphetamine, the effects of which were not modified by iprindole. Fig. 2b shows 5-hydroxyindoleacetic acid concentrations, which in all cases were affected much the same as were those of 5-hydroxytryptamine. All differences from control in Fig. 2a and b were statistically significant at the $P < 0·01$ level except for the effects of 3-chloroamphetamine at 1 week in saline-pretreated rats.

These results indicate that 3-chloroamphetamine is like 4-chloroamphetamine not only in having the ability to lower 5-hydroxyindole concentrations at short times but also at a long time (1 week) after drug treatment, when differences in metabolism between the two drugs are eliminated by treatment with iprindole to block ring hydroxylation. If some active metabolite is required for the long-term effect, the metabolite can apparently be formed from 3-chloroamphetamine as well as from 4-chloroamphetamine and can be formed from both drugs after iprindole treatment. We suspect that the postulated neurotoxic metabolite may be produced in the 5-hydroxytryptamine neuron. Whereas iprindole can inhibit the ring hydroxylation of amphetamines by rat liver, there is no reason to believe that iprindole would alter any metabolic transformation of the chloroamphetamines in brain neurons. This study adds information that may be useful in elucidating the mechanism by which chloroamphetamines lead to long-lasting depletion of 5-hydroxytryptamine concentrations in rat brain.

The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana, U.S.A.

RAY W. FULLER
JOHN C. BAKER

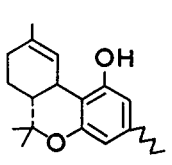
April 26, 1974

REFERENCES

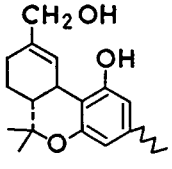
AXELROD, J. (1954). *J. Pharmac. exp. Ther.*, **110**, 315–326.
DRING, L. G., SMITH, R. & WILLIAMS, R. T. (1970). *Biochem. J.*, **116**, 425–435.
DUBNICK, B., LEESON, G. A., LEVERETT, R., MORGAN, D. F. & PHILLIPS, G. E. (1963). *J. Pharmac. exp. Ther.*, **140**, 85–92.
FREEMAN, J. J. & SULSER, F. (1972). *Ibid.*, **183**, 307–315.
FULLER, R. W., SNODDY, H. D. & MOLLOY, B. B. (1973). *Ibid.*, **184**, 278–284.
FULLER, R. W., SCHAFFER, R. J., ROUSH, B. W. & MOLLOY, B. B. (1972). *Biochem. Pharmac.*, **21**, 1413–1417.
MILLER, F. P., COX, R. H., JR., SNODGRASS, W. R. & MAICKEL, R. P. (1970). *Ibid.*, **19**, 435–442.
SANDERS-BUSH, E., BUSHING, J. A. & SULSER, F. (1972). *Eur. J. Pharmac.*, **20**, 385–388.

Binding of (+)- and (−)-Δ^1-tetrahydrocannabinols and (−)-7-hydroxy-Δ^1-tetrahydrocannabinol to blood cells and plasma proteins in man

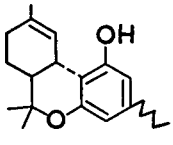
Previously we have studied the binding of (−)-Δ^1-tetrahydrocannbinol [(−)-Δ^1-THC*; I], the major psychoactive constituent of *Cannabis sativa* L. to human plasma proteins by using different electrophoretic techniques (Wahlqvist, Nilsson & others, 1970). We found that (−)-Δ^1-THC was associated 80–90% with lipoproteins, while Klausner, Wilcox & Dingell (1971), using ultracentrifugation, reported a binding of (−)-Δ^1-THC to both lipoproteins and albumin. Fehr & Kalant (1974) using electrophoresis also found a similar protein binding pattern of Δ^1-THC in rat serum. (−)-7-Hydroxy-Δ^1-tetrahydrocannabinol [(−)-7-hydroxy-Δ^1-THC†, II], which is a major primary metabolite of (−)-Δ^1-THC, with pharmacological activity, showed a binding of 94–99% to both lipoproteins and albumin by equilibrium dialysis, ultrafiltration, electrophoresis and ultracentrifugation (Widman, Nilsson & others, 1973).



I



II



III

We have now examined the distribution of (−)-Δ^1-THC and its metabolite in whole blood using equilibrium dialysis and centrifugation. We have also investigated the binding properties of the enantiomorphic (+)-Δ^1-THC (III) which does not occur naturally and has little pharmacological activity in rhesus monkeys and mice (Edery, Grunfeld & others, 1971; Jones, Pertwee & others, 1974).

Fresh human blood was collected in heparinized tubes and plasma was obtained by centrifugation. A suspension of washed blood cells was prepared according to McArthur, Dawkins & Smith (1971). After centrifugation, the blood cell fraction

* Designated as Δ^9-THC using the dibenzopyran numbering system.
† 11-Hydroxy-Δ^9-THC.