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DRUG DISPOSITION AS A FACTOR IN THE LOWERING OF BRAIN SEROTONIN BY CHLOROAMPHETAMINES IN THE RAT

RAY W. FULLER, ROBERT J. SCHAFFER, BETTY WARREN ROUSH and BRYAN B. MOLLOY

The Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Ind., U.S.A.

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Abstract—Brain serotonin content of rats treated 6 hr earlier was markedly reduced by 4-chloroamphetamine, slightly reduced by 3-chloroamphetamine, and not changed by 2-chloroamphetamine or amphetamine. In rats pretreated with desmethylimipramine (DMI), the reduction of serotonin caused by 4-chloroamphetamine was unchanged, but 3-chloroamphetamine was now equally as effective as 4-chloroamphetamine; amphetamine still had no effect, and 2-chloroamphetamine caused a slight elevation of serotonin. Levels of amphetamine, 2-chloroamphetamine and 3-chloroamphetamine were too low to detect in rat brain at 6 hr except in the rats pretreated with DMI to block *para*-hydroxylation. 4-Chloroamphetamine levels in brain were the same in controls and in DMI-pretreated rats. 4-Chloroamphetamine and 3-chloroamphetamine, which lowered serotonin equally in DMI-pretreated rats, were both present mainly in the particulate fraction after high speed centrifugation of brain homogenates, whereas 2-chloroamphetamine was evenly distributed between the supernatant and particulate fractions and amphetamine was present mainly in the supernatant fraction. The intrinsic ability of these compounds to lower serotonin is better demonstrated in DMI-pretreated rats in which metabolic differences between them are minimized; thus 3-chloro- and 4-chloroamphetamine are equally active. The association of those two drugs with brain particulate material is suggested to be related to their reduction of serotonin content.

4-CHLOROAMPHETAMINE, which lowers brain serotonin in rats,[1,2] differs from amphetamine (which does not) in respect to the disposition of the drugs in at least two ways. First, the half-life of 4-chloroamphetamine in rats is longer than that of amphetamine.[3] The difference seems to be due largely or entirely to the fact that rats metabolize amphetamine by *para*-hydroxylation;[4] the presence of the 4-chloro substituent blocks that conversion. Second, 4-chloroamphetamine is found mainly in the particulate fraction, whereas amphetamine is found mainly in the supernatant fraction, after high speed centrifugation of rat brain homogenates.[3] We have considered that both the long half-life and the association with the particulate fraction may be properties of 4-chloroamphetamine related to and required for its lowering of serotonin.

Previously we had shown that 2-chloro and 3-chloro derivatives of amphetamine did not lower brain serotonin in rats.[2] These compounds and others with the *para*-position available for enzymic hydroxylation had a half-life in rat brain similar to that of amphetamine.[3] The failure of 2-chloro- and 3-chloroamphetamine to lower brain serotonin might then have been due to their more rapid rate of disappearance as compared to 4-chloroamphetamine. The present study was designed to see what

effect the blockade of *para*-hydroxylation, expected to equalize the rate of metabolism of amphetamine, 2-chloro-, 3-chloro-, and 4-chloroamphetamine, would have on the action of these compounds on brain serotonin in rats. Desmethylimipramine (DMI) was chosen to inhibit *para*-hydroxylation,[5,6]

MATERIALS AND METHODS

Animals. Male Wistar rats from Harlan Industries were housed singly in wire mesh cages with food and water available *ad lib.*

Drugs. Desmethylimipramine HCl was supplied by Geigy Pharmaceuticals. *dl*-Amphetamine sulfate was purchased from Chemicals Procurement Laboratories, Inc., and *dl*-4-chloroamphetamine HCl from the Regis Chemical Company. *dl*-2-Chloroamphetamine HCl and *dl*-3-chloroamphetamine HCl were synthesized in The Lilly Research Laboratories; their identity and purity were verified by physicochemical methods.

Treatments. All drugs were injected i.p., the amphetamines at a dose of 0·1 m-mole/kg 6 hr before the rats were killed, and DMI at 10 mg/kg 7 hr before the rats were killed. The volume of injection in all cases was 1 ml/kg, and saline was injected in place of drug solutions in the control animals. The rats were decapitated, and the brains were rapidly removed, frozen on dry ice and stored frozen prior to analysis. Separate groups of animals were treated for serotonin and for drug level measurements.

Serotonin assay. The *o*-phthalaldehyde condensation method of Maickel and Miller[7] was used to measure serotonin content of whole brain.

Drug level assay. For analysis of the levels of the amphetamines, whole brains were homogenized in 9 vol. of 2·5 mM sucrose. After centrifugation for 30 min at 100,000 *g* in a Beckman/Spinco L2–65 ultracentrifuge, drug levels were measured in both the supernatant and the particulate fractions by the methyl orange method of Axelrod[8] as modified by Dubnick *et al.*[9] and adapted for use in our laboratory. Values were corrected for the slight blanks in saline-treated or DMI-treated groups (DMI treatment did not alter the blank value).

RESULTS

Serotonin levels. Figure 1 shows brain serotonin content of rats treated 6 hr earlier with amphetamine or the chloroamphetamines, either alone or after DMI pretreatment. Neither amphetamine nor 2-chloroamphetamine significantly affected brain serotonin when given alone. 3-Chloroamphetamine caused a slight but statistically significant reduction in serotonin at 6 hr, whereas we had detected no effect in our earlier study at 16 hr.[2] 4-Chloroamphetamine, as previously observed by numerous investigators, caused a marked reduction in serotonin.

In DMI-pretreated rats, the lack of effect by amphetamine was again observed. 2-Chloroamphetamine still did not lower serotonin and indeed, produced a slight but statistically significant elevation of serotonin content, perhaps due to monoamine oxidase inhibition.[10,11] 3-Chloroamphetamine, in marked contrast to its slight effect in control rats, caused a profound reduction in serotonin in DMI-pretreated rats to as great an extent as did 4-chloroamphetamine. The decrease of serotonin caused by 4-chloroamphetamine was not altered by DMI pretreatment.

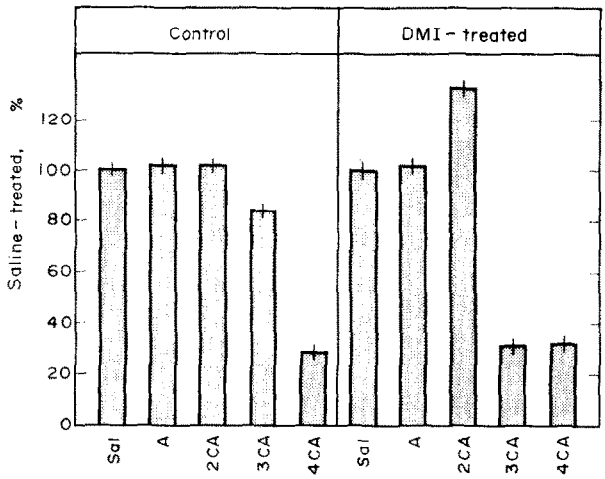

FIG. 1. Brain serotonin content in control and DMI-pretreated rats. Mean values ± S.E.M., expressed as per cent of the mean for the appropriate saline-treated group, are shown for rats treated with saline, amphetamine (A), 2-chloroamphetamine (2CA), 3-chloroamphetamine (3CA), or 4-chloroamphetamine (4CA) 6 hr before sacrifice and 1 hr after the injection of either saline (controls) or DMI. Serotonin levels in the control group averaged 0·59 μg/g for 10 saline-injected rats, and in the DMI-treated group averaged 0·58 μg/g for 10 saline-injected rats. All other experimental groups contained five rats. In the control group, the serotonin values for saline-treated rats were significantly different from those of rats treated with 3CA ($P < 0·005$) or 4CA ($P < 0·001$). In the DMI-treated group, the serotonin values for saline-treated rats were significantly lower than those for rats treated with 2CA ($P < 0·001$) and significantly higher than those for rats treated with 3CA ($P < 0·001$) or 4CA ($P < 0·001$).

Drug levels. In Fig. 2 are shown the concentrations of the drugs in brain at the time serotonin levels were measured. Without DMI pretreatment, amphetamine, 2-chloroamphetamine, and 3-chloroamphetamine had apparently been metabolized so that detectable levels were not present in brain by 6 hr. 4-Chloroamphetamine, on the other hand, was present and was mainly in the particulate fraction as we had found earlier at 1 hr.[3]

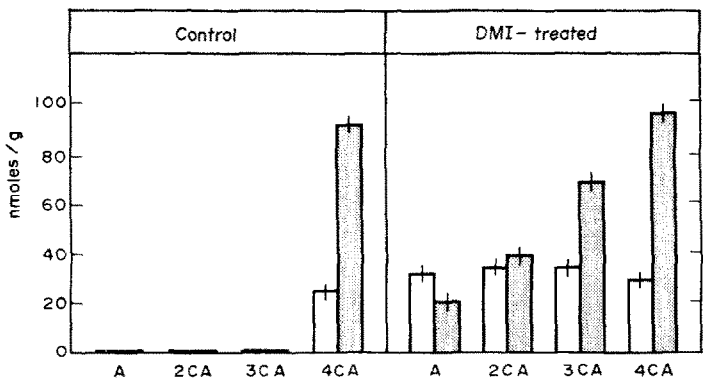

FIG. 2. Drug levels in the supernatant fraction (open bars) and particulate fraction (shaded bars) of rat brain homogenates. All treatments were as in Fig. 1. In those cases where no bars appear, drug levels were below the limits of detection. Mean(s) ± S.E.M. for five rats per group are shown.

In DMI-pretreated rats, all of the amphetamines were present in brain at 6 hr in substantial amounts. Amphetamine, in accord with past observations at 1 hr,[3] was found mainly in the supernatant fraction. 2-Chloroamphetamine was equally distributed between the supernatant and particulate fractions. 3-Chloroamphetamine was like 4-chloroamphetamine in that the particulate fraction contained substantially more of the drug than did the soluble fraction. 4-Chloroamphetamine levels and distribution were not altered by the DMI pretreatment.

DISCUSSION

These results indicate that 3-chloroamphetamine was equally as effective as 4-chloroamphetamine in lowering brain serotonin when the faster rate of disappearance of the 3-chloro compound from rat brain was prevented. Whatever the mechanism by which 4-chloroamphetamine lowers brain serotonin, whether it be by impairment of serotonin binding[10,12] or by inhibition of tryptophan hydroxylation,[13,14] 3-chloroamphetamine appears to be capable of acting in a similar manner.

The data reported here strengthen the idea that the serotonin lowering by 4-chloroamphetamine is linked to the association of that drug with particulate material. Although the presence of the drugs in the particulate fraction does not indicate any specific association with serotoninergic synaptosomes, 3-chloroamphetamine and 4-chloroamphetamine may be present in such particles to a greater extent than either 2-chloroamphetamine or amphetamine, since higher proportions of both of the latter compounds were in the supernatant fraction. Thus, greater concentration of 4-chloro- and 3-chloroamphetamine in serotoninergic neurons might be responsible for their activity in lowering serotonin and the inactivity of amphetamine and 2-chloroamphetamine, irrespective of the mechanism of the serotonin lowering. Alternatively, 4-chloro- and 3-chloroamphetamine may exert an action that amphetamine and 2-chloroamphetamine do not exert. Carlsson[15] has shown that 4-chloromethamphetamine was a potent inhibitor of serotonin uptake into brain slices. It would be instructive to know the relative effects of the 2-chloro and 3-chloro derivatives in such a system *in vitro*.

Meek *et al.*[16] have reported that chloroimipramine and related tricyclic drugs antagonize the lowering of serotonin by 4-chloromethamphetamine. Perhaps, as they suggested, the tricyclic drugs prevented the entry of 4-chloromethamphetamine into serotoninergic neurons. DMI was among the drugs they studied. The failure of DMI in our experiments to alter the lowering of serotonin by 4-chloroamphetamine or the gross subcellular distribution of the drug is not contradictory to their results, inasmuch as they obtained only minimal antagonism by DMI at 2·5 times the dose we used.

Sanders-Bush and Sulser[13,14] have presented evidence that 4-chloroamphetamine inhibits the biosynthesis of serotonin by interfering with tryptophan hydroxylation. If indeed that enzymic step is the primary site of action, the precise mechanism by which enzyme activity is inhibited needs to be elucidated. Perhaps the knowledge that 3-chloroamphetamine can act like 4-chloroamphetamine when differences in their biological disposition are removed will be useful in attempts to establish that mechanism.

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