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LOWERING OF BRAIN SEROTONIN LEVEL BY CHLORAMPHETAMINES*

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Abstract—4-Chloroamphetamine and several structurally similar compounds lowered serotonin level in the whole brain of rats without lowering the level of norepinephrine. Relationships between chemical structure of chloroamphetamines and their effects on brain serotonin level are presented. There was no difference between the effects of dextrorotatory and levorotatory isomers of 4-chloromethamphetamine. 4-Chloroamphetamine lowered serotonin but not norepinephrine level in the septum–diencephalon–midbrain area of dog brain. 4-Chloroamphetamine did not inhibit tryptophan hydroxylation in rat liver or bacterial preparations nor did it inhibit the uptake of ^{14}C-5-hydroxytryptophan into rat brain. The lowering of brain serotonin level does not appear to be a result of decreased serotonin synthesis but may be due to an unusual type of release of serotonin.

A ROLE for serotonin and catecholamines in the activity of the central nervous system has been suggested by a large body of evidence. Consequently, drugs that alter the levels of these amines in the brain are of interest. Reserpine,[1] α-methyldopa,[2] and α-methyl-*m*-tyrosine[2] are examples of agents that lower tissue levels of both catecholamines and serotonin. Pletscher *et al.*[3] found that serotonin but not norepinephrine level in the brain of rats was decreased by 4-chloromethamphetamine, apparently the first compound reported to have this specific effect on serotonin. This paper describes further studies of the effects of chlorinated amphetamines on serotonin metabolism.

EXPERIMENTAL

Male white Harlan rats weighing 130–160 g were used. Compounds (*dl* mixtures unless otherwise noted) were dissolved in distilled water and injected i.p. The animals were decapitated, and the whole brains were removed and frozen between slabs of dry ice. The frozen brains were stored at — 15° prior to analysis.

Dogs were sacrificed and brain samples that included the septum, diencephalon, and midbrain were prepared as described by Stark *et al.*[4]

Serotonin and norepinephrine were determined by the Mead and Finger[5] modification of the method of Shore and Olin.[6] A Farrand spectrofluorometer was used.

Tryptophan hydroxylation by whole cells of *Chromobacterium violaceum* was measured by the method of Mitoma and Weissbach.[7] Tryptophan hydroxylation by rat liver was measured by the method of Burkard *et al.*[8]

* A partial account of this work appeared in *Fed. Proc.* **23**, 146 (1964).

Total radioactivity in the brain of rats was measured 20 min after the i.p. injection of 5 μmoles DL-5-hydroxytryptophan-3-^{14}C/kg (New England Nuclear Corp.; specific activity 2·5 mc/mmole). The animals were sacrificed by decapitation and the whole brains were removed and homogenized in two volumes of 10% (w/v) trichloroacetic acid. After centrifugation, 1-ml aliquots of the supernatant fluid were shaken 5 min with 1 g sodium chloride and 3 ml *n*-butanol. One-ml aliquots of the butanol layer were added to 3 ml absolute ethanol in counting vials. After addition of 10 ml phosphor solution,[9] the samples were counted in a Packard Tri-Carb scintillation spectrometer.

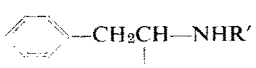
RESULTS

Structure–activity relationships

The lowering of serotonin level in the whole brain of rats by compounds that may be regarded as substituted phenethylamines was studied. Brain serotonin level was measured 16 hr after administration of the test compounds, since this was shown to be the time of lowest brain serotonin level after 4-chloromethamphetamine administration.[3] We have found a similar time course for 4-chloroamphetamine. The serotonin level in the brain of saline-treated rats was 0·52 ± 0·05 μg/g tissue (wet weight).

The effects of substitutions on the side chain of 4-chlorophenethylamine are shown in Table 1. The methyl group alpha to the amino function was necessary for lowering

TABLE 1. EFFECT OF SIDE-CHAIN SUBSTITUTION ON LOWERING OF RAT BRAIN SEROTONIN LEVEL BY CHLOROAMPHETAMINES



R	R′	Serotonin level (% of control)
H	H	114
H	$CH(CH_3)_2$	89
CH_3	H	17
CH_3	CH_3	36
CH_3	C_2H_5	40
CH_3	$CH(CH_3)_2$	69
CH_3	$(CH_3)_2$	44
C_2H_5	H	58

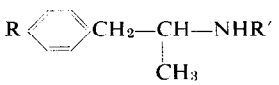
All compounds were injected at a dose of 0·1 mmole/kg 16 hr prior to sacrifice. Two groups of 3 animals each were used for serotonin determination; the average results are shown.

of serotonin level. Compounds without this branching in the chain would presumably be substrates for monoamine oxidase and would probably be metabolized rapidly. The most effective compound had a free amino group, although substitution with methyl, ethyl, isopropyl, or dimethyl reduced activity only slightly, as did replacement of the α-methyl with an ethyl group.

Table 2 shows the effects of aromatic halogenation. Unchlorinated amphetamines did not lower serotonin level. The most effective isomer had a single chloro substitution in the 4-position. Neither 3-chloroamphetamine nor 4-fluoroamphetamine

decreased the amount of serotonin in the brain. 2,4-Dichloroamphetamine did not lower serotonin level and 3,4-dichloroamphetamine had less effect than did 4-chloroamphetamine. The 2,6-dichloro compound, like the 2-chloro analog, had only marginal effect.

TABLE 2. EFFECT OF RING SUBSTITUTION ON LOWERING OF RAT BRAIN SEROTONIN LEVEL BY CHLOROAMPHETAMINES



R =	R′ =	Serotonin level (% of control)
H	CH_3; H	96; 99
2–Cl	CH_3	80
3–Cl	H	98
4–Cl	CH_3; H	36; 17
4–F	H	114
2, 4–Cl	H	101
3, 4–Cl	H	41
2, 6–Cl	H	80

All compounds were injected at a dose of 0·1 mmole/kg 16 hr prior to sacrifice. Two groups of 3 animals each were used for serotonin determination; the average results are shown.

In all experiments in which chloroamphetamines were adminstered, norepinephrine level was not appreciably affected.

The degree to which serotonin was lowered in the brain was the same for both the *d* and *l* isomers of 4-chloromethamphetamine (Table 3). Similar results were obtained with other compounds when *dl* mixtures were compared to either the *d* or the *l* isomers.

TABLE 3. LOWERING OF RAT BRAIN SEROTONIN LEVEL BY ISOMERS OF 4-CHLOROMETHAMPHETAMINE

Dose (mg/kg)	Isomer	Serotonin level (% of control)
2	*dl*	102
	d	96
	l	91
5	*dl*	74
	d	69
	l	65
22	*dl*	36
	d	36
	l	35

All compounds were injected 16 hr prior to sacrifice. Two or three groups of 3 animals each were used for each determination; the average results are shown.

Brain amines in dogs

4-Chloromethamphetamine was reported to lower brain serotonin level in rats and guinea pigs, but not in mice and rabbits.[3] Table 4 shows the effect of 4-chloroamphetamine on brain amine levels in dogs. There was a significant fall in the amount of serotonin in the septum–diencephalon–midbrain area of the brain. The lowest level was observed 8 hr after injection, indicating a somewhat different time course in this species. The norepinephrine level was not significantly altered. Thus the dog is another species in which chloroamphetamines selectively decrease brain serotonin and not norepinephrine level.

TABLE 4. EFFECT OF 4-CHLOROAMPHETAMINE ON SEROTONIN AND NOREPINEPHRINE LEVELS IN DOG BRAIN

Hours after administration	Amine level	
	Serotonin	Norepinephrine
0	0·78 ± 0·04	1·45 ± 0·20
8	0·51 ± 0·14*	1·32 ± 0·13
16	0·54 ± 0·06†	1·30 ± 0·13
24	0·63 ± 0·14	1·34 ± 0·19

* $0{\cdot}05 > P > 0{\cdot}01$.
† $0{\cdot}01 > P > 0{\cdot}005$.
4-Chloroamphetamine was injected i.p. at a dose of 0·025 mmole/kg. There were 3 dogs in each group. Amine levels in micrograms per gram (wet weight) of tissue are shown with standard deviations.

TABLE 5. UPTAKE OF ^{14}C-5-HYDROXYTRYPTOPHAN INTO RAT BRAIN

Treatment	Average (cpm/g brain)	Range
Saline 10 min before 5HTP	756	702–792
4-chloroamphetamine 10 min before 5HTP	1,041	954–1,215
4-chloroamphetamine 16 hr before 5HTP	809	837–927

Three animals in each group were injected i.p. with saline or 4-chloroamphetamine at a dose of 0·1 mmole/kg and then with ^{14}C-5HTP.

Tryptophan hydroxylation

4-Chloroamphetamine at a concentration of 10^{-3} M did not inhibit tryptophan hydroxylation by a rat liver preparation or by whole cells of *C. violaceum.*

Uptake of ^{14}C-5HTP into rat brain

4-Chloroamphetamine did not decrease brain uptake of ^{14}C-5HTP injected i.p. into rats (Table 5). In fact, there was a higher level of radioactivity in the brain of animals receiving the drug.

DISCUSSION

Changes in behavior are often associated with alterations in the level of brain serotonin.[10] After administration of chloroamphetamines to rats at the high dose levels

used, there was marked stimulation of activity. The serotonin level in the brain was not the primary determining factor in the behavioral effects observed, since there was no temporal relationship between serotonin level and behavioral stimulation. The increase in activity was apparent within minutes after injection and persisted throughout the 16-hr period prior to sacrifice, while the brain serotonin level decreased slowly to the lowest value at 16 hr. Further, stimulation of activity was present after injection of compounds that had no effect on brain serotonin level.

Structurally the chloroamphetamines are α-methyl amines similar to those produced by decarboxylation of α-methyl amino acids like α-methyldopa.[2] The central effects of α-methyldopa may be due to the α-methyl amines formed but, since the latter do not penetrate well into the brain, the precursor amino acid is given. One effect of the chloro groups may be to increase passage through a lipid barrier by increasing lipid solubility. Pletscher *et al.*[11] found that 4-chloromethamphetamine reached levels in rat brain about three times higher than did amphetamine after administration of equimolar amounts of the two drugs and that the chlorinated compound persisted for a longer time in the brain. Since α-methyldopa also produces a decrease in cerebral 5-hydroxyindoleacetic acid and serotonin,[12, 13] the possibility is raised that the lowering of serotonin by α-methyldopa and by chloroamphetamines occurs by the same mechanism.

It is noteworthy that the ability of the chloroamphetamines to lower serotonin level is not influenced by steric arrangement of the methyl group. The effect on appetite-controlled behavior produced by some of these compounds[14] and the inhibition *in vitro* of monoamine oxidase by chloroamphetamines and amphetamine[15] are affected by steric differences. Other effects by α-methyl amines are influenced by stereoisomerism of the asymmetric carbon. The toxicity and lowering of norepinephrine levels by amphetamine[16] and the ability of α-methyl amines to act as substrates for dopamine β-hydroxylase[17] are examples.

Mechanisms by which serotonin levels in tissues could be lowered include depression of rate of synthesis or depletion of serotonin stores. A lowered rate of serotonin synthesis could occur by inhibition of anabolic enzymes or by interference with uptake into the brain of a precursor. Serotonin is formed from tryptophan by 5-hydroxylation and subsequent decarboxylation. Pletscher *et al.*[11] have reviewed the evidence that chloroamphetamines do not inhibit either of these enzymic steps leading to serotonin. We have confirmed that 4-chloroamphetamine does not inhibit tryptophan hydroxylation by rat liver or by whole cells of *C. violaceum*. Likewise, chloroamphetamines do not inhibit the uptake into the brain of 5-hydroxytryptophan. Table 5 shows that 4-chloroamphetamine produced some increase in the amount of radioactivity in the brain after 5-hydroxytryptophan administration. (This may explain the finding of Pletscher *et al.* that 4-chloromethamphetamine slightly enhances the increase of cerebral serotonin produced by 5-hydroxytryptophan administration.) Thus, all available evidence would indicate that there is no inhibition of serotonin biosynthesis by chloroamphetamines.

Another possible mechanism by which chloroamphetamines could lower brain serotonin level is by release of the bound amine. When amines are depleted from their stores, they are usually metabolized, and increased levels of their metabolic products are found. After administration of reserpine, for example, brain level of 5-hydroxyindoleacetic acid increases as serotonin level decreases,[18, 19] indicating release of

bound serotonin and metabolism by monoamine oxidase. After administration of chloroamphetamines, however, there is a decrease in 5-hydroxyindoleacetic acid in the brain. It follows that a release of serotonin of the type produced by reserpine does not occur after chloroamphetamine administration. Bartholini and Pletscher[20] reported that rabbit platelet serotonin was released by 4-chloromethamphetamine but was not metabolized, although it was metabolized after reserpine release. It is interesting that the platelets were from a species[3] in which brain serotonin levels are not affected by chloroamphetamines. Nevertheless, a similar type of release of unmetabolized serotonin may occur in the brain of other species. The actual mechanism by which chloroamphetamines lower brain serotonin demands further study.

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