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THE ACTION OF 2-AMINO-TETRALIN (β-TETRAHYDRONAPHTHYLAMINE) ON THE METABOLISM OF 5-HYDROXYTRYPTAMINE IN THE BRAIN OF THE MOUSE

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It has been shown that the administration of 2-amino-tetralin (β-tetrahydronaphthylamine) to the mouse results in a decrease in the concentration of 5-hydroxyindol-3-ylacetic acid, an acid metabolite of 5-hydroxytryptamine, in the brain (Sharman, 1966). This report attempts to explain the mechanism by which this change is brought about.

METHODS

All chemicals and reagents were of analytical reagent quality.

The following drugs were used: L-α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa), Merck, Sharp and Dohme. 2-Amino-tetralin hydrochloride (2-amino-1,2,3,4-tetrahydronaphthalene hydrochloride; β-tetrahydronaphthylamine hydrochloride), Dr. Theodor Schuchardt G.m.b.H. Pheniprazine (JB 516; β-phenylisopropylhydrazine hydrochloride), Lakeside Laboratories Inc. L-tryptophan, Hopkins & Williams Limited.

Drugs were injected intraperitoneally dissolved in 0.9% sodium chloride solution except for L-tryptophan which was triturated in 0.9% sodium chloride in an agate mortar to yield a very fine suspension of the amino acid (Ashcroft, Eccleston & Crawford, 1965).

Male albino mice, 20–35 g wt. were used throughout. The tissue used for analysis was obtained as follows: the animals were stunned, decapitated and the brains taken out and placed on a glass plate in an ice bath. Olfactory bulbs, cerebellum, medulla and pons were removed, and the remaining brain tissue from three mice was combined for each estimation. The tissue was homogenised in 2 vol. of 0.1 N-hydrochloric acid together with 5–10 mg ascorbic acid in a Griffith pattern glass tissue grinder and the homogenate diluted with 1 vol. of water. One millilitre of 16% zinc sulphate ($ZnSO_4.7H_2O$) and 0.1 ml. 20% sodium hydroxide were added and the homogenate was centrifuged for 5 min at 3,500 rev/min. The supernatant fluid was filtered to remove floating fatty material and the precipitate washed with water. After centrifuging, the washings were also filtered and combined with the first supernatant fluid. The total volume was 6 ml. Of this, 4 ml. was used to estimate 5-hydroxyindol-3-ylacetic acid and 2 ml. was used to estimate 5-hydroxytryptamine. 5-Hydroxyindol-3-ylacetic acid was estimated fluorimetrically as acidic 5-OR indolyl compounds (Ashcroft & Sharman, 1962). 5-Hydroxytryptamine was estimated, in principle, as described by Bogdanski, Pletscher, Brodie & Udenfriend (1956).

RESULTS

The mean and standard error of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations were estimated from all of the control observations to be 0.62 ± 0.03 μg/g (n=60) and 0.37 ± 0.03 μg/g (n=66) respectively. These figures are corrected for mean recovery values for added 5-hydroxytryptamine of $65.7\% \pm 1.4\%$ (n=40) and for added 5-hydroxyindol-3-ylacetic acid of $44.8\% \pm 1.1\%$ (n=51). Small differences in the mean estimates of the normal concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine were observed between the groups of control animals used during this work. The differences between the highest and lowest mean control values for both substances were significant at the 2% level. To reduce the effect of these differences the further results are expressed as percentages of the control values obtained simultaneously with the observations on the drug-treated animals.

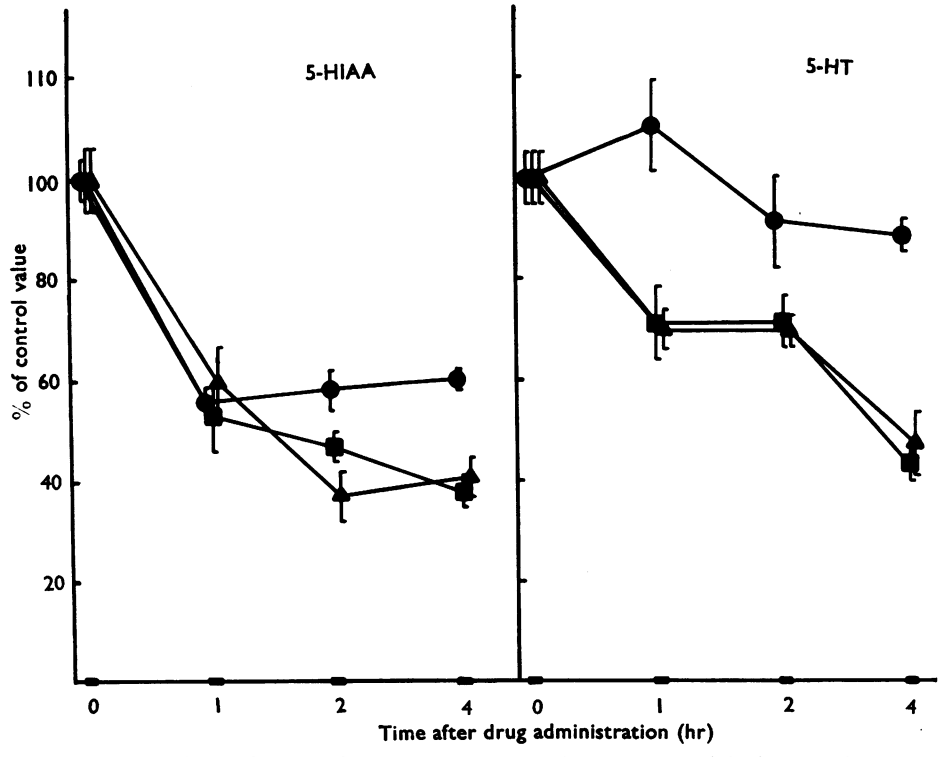

Fig. 1. The effect of 2-amino-tetralin and α-methyl-3,4-dihydroxyphenylalanine on the concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in the brain of the mouse. ●——● 2-amino-tetralin (30 mg/kg); ■——■ α-methyldopa (400 mg/kg); ▲——▲ 2-amino-tetralin (30 mg/kg) and α-methyldopa (400 mg/kg).

		5-HIAA				5-HT			
Time after drug administration (hr)		0	1	2	4	0	1	2	4
No. of observations	●	21	16	14	8	17	16	8	8
	■	19	8	8	8	19	8	8	8
	▲	12	8	8	8	10	8	8	4

Results are given as means ± s.e.m. expressed as a percentage of the corresponding mean control value.

The effects of 2-amino-tetralin and α-methyldopa

Figure 1 shows the effects of 2-amino-tetralin (30 mg/kg), α-methyldopa (400 mg/kg) and a combination of these two drugs on the concentration of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in the brain of the mouse. Both drugs when given alone reduce the concentration of the acid, but only α-methyldopa lowers the concentration of the amine. The changes seen after a combination of the two drugs are similar to those seen after α-methyldopa alone.

The effect of 2-amino-tetralin on the response to tryptophan

The effect of 2-amino-tetralin on the concentrations of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid after a loading dose of tryptophan was then examined.

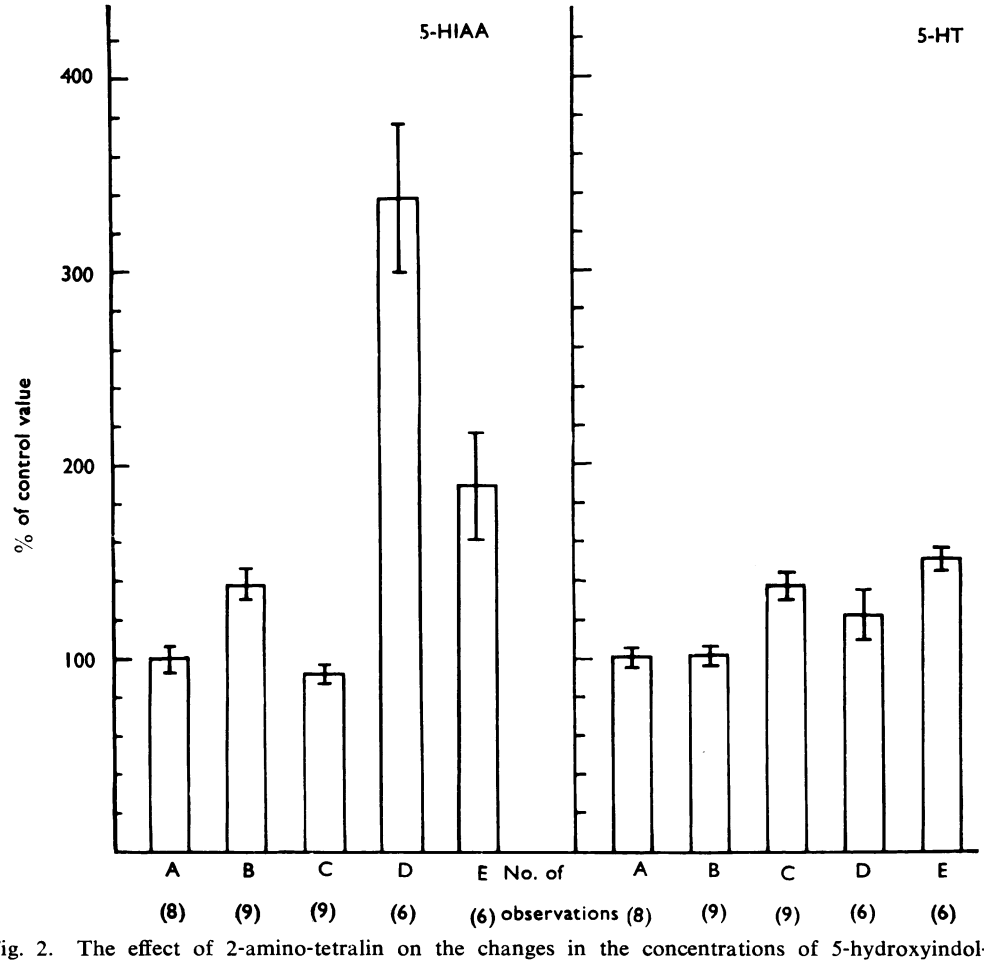

Fig. 2. The effect of 2-amino-tetralin on the changes in the concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in the brain of the mouse produced by a tryptophan load. A=control; B=tryptophan (100 mg/kg); C= tryptophan (100 mg/kg) and 2-amino-tetralin (30 mg/kg); D=tryptophan (400 mg/kg); E=tryptophan (400 mg/kg) and 2-amino-tetralin 30 mg/kg). Results are given as means ± s.e.m. expressed as a percentage of the mean control value. The observations were made 2 hr after the administration of the drugs.

The results are shown in Fig. 2. Tryptophan given in doses of 100 mg/kg and 400 mg/kg caused a significant increase in the concentration of 5-hydroxyindol-3-ylacetic acid ($P<0.01$) but only the larger dose of the amino acid produced a small but significant ($P<0.01$) increase in 5-hydroxytryptamine. The administration of 2-amino-tetralin in combination with tryptophan reduced the increase in 5-hydroxyindol-3-ylacetic acid. In addition there was now a significant increase ($P<0.01$) in 5-hydroxytryptamine above control values with both doses of tryptophan.

The effect of pheniprazine and α-methyldopa

The effect of the monoamine oxidase inhibitor pheniprazine (2 mg/kg and 5 mg/kg) and of α-methyldopa (400 mg/kg), alone and combined, on the concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in the brain of the mouse are

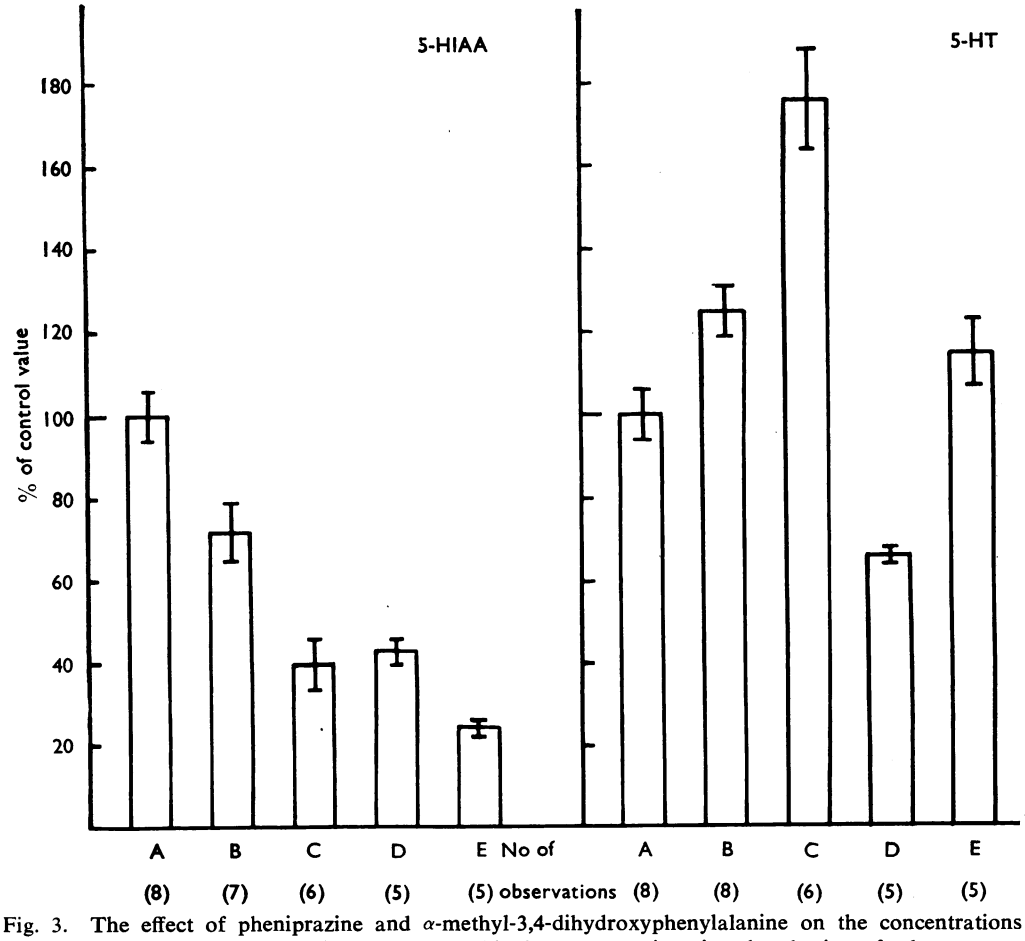

Fig. 3. The effect of pheniprazine and α-methyl-3,4-dihydroxyphenylalanine on the concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in the brain of the mouse. A=control; B=pheniprazine (2 mg/kg); C=pheniprazine (5 mg/kg); D=α-methyldopa (400 mg/kg); E=α-methyldopa (400 mg/kg) and pheniprazine (5 mg/kg). Results are given as means±s.e.m. expressed as a percentage of the mean control value. The observations were made 2 hr after the administration of the drugs.

shown in Fig. 3. Like 2-amino-tetralin (Fig. 1) pheniprazine lowered the tissue content of 5-hydroxyindol-3-ylacetic acid, but produced a significant accumulation of 5-hydroxytryptamine, which did not occur after 2-amino-tetralin. When doses of α-methyldopa and of pheniprazine, which alone produced equal falls in the concentration of 5-hydroxyindol-3-ylacetic acid, were combined the effects on the acid were additive (Fig. 3). The same combination of drugs also had additive effects on the tissue content of 5-hydroxytryptamine, the rise produced by pheniprazine and the fall caused by α-methyldopa cancelling each other out. This was not observed when 2-amino-tetralin and α-methyldopa were combined.

DISCUSSION

5-Hydroxyindol-3-ylacetic acid is formed from 5-hydroxytryptamine by the action of the enzymes monoamine oxidase and aldehyde dehydrogenase. The simplest explanation for a decrease in this acid metabolite after 2-amino-tetralin would be inhibition of these enzymes. However, the fall in the concentration of 5-hydroxyindol-3-ylacetic acid in the brain should then be accompanied by an increase in 5-hydroxytryptamine. The administration of the known monoamine oxidase inhibitor pheniprazine caused a significant increase in the concentration of 5-hydroxytryptamine even with a dose that produced a fall in the concentration of 5-hydroxyindol-3-ylacetic acid smaller than that seen after 2-amino-tetralin. However, no increase in the amine was seen after 2-amino-tetralin. That the effect of 2-amino-tetralin is due to inhibition of the enzyme aldehyde dehydrogenase is unlikely since it has been shown that the formation of homovanillic acid, an acid metabolite of dopamine is, in fact, accelerated in the mouse brain by the administration of 2-amino-tetralin (Sharman, 1966). The evidence thus suggests that the enzymic processes by which 5-hydroxytryptamine is oxidized to 5-hydroxyindol-3-ylacetic acid are unaffected by 2-amino-tetralin.

Ashcroft *et al.* (1965) and Eccleston, Ashcroft & Crawford (1965) have shown how tryptophan loading of animals can be used to study the intermediate metabolism of 5-hydroxyindole compounds in tissues. The present results with tryptophan loading suggest that the ability to synthesize 5-hydroxytryptamine in the brain is not arrested by 2-amino-tetralin since there is an increased level of 5-hydroxytryptamine when both tryptophan and 2-amino-tetralin are given together. The reduced formation of 5-hydroxyindol-3-ylacetic acid, however, suggests that some step in the conversion of 5-hydroxytryptamine to 5-hydroxyindol-3-ylacetic acid is impaired by the administration of 2-amino-tetralin.

A possible point in this conversion which might be affected is the rate of release and transport of the amine to a site where it can be metabolized to the acid. The rate at which an amine is being used in the brain can be studied by blocking its synthesis and measuring the rate of its disappearance from the tissue. α-Methyldopa blocks the synthesis of 5-hydroxytryptamine in the brain (Smith, 1960). The present experiments show that the rate of fall of 5-hydroxytryptamine after α-methyldopa is unaffected by the simultaneous administration of 2-amino-tetralin, and thus it would appear that the rate of release of 5-hydroxytryptamine from its storage sites is proceeding at a normal rate.

Yet this need not be so when 2-amino-tetralin is given alone. Assuming this compound inhibited the release of 5-hydroxytryptamine, one would not only have expected that the decrease in tissue 5-hydroxytryptamine following the administration of α-methyldopa would be checked by 2-amino-tetralin, but also that there would have been a larger fall in tissue 5-hydroxyindol-3-ylacetic acid when the two drugs were given together.

No such additive effects would be expected, however, if the release of 5-hydroxytryptamine by 2-amino-tetralin were inhibited by a reflex response which counteracts or balances a direct effect of the drug on some other neural mechanism. Such a reflex response may not be elicited in the presence of α-methyldopa because the release of 5-hydroxytryptamine is already sufficiently lowered by inhibition of synthesis. Thus the effect of a combination of the two drugs on the concentration of 5-hydroxytryptamine would not be additive.

The effects of 2-amino-tetralin on the metabolism of monoamines in the brain are not the same in different species of animals. Juorio & Vogt (unpublished) have shown that in the rat this drug produces changes similar to those seen in the mouse, whereas in the pigeon the levels of both dopamine and 5-hydroxytryptamine and their acid metabolites were depressed.

SUMMARY

1. The administration of 2-amino-tetralin (β-tetrahydronaphthylamine) to the mouse causes a fall in the concentration of 5-hydroxyindol-3-ylacetic acid in the brain without changing the concentration of 5-hydroxytryptamine.

2. A comparison of the effect of 2-amino-tetralin with the fall in 5-hydroxyindol-3-ylacetic acid and the increase in 5-hydroxytryptamine produced by pheniprazine, a known inhibitor of monoamine oxidase, shows that the change in 5-hydroxyindol-3-ylacetic acid caused by 2-amino-tetralin is not a result of inhibition of this enzyme.

3. The effect of 2-amino-tetralin on the cerebral concentrations of 5-hydroxyindol-3-ylacetic acid and 5-hydroxytryptamine in mice given a loading dose of tryptophan suggested that it is a slowing rate of utilization of 5-hydroxytryptamine that plays the major role in causing the reduction of 5-hydroxyindol-3-ylacetic acid. However, when the synthesis of 5-hydroxytryptamine was inhibited by α-methyl-3,-4-dihydroxyphenylalanine the administration of 2-amino-tetralin did not appear to reduce the rate at which 5-hydroxytryptamine disappeared from the brain.

4. It is suggested that the effect of 2-amino-tetralin on the cerebral metabolism of 5-hydroxytryptamine in the mouse might be an indirect effect of the drug. Because the inhibition of synthesis of 5-hydroxytryptamine by α-methyl-3,4-dihydroxyphenylalanine brings about a reduction in the rate of utilization of the amine, the effects of a combination of these two drugs need not be additive.

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