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Evidence for inhibition of the reuptake of 5-hydroxytryptamine and noradrenaline by tetrahydronaphthylamine in rat brain

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Summary

1. The concentrations of 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA) and noradrenaline (NA) in homogenized rat brains were determined after intraperitoneal injection of 1,2,3,4-tetrahydro-2-naphthylamine (THN).

2. THN caused a decrease in the concentration of brain 5-HIAA without altering its 5-HT content, but the percentage of ' free 5-HT ' in the supernatant increased. The decrease in 5-HIAA and the increase in free 5-HT were negatively correlated, suggesting inhibition of the reuptake of 5-HT.

3. THN decreased brain NA content without changing free NA. The fact that no increase in free NA occurred is ascribed to the action of catechol-*O*-methyl-transferase.

4. Inhibition of the reuptake of NA and of 5-HT was further studied by using the compounds 5-methyl-α-ethyl-meta-tyramine (H 75/12) and 4,α-dimethyl-meta-tyramine (H 77/77). The results of these studies also suggested inhibition by THN of the reuptake of 5-HT as well as of NA.

5. The action of THN is explained by inhibition of the reuptake of NA and of 5-HT and by release of NA from its stores. However, the possibility is not excluded that, instead of releasing NA from its stores, THN inhibits the enzyme dopamine-β-hydroxylase.

Introduction

The effect of 1,2,3,4-tetrahydro-2-naphthylamine (THN) on body temperature in rats depends on the availability of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) (Kemper & Bruinvels, 1969). However, the mechanism by which THN acts is not clear. According to Vogt (1954) and Laverty & Sharman (1965) administration of THN to cats decreased the concentration of NA in the hypothalamus. This might indicate that THN released NA from its stores. The effect of THN on 5-HT metabolism was studied by Sharman (1966) and Robinson & Sharman (1967). They showed that administration of THN decreased the concentration of 5-hydroxyindolacetic acid (5-HIAA) in the brain but not the 5-HT content. The decrease in 5-HIAA did not result from inhibition of MAO, nor from inhibition of 5-HT bio-

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synthesis ; it was ascribed to an unknown indirect effect of the drug. These experiments were undertaken to obtain more information about the action of THN on 5-HT and NA in rat brain.

Methods

Male albino rats, weighing 100–110 g, were used. 1,2,3,4-tetrahydro-2-naphthylamine (THN ; Suchardt) dissolved in saline was administered intraperitoneally in a volume of 1 ml. Rats were decapitated at different times after the injection of THN. Brain without cerebellum was used for analysis. The tissue was homogenized in 2 volumes of 0·32 M sucrose (Teflon pestle and glass homogenizing tube). The homogenate was transferred to a 15 ml centrifuge tube. The homogenizer was rinsed with another 2 volumes of 0·32 M sucrose and this washing was added to the homogenate. The homogenate was centrifuged for 20 min at 100,000 *g* or 30 min at 75,000 *g*. The supernatant (S) was transferred to a 30 ml glass stoppered centrifuge tube and after adding two drops of 5 N HCl, stored at 4° C. The pellet was resuspended in 4 volumes of 0·1 N HCl. After allowing the suspension to stand for 30 min at 4° C, it was centrifuged for 20 or 30 min at 100,000 or 75,000 *g* respectively. The supernatant (P) was transferred to a 30 ml glass stoppered centrifuge tube. The amines in S and P were regarded as the 'free' and 'bound' fractions, respectively.

Determination of 5-hydroxyindolacetic acid, 5-hydroxytryptamine and noradrenaline

The acidified supernatants were extracted with ether as described by Ashcroft & Sharman (1962), and 5-HIAA was estimated fluorimetrically by the method of Udenfriend, Weissbach & Brodie (1958). After removal of the ether, the aqueous layer was used for the determination of 5-HT (Bogdanski, Pletscher, Brodie & Udenfriend, 1956). Noradrenaline was determined as described by Sourkes & Murphy (1961). In all determinations internal standards were used. These were added to the supernatants S and P before the extraction was carried out.

Measurement of inhibition of the reuptake of 5-HT and NA

Inhibition of the reuptake of 5-HT and NA was measured with the compounds H 75/12 (4-methyl-α-ethyl-meta-tyramine) and H 77/77 (4,α-dimethyl-meta-tyramine) as described by Carlsson, Corrodi, Fuxe & Hökfelt (1969a, b). The method is based on the prevention of the depleting action of H 75/12 and H 77/77 on intraneuronal 5-HT and NA, respectively. H 75/12 and H 77/77 were obtained from Kistner Labtjänst AB, Sweden. The formula used for the calculation of the percentage of inhibition of the uptake of H 75/12 and of H 77/77 was a modification of that proposed by Carlsson *et al.* (1969b) in order to obtain the percentage of inhibition of the uptake also when the drug altered the monoamine content of the tissue.

The formula proposed by Carlsson *et al.* is:

$$\% \text{ inhibition} = \frac{^{(NA)}\text{drug} + \text{H } 77/77 - {}^{(NA)}\text{H } 77/77}{^{(NA)}\text{drug} - {}^{(NA)}\text{H } 77/77} \times 100$$

The formula used in this paper is:

$$\% \text{ inhibition} = \frac{\frac{^{(NA)}\text{saline} - ^{(NA)}\text{H } 77/77}{^{(NA)}\text{saline}} - \frac{^{(NA)}\text{drug} - ^{(NA)}\text{drug} + \text{H } 77/77}{^{(NA)}\text{drug}}}{\frac{^{(NA)}\text{saline} - ^{(NA)}\text{H } 77/77}{^{(NA)}\text{saline}}} \times 100$$

which can be simplified as:

$$\% \text{ inhibition} = \frac{\frac{^{NA)}\text{drug} + \text{H } 77/77}{^{(NA)}\text{drug}} - \frac{^{(NA)}\text{H } 77/77}{^{(NA)}\text{saline}}}{1 - \frac{^{NA)}\text{H } 77/77}{^{(NA)}\text{saline}}} \times 100$$

For calculation of the percentage of inhibition of the uptake of H 75/12, H 77/77 must be replaced by H 75/12 and (NA) by (5-HT). The drug, in this case THN, was injected intraperitoneally 0·5 h before H 77/77 or H 75/12. Rats were decapitated 1 h later.

Results

Effect of THN on 5-HT metabolism

One hour after intraperitoneal injection of THN in rats there was a decrease in the concentration of brain 5-HIAA, without a change in brain 5-HT. As shown in Table 1, the decrease in 5-HIAA was significant for all three doses used (9·9, 19·7 and 39·4 mg/kg) and although total 5-HT did not change, there was a shift in the distribution of 5-HT between its bound and free forms in favour of the latter. To follow the time course of these changes, brains were analysed for 5-HIAA, total 5-HT and free 5-HT at 0·5, 1·0, 2·0 and 4·0 h after the injection of THN (40 mg/kg). The results are given in Table 2. Until 4 h after the administration of THN the concentration of brain 5-HT did not change, while the concentration of 5-HIAA

TABLE 1. *Effect on brain 5-HT and 5-HIAA 1 h after intraperitoneal injections of graded doses of THN*

Treatment	Total 5-HT μg/g	% of total 5-HT in supernatant	Total 5-HIAA μg/g	
Saline	0·40±0·02	28±1	0·85±0·03	(16)
THN 9·9 mg/kg	0·45±0·01	29±3	0·63±0·03†	(3)
THN 19·7 mg/kg	0·40±0·03	35±2†	0·56±0·04‡	(10)
THN 39·4 mg/kg	0·36±0·03	34±2*	0·56±0·04‡	(9)

*$P<0·02$; †$P<0·01$; ‡$P<0·001$. Numbers in brackets indicate number of experiments.

TABLE 2. *Distribution of brain 5-HT and 5-HIAA at different times after an intraperitoneal injection of THN (40 mg/kg)*

Time after injection of THN (min)	Total 5-HT μg/g	% of total 5-HT in supernatant	Total 5-HIAA μg/g	
0	0·42±0·05	30±2·0	0·76±0·03	(6)
30	0·48±0·04	36±1·9	0·62±0·04*	(6)
60	0·45±0·04	42±3·2†	0·53±0·05†	(6)
120	0·42±0·04	42±3·5*	0·42±0·03‡	(6)
240	0·46±0·03	38±4·2	0·31±0·02‡	(6)

*$P<0·02$; †$P<0·01$; ‡$P<0·001$. Numbers in brackets indicate number of experiments.

declined during this period. The percentage of total 5-HT in the supernatant, which was regarded as free 5-HT, showed a gradual increase which became significant 1 and 2 h after the injection of THN. Plotting the logarithm of the 5-HIAA concentration versus time, a straight line was obtained for the first hour. Equation of the regression line was $ln\ y = -0{\cdot}38 \times -0{\cdot}48$. Such a linear relationship was also found when the logarithm of the percentage of total 5-HT in the supernatant was plotted versus time: $ln\ y = 0{\cdot}34 \times +3{\cdot}39$. The two regression coefficients were not significantly different (Fig. 1).

Effect of THN on the distribution of NA

Administration of THN (40 mg/kg i.p.) resulted in a decrease in the concentration of brain NA without modifying the distribution between free and bound NA (Table 3).

Inhibition of the reuptake of 5-HT and NA

Figure 2 shows that THN inhibited the uptake of both 5-HT and NA. With 9·9 and 19·7 mg/kg THN, uptake of H 77/77 but not of H 75/12, was inhibited ; with 40 mg/kg THN, however, the uptake of H 77/77 and H 75/12 was inhibited to the same degree.

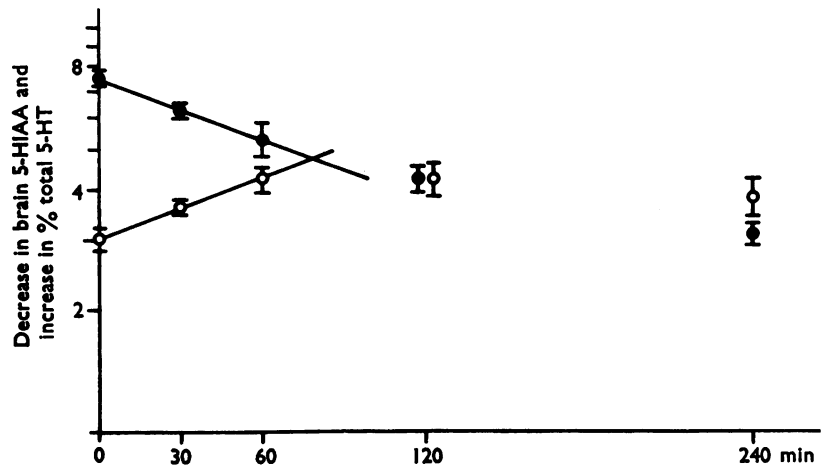

FIG. 1. Semilogarithmic plot of the decrease in the concentration of brain 5-HIAA (●) (μg-5-HIAA/g) and the increase in percentage of total 5-HT in the supernatant (○) at different times after the administration of THN (40 mg/kg intraperitoneally). Dots represent the mean of six experiments. Bars indicate S.E. ^{b}5-HIAA = −0·38 ; ^{b}%5-HT = 0·34. 95% confidence limits for ^{b}5-HIAA: −0·16 ; −0·61, and for ^{b}%5-HT: 0·12 ; 0·53.

TABLE 3. *Distribution of brain NA at different times after an intraperitoneal injection of THN (40 mg/kg)*

Time after injection of THN (min)	Total NA g/g	% of total NA in supernatant
0	0·43±0·03	40±5 (6)
30	0·33±0·02‡	45±5 (6)
60	0·34±0·04*	43±5 (6)
120	0·33±0·03†	47±4 (5)
240	0·39±0·03	48±4 (5)

*$P<0{\cdot}1$; †$P<0{\cdot}05$; ‡$P<0{\cdot}02$. Numbers in brackets indicate number of experiments.

Discussion

The finding that intraperitoneal administration of 1,2,3,4-tetrahydro-2-naphthylamine (THN) to rats resulted in a decrease in the formation of 5-HIAA without affecting the concentration of 5-HT in brain is in agreement with the results obtained in mice by Robinson & Sharman (1967). They suggested that the effect of THN on the metabolism of cerebral 5-HT might be an indirect effect of THN. However, from their experiments no conclusion could be drawn as to the mechanism of this effect. My results show that besides a decrease in the concentration of 5-HIAA there was also a shift in the distribution between bound and free 5-HT in favour of the latter. The changes of 5-HIAA and of free 5-HT in time were negatively correlated; therefore the decrease in 5-HIAA might be the result of an increase in free 5-HT.

Since intraneuronal monoamine-oxidase (MAO) is held responsible for the conversion of 5-HT to 5-HIAA the simplest explanation would be that the increase in free 5-HT is caused by inhibition of the reuptake of extraneuronal 5-HT. This conclusion would be justified only if THN did not inhibit 5-HT biosynthesis and if intraneuronal release of 5-HT from its stores were slow. However, total 5-HT did not change after THN administration so it is improbable that the formation of 5-HT is slowed down. The fact that the decrease in 5-HIAA as well as the increase in free 5-HT occurred during the first hour after the administration of THN, and then remained constant, indicates that a new equilibrium had been reached between intraneuronally bound and free 5-HT.

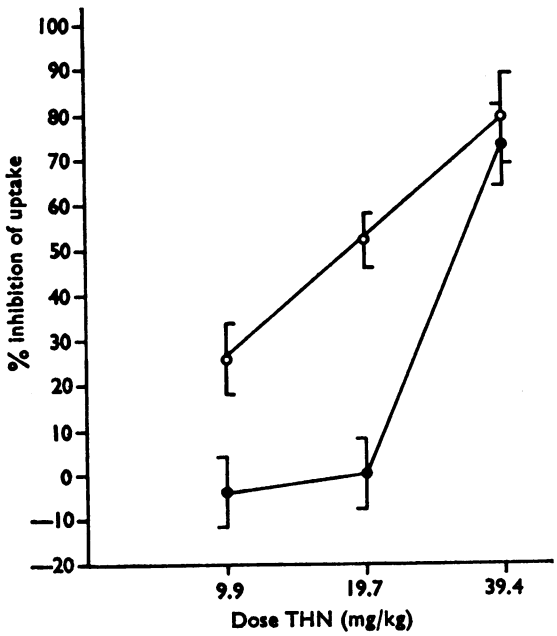

FIG. 2. Dose-response curves of the inhibition of the uptake of H 75/12 (●) and H 77/77 (○) after intraperitoneal administration of graded doses of 1,2,3,4-tetrahydro-2-naphthylamine (THN). THN was injected 0·5 h before H 75/12 or H 77/77 and the rats were decapitated 1 h thereafter. Dots represent the mean of six to eight experiments. Bars indicate S.E.

Evidence for an inhibition of the reuptake of 5-HT and NA was also obtained using the method developed by Carlsson *et al.* (1969a, b). Although this method does not show a direct effect of THN on the reuptake of 5-HT and NA, it shows in an indirect manner that the reuptake mechanism for 5-HT and NA in monoaminergic nerve fibres is inhibited. The fact that no shift was found in the distribution between free and bound NA can be ascribed to the enzyme catechol-*O*-methyltransferase, which is generally accepted to be localized extraneuronally. Thus an increase in extraneuronal NA will result in an increase in the formation of its *O*-methylated derivative without any detectable change in the concentration of free NA. The decrease in the concentration of brain NA which was found after the administration of THN is in agreement with the results obtained in cats and dogs (Vogt, 1954; Laverty & Sharman, 1965). The mechanism by which this decrease is obtained is not known. The finding that dopamine (DA) turnover is increased in the cat and the mouse after THN administration (Laverty & Sharman, 1965 ; Sharman, 1966) excludes an inhibition of the enzyme tyrosine hydroxylase, which is the rate-limiting enzyme in the biosynthesis of NA as well as that in DA (Udenfriend, Zaltzman-Nirenberg, Gordon & Spector, 1966). This leaves two other possible mechanisms, namely, inhibition of dopamine-β-hydroxylase or a release of NA from its stores. Since the depletion of brain NA was obtained as soon as 30 min after the administration of THN, it is more likely that the decrease of brain NA is caused by release of NA from its storage sites. These results therefore suggest that THN acts on rat brain by releasing NA as well as inhibiting the uptake of both 5-HT and NA.

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