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Replenishment by 5-hydroxytryptophan of the amine stores in the central 5-hydroxytryptamine neurons after depletion induced by reserpine or by an inhibitor of monoamine synthesis

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A combined biochemical and histochemical analysis of central 5-hydroxytryptamine distribution has produced further evidence to support the theory that 5-hydroxytryptophan is specifically taken up and decarboxylated in 5-hydroxytryptamine neurons of rat brain. After treatment with an inhibitor of monoamine synthesis, α-propyldopacetamide, but not after treatment with reserpine, the intraneuronal 5-hydroxytryptamine stores could be replenished by injected 5-hydroxytryptophan. This appears to indicate that the uptake-storage mechanism of amines in the 5-hydroxytryptamine neurons is intact after α-propyldopacetamide. Pretreatment with a monoamine oxidase inhibitor, nialamide, allowed repletion of the intraneuronal 5-hydroxytryptamine stores by 5-hydroxytryptophan even after treatment with reserpine. This further stresses the importance of monoamine oxidase in regulating the amine levels of the 5-hydroxytryptamine neurons.

IN previous experiments (Corrodi, Fuxe & Hökfelt, 1966; Corrodi & Fuxe, 1967) it was shown that L-3,4-dihydroxyphenylalanine (dopa) can replenish the intraneuronal catecholamine stores in the brain after depletion by the methyl ester of α-methyltyrosine (H 44/68), which is a tyrosine hydroxylase inhibitor (see Andén, Corrodi & others, 1966; Corrodi & Hanson, 1966), but not after depletion by reserpine, which blocks the uptake-storage mechanism of the amine granules (Dahlström, Fuxe & Hillarp, 1965; Carlsson, 1966). When the monoamine oxidase inhibitor nialamide was injected before dopa, a replenishment of the intraneuronal catecholamine stores occurred even after depletion by reserpine. In the dopamine neurons, levels above normal were obtained, whereas the amine stores in the noradrenaline neurons were only partly replenished. The exceptionally high amounts of dopamine formed after nialamide-dopa treatment in the brains of rats pretreated with H 44/68 or reserpine, were found to be localized partly in the cells around the capillary walls (pericytes and endothelial cells).

The present study was made to establish how the 5-hydroxytryptamine (5-HT) neurons, depleted either by reserpine or an inhibitor of monoamine synthesis, react to 5-hydroxytryptophan (5-HTP) with or without nialamide pretreatment. The catecholamine and 5-HT biosynthesis was inhibited by α-propyldopacetamide (H22/54) (Carlsson, Corrodi & Waldeck, 1963).

Experimental

MATERIAL AND METHODS

About 100 male Sprague–Dawley rats (150–250 g) were treated either with reserpine (5 mg/kg, i.p.) or α-propyldopacetamide (500 mg/kg, i.p.) to lower the 5-HT stores in the brain. The reserpinized rats were injected 3 hr later with 5-HTP (50 mg/kg, i.p.), half these rats being treated also

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with nialamide (100 mg/kg, i.p.) ½ hr before the 5-HTP injection. The group of rats receiving α-propyldopacetamide were treated with 5-HTP (20 or 100 mg/kg, i.p.), half of the rats treated with the lower dose of 5-HTP (20 mg/kg) being pretreated with nialamide (100 mg/kg, i.p.) ½ hr before the 5-HTP injection. Rats were killed ½, 1 and 2 hr after the 5-HTP injection by decapitation under light chloroform anaesthesia. The brains and spinal cords of some of the animals were taken for histochemical analysis of dopamine, noradrenaline and 5-HT according to the procedure previously described (Dahlström & Fuxe, 1964; Hamberger, Malmfors & Sachs, 1965) but as modified by Fuxe & Jonsson (1967). The brains and spinal cords of others were taken for biochemical analysis of 5-HT (Bertler, 1961). Control rats received saline instead of 5-HTP. Some rats pretreated with reserpine or reserpine-nialamide as described above were also injected with doses of 20 or 100 mg/kg of 5-HTP (i.p.) 1 or 2 hr before killing. Some of the rats treated with α-propyldopacetamide-nialamide as described above received the higher dose of 5-HTP (100 mg/kg). These animals, however, were used only for histochemical analysis.

Results

REPLETION AFTER RESERPINE

Histochemistry. In the rats treated with reserpine alone, practically no yellow fluorescent terminals were observed in the spinal cord and in various parts of the brain (e.g. in the medulla oblongata and the hypothalamus). The 5-HT cell bodies also did not show any definite yellow fluorescence. Furthermore, there were marked reductions in the number and fluorescence intensity of the noradrenaline and dopamine nerve terminals of the brain, and the catecholamine cell bodies became almost completely non-fluorescent. After reserpine-nialamide treatment, the fluorescence microscopical picture did not change except after 2 hr, when a weak yellow fluorescence began to appear in the 5-HT cell bodies of the lower brain stem and a very weak to weak yellow fluorescence of the 5-HT non-terminal axons and terminals became visible.

After administration of 5-HTP (20–100 mg/kg) to reserpinized rats, no restoration of fluorescence was observed in the 5-HT neurons, either in the terminals or in the cell bodies. The only change observed was that with the highest doses of 5-HTP (50–100 mg/kg) the endothelial sheath and the pericytes around the capillary walls showed a weak yellow fluorescence. The yellow fluorescence in the pericytes was most pronounced 30 min after injection. The small amounts of 5-HT found biochemically in the reserpine-5-HTP treated rats (see Fig. 1B) are probably localized in these cells.

The fluorescence microscopical picture was dramatically changed by pretreatment with nialamide before the 5-HTP injection. In the reserpine-nialamide treated rats, 5-HTP in doses of 50–100 mg/kg i.p. produced a moderate to bright yellow fluorescence of the 5-HT nerve terminals in many areas in the brain (e.g. in the medulla oblongata and pons) and in the spinal cord. The catecholamine nerve terminals, however, did not

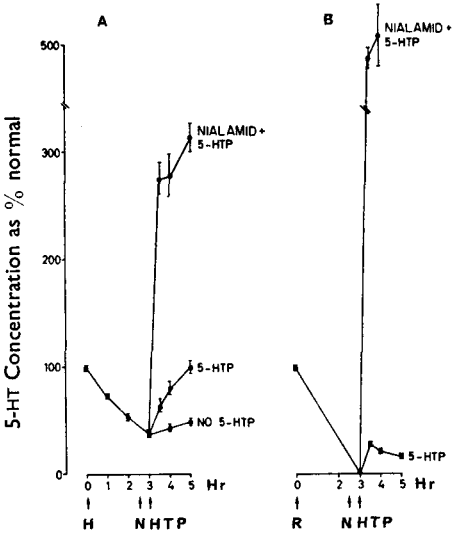

FIG. 1. Effect of 5-HTP with or without nialamide pretreatment on brain 5-HT concentrations in rats pretreated with α-propyldopacetamide (500 mg/kg, i.p.) in (A), or reserpine (5 mg/kg, i.p.) in (B). Each point is an average of 3–4 separate experiments and is expressed as a percentage of normal values ± s.e.m. Arrows indicate time after α-propylacetamide (H) or reserpine (R) when (A) nialamide (N) (100 mg/kg, i.p.) and 5-HTP (HTP) (20 mg/kg, i.p.), or (B) nialamide (100 mg/kg, i.p.) and 5-HTP (50 mg/kg, i.p.), were injected. The normal content of 5-HT was found to be 0·45 ± 0·009 μg/g wet weight (12 experiments).

show any signs of yellow fluorescence. Furthermore, the 5-HT cell bodies, but not the catecholamine cell bodies, showed a moderate to bright yellow fluorescence. Thus, the 5-HT neurons possessed a fluorescence intensity which was clearly above normal. The cells in the capillary walls showed a bright yellow fluorescence and this was observed in all parts of the brain. Yellowish background fluorescence was also noticed especially with the highest dose of 5-HTP (100 mg/kg). This fluorescence may be due to the diffuse presence of the amino-acid itself in the brain tissue. Similar results have been obtained previously in nialamide-5-HTP treated rats (Fuxe, 1965). After the dose of 20 mg/kg of 5-HTP, however, the main observation was an increase in the number and intensity of yellow fluorescence of the 5-HT nerve terminals and cell bodies. The cells in the capillary walls showed only a weak yellow fluorescence.

Biochemical findings. Biochemical results (Fig. 1B) also revealed dramatic changes after 5-HTP administration to reserpine-nialamide pretreated rats when compared with reserpine pretreatment alone. There was an increase in the 5-HT content of the brain from practically zero to a level five-fold the normal value. From the histochemical findings it is probable (see discussion) that these high amounts of 5-HT are present partly intraneuronally in the 5-HT neurons and partly in the pericytes and the endothelial cells along the capillary vessels.

REPLETION AFTER α-PROPYLDOPACETAMIDE

Histochemical findings. After treatment with α-propyldopacetamide alone, there was a large decrease in the number and fluorescence intensity of the 5-HT nerve terminals. The 5-HT cell bodies, which normally emit only a weak yellow fluorescence (Dahlström & Fuxe, 1964), showed a weaker yellow fluorescence or no specific fluorescence at all. In addition the catecholamine cell bodies and terminals showed markedly decreased fluorescence intensity. The brains from the rats treated with α-propyldopacetamide-nialamide showed a similar fluorescence microscopical picture as those from animals receiving α-propyldopacetamide alone.

When 5-HTP (20 mg/kg) was given to rats pretreated with α-propyldopacetamide, the intensity of the yellow fluorescence in the 5-HT nerve terminals and cell bodies was restored to normal levels after 1 hr. The cells in the capillary walls remained practically non-fluorescent. A dose of 100 mg/kg of 5-HTP caused a noticeable restoration of fluorescence in the 5-HT nerve terminals and cell bodies after only 30 min. The cells around the capillary walls now also showed a distinct yellow fluorescence and a yellowish fluorescence of low intensity was seen diffusely in the brain tissues. This fluorescence microscopical picture did not change to any marked degree during the following $1\frac{1}{2}$ hr. The catecholamine neurons were not affected by the 5-hydroxytryptophan injection.

Biochemical findings. Treatment with α-propyldopacetamide produced a decrease in 5-HT levels to about 40% of their normal values (see Fig. 1A and 2). A progressive restoration of the 5-HT contents in brains of α-propyldopacetamide-treated rats occurred after a dose of 20 mg/kg of

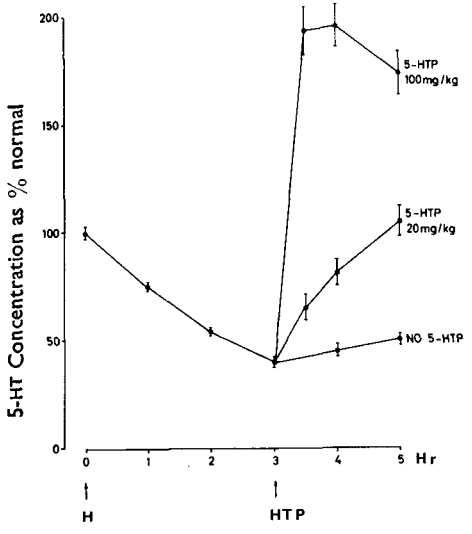

FIG. 2. Effects of two different doses of 5-HTP on brain 5-HT concentrations in rats pretreated with α-propyldopacetamide (500 mg/kg, i.p.). Each point is an average of normal values ± s.e.m. Arrow indicates time after α-propyldopacetamide(H) when 5-HTP was injected.

5-HTP. Normal levels were reached after 2 hr. If a high dose of 5-HTP (100 mg/kg) was injected, the 5-HT content of the brain increased to twice the normal value within 30 min. In view of the histochemical findings, these amounts of 5-HT were probably present in the pericytes and the endothelial cells of the capillary walls.

When the low dose (20 mg/kg) of 5-HTP was given to α-propyldopacetamide-pretreated rats in which monoamine oxidase had been inhibited with nialamide there was a marked restoration of the amine levels in the 5-HT nerve terminals and cell bodies after only 30 min. In fact, 5-HT terminals were even observed in large numbers in the reticular formation, the tectum and some of the cortical areas, where normally they are difficult to detect. The catecholamine neurons were not affected. A distinct yellow fluorescence also appeared in the pericytes and in the endothelial cells, and there was a slight increase in yellow background fluorescence. If a high dose of 5-HTP (100 mg/kg) was used there was no further increase in the intensity of the yellow fluorescence present intraneuronally but the yellow fluorescence of the pericytes and endothelial cells became brighter and an increased background fluorescence was observed. Nialamide pretreatment produced a marked increase in the 5-HT levels of the brain when compared with α-propyldopacetamide-5-HTP treated animals. After the low dose of 5-HTP (20 mg/kg) levels three times the normal value of 5-HT were obtained within 30 min in nialamide-pretreated rats. In view of the histochemical findings this peak level of 5-HT probably lies both in the 5-HT nerve terminals and in the cells of the capillary walls.

Discussion

There is good evidence that most of the 5-HTP decarboxylase is present in the monoamine neurons and is highly active there (Andén, Magnusson & Rosengren, 1965; Heller, Seiden & others, 1965; Andén, Dahlström & others, 1966). Thus, the restoration of yellow fluorescence in the 5-HT neurons after 5-HTP is probably due to 5-HT, formed intraneuronally by decarboxylation (Fuxe, 1965). Furthermore, the specific yellow fluorescence observed in the pericytes and the endothelial cells is also probably due to 5-HT and not to 5-HTP, since dopa-5-HTP decarboxylase is present in these cells (Bertler, Falck & Rosengren, 1964). The present combined biochemical and histochemical study provides further evidence supporting the view that 5-HT is responsible for the yellow fluorescence observed in these structures. Thus, changes in the 5-HT levels determined biochemically were always parallelled by corresponding changes in the yellow fluorescence in 5-HT nerve terminals and cells of the capillary walls. The diffuse yellowish fluorescence observed after high doses of 5-HTP in the brain tissue, however, is probably due to the amino-acid itself.

The fact that after depletion of 5-HT stores by α-propyldopacetamide a low dose of 5-HTP (20 mg/kg) was able to refill the intraneuronal 5-HT stores, provides evidence that α-propyldopacetamide acts by blocking only the first rate-limiting step in monoamine biosynthesis (Carlsson & others, 1963) and does not block the uptake-storage mechanism of the

5-HT granules. After depletion induced by reserpine, not even a high dose of 5-HTP could effect any repletion of the amine in the 5-HT neurons. The 5-HT formed appeared to be present extraneuronally. It is evident, however, that after pretreatment with nialamide, an injection of 5-HTP causes a marked and rapid replenishment of the amine stores in the 5-HT neurons to levels above normal, irrespective of whether the initial depletion had been caused by α-propyldopacetamide or by reserpine. These results agree with previous findings that monoamine oxidase plays an important role in the regulation of intraneuronal 5-HT levels (Carlsson, Lindqvist & Magnusson, 1959; Dahlström & Fuxe, 1964). Furthermore, regardless of whether the uptake-storage mechanism of the 5-HT storage granules is blocked or not, large amounts of 5-HT can be formed and accumulated in the 5-HT neurons after 5-HTP, provided that the monoamine-oxidase has been inhibited. Thus the 5-HT neurons behave like the dopamine neurons, since in the latter pretreatment with nialamide leads to the formation and accumulation of large amounts of dopamine from dopa (Corrodi & Fuxe, 1967). In addition, a large proportion of the high level of 5-HT formed in brain after 5-HTP and nialamide pretreatment is present in extra-neuronal stores, situated mainly in the pericytes and endothelial cells. The present findings also further underline the view (Fuxe, 1965) that there is a fairly specific uptake into, and/or a specific decarboxylation of 5-HTP in the 5-HT neurons, since the catecholamine neurons were hardly affected by the 5-HTP treatment.

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References

Andén, N.-E., Corrodi, H., Dahlström, A., Fuxe, K. & Hökfelt, T. (1966). *Life Sci.*, **5**, 561–568.
Andén, N.-E., Dahlström, A., Fuxe, K., Larsson, K., Olson, L. & Ungerstedt, U. (1966). *Acta physiol. scand.*, **67**, 313–326.
Andén, N.-E., Magnusson, T. & Rosengren, E. (1965). *Ibid.*, **64**, 127–135.
Bertler, Å. (1961). *Ibid.*, **51**, 75–83.
Bertler, Å., Falck, B. & Rosengren, E. (1964). *Acta pharmac. tox.*, **20**, 317–321.
Carlsson, A. (1966). In *Handbuch der experimentellen Pharmakologie*, editor Erspamer, V., pp. 529–592, Berlin-Heidelberg-Göttingen: Springer.
Carlsson, A., Corrodi, H. & Waldeck, B. (1963). *Helv. chim. Acta*, **46**, 2270–2285.
Carlsson, A., Lindqvist, M. & Magnusson, T. (1959). *Jorn. Soc. Sci. méd. Lisb.*, **123**, Suppl., 96–98.
Corrodi, H. & Fuxe, K. (1967). *Life Sci.*, in the press.
Corrodi, H., Fuxe, K. & Hökfelt, T. (1966). *Ibid.*, **5**, 605–611.
Corrodi, H. & Hanson, L. (1967). *Psychopharmacologia*, **10**, 116–125.
Dahlström, A. & Fuxe, K. (1964). *Acta physiol. scand.*, **62**, Suppl. 232, 1–55.
Dahlström, A., Fuxe, K. & Hillarp, N.-Å. (1965). *Acta pharmac. tox.*, **22**, 277–292.
Fuxe, K. (1965). *Acta physiol. scand.*, **64**, Suppl. 247, 37–85.
Fuxe, K. & Jonsson, G. (1967). *J. Histochem. Cytochem.*, in the press.
Hamberger, B., Malmfors, T. & Sachs, Ch. (1965). *Ibid.*, **13**, 147.
Heller, A., Seiden, L. S., Porcher, W. & Moore, R. T. (1965). *Science, N.Y.*, **5**, 91–101.