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EFFECT OF MONOAMINE RELEASERS AND DECARBOXYLASE INHIBITORS ON ENDOGENOUS 5-HYDROXYINDOLE DERIVATIVES IN BRAIN

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Abstract—The effect of α-methyl-dopa on endogenous cerebral 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) was compared with that of two monoamine releasers (reserpine and the benzoquinolizine derivative Ro 4-1284) as well as a potent, rather specific inhibitor of decarboxylase of aromatic amino acids (DC), i.e. the hydrazine derivative Ro 4-4602.

The monoamine releasers decreased 5HT and increased 5HIAA, whereas α-methyl-dopa and Ro 4-4602 diminished both indolyl compounds. Thereby, the effect of α-methyl-dopa was at least as pronounced and of somewhat longer duration than that of Ro 4-4602, although Ro 4-4602 caused a considerably more marked and longer lasting inhibition of DC than α-methyl-dopa.

It is concluded that the mechanism of action of α-methyl-dopa on the cerebral 5HT metabolism is different from that of reserpine and Ro 4-1284 and does not consist in a mere inhibition of DC.

THE decrease of norepinephrine (NE) in various tissues by α-methylated aromatic amino acids like α-methyl-3,4-dihydroxyphenylalanine (α-methyl-dopa) is probably related to a displacement or a release mechanism rather than to inhibition of decarboxylase of aromatic amino acids (DC).[1–5] The diminution of the level of 5-hydroxytryptamine (5HT) in brain induced by α-methyl-dopa,[6] might, however, be connected with DC inhibition. Thus, in brain α-methyl-dopa decreases acidic 5-hydroxyindole derivatives, whereas the monoamine releaser reserpine causes an increase of these 5HT metabolites.[7, 8, 15, 19]

To clarify the mechanism of action of α-methyl-dopa, this drug has been compared with a strong and rather specific DC inhibitor (Ro 4-4602*) as well as with monoamine releasers of long and short duration of action (reserpine and the benzoquinolizine derivative Ro 4-1284†). Thereby, measurements of 5HT and 5-hydroxyindoleacetic acid (5HIAA) in rat brain were carried out.

METHODS

Male Wistar rats of 60–90 g were injected i.p. with reserpine, the benzoquinolizine derivative Ro 4-1284, α-methyl-dopa, or Ro 4-4602 and decapitated at various intervals thereafter. Untreated animals served as controls. The following determinations were carried out:

(a) DC in the supernatant of brain homogenates (prepared in M/15 phosphate

* Ro 4-4602 = N-(DL-Seryl)-N′-(2,3,4-trihydroxybenzyl)hydrazine.[9, 11]

† Ro 4-1284 = 2-Hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo(a) quinolizine.[12]

buffer, pH = 8·0) with 5-hydroxytryptophan (5HTP) as a substrate. Thereby, the newly formed 5HT was measured by a spectrophotometric method.[9, 10]

(b) 5HT in hydrochloric acid extracts of homogenates of total brain by a spectrophotofluorimetric method.[13]

(c) 5HIAA in the supernatant of homogenates of brain stem by spectrophotofluorimetry after extraction into ethyl ether and reextraction into phosphate buffer.[7, 14] The tissue was homogenized with 4 vol perchloric acid, centrifuged and the supernatant adjusted to pH 1. All operations were carried out in the cold. It was shown by thin layer chromatography* that the fraction of acidic 5-hydroxyindolyl compounds extracted by this method consisted mainly of 5HIAA. The presence of small amounts of other acidic 5-hydroxyindole derivatives can, however, not be excluded.

RESULTS

(1) The DC inhibitor Ro 4-4602 caused a more marked inhibition of 5HTP-decarboxylation than α-methyl-dopa. Thus after 1 hr the dose for 50 per cent inhibition of DC (ED_{30}) was found to be approx. 0·015 mmole/kg for Ro 4-4602, but approx. 1 mmole/kg for α-methyl-dopa. 100 per cent inhibition of DC was induced by

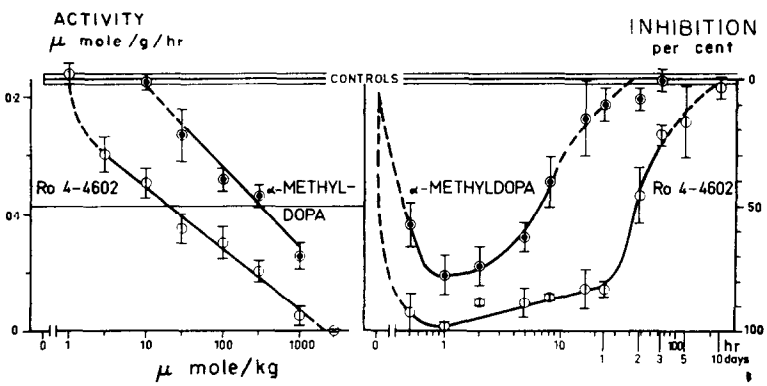

FIG. 1. Inhibition of DC in rat brain by α-methyl-dopa and Ro 4-4602.
Ordinate: left: enzyme activity, μmole 5HT formed per gram fresh tissue and hour.
right: per cent inhibition.
Abscissa: left: i.p. dose of the inhibitors administered 1 hr prior to decapitation.
right: time after i.p. administration of 2·4 mmole/kg of the inhibitors (500 mg/kg α-methyl-dopa; 693 mg/kg Ro 4-4602).
Each point represents an average with standard error of 2–5 (21 controls) double determinations in 5 pooled brains.

about 1 mmole/kg of Ro 4-4602, whereas even 3·0 mmole/kg of α-methyl-dopa did not reduce the enzyme activity by more than 75 per cent. The inhibition of DC was maximal 1 hr after administration of both drugs. Normal enzyme activities were restored 20–30 hr after 2·4 mmole α-methyl-dopa and 100–200 hr after the equivalent dose of Ro 4-4602 (Fig. 1).

* Carried out by Dr. G. Bartholini, Medical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basle.

(2) The monoamine releasers reserpine and Ro 4-1284 caused a rapid decrease of 5HT which was of long and short duration respectively. Both drugs increased 5HIAA concomitantly with the 5HT decrease. Maximal levels of the acid were reached 4 and 8 hr after Ro 4-1284 and reserpine respectively. The subsequent decline of 5HIAA was slower after reserpine than after Ro 4-1284. Following Ro 4-1284, the restoration of normal contents of 5HT and 5HIAA proceeded at a similar rate, whereas after reserpine the 5HIAA content was normalized several days before the 5HT had recovered (Fig. 2).

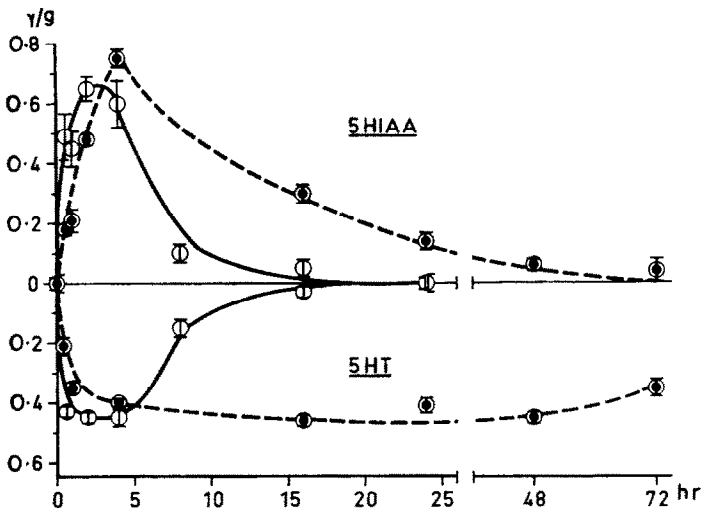

FIG. 2. Effect of reserpine and Ro 4-1284 on endogenous 5HT and 5HIAA of rat brain.
Ordinate: Changes of 5HT and 5HIAA resp., γ/g fresh brain. O levels = control values (absolute values of controls: 5HT = 0·60 ± 0·02 γ/g; 5HIAA = 0·39 ± 0·01 γ/g).
Abscissa: Hours after i.p. injection of 2·5 mg/kg reserpine and 15 mg/kg Ro 4-1284.
– – – – – reserpine.
———— Ro 4-1284.
Each point represents an average of 4–8 experiments with standad error.

(3) Both α-methyl-dopa and Ro 4-4602 decreased 5HT as well as 5HIAA. These effects were dose-dependent; the maximal decrease of the indolyl compounds did, however, not exceed 50 per cent. The diminution of 5HT and 5HIAA induced by Ro 4-4602 showed a similar time course, minimal levels being reached after about 2 hr, full recovery within about 16 hr. α-Methyl-dopa decreased the 5HT to minimal levels within 2–4 hr, but the 5HIAA content declined to minimal values only after 4–8 hr. Control values of both 5HT and 5HIAA were restored 16–24 hr after administration of α-methyl-dopa. The initial decrease of 5HT induced by both α-methyl-dopa and Ro 4-4602 was slower than that due to the monoamine releasers (Figs. 3 and 4).

DISCUSSION

The present findings of a decrease of 5HT and a concomitant increase of 5HIAA in rat brain by reserpine and Ro 4-1284 correspond to earlier experiments with reserpine.[7, 15] These results are in agreement with the hypothesis that both monoamine

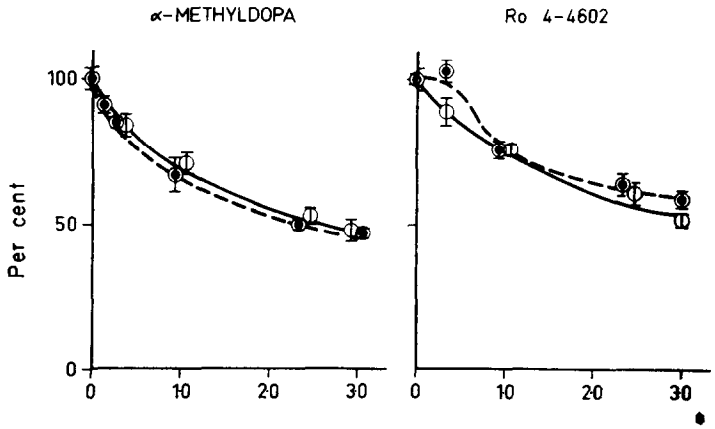

FIG. 3. Effect of various i.p. doses of α-methyl-dopa and Ro 4-4602 on 5HT and 5HIAA of rat brain.
Ordinate: 5HT and 5HIAA, per cent of controls.
Abscissa: Dose of the inhibitors, mmole/kg.
Measurement of 5HT 2 hr after α-methyl-dopa and Ro 4-4602; determinations of 5HIAA 2 hr after Ro 4-4602 and 8 hr after α-methyl-dopa.
———— 5HT.
– – – – – 5HIAA.
Each point represents an average of 4–6 experiments with standard error.

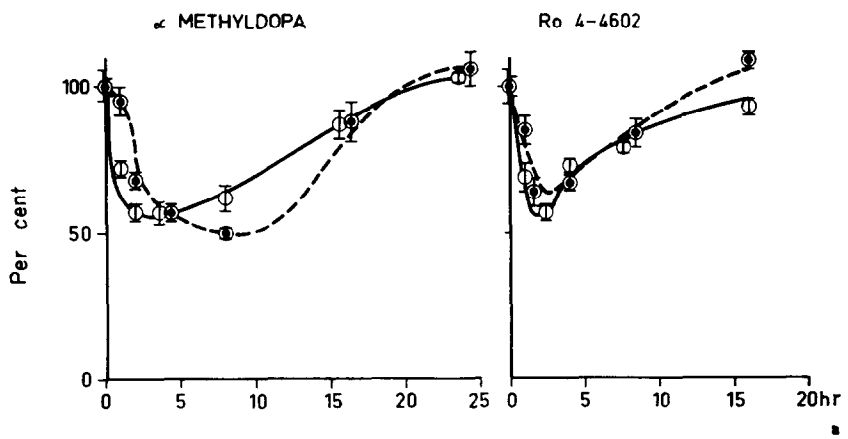

FIG. 4. Effect of 2·4 mmole α-methyl-dopa (500 mg/kg) and Ro 4-4602 (693 mg/kg) on 5HT and 5HIAA of rat brain after various time intervals.
Ordinate: 5HT and 5HIAA, per cent of controls.
Abscissa: Hours after i.p. injection of the drug.
———— 5HT.
– – – – – 5HIAA.
Each point represents an average of 4–10 experiments with standard error.

releasers render the stored 5HT available to the catabolizing enzyme MAO. With the short-acting Ro 4-1284, the decrease of 5HT and the rise of its metabolite 5HIAA show a similar time course indicating a rapid release and oxidation of stored 5HT

followed by a rapid repletion of the 5HT depots. The long-acting reserpine, however, causes an increase of 5HIAA only during the initial phase (0–24 hr) of 5HT diminution, whereas later (24–72 hr) the 5HIAA content is normal. This indicates also a rapid degradation of released 5HT, but only a slow restoration of the 5HT depots.

Ro 4-4602 differs from reserpine and Ro 4-1284 because it decreases 5HT as well as 5HIAA. These effects of Ro 4-4602 can be explained by DC inhibition resulting in a reduced formation of 5HT and 5HIAA.

α-Methyl-dopa has a similar qualitative action on 5HT and 5HIAA as Ro 4-4602. Nevertheless, it is unlikely that α-methyl-dopa acts merely by DC inhibition. Thus, Ro 4-4602 causes more potent and longer lasting inhibition of the enzyme than α-methyl-dopa as shown by the effects on DC activity (Fig. 1) and on the 5HTP-induced 5HT-increase in brain.[16] In spite of this higher potency of Ro 4-4602, α-methyl-dopa decreases 5HT and 5HIAA at least as markedly as Ro 4-4602. In addition, after α-methyl-dopa the decrease of indolyl compounds, especially of 5HIAA, persists for a longer period of time than following Ro 4-4602 (Figs. 3 and 4). In consequence, a mechanism other than DC inhibition has to be considered for the effect of α-methyl-dopa. The existence of such a mechanism is supported by recent findings in our laboratories with various ring-substituted phenylalkylamines. These compounds, similarly to α-methyl-dopa, decrease both 5HT and 5HIAA in the brain, although they do not inhibit other enzymes (e.g. DC, monoamine oxidase, dopamine-β-hydroxylase) in the brain *in vivo*.

The following mechanisms of action of α-methyl-dopa have to be considered and further elucidated:

(a) *Displacement or release of stored amines.* This would imply that α-methyl-dopa acts differently from reserpine and the benzoquinolizine Ro 4-1284 since α-methyl-dopa, in contrast to the other drugs, diminishes both 5HT and 5HIAA. The decrease of 5HIAA suggests that 5HT released by α-methyl-dopa is not metabolized by monoamine oxidase but via an alternative metabolic pathway not leading to 5HIAA. Such an effect is conceivable since NE, released by sympathicomimetic amines, is also metabolized by an enzyme other than monoamine oxidase (O-methyl-transferase).[17, 18]

(b) *Reduction of the cerebral supply of 5HTP.* α-Methyl-dopa (in contrast to Ro 4-4602) might inhibit the 5-hydroxylation of tryptophan* or interfere with the uptake of amino acids by brain.[19]

* *Added in press*—Marked inhibition of tryptophan hydroxylase in rat liver by α-methyl-dopa has recently been demonstrated *in vitro* and *in vivo*.[20]

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