Biochemical Pharmacology, 1963, Vol. 12, pp. 223–228. Pergamon Press Ltd., Printed in Great Britain.

THE EFFECT OF A NEW DECARBOXYLASE INHIBITOR ON ENDOGENOUS AND EXOGENOUS MONOAMINES

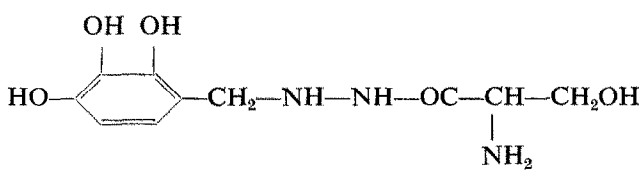
A. PLETSCHER and K. F. GEY

Medical Research Department of F. Hoffmann-La Roche & Co. Ltd.,
Basle, Switzerland

(*Received* 9 *November* 1962; *accepted* 13 *November* 1962)

Abstract—In various animal species the hydrazide Ro 4-4602, a potent irreversible inhibitor of decarboxylase of aromatic amino acids, decreases 5-hydroxytryptamine (5HT) and norepinephrine (NE) in the brain as well as NE in the heart by a maximum of about 50 per cent. The 5HT-increase in brain and heart induced by 5-hydroxytryptophan as well as the recovery of 5HT in the brain after its depletion by a monoamine releaser (Ro 4-1284) is, however, completely inhibited by the drug. The possibility is discussed that Ro 4-4602 might interfere mainly with the metabolism of "free" and relatively little with that of "stored" amines.

IT HAS been reported that the hydrazine derivative Ro 4-4602 (I) markedly inhibits decarboxylase of aromatic amino acids (DCO) without inhibiting monoamine oxidase.[1] Intraperitoneal injection of doses as low as 0·5 and 12 mg/kg (1·7 and 41 μM/kg) of the hydrochloride causes a 50 per cent inhibition of the enzyme in homo-



(I)

genates of kidney and brain, respectively. The new inhibitor differs from older ones (e.g. α-alkylated aromatic amino acids) in that its effect cannot be reversed by pyridoxal-5′-phosphate. In spite of marked DCO inhibition, the action of Ro 4-4602 on the total content of endogenous monoamines is rather weak. Even repeated administration of high doses of the compound reduces the monoamine content of various organs only to a limited extent. Similar results have been reported with another decarboxylase inhibitor of the hydrazide type (NSD 1034).[2]

The discrepancy between DCO inhibition and the decrease of endogenous monoamines remains to be explained. It has, for example, not been clarified whether the effect on DCO as measured in tissue homogenates of animals pretreated with Ro 4-4602 reflects a true *in vivo* situation. Ro 4-4602 present in the blood and in the extracellular fluid (e.g. in the brain) might cause DCO inhibition only after homogenizing the tissues (*in vitro* inhibition), whereas *in vivo* the penetration of the drug to the site of the enzyme could possibly be hindered.

In the present paper investigations on the mode of action of Ro 4-4602 *in vivo* were carried out. For this purpose, the effect of the drug on endogenous and on exogenous monoamines was compared.

EXPERIMENTAL PROCEDURE

In albino mice (10–15 g), Wistar rats (60–80 g), and guinea pigs (250–350 g) fasted for 16 hr, endogenous 5-hydroxytryptamine (5HT) in brain and norepinephrine (NE) in brain and heart were measured spectrophotofluorometrically[3, 4] after i.p. administration of the hydrochloride of Ro 4-4602. Furthermore, the following investigations were carried out in rats:

(a) Influence of Ro 4-4602 on the 5HT-increase induced by 5-hydroxytryptophan (5HTP) in heart and brain.

(b) Influence of Ro 4-4602 on the restoration of 5HT in brain previously depleted by a monoamine releaser (benzoquinolizine Ro 4-1284*).

RESULTS

(1) Single i.p. doses of Ro 4-4602 moderately diminished the 5HT and NE content in the brain of rats and mice as well as the NE content in the heart of rats, mice, and guinea pigs. This effect was related to the dose of the drug. In the brain

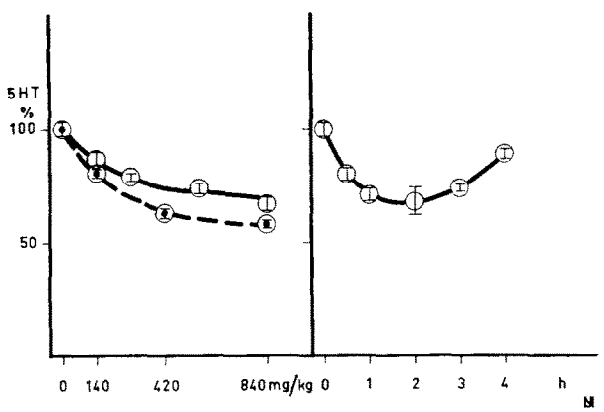


FIG. 1. Decrease of the 5-hydroxytryptamine content in the brain by i.p. administration of Ro 4-4602. Ordinate: 5-hydroxytryptamine content of brain in per cent of controls. Abscissa: *left*, dose of Ro 4-4602 in mg/kg administered 1 hr before sacrificing the animals; *right*, time in hours after i.p. administration of 555 mg/kg Ro 4-4602. ———— rats; – – – – mice. Each point represents the average value of 5–9 experiments ± standard error. Absolute 5-hydroxytryptamine values of brains of controls: rats, 0·61 ± 0·02 μg/g; mice, 0·72 ± 0·02 μg/g.

the maximal depression of 5HT occurred 1–2 hr after injection of Ro 4-4602, the control levels being restored after 4–6 hr. In the heart the NE dropped after an initial, non-significant rise ($p > 0{\cdot}05$); minimal levels were reached 16 hr after administration of Ro 4-4602 and still persisted after 24 hr. The decrease of 5HT and NE never exceeded 50 per cent (Figs. 1 and 2). In the brain of mice

* Ro 4-1284 = 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo (a) quinolizine.[5]

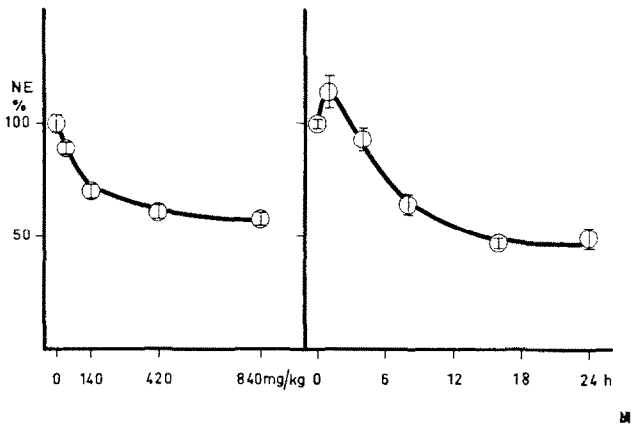


FIG. 2. Decrease of the norepinephrine content of the heart by i.p. administration of Ro 4-4602. Ordinate: norepinephrine content of the heart in per cent of controls. Abscissa: *left*, dose of Ro 4-4602 in mg/kg administered 16 hr before sacrificing the animals (rats); *right*, time in hours after i.p. administration of 840 mg/kg Ro 4-4602 (guinea pigs). Each point represents the average of 3–9 experiments ± standard error. Absolute norepinephrine values of hearts of controls: rats, 0·72 ± 0·03 μg/g; guinea pigs, 1·48 ± 0·03 μg/g.

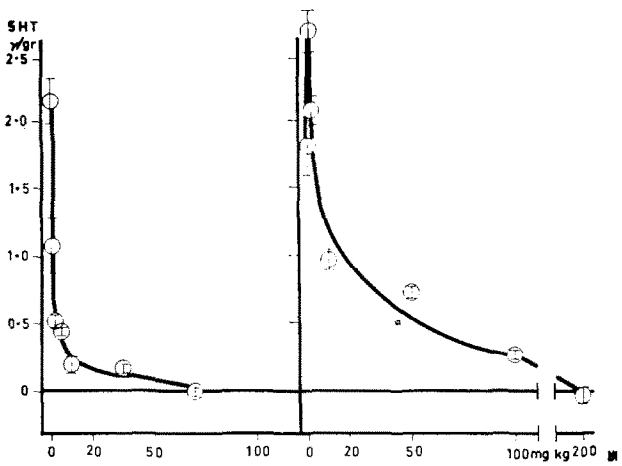


FIG. 3. Inhibition by Ro 4-4602 of the increase of 5-hydroxytryptamine induced by 5-hydroxytryptophan in rats.

Ordinate: increase of the 5-hydroxytryptamine content over the control level in μg/g tissue. Abscissa: dose of Ro 4-4602 in mg/kg. Ro 4-4602 was administered i.p. 1 hr before 75 mg/kg 5-hydroxytryptophan s.c. The animals were sacrificed ½ hr after injection of 5-hydroxytryptophan. *Left hand graph:* heart. *Right hand graph:* brain; these animals were pretreated with the monoamine oxidase inhibitor Pargyline (100 mg/kg i.p.) 16 hr before administration of Ro 4-4602 in order to obtain 5-hydroxytryptamine levels comparable to those in the heart. Each point represents the average of 4–6 experiments ± standard error. Control values of 5-hydroxytryptamine (= zero levels): *heart** (no treatment), 0·75 ± 0·03 μg/g; brain (pretreated with Pargyline): 1·40 ± 0·02 μg/g.

* It has not been proved whether all the fluorescence measured in the extract of hearts of controls corresponds to 5-hydroxytryptamine.

and rats doses as high as 840 mg/kg Ro 4–4602 i.p. caused a small but significant decrease of NE (about 20 per cent, $p < 0{\cdot}01$) only after 4 hr.

(2) The 5HTP-induced increase of 5HT in brain and heart of rats was markedly diminished by Ro 4-4602. In the heart 0·5 mg/kg Ro 4-4602 caused a 50 per cent reduction of the 5HT-increase (ED_{50}), whereas in the brain the ED_{50} of Ro 4-4602 was approx. 20 mg/kg. For a complete abolishment of the 5HTP-induced rise of 5HT in the brain and in the heart respectively 200 and 70 mg/kg Ro 4-4602 were necessary (Fig. 3). Low doses of Ro 4-4602 (5 mg/kg) caused a significant enhancement of the 5HTP-induced 5HT-increase in the brain ($p < 0{\cdot}01$).

(3) After depletion of the 5HT-content of the brain by a rapidly acting monoamine releaser (benzoquinolizine derivative Ro 4-1284) the restoration of the amine content could be completely abolished by subsequent administration of repeated large doses of Ro 4-4602. Without the monoamine releaser, however, Ro 4-4602 diminished the endogenous 5HT only slightly (Table 1).

TABLE 1. EFFECT OF REPEATED DOSES OF Ro 4-4602 ON THE 5-HYDROXYTRYPTAMINE CONTENT OF BRAIN AFTER MAXIMAL DEPLETION OF THE AMINE BY THE MONOAMINE RELEASER Ro 4-1284

Ro 4-1284 + Ro 4-4602	Ro 4-4602	Ro 4-1284 ¾ hr	Ro 4-1284 16¾ hr	Controls
27 ± 5	66 ± 1	30 ± 2	97 ± 2	100 ± 7

1680 mg/kg Ro 4-4602 were administered i.p. in three divided doses alone or ¾ hr after 10 mg/kg Ro 4-1284 s.c. at the time of maximal depletion of 5-hydroxytryptamine. The rats were sacrificed 16 hr after the first and 3½ hr after the last dose of Ro 4-4602. It may be seen that with Ro 4-1284 + Ro 4-4602 the 5-hydroxytryptamine content still shows maximal depression at the end of the experiment (16¾ hr after Ro 4-1284 administration), whereas 16¾ hr after Ro 4-1284 alone the 5-hydroxytryptamine has totally recovered. Ro 4-4602 alone causes only a relatively slight decrease of 5-hydroxytryptamine.

The figures represent averages of 4–6 experiments each in per cent of untreated controls ± standard error.

Absolute 5-hydroxytryptamine values of brains of controls: 0·58 ± 0·04.

DISCUSSION

The strong reduction of the 5HTP-induced increase of 5HT in brain and heart by small doses of Ro 4-4602 indicates that the compound is a potent DCO inhibitor *in vivo*. The results also demonstrate that marked inhibition of DCO *in vivo* does not necessarily lead to a considerable decrease of endogenous amines in these organs. This discrepancy is not fully understood, but might, however, be due to the following reasons:

(a) Decarboxylation is possibly not rate limiting in the biosynthesis of endogenous monoamines, but in the excessive formation of 5HT caused by exogenous 5HTP. In consequence, though DCO is inhibited even to a high degree, the residual activity of the enzyme might be sufficient to sustain control levels of endogenous amines under normal conditions. On the other hand, 5HT-formation

from exogenous 5HTP might already be diminished by relatively little DCO inhibition.

(b) The endogenous monoamines of the tissues are probably localized in different cellular compartments (e.g. as stored and "free"* amines).[6–9] The "free" amines may be assumed to represent a relatively small portion of the total amines, but to have a high turnover, whereas for the stored amines the reverse is possibly true. DCO inhibition by Ro 4-4602 might therefore preferentially reduce the pool of "free" amines, but not markedly decrease that of stored amines. However, after administration of large amounts of 5HTP, which probably increases especially the pool of "free" amines, a relatively weak inhibition of DCO might have a marked effect on amine formation.

According to the present findings, Ro 4-4602 completely suppresses the recovery of endogenous 5HT once the amine has been depleted from the cerebral stores by Ro 4-1284. In consequence, the rate of biosynthesis is probably reduced to such a degree that the normal amine level can no longer be restored. This suggests that the relatively weak effect of Ro 4-4602 on total endogenous monoamines is not due to incomplete inhibition of DCO, but rather to a slow turnover of stored monoamines.

In order to support the above hypothesis, it has to be excluded that Ro 4-4602 induces release of monoamines from the tissues, Such an effect has been shown for other DCO inhibitors, e.g. α-methyl-3,4-dihydroxyphenylalanine (α-methyl-dopa).[10–12] Preliminary experiments in which the endogenous NE of the heart was pooled with ^{14}C-NE[13] suggest, however, that Ro 4-4602, in contrast to α-methyl-dopa, causes at the most slight liberation of the amine.

The finding that Ro 4-4602 has a more marked effect on the 5HTP-induced increase of 5HT in the heart than in the brain remains to be explained. The following possibilities have to be considered:

(a) DCO of heart and brain is inhibited to various degrees by Ro 4-4602. This might be due either to a different susceptibility of the enzymes in the two organs or to a better penetration of the drug into the heart than into the brain. Experiments with rabbits, however, do not support these hypotheses, since heart and brain normally show corresponding DCO activity and since the drug inhibits DCO of both organs *in vivo* to a similar extent.[13]

(b) Heart, in contrast to brain, takes up 5HT from the blood. Therefore the 5HTP-induced 5HT-increase in the heart might be partly due to uptake of 5HT which has been formed by decarboxylation of 5HTP in other organs, e.g. kidney, liver. Since DCO of the kidney, for instance, is highly sensitive to Ro 4-4602,[1] the effect of the drug on the 5HTP-induced 5HT-increase in the heart might partly reflect DCO inhibition in other organs.

The initial small increase of catecholamines in the heart by 840 mg/kg Ro 4-4602 is possibly due to inhibition of catechol-*o*-methyl transferase. Thus, i.p. injection of 900 mg/kg of the drug similar to other polyphenols, like pyrogallol[14, 15] diminishes the activity of the enzyme markedly in the supernatant of liver homogenate, but not in that of brain.[13] The enhancement of the 5HTP-induced 5HT-increase in brain by small doses of Ro 4-4602 (5 mg/kg; $p < 0{\cdot}01$) cannot be explained. It might be

* "Free" is used for lack of a better expression. It means: more easily available for the metabolizing enzymes as compared with the stored amines.

caused by an activation of DCO, because enzyme activation has also been seen in the case of MAO.[16]

REFERENCES

1. W. P. BURKARD, K. F. GEY and A. PLETSCHER, *Experientia.* **18**, 512 (1962).
2. B. B. BRODIE, R. KUNTZMAN, C. W. HIRSCH and E. COSTA, *Life Sci.* (3) 81 (1962).
3. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **117**, 82. (1956)
4. P. A. SHORE and J. S. OLIN, *J. Pharmacol. exp. Ther.* **122**, 295 (1958).
5. A. PLETSCHER, H. BESENDORF and K. F. GEY, *Science* **129**, 844 (1959).
6. B. B. BRODIE and P. A. SHORE, *Ann. N.Y. Acad. Sci.* **66**, 631 (1957).
7. J. P. GREEN, *Advances in Pharmacology* Vol. 1, p. 349. Academic Press New York (1962).
8. L. T. POTTER, J. AXELROD and I. J. KOPIN, *Biochem. Pharmacol.* **11**, 254 (1962).
9. R. KUNTZMAN, E. COSTA, G. CREVELING, C. W. HIRSCH and B. B. BRODIE, *Life Sci.* (3) 85 (1962).
10. S. M. HESS, R. H. CONNAMACHER, M. OZAKI and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **134**, 129 (1961).
11. A. CARLSSON and M. LINDQVIST, *Acta physiol. scand.* **54**, 87 (1962).
12. S. M. HESS, *Pharmacologist* **3**, 77 (1961).
13. W. P. BURKARD, K. F. GEY and A. PLETSCHER. In preparation.
14. Z. M. BACQ, L. GOSSELIN, A. DRESSE and J. RENSON, *Science* **130**, 453 (1959).
15. J. AXELROD and M.-J. LAROCHE, *Science* **130**, 800 (1959).
16. K. F. GEY and A. PLETSCHER, *J. Neurochem.* **6**, 239 (1961).