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FORMATION OF MONOAMINES FROM VARIOUS AMINO ACIDS IN THE BRAIN AFTER INHIBITION OF EXTRACEREBRAL DECARBOXYLASE

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Abstract—The effect of an inhibitor of extracerebral decarboxylase (Ro 4-4602) on the formation of cerebral monoamines from various amino acids has been investigated in rats by biochemical and histochemical methods. Pretreatment with Ro 4-4602 markedly increases the L-3,4-dihydroxyphenylalanine (DOPA)-induced formation of dopamine (DA), slightly enhances the DL-5-hydroxytryptophan (5HTP)-induced elevation of 5-hydroxytryptamine (5HT) and diminishes the DL-threo-3,4-dihydroxyphenylserine (DOPS)-induced rise of norepinephrine (NE) in the brain. Correspondingly, Ro 4-4602 abolishes the typical fluorescence caused by the amines within the walls of brain capillaries but markedly increases the parenchymal fluorescence after DOPA, and slightly that after 5HTP. The DOPS-induced weak parenchymal fluorescence is not modified by the inhibitor.

It is concluded that a) the effect of Ro 4-4602 varies according to differences in the affinity of the amino acids for the decarboxylase and possibly in their penetration through the blood–brain barrier, and b) the cerebral norepinephrine formed after administration of DOPS is mainly localized in the capillaries of the brain.

VARIOUS aromatic amino acids e.g. 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, 3,4-dihydroxyphenylserine, histidine have been used in order to selectively increase the concentration of the corresponding amines, i.e. dopamine (DA), serotonine (5HT), norepinephrine (NE) and histamine in the brain. The 3,4-dihydroxyphenylalanine-induced increase of cerebral DA is markedly enhanced by compounds which inhibit the decarboxylase of aromatic amino acid (decarboxylase) in extracerebral tissues. These inhibitors, e.g. Ro 4-4602(*N*-(DL-seryl)-N^1-(2,3,4-trihydroxybenzyl)-hydrazine), also interfere with the decarboxylase of the brain capillaries where this enzyme functions as a barrier for the penetration of 3,4-dihydroxyphenylalanine into the brain parenchyma.[1–4]

This paper presents biochemical and histochemical evidence that in brain the decarboxylase inhibitor Ro 4-4602 influences the formation of amines from various precursors in a different way. Reasons for these differences will be discussed.

MATERIAL AND METHODS

Albino rats of both sexes, of Wistar origin (Füllinsdorf), weighing 80–120 g were injected i.p. with L-3,4-dihydroxyphenylalanine (DOPA, 200 mg/kg), DL-threo-3,4-dihydroxyphenylserine (DOPS, 500 mg/kg) or DL-5-hydroxytryptophan (5HTP, 225 mg/kg) respectively 1 and 2 hr before sacrifice by decapitation. The amino acids

were given either alone or 30 min after 50 mg/kg Ro 4-4602 i.p. Animals injected with saline served as control.

DA, NE and 5HT were measured biochemically in the whole brain (NE also in the heart) as previously described.[2,5] In some of the animals the brains were investigated by a histofluorimetric method in which the catecholamines (DA, NE) and 5HT show a typical green and yellow fluorescence respectively.[6] For these experiments, the animals were treated as described above with the exception that only 100 mg/kg 5HTP or 75 mg/kg DOPA were administered.

RESULTS

(1) In the brain DA, 5HT and NE are increased 1 and 2 hr following administration of DOPA, 5HTP or DOPS respectively. Maximal values are reached after 1 hr. In the heart, too, the NE rises after administration of DOPS and follows a time course similar to that of the amine in the brain (Table 1).

(2) The decarboxylase inhibitor Ro 4-4602 markedly enhances the DOPA-induced increase of DA in the brain at both times. The 5HTP induced rise of cerebral 5HT is slightly enhanced only after 2 hr, whereas the NE elevation due to DOPS is completely abolished by Ro 4-4602 (Table 1, Fig. 1).

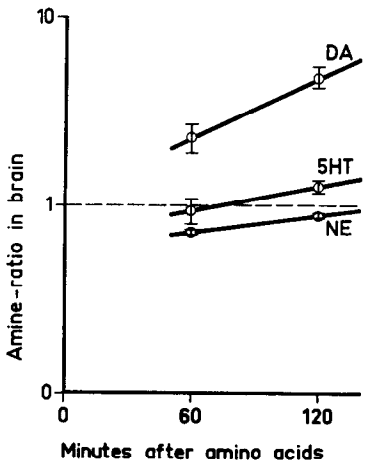

FIG. 1. Effect of Ro 4-4602 on the increase of brain monoamines induced by i.p. injection of various amino acids. Rats have been treated as explained in the Table. The values are expressed by the following ratios: cerebral amine concentration in animals treated with Ro 4-4602 plus an amino acid versus the cerebral amine concentration in animals treated with this amino acid alone. Average with S.E. of three to five duplicate experiments. DA = dopamine, 5HT = 5-hydroxytryptamine, NE = norepinephrine.
Significance: DA: $P < 0.01$ (both values).
5HT: $P > 0.05$ (60 min).
$P < 0.01$ (120 min).
NE: $P < 0.01$ (both values).

(3) The histofluorimetric experiments show that administration of 5HTP or DOPA causes a yellow and a green fluorescence respectively in the endothelial cells and pericytes of the capillary walls in all the brain regions. Furthermore, a slight increase of the diffuse parenchymal fluorescence is also to be seen in the striatum and

in the locus niger. Pretreatment with Ro 4-4602 abolishes the fluorescence of the capillaries induced by both amino acids in all the regions of the brain with the exception of the magnocellular nuclei of the hypothalamus. Concomitantly, the

TABLE 1.

	Controls	Amino acids alone		Ro 4-4602 + amino acids	
		1 hr	2 hr	1 hr	2 hr
Brain DA after DOPA	0·87 ± 0·05	2·41 ± 0·03*	1·56 ± 0·21*	5·33 ± 0·58†	6·14 ± 1·01†
Brain 5HT after 5HTP	0·36 ± 0·03	1·29 ± 0·11*	0·89 ± 0·04*	1·19 ± 0·09§	1·11 ± 0·06†
Brain NE after DOPS	0·46 ± 0·01	0·63 ± 0·03*	0·55 ± 0·02*	0·45 ± 0·02‡	0·48 ± 0·01‡
Heart NE after DOPS	1·09 ± 0·01	2·33 ± 0·15*	1·96 ± 0·16*	1·09 ± 0·05‡	1·01 ± 0·10‡

L-3,4-dihydroxyphenylalanine (DOPA), DL-5-hydroxytryptophan (5HTP) and DL-threo-3,4-dihydroxyphenylserine (DOPS) have been administered, i.p. alone, or 30 min after 50 mg/kg RO 4-4602 i.p. respectively. Sacrifice 1 and 2 hr after administration of the amino acids. The values are indicated in μg/g tissue and represent averages with S.E. of three to five duplicate experiments. DA = dopamine, 5HT = 5-hydroxytryptamine, NE = norepinephrine.

Significance: *$P < 0·01$ vs. controls.
†$P < 0·01$ vs. controls and vs. amino acid alone.
‡$P > 0·05$ vs. controls.
*$P < 0·01$ vs. amino acid alone.
§$P < 0·01$ vs. controls.
§$P > 0·05$ vs. amino acid alone.

inhibitor slightly enhances the 5HTP induced yellow fluorescence and markedly increases the DOPA induced diffuse green fluorescence of the parenchyma of the striatum and the locus niger. Moreover, a slight yellow and a marked green fluorescence appears in the rest of the parenchyma after 5HTP and DOPA respectively. This effect is more pronounced in the grey than in the white matter.

Administration of DOPS induces the appearance of a green fluorescence in the capillaries of all the brain regions. It is partly localized within the endothelial cells and pericytes but also in the lumen. Moreover, a very slight green fluorescence appears in the parenchyma of the tuber cinereum, striatum, locus niger, locus coeruleus, nuclei laterales bulbi. Ro 4-4602 markedly diminishes the fluorescence in the cells of the capillaries but only slightly that in the lumen and does not modify the parenchymal fluorescence.

DISCUSSION

The present results with the combination of Ro 4-4602 + DOPA confirm earlier findings. Accordingly, Ro 4-4602 enhances the DOPA-induced rise of cerebral DA due to a preferential inhibition of extracerebral decarboxylase (e.g. in heart, liver and kidney). As a consequence, DOPA accumulates in the blood and increased amounts of the amino acid are available for the brain. Since the cerebral decarboxylation is not inhibited, major amounts of dopamine are formed and accumulate in the brain.[3,4] Ro 4-4602 also acts on the cerebral capillaries. Under normal conditions considerable amounts of DOPA are decarboxylated within the capillary walls of the brain. The

resulting catecholamines cannot penetrate into the brain parenchyma, and the major part is probably further metabolized. Some catecholamines, however, accumulate in the capillary walls inducing a typical green fluorescence on histofluorimetric examination. Ro 4-4602 inhibits the decarboxylase of the capillaries which behave like extracerebral tissues. As demonstrated before[7,8] the capillaries of the various brain regions show differences with regard to Ro 4-4602. Thus, the inhibitor interferes less markedly with the capillary decarboxylase in the hypothalamus than in other parts of the brain. As a consequence of the decarboxylase inhibition in the walls of the brain capillaries, the DOPA which has penetrated into these structures is no longer decarboxylated but enters the brain parenchyma where decarboxylation takes place. Therefore, the green capillary fluorescence disappears in most of the brain regions, and a diffuse parenchymal fluorescence appears,[2] which, as shown by the biochemical experiments (Table 1, Fig. 1), is at least in part due to DA.

The decarboxylation of 5HTP and DOPS is also inhibited in extracerebral organs by Ro 4-4602. This has been previously shown for 5HTP[9] and is demonstrated in the present investigation for DOPS, since the inhibitor abolishes the DOPS-induced rise of NE in the heart. In the brain, however, both 5HTP and DOPS in the doses used, behave differently from DOPA. Thus, Ro 4-4602 does not markedly enhance the 5HTP-induced rise of cerebral 5HT although the decarboxylation of 5HTP in the capillary walls is abolished as judged by the disappearance of the yellow 5HT fluorescence. Furthermore, the parenchymal yellow fluorescence only shows a slight increase. Therefore, the penetration of 5HTP into the brain can probably not be as markedly improved as that of DOPA by inhibition of the decarboxylase in extracerebral organs including the capillary walls of the brain. This finding may be due to the fact that 5HTP has a lower affinity for the decarboxylase than DOPA.[10] Thus, 5HTP is decarboxylated to a lesser extent than DOPA and therefore inhibition of decarboxylase probably increases less markedly the content of 5HTP in the blood than that of DOPA. As a consequence the amounts of 5HTP which penetrate into the brain are not greatly enhanced by decarboxylase inhibition, whereas the DOPA penetration is considerably increased. Accordingly the slight increase of cerebral histamine induced by histidine, which has a low affinity for decarboxylase[10] is not influenced by Ro 4-4602 (W. P. BURKARD, personal communication).

The enhancing effect of Ro 4-4602 on the 5HTP-induced increase in cerebral 5HT seems to depend on the dose of 5HTP. Thus, in preliminary experiments in rats with low doses (16 mg/kg) of the amino acid, a more pronounced enhancement of the 5HTP-induced rise of brain 5HT by Ro 4-4602 was observed as in the present investigation. Similar findings have been reported for rabbits treated with another decarboxylase inhibitor (DL-α-hydrazino-α-methyldopa) plus 35 mg/kg 5HTP.[11] However, the 5HTP-induced increase of 5HT is markedly less enhanced by the decarboxylase inhibitors than the rise of DA induced by DOPA.[3,4]

The relatively slight increase in cerebral NE by administration of DOPS is completely abolished by Ro 4-4602. Moreover, no increase in parenchymal green fluorescence occurs. Therefore, since Ro 4-4602 preferentially acts in extracerebral tissues including brain capillaries, the bulk of the NE that appears in the brain after administration of DOPS is probably located in the capillary walls. The effect of Ro 4-4602 might not only be the consequence of a low affinity of DOPS for decarboxylase, but possibly indicates that DOPS penetrates less easily through the brain capillaries into

the parenchyma than DOPA and 5HTP. Nevertheless, the slight increase of parenchymal green fluorescence observed after DOPS alone as well as with DOPS plus Ro 4-4602 in certain brain areas (e.g. striatum, tuber cinereum, locus coeruleus, locus niger) reflects some (probably very small) penetration of the amino acid into these regions.

It might be argued that the missing enhancement of the 5HTP- and DOPS-induced accumulation of cerebral amines by Ro 4-4602 is due to a decreased decarboxylation of these amino acids in the brain parenchyma. Thereby, one would have to assume that in the relatively small doses used Ro 4-4602 inhibits parenchymal decarboxylase to some extent and that 5HTP and DOPS might be more sensitive to this inhibition than DOPA. These possibilities are unlikely, since, as previously shown, Ro 4-4602 in the presently used doses does not significantly interfere with cerebral decarboxylase. Furthermore, *in vitro* the drug inhibits the decarboxylation of DOPA to about the same degree as that of 5HTP.[3,12]

In conclusion, the described differences in the effect of Ro 4-4602 are probably due to a different affinity of the various amino acids for the decarboxylase and to differences in their penetration through the walls of the brain capillaries into the cerebral parenchyma.

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