A New Inhibitor of Decarboxylase of Aromatic Amino Acids

The main compounds so far known to inhibit decarboxylase of aromatic amino acids are caffeic acid derivatives[1], α-methylated aromatic amino acids, and carbonyl reagents[2]. For the first group, *in vivo* inhibition of decarboxylase has been demonstrated only by indirect methods (reduction of the 3,4-dihydroxyphenyl-alanine-induced rise in blood pressure of pithed cats[3] or rats[4]). α-Methylated aromatic amino acids, e.g. α-methyl-3,4-dihydroxy-*DL*-phenylalanine, α-methyl-m-tyrosine seem to release monoamines and to inhibit decarboxylase[5–8]. Carbonyl reagents, e.g. isoniazid[9], hydroxylamine[10], are weak inhibitors and interfere probably only with the coenzyme pyridoxal-5′-phosphate[11–14]. Potent *in vivo* inhibitors of decarboxylase without further influence on monoamine metabolism are therefore of distinct interest. The recently described N-(3-hydroxybenzyl)-N-methylhydrazine (NSD 1034) is a strong *in vivo* inhibitor of decarboxylase, but still interferes to some extent with monoamine oxidase[8,15,16].

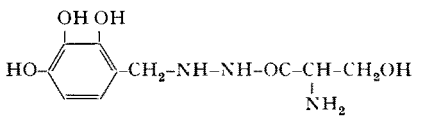
The present communication deals with N-(*DL*-seryl)-N′-(2,3,4-trihydroxybenzyl)-hydrazine (Ro 4–4602) (I)[17], a new and potent decarboxylase inhibitor, which is not influenced by pyridoxal-5′-phosphate and which has no effect on monoamine oxidase in brain.

Methods. In vitro: Supernatant of homogenized hog kidney cortex or rat kidney with or without addition of pyridoxal-5′-phosphate was incubated with 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan respectively. Decarboxylation was measured manometrically (CO_2 formation) or by spectrophotometric determination of the newly formed amines[18] as described earlier[19]. In some experiments a mixture of 10 ml supernatant of rat kidney and Ro 4–4602 (final concentration 10^{-6} *M*) was twice dialyzed against 2 l of phosphate buffer (pH 6.8) within 24 h.

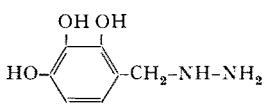
In vivo: Female Wistar rats of 60–80 g were pretreated with Ro 4–4602 and decapitated in order to carry out the following measurements: (a) Decarboxylase activity in the supernatant of brain or kidney as indicated above. (b) Monoamine oxidase activity in total homogenate of brain as described before[20]. (c) 5-Hydroxytryptamine and catecholamines of various organs by spectrophotofluorometric procedures[21,22]. Untreated rats served as controls.

Results and Discussion. In vitro: 10^{-5} *M* Ro 4–4602 diminished decarboxylase activity to about 50% if inhibitor and substrate (5-hydroxytryptophan, 0.05 *M*) were added simultaneously. Addition of pyridoxal-5′-phosphate was without effect on the inhibition, which suggests that Ro 4–4602 does not act through interference with the coenzyme.

Preincubation of the enzyme together with the inhibitor without substrate (5-hydroxytryptophan) increased decarboxylase inhibition progressively for 2 h. This rise has probably to be attributed to hydrolysis of the seryl hydrazide link, since the inhibition caused by 2,3,4-trihydroxybenzylhydrazine (II)[17] (aralkyl-hydrazine part of Ro 4–4602 (I)) was not progressive.



I



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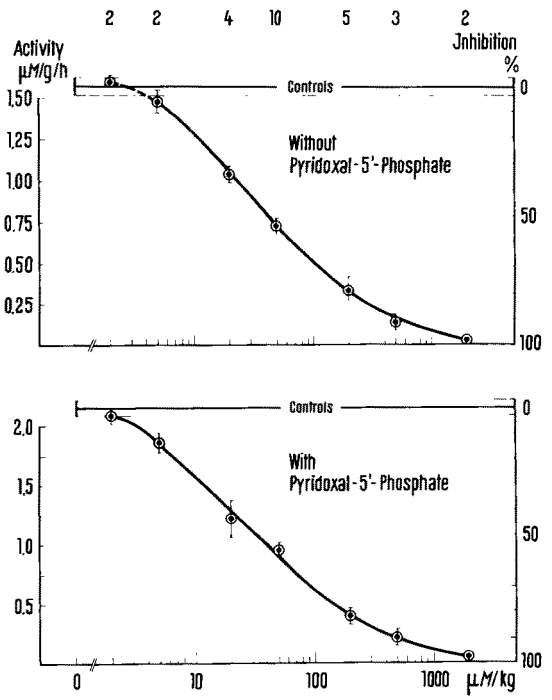

Fig. 1. Inhibition of 3,4-dihydroxyphenylalanine decarboxylation in rat brain *in vivo* with and without coenzyme addition to the incubation medium. *Ordinate:* left: enzyme activity (formation of 3-hydroxytyramine in µM/g/h); right: inhibition in % as compared to untreated controls. *Abscissa:* i.p. dose of Ro 4-4602 1 h prior to decapitation (logarithmic scale). *Vertical lines:* standard errors of mean values. The mean values are derived from 2–10 experiments (as indicated on the top), each with 5 pooled brains.

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Dialysis of supernatant and Ro 4–4602 against phosphate buffer did not significantly reduce the inhibition of 3,4-dihydroxyphenylalanine decarboxylation (inhibition without dialysis: 89 ± 4%; inhibition after dialysis: 78 ± 2%; $p > 0.01$).

In vivo: Intraperitoneal injection of 30–200 μM/kg Ro 4–4602 induced maximal inhibition of 3,4-dihydroxyphenylalanine decarboxylation in brain and kidney within 20–60 min. The decarboxylase activity was restored after about 3 days with and without addition of pyridoxal-5'-phosphate. Within 1 h a 50% inhibition of decarboxylase was obtained by 42 μM/kg Ro 4–4602 (corresponding to about 12 mg/kg) in brain (Figure) and by 0.5 μM/kg Ro 4–4602 in kidney. Addition of pyridoxal-5'-phosphate to the incubation medium had no influence on the effect of the inhibitor.

Monoamine oxidase activity in brain was not inhibited significantly ($p < 0.01$) within 16 h after i.p. application of doses as high as 1.0 mM/kg (293 mg/kg) Ro 4–4602.

According to the above-mentioned results, Ro 4–4602 probably inhibits decarboxylase *in vivo*. It can, however, not be excluded that an *in vitro* effect might be involved. Ro 4–4602 present in the blood and the extracellular fluid possibly causes or enhances decarboxylase inhibition only after homogenization of the tissues, whereas *in vivo* the penetration of the drug to the site of the enzyme might be hindered.

Preliminary measurements of the 5-hydroxytryptamine and catecholamine content of various organs showed, however, that Ro 4–4602 has at least some activity *in vivo*. Thus, after single and repeated doses of the compound the level of the amines in different tissues decreased (Table). Furthermore, the increase of 5-hydroxytryptamine in heart and brain induced by 5-hydroxytryptophan could be completely inhibited by Ro 4–4602[23]. These effects are probably produced by inhibition of the decarboxylation of the monoamine precursors, such as 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine. It remains to be elucidated why repeated high doses of Ro 4–4602 decreased the content of endogenous monoamines only moderately. The following explanations have to be considered: (a) Only a small fraction of the decarboxylase activity available in normal tissues might be sufficient for the physiological rate of decarboxylation. (b) The amine stores cannot be completely depleted despite marked reduction of monoamine formation.

Effect of repeated i.p. administration of Ro 4-4602 (6 × 500 mg/kg within 4 days) on catecholamine and 5-hydroxytryptamine content of various organs.

		Endogenous monoamines in % of controls	
		Catecholamines	5-Hydroxytryptamine
Mice	Brain	88 ± 5[a]	58 ± 6
	Heart	48 ± 5	
Guinea pigs	Brain	64 ± 5	64 ± 6
	Heart	41 ± 10	
	Duodenum		65 ± 8
	Adrenals	26 ± 5	

[a] $0.01 < p < 0.05$; p of all other values < 0.01.

Zusammenfassung. N-(*DL*-Seryl)-N'-(2,3,4-trihydroxybenzyl)hydrazin (Ro 4–4602) bewirkt *in vitro* starke Hemmung der Decarboxylase, die durch Pyridoxal-5'-phosphat nicht und durch Dialyse nur wenig vermindert werden kann. Nach i.p. Applikation der Substanz kommt es zu starker Verminderung der Decarboxylase-Aktivität und zu mässiger Herabsetzung von 5-Hydroxytryptamin und Noradrenalin in verschiedenen Organen. Monoaminoxydase des Gehirns wird *in vivo* durch Ro 4–4602 nicht beeinflusst.

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Medizinische Forschungsabteilung der F. Hoffmann-La Roche & Co. A.G., Basel (Switzerland), June 1, 1962.

[23] A. PLETSCHER et al., in preparation (1962).

The Golgi Apparatus in the Male Germ-Cells of *Vaginula* (Pulmonata)

NATH[1], in his review of work on spermatogenesis, refers to my paper on the spermatogenesis of *Vaginula*[2] (a pulmonate) and states that the Golgi body described by me as such is not Golgi body 'as all the other previous workers on the spermatogenesis of the pulmonate gastropods have described the Golgi body as a dictyosome'. He refers to his earlier view[3] that the inclusion I have described as Golgi body is a chromatoid body. It is difficult to make out what has led NATH to hold this view. Is it because I find this body to be spherical rather than plate-like? If the body I have described is not a Golgi body, then this organelle must be represented by some other cytoplasmic inclusion. The illustrations in my fuller paper[4], to which NATH does not refer, make it abundantly clear that there is no other cytoplasmic body which can be taken to represent the Golgi apparatus. Surely NATH would not go to the length of thinking that there is no Golgi body in these cells at all. Also, my sketch of the Golgi apparatus of *Vaginula*[4] shows it to be structurally similar to the Golgi elements of other pulmonates as described by GATENBY, BAKER et al. It is correct that I find it spherical rather than a flat platelet, but so does BAKER[5], in *Helix*. I have again studied the spermatocytes of *Vaginula* with phase-contrast microscopy and find I can only confirm my previous description.

NATH further states that to the best of his knowledge I am the only worker 'who has denied any connection between the acrosome and the Golgi complex'. This is incorrect. One of the works listed by NATH in his references is BAKER's paper on *Helix*[5], in which he clearly states that there is no connection between the Golgi apparatus and acrosome-formation. BAKER[5] (p. 303) states 'the

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