Accumulation of Dopamine in the Parenchyma after Decarboxylase Inhibition in the Capillaries of Brain

In the brain of rats, relatively small doses (50 mg/kg i.p.) of Ro 4-4602[1], an inhibitor of decarboxylase of aromatic amino acids, markedly enhance the increase of ^{14}C-catecholamines induced by ^{14}C-3,4-dihydroxyphenylalanine (^{14}C-dopa), whereas in the heart and blood the drug antagonizes the rise of these amines[2-4]. This action of Ro 4-4602 seems to be due to a relatively selective inhibition of the decarboxylation of ^{14}C-dopa in extracerebral tissues leading to an accumulation of ^{14}C-dopa in the blood and in consequence to an increased penetration of the amino acid into the brain. In this organ, ^{14}C-dopa is probably transformed into ^{14}C-catecholamines (especially ^{14}C-dopamine), since Ro 4-4602 does not markedly inhibit cerebral decarboxylase. It has not yet been shown, however, whether the accumulation of catecholamines caused by Ro 4-4602 + dopa takes place in the parenchyma or in the capillaries of the brain. In fact, after injection of dopa, the transformation of the amino acid into dopamine and the accumulation of dopamine (especially in mice pretreated with a monoamine oxidase (MAO) inhibitor) occurs to a great part in the walls of the cerebellar capillaries which probably constitute the blood/brain barrier for dopamine[5-8].

In the present work, attempts were made to localize the accumulation of catecholamines in the brain after administration of 50 mg/kg Ro 4-4602 together with dopa.

Experimental. Albino rats of 200–250 g were pretreated with 50 mg/kg of Ro 4-4602 or 250 mg/kg of the MAO inhibitor nialamide i.p. $^1/_2$ and 2 h respectively before administration of 75 mg/kg of unlabelled dopa i.p. Animals treated with dopa alone as well as untreated rats served as controls. One hour after dopa injection, the animals were sacrificed by decapitation, and dopamine as well as norepinephrine were determined in the pallium and in the caudate nucleus according to spectrophotofluorimetric procedures[9,10]. In order to get optimal dopamine fluorescence, the final solution was heated for 5 min in a boiling water bath[11]; irradiation with UV-light was omitted. Furthermore, in the same brain parts, the catechol derivatives were made visible by a specific fluorescence microscopic method whereby catechol com-

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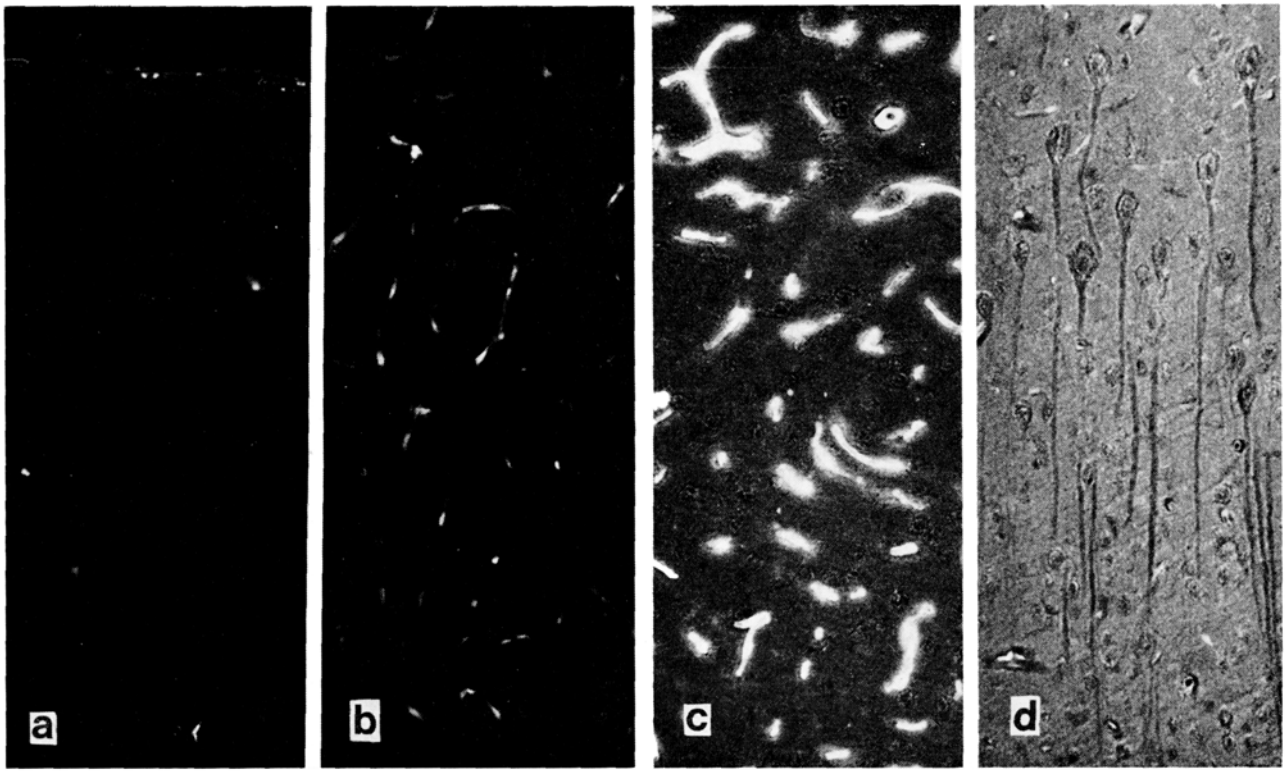

Fluorescence micrographs of the cortex of rat's brain. Frontal sections. Exposure time 10 sec. (a) no treatment, (b) dopa alone, (c) nialamide + dopa, (d) Ro 4-4602 + dopa. 75 mg/kg dopa was injected i.p. either alone or 30 min after 50 mg/kg Ro 4-4602 i.p. or 120 min after 250 mg/kg nialamide i.p. Sacrifice 1 h after dopa.

Effect of Ro 4-4602 or nialamide on the dopa-induced increase of dopamine and norepinephrine in different parts of the rat's brain

Treatment	Pallium		Caudate nucleus	
	Dopamine	Norepinephrine	Dopamine	Norepinephrine
Untreated	0.25 ± 0.06	0.26 ± 0.01	6.36 ± 0.90	0.48 ± 0.03
Dopa alone	0.56 ± 0.10	0.44 ± 0.04	8.00 ± 0.30	0.51 ± 0.07
Ro 4-4602 + dopa	1.01 ± 0.10	0.32 ± 0.01	18.56 ± 1.20	0.57 ± 0.04
Nialamide + dopa	8.50 ± 0.07	0.69 ± 0.04	24.90 ± 0.75	0.90 ± 0.04

Each value represents an average ± S.E. of 3–9 experiments. Experimental details see Figure.

pounds, e.g. dopamine, norepinephrine and dopa, exhibited a green fluorescence[12,13].

Results. Histochemical (Figure): brains of untreated rats show a faint diffuse (parenchymal) green fluorescence of the striatum, but no fluorescence of the pallium. One hour after i.p. injection of 75 mg/kg dopa, the diffuse fluorescence of the striatum increases. Furthermore, capillary walls exhibiting a green fluorescence appear in the cortex as well as in the caudate nucleus with adjacent white matter (capsula interna, radiations of the corpus callosum), but no diffuse fluorescence of the cortex is visible. Following combined treatment with nialamide plus dopa (without Ro 4-4602), the green fluorescence of the parenchyma of the caudate nucleus as well as of the capillaries becomes more marked than after dopa alone, but at most a faint diffuse fluorescence appears in the cortical region. Nialamide alone has no marked effect. With Ro 4-4602 + dopa practically no fluorescence of the capillary walls is to be seen, neither in the cortex nor in the caudate nucleus. A marked diffuse green fluorescence appears, however, in the cortex, and the fluorescence of the caudate nucleus is somewhat increased compared with animals treated with dopa alone. In the pallium, numerous neuronal cells including their axons are visible as dark structures in a diffusely fluorescent background. The cell bodies, but not the axons, contain some granules which exhibit green fluorescence. Ro 4-4602 alone does not change the fluorescence picture of the normal brain.

Biochemical (Table): the combination of Ro 4-4602 plus dopa elevates the dopamine content in the pallium and caudate nucleus by several times compared with controls not treated at all or administered dopa only. In contrast, the norepinephrine content is not significantly increased ($p > 0.05$). The combination of nialamide plus dopa also causes a considerable elevation of dopamine and a much less marked but significant rise of norepinephrine in the pallium as well as in the caudate nucleus ($p < 0.01$). The increase of dopamine is more pronounced with nialamide + dopa than with Ro 4-4602 + dopa. Ro 4-4602 alone does not influence the endogenous catecholamines in the brain, whereas nialamide causes a relatively slight rise.

Discussion. According to previous experiments, relatively high doses of Ro 4-4602 (100–200 mg/kg i.p.) diminish the dopa-induced appearance of the green fluorescence in the capillaries in the cerebellum of mice. Simultaneously, the drug enhances the diffuse background fluorescence which seems to be mainly due to an accumulation of dopa in the brain parenchyma[5,6]. The present findings demonstrate that in rats with relatively low doses of Ro 4-4602 (50 mg/kg) + dopa, a similar histological picture can be produced. In contrast to the previous results, however, the dopamine is markedly increased (Table). Since the capillaries show practically no green fluorescence, most of the amine is probably localized together with dopa in the brain parenchyma. The marked enhancement of the rise of cerebral dopamine after peripheral inhibition of decarboxylase (by 50 mg/kg Ro 4-4602) as found in earlier experiments[2–4] seems therefore to be mainly due to an accumulation of the amine in the brain parenchyma. This localization is different from that after combined treatment with MAO inhibitors + dopa which induces a considerable accumulation of the amine in the capillaries[5–8] (Figure).

The fact that previous authors did not find a major increase of cerebral dopamine after Ro 4-4602 + dopa in mice[5–7] might be due to the relatively high doses of the inhibitor which probably also interfered with decarboxylase of the brain parenchyma. In fact, after 200 mg/kg of Ro 4-4602 i.p. a marked inhibition of decarboxylase in homogenates of rat brain was found, whereas with 50 mg/kg no inhibition of the enzyme occurred[2].

The relatively selective inhibition of extracerebral decarboxylase by low doses of Ro 4-4602 has been attributed to a poor penetration of the drug from the blood into the brain[2]. According to the present investigation, the barrier mechanism for Ro 4-4602 might be located in the wall of the brain capillaries.

In conclusion, low doses of Ro 4-4602 probably inhibit dopa decarboxylase not only in extracerebral organs[2–4], but also in the walls of the brain capillaries, whereas the enzyme seems to remain active in the brain parenchyma. After administration of Ro 4-4602 + dopa, the amino acid which accumulates in the blood[2–4] probably penetrates the brain capillaries and is decarboxylated into dopamine within the parenchyma. This method of increasing cerebral dopamine might be of use in the treatment of parkinsonism since in this condition a marked decrease of the amine in the extrapyramidal brain centres has been observed[14].

Zusammenfassung. Im Pallium und im Nucleus caudatus des Rattenhirns verstärkt der Decarboxylasehemmer Ro 4-4602 den durch Dopa bedingten Anstieg von Dopamin. Mit einer fluoreszenzmikroskopischen Methode wird gezeigt, dass Dopamin wahrscheinlich im Parenchym und nicht in den Kapillaren des Gehirns akkumuliert.

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