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Passage into the rat brain of dopa and dopamine injected into the lateral ventricle

J. CONSTANTINIDIS, J. M. GAILLARD, F. GEISSBUHLER AND R. TISSOT

The University Psychiatric Clinic of Geneva, Bel-Air, 1225, Geneva, Switzerland

Summary

1. The green fluorescence of catechols of the brain was studied in rats after intraventricular injection of L-dopa or dopamine in untreated rats as well as in rats in which dopa decarboxylase (DC) was inhibited by Ro 4602, or the monoamine oxidase by nialamide.

2. From the patterns of fluorescence obtained in these conditions, it is concluded that in the areas close to the liquor space dopa is rapidly taken up by the endothelium of the brain capillaries and then converted into dopamine ; when the DC is inhibited the dopa passes freely from the endothelium into the brain tissue.

3. On the other hand, dopamine passes from the liquor space via the ependyma directly into the brain tissue and from there into the capillary endothelium which is thus permeable to the amine in the direction from the brain tissue, in contrast to the impermeability in the direction from the capillary lumen.

Introduction

Although the concentration of homovanillic acid (HVA) increases in the cerebrospinal fluid (c.s.f.) of animals, including man, on oral or parenteral administration of dopa, this does not happen when its administration is coupled with that of Ro 4602, an inhibitor of decarboxylase (DC) in amounts which are sufficient to block peripheral DC, but insufficient to block the enzyme in the nervous tissue of the brain. Under this condition it was found that the HVA concentration fell in the c.s.f. of rabbits, that the fall was associated with a rise in the dopamine concentrations of the brain (Bartholini, Pletscher & Tissot, unpublished results), and that in man, too, there was a fall in the HVA concentration of the c.s.f. (Tissot & Geissbühler, unpublished results).

Bertler, Falck & Rosengren (1964) have shown that apart from the usual blood-brain barrier for amines, which is probably of a physical nature (Weil-Malherbe, Axelrod & Tomchick, 1959), an enzymatic blood-brain barrier exists for dopa. At the endothelial level of the cerebral capillaries most of the blood borne dopa is decarboxylated to dopamine by DC and then further metabolized into acids, most likely into HVA, so that only a small fraction of the dopa passes into the nervous tissue of the brain, where it undergoes catecholamine synthesis. After the administration of Ro 4602 in amounts which block DC in the periphery and endothelium of the brain capillaries, but not in the nervous tissue of the brain, the situation is

changed: dopa is now poorly metabolized at the peripheral level and at the endothelium of the brain capillaries; it thus passes freely into the nervous tissue of the brain where it is synthesized to catecholamines. There is therefore a greater rise in the catecholamine concentrations of the brain when dopa is given after the administration of Ro 4602 than when given alone (Bartholini, Bates, Burkard & Pletscher, 1967; Bartholini & Pletscher, 1968; Constantinidis, Bartholini, Tissot & Pletscher, 1967, 1968).

In these experiments described here L-dopa and dopamine were injected into the cerebral ventricles of rats and their brains were subsequently examined under the fluorescence microscope. From the pattern of green fluorescence obtained after the injections in otherwise untreated rats, or in rats in which the MAO was inhibited by nialamide or the DC was inhibited by Ro 4062, it was concluded that dopa behaved in the same way when applied by the intraventricular route as when given systemically. It passed into the endothelium of the brain capillaries before passing into the brain substance. This, however, is not the pathway for dopamine.

Methods

Ten-week-old Wistar NW albino rats weighing about 250 g were used. Under general anaesthesia (atropine (8μg/kg) + pentobarbitone sodium (55 mg/kg i.p.)) and aseptic conditions, a cannula was implanted in the right lateral ventricle according to the procedure described by Hayden, Johnson & Maickel (1966). Two days later, when the rats had recovered, 50 μg of L-dopa or a massive dose of dopamine (1 mg), was injected without anaesthesia through the indwelling cannula into the ventricles in a volume of 10 μl. The animals were killed 1 h later by decapitation, the brains were removed, lyophilized and treated according to the method of Falck, Hillarp, Thieme & Torp (1962) before examination under the fluorescence microscope for green fluorescence.

Some rats received an intraperitoneal injection of 250 mg/kg nialamide 2 h before, others of 50 mg/kg Ro 4602 [N^1- (DL-seryl)-N^2 (2,3,4-trihydroxybenzyl)-hydrazine], 0·5 h before, the intraventricular injection of either L-dopa or dopamine.

Results

L-*Dopa*

Intraventricular injection of L-dopa resulted in a green fluorescence of the endothelium of the capillaries in the walls of the lateral ventricles, third ventricle, aqueduct and fourth ventricle. The capillary fluorescence was most intense in the regions adjacent to the ventricular lumen and decreased progressively as the distance from the lumen increased. This is illustrated in Fig. 1a and Fig. 3a for the walls of the right lateral ventricle, and in Fig. 2a for the walls of the third ventricle. In addition to this capillary fluorescence there was a diffuse green background fluorescence of the brain tissue, more pronounced in grey than in white matter. In Fig. 1a the more pronounced fluorescence of grey matter is illustrated for the head of the corpus striatum and for the septum, whereas in Fig. 1b the weaker fluorescence of white matter is shown for the corpus callosum and the superficial layer of the hippocampus. Pronounced diffuse green fluorescence was also present in the nucleus coeruleus, in the 5-hydroxytryptamine containing nuclei of the raphe dorsalis and obscurus and, though less intense, in the substantia nigra, regions

probably reached by penetration of the dopa from the aqueduct and floor of the fourth ventricle.

Little fluorescence was produced in the choroid plexus (Fig. 2a) and none in the ependyma (Fig. 1). The slight fluorescence of the ependyma seen in Fig. 1a is natural, visible without injection or after an injection into the ventricles of 10 μl of saline solution instead of dopa.

As the dopa injected intraventricularly passed through the foramina of Luschka into the subarachnoid space it produced capillary and diffuse background fluorescence also in the superficial structures of the brain stem, particularly in the basal part of the ponto-bulbar region, and in the vermis of the cerebellum. The capillary fluorescence of the vermis is illustrated in Fig. 3c.

After pretreatment with nialamide, the only change produced by the dopa injection was a more intense capillary fluorescence which extended deeper into the ventricular walls and the tissues adjoining the subarachnoid space.

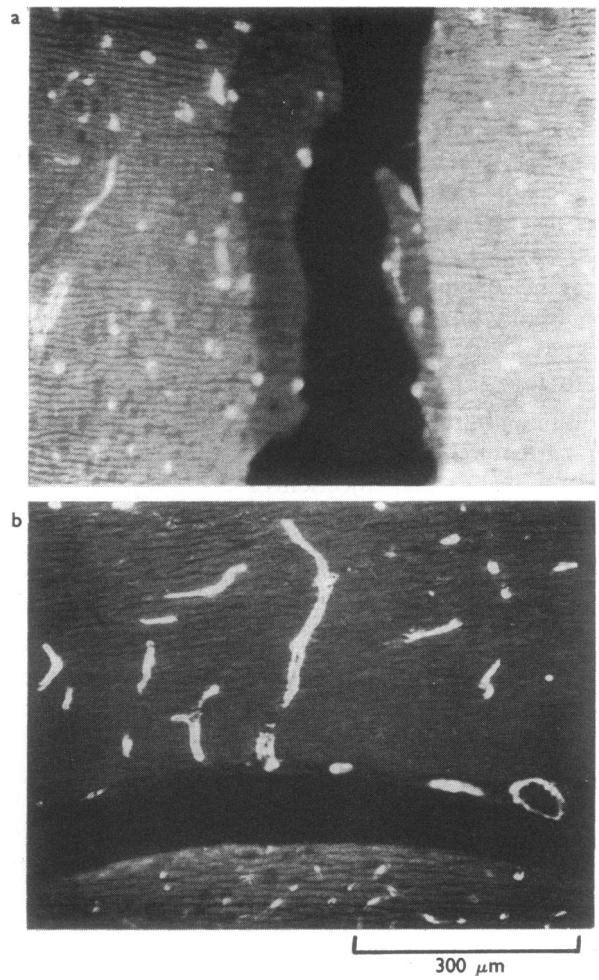

FIG. 1. Section of rat brain seen under the fluorescence microscope after an intraventricular injection of 50 μg L-dopa. (a) Frontal section passing through left lateral ventricle shown in black; right, head of striatum; left, septum. (b) Frontal section passing through left lateral ventricle shown in black. Above, corpus callosum; below, hippocampus. (×100.)

After pretreatment with the DC inhibitor Ro 4602, the dopa no longer produced capillary fluorescence, but the diffuse, green fluorescence of the brain tissue near the ventricular cavities and subarachnoid space was more intense. This fluorescence is illustrated for the hippocampus in Figs. 2b and 3b, and for the vermis in Fig. 3d.

Dopamine

The intraventricular injection of 1 mg resulted in intense diffuse fluorescence of the choroid plexus, the ependyma, the ventricular walls and the superficial layers of the brain stem and vermis. This fluorescence is shown in Fig. 4a for the walls of the third ventricle, in Fig. 5a for the walls of the right lateral ventricle and for the choroid plexus, and in Fig. 4b for the brain stem. As the distance from the liquor spaces increased the intensity of the diffuse fluorescence of the brain tissue

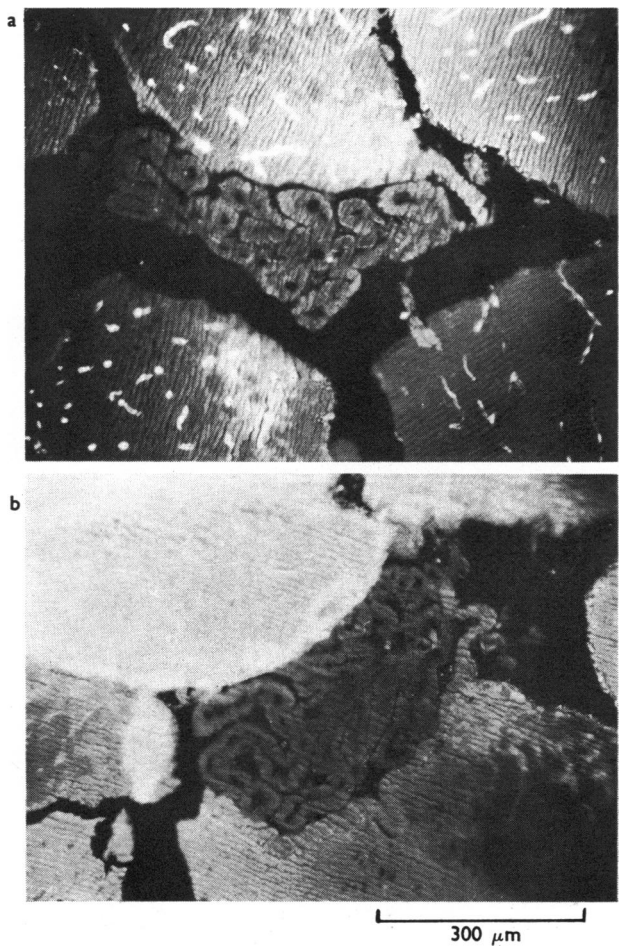

FIG. 2. Frontal sections of rat brain seen under the fluorescence microscope after an intraventricular injection of 50 μg L-dopa without (a) and with (b) pretreatment by Ro-4602. The section passes through the upper part of the third ventricle shown in black. Above, hippocampus; below, thalamus; inside ventricle, choroid plexus (×75).

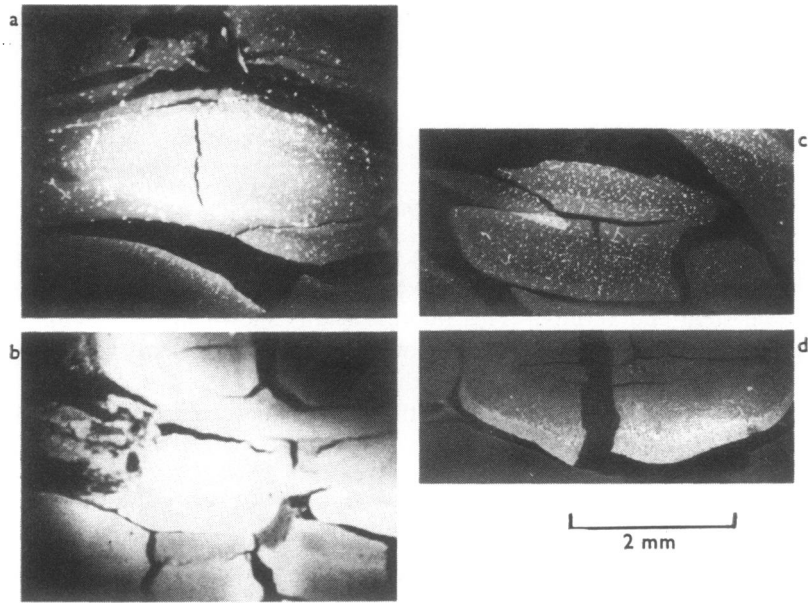

FIG. 3. Sections of rat brain seen under the fluorescence microscope. (a and b), Frontal sections passing through the hippocampus; (c and d), frontal sections passing through the vermis. After an intraventricular injection of 50 μg L-dopa without (a and c) and with (b and d) pretreatment with Ro-4602 (×11).

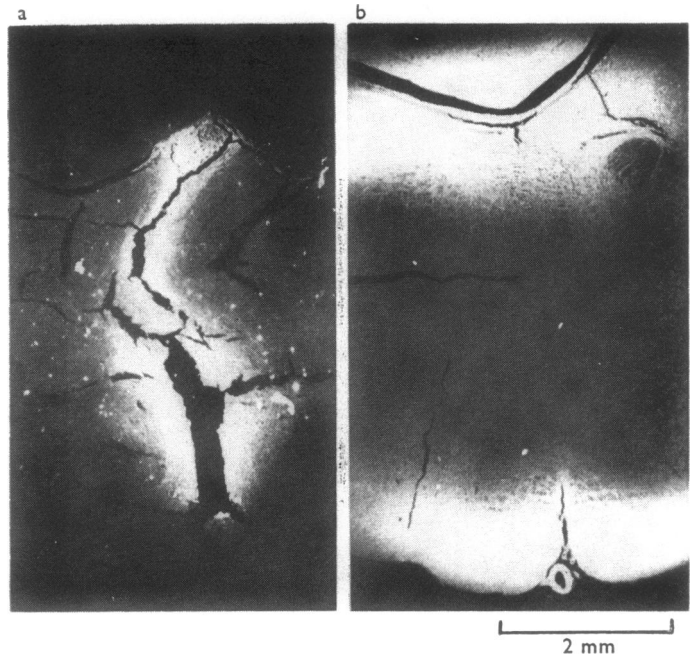

FIG. 4. Sections of rat brain seen under the fluorescence microscope after an intraventricular injection of 1 mg dopamine. (a), Frontal section passing through the third ventricle shown in black; in the upper part of the ventricle choroid plexus. (b) Frontal section of medula oblongata; above, IVth ventricle shown in black; below, pyramidal tracts and basilar artery (×11).

diminished progressively and finally disappeared ; there was then a zone with green fluorescence of the capillary endothelium, but with no fluorescence in the capillary lumen. The fluorescence of the endothelium is illustrated in Fig. 5b.

After pretreatment with nialamide the only difference was that the fluorescence produced by the intraventricular injection of dopamine was more intense and more widespread. After pretreatment with Ro 4602 there was no difference. As shown in Fig. 5c, the endothelial fluorescence persisted.

Discussion

The pattern of green fluorescence seen in the brain after an intraventricular injection of L-dopa suggests that only a small part of the acid passes from the liquor spaces into the brain tissue and remains there, but that most of it is rapidly taken up by the capillary endothelium and converted into dopamine by the endothelial decarboxylase. This is similar to the fate which befalls L-dopa when injected intravenously or intraperitoneally. On the other hand, when the endothelial decarboxylase is inhibited by an intraperitoneal injection of Ro 4602, the dopa injected into the ventricles passes freely from the capillary endothelium into the brain tissue. Thus the passage of dopa from the liquor spaces into the brain on intraventicular injection appears to be similar to that from the blood stream into the brain on intravenous or intraperitoneal injection. This passage occurs across the endothelial cell layer and its decarboxylase, which transforms L-dopa into dopamine, constitutes an enzymatic barrier between blood and brain, and between cerebrospinal fluid and brain.

L-Dopa undergoes the same fate when injected directly into the cerebral parenchyma, as shown by Bertler, Falck, Owman & Rosengren (1966) and confirmed by us (unpublished experiments). At the injection site there is an intense green fluo-

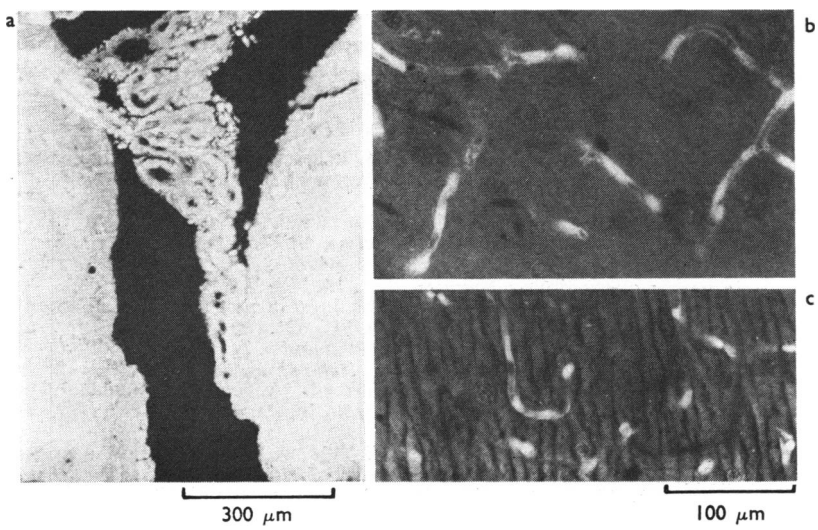

FIG. 5. Sections of rat brain seen under the fluorescence microscope after an intraventricular injection of 1 mg dopamine. (a) Frontal section passing through left lateral ventricle shown in black ; left, striatum ; right, septum ; inside ventricle, choroid plexus. (b), Section from deeper area of striatum. (c) Similar section to (b) but from a rat pretreated with Ro-4602 (×173).

rescence of the brain tissue which diminishes as the distance from the injection site increases ; in addition, there is strong fluorescence of the endothelium but not in the lumen of the capillaries. The endothelial fluorescence occurs only around the needle tract and a few millimetres away from it, but not in more distant regions. Therefore, it does not result from passage of the L-dopa into the general circulation.

The situation is different for dopamine. Bertler *et al.* (1966) have shown that an intraperitoneal injection of a large dose produces green fluorescence in the lumen of the capillaries, but not in its endothelium, and that an injection into the brain substance causes diffuse fluorescence near the injection site as well as endothelial fluorescence. There are further observations on reserpinized rats by Fuxe & Ungerstedt (1968) who studied catecholamine uptake after an intraventricular injection of dopamine and noradrenaline by examining the green fluorescence of the brain. In a zone close to the ventricles and subarachnoid space, an uptake occurred in those neurones which normally contained catecholamines or 5-HT and had been depleted of their amines by the reserpine, but no uptake occurred in other neurones. There was in this zone, however, a diffuse, green background fluorescence due to diffuse extraneural localizations and an intense green fluorescence of the capillary endothelium as well as of the ependyma.

The pattern of fluorescence found in these experiments after an intraventricular injection of a massive dose of dopamine also suggested that the amine passes from the liquor space via the ependyma directly into the brain tissue and from there into the endothelium of the brain capillaries. This explains the intense fluorescence found in the ependyma and in the brain tissue surrounding the ventricular cavities and adjacent to the subarachnoid space, a fluorescence that was not affected by pretreatment with Ro 4602 and somewhat increased by pretreatment with nialamide. The finding that inhibition of the DC with Ro 4602 did not abolish the endothelial fluorescence is good evidence for the view that it results from dopamine that has reached the endothelium from the brain parenchyma and not from the capillary lumen. The endothelial membrane which is impermeable to dopamine from the blood or capillary lumen is thus permeable to the amine in the opposite direction, that is from the brain parenchyma.

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