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B. THE LOCALIZATION OF MONOAMINERGIC BLOOD-BRAIN BARRIER MECHANISMS

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It has recently been demonstrated that dopamine, norepinephrine (NE), and 5-hydroxytryptamine (5-HT) occur in neurons within the central nervous system (2, 5, 11, 16, 17, 30), where they probably serve as transmitters (3, 4, 34). Such neurons seem to be the storage sites of enzymes, *e.g.*, dopa decarboxylase and monoamine oxidase (MAO), which are involved in the synthesis and breakdown of these amines (1, 6, 35, 49, 50). However, there are reasons to assume that both the decarboxylating enzyme and MAO have also an extraneuronal location. Thus, it has been shown that after transection of the spinal cord a complete loss of monoamines occurs below the lesion (16, 19, 44) but only a partial disappearance of dopa decarboxylase, this enzyme still being present at a stage when the spinal adrenergic neurons must be assumed to have undergone a complete degeneration (7). Furthermore, significant activities of both dopa decarboxylase and MAO are found in brain areas, *e.g.*, the cerebral cortex and the cerebellum, that contain only a small amount of catecholamines and 5-HT (14, 15, 51).

Preliminary observations have suggested that part of the dopa decarboxylase and MAO present in the central nervous system of the rat and mouse is located in the capillary walls (12). The present report deals in greater detail with the problem whether these enzymes constitute a specific barrier mechanism in the capillaries of the central nervous system (including the optic tract and retina). A combination of chemical and histochemical methods has been applied which allows a direct demonstration of certain monoamines and their immediate precursors at the cellular level.

MATERIALS AND METHODS

Histochemical investigations. Most of the experiments in the histochemical part of the present study were performed on adult mice of both sexes, weighing 20 to 35 g. The animals were subjected to the treatments summarized in table 1.

In 9 other (etherized) mice, 0.01 ml of a saline solution of 0.5% L-dopa was deposited in the cerebellar parenchyma by a fine cannula (outer diameter 0.5 mm) through a burr hole in the calvarium. A further 9 mice were similarly given a 5% solution of dopamine. The animals were killed 1 hr after the administration.

The spinal cords of 6 rats weighing 200 to 250 g were transected at the Th 3 level under ether anesthesia. After 10 days 4 of the animals received nialamide and L-dopa and 2 were given nialamide alone (doses and times as in table 1).

The cellular localization of dopa and catecholamines was studied with the fluorescence method of Falck and Hillarp (22, 23, 28, 29). Immediately after decapitation of the animals, performed under light ether anesthesia, the following tissues were rapidly dissected out: eye (including the distal portion of the optic nerve), hypothalamus, area postrema, cerebellum, and cervical (in the rats also

TABLE 1

Experimental design of histochemical investigation

Exp. No.	Pretreatment[a]	Dose	Time[b]	Treatment[a]	Dose	Time[c]	No. of animals
		mg/kg	*min*		*mg/kg*	*min*	
I				Untreated			34
II				L-Dopa	50	20	11
III a				L-Dopa	10, 20, 40, 80, 160	20	4/group
III b				L-Dopa i.v.	10, 20, 40, 80, 160	20	4/group
IV				D-Dopa	500	20	6
V	*m*-Hydroxybenzyl hydrazine[d]	100	60	L-Dopa	50	20	9
VI	*m*-Hydroxybenzyl hydrazine	100	80				6
VII a	Nialamide[e]	100	60	L-Dopa	50	20	13
VII b	Pargyline[f]	100	60	L-Dopa	50	20	4
VII c	NSD 2023[g]	30	10	L-Dopa	50	20	4
VIII	Nialamide	100	80				6
IX	Nialamide + *m*-Hydroxybenzyl hydrazine	100 100	240 60	L-Dopa	50	20	6
X	Reserpine[h]	5	60	L-Dopa	50	20	4
XI				Dopamine	50, 100, 300, 500	20	4/group
XII	Nialamide	100	60	Dopamine	500	20	16

[a] Intraperitoneal if nothing else stated.
[b] Interval between pretreatment and treatment.
[c] Interval before killing.
[d] NSD-1015 (Smith and Nephew).
[e] Niamid (Pfizer).
[f] MO 911 (Eutonyl, Abbott).
[g] Butyrophenone derivative (Ferrosan).
[h] Serpasil (Ciba).

lumbar) spinal cord. The whole brain was removed in 21 mice. A peripheral preparation consisting of trachea, thyroid, larynx, and surrounding muscle tissue was also removed. All specimens were freeze-dried, treated in formaldehyde gas, embedded in paraffin, sectioned at 6 μ, and mounted for fluorescence microscopy, all according to the descriptions by Falck and Owman (31) and Hamberger *et al.* (37).

Chemical investigations. The experiments in this section were performed in mice divided into four groups: (a) normal untreated; and treatment with (b) L-dopa; (c) *m*-hydroxybenzyl hydrazine and L-dopa; and (d) nialaminde and L-dopa. Doses and times used were the same as those appearing in table 1. The animals were decapitated under light ether anesthesia. Dopamine and NE were determined fluorimetrically in the cerebellum as described previously (10, 13, 20). The content of dopa was estimated chromatographically (9) in four ex-

periments with extract from the cerebella of 15 mice. The animals were either (a) untreated or treated with (b) L-dopa alone, or (c) *m*-hydroxybenzyl hydrazine and L dopa (doses and times as in table 1). The spots obtained on the paper chromatogram were evaluated semiquantitatively by comparison with a set of references.

RESULTS

1. Normal animals. Fluorescence microscopy of the central nervous system of the mouse revealed widespread systems of nerve-cell bodies and varicose nerve terminals emitting a green or yellow fluorescence due to their content of catecholamines or 5-HT, respectively. The general pattern of distribution of the monoaminergic neurons conforms to previous extensive investigations on the central nervous system (2, 5, 11, 16, 17, 30) including the median eminence (28a, 32), the area postrema (36), as well as the retina (26, 45). No fluorescent nerves were found in the optic tract. A specific yellow 5-HT fluorescence developed also in mast cells located along intracerebral vessels. The number of cells was usually quite small and was subject to individual variations, mast cells being practically absent in some animals.

The monoaminergic nerves of the cerebellum emitted a green fluorescence. Characteristically, only the terminal varicose portion of the fibre was demonstrable. These varicose nerves were mainly distributed in the granular layer, coursing in an irregular manner among the nonfluorescent pericarya, sometimes contiguous to the latter in a manner suggesting a synaptic arrangement. Such fibres were occasionally also seen in close contact with Purkinje cells. Few green-fluorescent fibres issued into the molecular layer, and only scattered axons were found in the medullary layer. A greenish background fluorescence occurred throughout the parenchyma in all specimens; this was very low, however, and did not interfere with the observation of specifically fluorescent neurons. It faded rather rapidly upon ultraviolet irradiation. The choroid plexus appeared dark. The adrenergic innervation of the vessels within the central nervous system was sparse, larger vessels occasionally being accompanied by single axons. The pial vessles, on the other hand, were enclosed by a typical adrenergic ground-plexus (fig. 1) of moderate density (*cf.* 30).

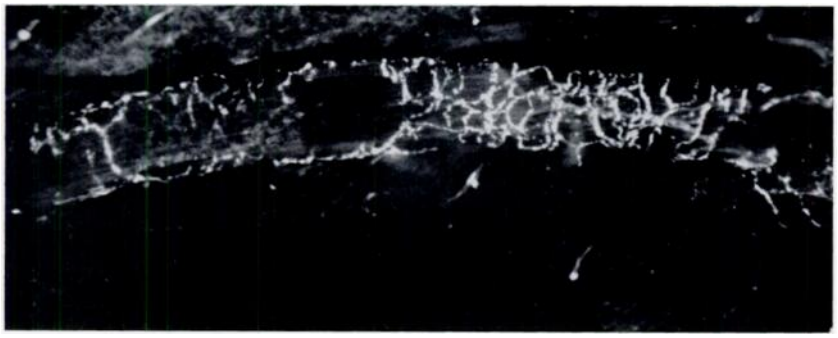

FIG. 1. Pial artery enclosed by a characteristic adrenergic ground-plexus. × 115.

TABLE 2

Summary of pertinent data from results of the histochemical and chemical investigations

Exp No.	Treatment	Capillary fluorescence[a]		Parenchymal fluorescence[a]	NE μg/g ± SE (n)	Dopamine μg/g ± SE (n)	Dopa (n)
		Wall	Lumen				
	dose in mg/kg						
I	Untreated	0	0	(+)	0.18 ± 0.02 (11)	0.00 (8)	Absent (4)
II	L-Dopa (50)	++	0	(+)	0.17 ± 0.02 (11)	0.43 ± 0.01 (5)	Small amounts (4)
V	*m*-Hydroxybenzyl hydrazine (100), L-dopa (50)	(+)	0	++	0.25 ± 0.01 (6)	0.12 ± 0.03 (6)	Large amounts (4)
VI	*m*-Hydroxybenzyl hydrazine (100)	0	0	(+)			
VII a	Nialamide (100), L-dopa (50)	++++	0	(+)	0.40 ± 0.03 (5)	3.5 ± 0.08 (4)	
VIII	Nialamide (100)	0	0	(+)	0.28 ± 0.03 (4)	0.00 (4)	
IX	Nialamide, *m*-hydroxybenzyl hydrazine, L-dopa	(+)	0	++			
X	Reserpine (5), L-dopa (50)	++	0	(+)			
IV	D-Dopa (500)	(+) or 0	+++	(+)			
XI	Dopamine (500)	0	+++	(+)			
XII	Nialamide (100), Dopamine (500)	(+) or 0	+++	(+)			

[a] Fluorescence intensity: 0, nonfluorescent; (+), scarcely any; +, slight; ++, moderate; +++, high; ++++, very high.

In accordance with the amount of catecholamine-containing nerve fibres in the mouse cerebellum, chemical determinations revealed 0.18 μg per g of NE; no dopamine or dopa was present (table 2, I).

2. Effect of L*-dopa.* After the injection of L-dopa (50 mg/kg; table 1, II) no definite change was noted in the fluorescence of the adrenergic nerves and mast cells in the mouse cerebellum, and the cerebellar parenchyma showed the same low greenish background fluorescence as that seen in untreated animals. On the other hand, the larger pial arteries now exhibited a diffuse and relatively strong fluorescence in their media; a weak green light was also observed in the vascular walls and epithelial cells (including the nuclei) of the choroid plexus.

A striking feature, however, was that a moderate green fluorescence had developed in the wall of capillaries distributed throughout the whole cerebellum (*cf.* fig. 3). They were numerous in the gray matter, but were more sparse in the white matter. The fluorescence was diffusely located in the cytoplasm as well as in the nucleus of both the endothelial cells and the pericytes: the slender nucleus of the endothelial cells bulging into the capillary lumen was clearly distinguishable from the short rounded nucleus of the pericytes which bulge outward from the lumen (fig. 2) (*cf.* 41). The content of the capillary lumina was nonfluorescent.

Chemically, no significant change occurred in the NE content (0.17 μg/g) of mouse cerebellum, but it was now possible to demonstrate the presence of dopamine (0.43 μg/g). Only small amounts of dopa could be found (table 2, II).

In the control preparations of peripheral tissues, there was found at the most a weak green overall fluorescence. No accumulation of fluorescent material occurred within the capillary walls. A more pronounced, cytoplasmic fluorescence appeared, however, in parafollicular cells of the thyroid and in many flask-shaped cells located in the tracheal epithelium. It has recently been demonstrated that these special cell systems, which in normal mice are nonfluorescent, readily take up exogenous L-dopa, which is decarboxylated to dopamine within the cells (42, 43).

The difference in response to L-dopa between central and peripheral capillaries was especially apparent in the eye. In all peripheral ocular tissues a weak green

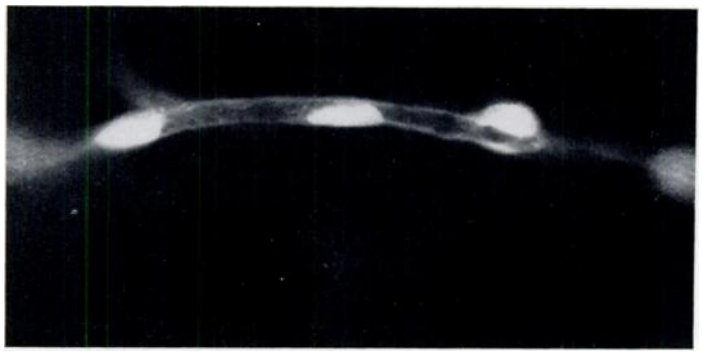

FIG. 2. L-Dopa. Fluorescence in cerebellar capillary wall. The fluorescence is located in cytoplasm as well as nucleus of the endothelial cells, and a pericyte, bulging outward from the lumen (*cf.* 41). × 790.

fluorescence appeared diffusely without any accumulation in capillaries whereas endothelium and pericytes of the capillaries in the retina and optic nerve fluoresced in the same manner as in the other central nervous tissues.

In the dose-response studies using the intraperitoneal route (III a) a fluorescence in the brain capillaries was registered first at the dose level of 40 mg per kg; the intensity was very weak. An increased fluorescence intensity was registered at the two higher dose levels. The outcome of the experiments with intravenous injections (III b) was essentially the same, although a weak capillary fluorescence appeared even at the 20 mg per kg level, and the intensity at the different dose levels was somewhat higher than with the corresponding intraperitoneal injections. With increasing dosages, the fluorescence increased simultaneously in both the endothelial cells and the pericytes. After both the intraperitoneal and the intravenous administrations, a clear-cut increase in the diffuse parenchymal fluorescence occurred first at the 80 mg per kg level.

The cerebellum of animals given L-dopa directly into the cerebellar parenchyma was analyzed in serial sections. The stich channel was easily localized. Around its end-point was found a narrow region of parenchyma having a diffuse green fluorescence of very high intensity. In spite of this it was possible to distinguish a considerable accumulation of fluorescence in the capillary walls, whereas the luminal content seemed to be nonfluorescent. The diffuse fluorescence showed a gradual but rather rapid decrease out to parenchyma with only a very weak background fluorescence, characteristic of untreated animals. Numerous capillaries in this fluorescent area, as well as in the adjacent areas of essentially nonfluorescent parenchyma, also displayed a wall fluorescence varying from moderate to weak. Farther away from the point of injection, however, no fluorescence was found in the capillaries. This shows that the accumulation in the endothelium and pericytes is not due to L-dopa taken up from the circulation. Noncapillary vessels never accumulated any fluorescence.

3. Effect of L-dopa after decarboxylase inhibition. When L-dopa was injected after previous inhibition of dopa decarboxylase (V) there was detected only a very weak capillary fluorescence, often barely visible and confined to the walls. A slight green fluorescence, clearly of higher intensity than after L-dopa administration alone, had developed diffusely throughout the brain parenchyma, including such regions as the median eminence and area postrema. Because of this increased background fluorescence it was not possible to establish whether the fluorescence intensity of monoaminergic neuronal structures had changed. A diffuse light, somewhat more intense than after L-dopa alone, was seen in the choroid plexus.

The NE content (0.25 μg/g) measured fluorimetrically in the mouse cerebellum was slightly higher ($P < .01$) Than after L-dopa injection alone, while the level of dopamine was considerably lower ($P < .001$), although it did not reach zero (0.12 μg/g; $P < .01$). In this experiment a large amount of dopa was found (table 2, V). Thus, the decarboxylase seemed to be sufficiently although not completely inhibited (see Discussion, below).

In the periphery, the general diffuse tissue fluorescence was slightly more

intense than when L-dopa was given alone. There was no accumulation of fluorescent material in the special cells in the thyroid and tracheal epithelium (42, 43).

Central and peripheral tissues from animals receiving only the decarboxylase inhibitor (VI) appeared quite similar to those from untreated animals.

4. Effect of L-dopa after MAO inhibition. After treatment with nialamide, pargyline, or NSD 2023 combined with L-dopa (VII), the parenchyma of the central nervous tissue exhibited the same very slight fluorescence as after L-dopa alone. In the capillary walls as well as in the choroid plexus, the fluorescence intensity was highly increased. Again the content of the brain capillaries seemed completely "drained" of fluorescent material, in contrast to the picture found after D-dopa or dopamine (see below). In the controls given MAO inhibitor only (VIII), no overt changes were registered in comparison with untreated animals.

In the rat, the same picture of highly fluorescent capillaries was seen in the cerebellum and in the spinal cord, above as well as below the transection. The fluorescence of the monoaminergic nerve fibres had almost completely disappeared in the lumbar spinal cord tissue below the lesion (*cf.* 16).

It was now evident that the fluorescent material occurred throughout the capillary walls giving almost a three-dimensional appearance of the capillary tubes in the sections (fig. 3). The distribution of the fluorophore was, however, not quite even; slender, more intensely fluorescent striations were often found to run everywhere in the wall. These striations may represent the processes of

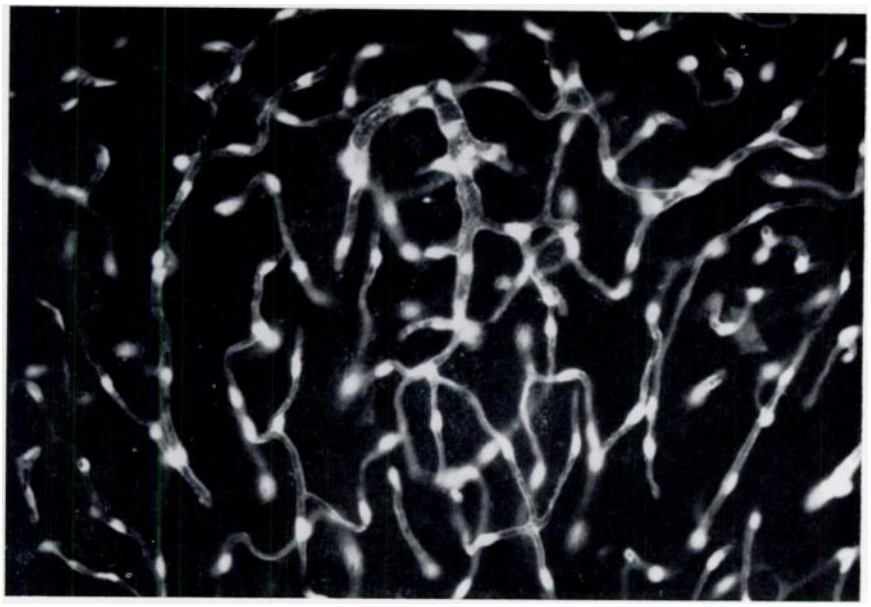

FIG. 3. Nialamide and L-dopa. Three-dimensional network of capillary tubes in cerebellum. Fluorescence of high intensity in cytoplasm and nucleus of both endothelial cells and pericytes. × 250.

the pericytes known to form a discontinuous layer superimposed upon the endothelium.

No fluorescence was observed in the walls of the larger intracerebral vessels, although they contained a certain amount of fluorescent substance in their lumen. On the other hand, the pial vessels, including the thickest arteries, showed a clear-cut fluorescence in their walls and, to some extent, also in their luminal content. The pial membrane itself was moderately fluorescent. A narrow zone of the brain parenchyma located just underneath the pia exhibited a somewhat higher fluorescence intensity than the rest of the brain. It was often possible to follow how from a pial artery with a green fluorescence within the lumen as well as in the wall there issued a radial artery that still possessed luminal fluorescent material but, immediately inside the brain surface, no longer any wall fluorescence.

No capillaries were seen in the eye preparation, except in the retina and optic

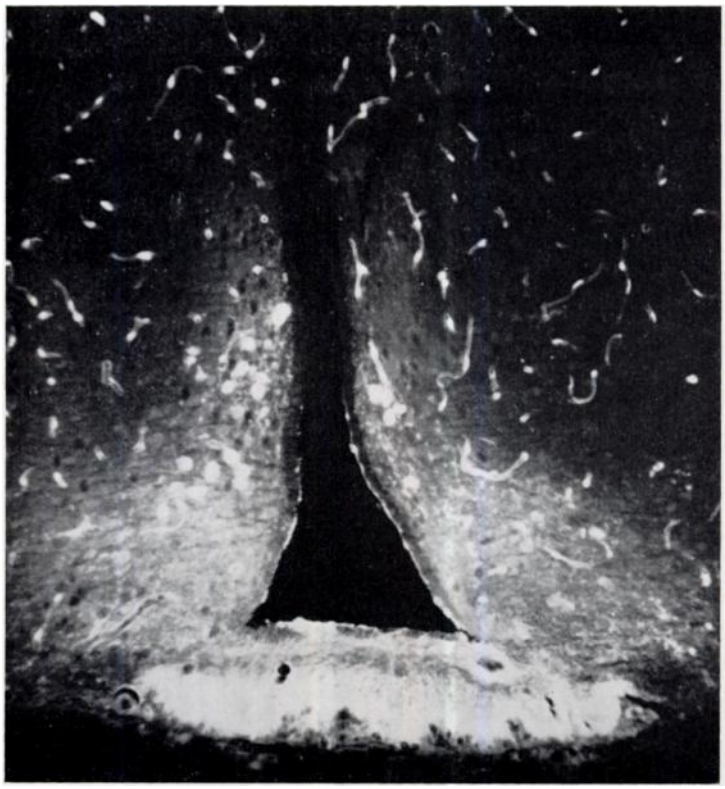

FIG. 4. Nialamide and L-dopa. Frontal section through median eminence. Considerable fluorescence related to portal system (below), seen also in untreated animals (*cf.* 32). Intense, diffuse fluorescence in the parenchyma in and above the median eminence. Fluorescent nerve-cell bodies, but little or no capillary fluorescence. Outside this area practically no parenchymal fluorescence, but several capillaries with wall fluorescence. Note fluorescence at ependymal surface. × 80.

tract, which contained a considerable number of capillaries showing a high fluorescence in both their endothelial cells and their pericytes.

Within and immediately around the median eminence and the area postrema, the fluorescence of the parenchyma was more intense than in the rest of the central nervous system (fig. 4), whereas that of the capillaries was weak or absent. Nerve-cell bodies and varicose fibres with considerably increased fluorescence intensity were found in these areas (*cf.* 36). Highly fluorescent material was present at the surface of the ventricular ependyma (fig. 4).

A somewhat higher parenchymal fluorescence, though only a comparatively weak capillary fluorescence, was also found in a narrow region at the median surfaces of the olfactory bulbs (fig. 5). In this area, a large number of nerve-cell bodies appeared, exhibiting a green fluorescence of moderate intensity in the cytoplasm and nuclei. These nerve cells, which were intermingled with nonfluorescent ones, do not display a specific fluorescence in the normal brain even after MAO inhibition. No accumulation of fluorescent material occurred in the capillaries of the peripheral tissues.

L-Dopa given to mice pretreated with nialamide caused a very marked increase in cerebellar dopamine (3.5 μg/g) compared with L-dopa alone; nialamide itself does not cause any dopamine to appear (table 2, VIIa and VIII). The increase in the NE level was less prominent (0.40 μg/g) and differed with only slight significance ($.01 < P < .02$) from that after nialamide injection alone (0.28 μg/g).

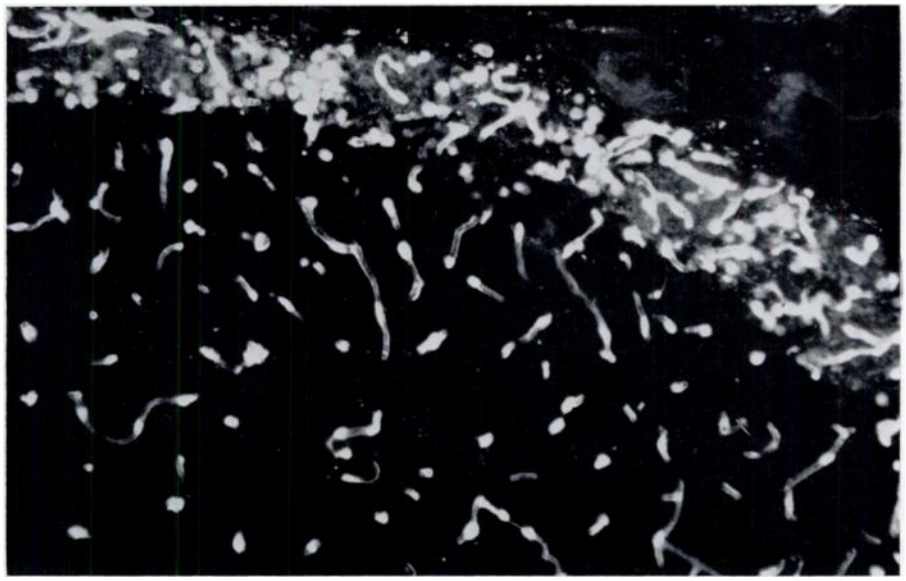

FIG. 5. Nialamide and L-dopa. Medial surface of olfactory bulb (above), frontal section. Increased parenchymal fluorescence in a narrow zone, containing fluorescent nerve-cell bodies and capillaries. Below this zone, capillaries with wall fluorescence in essentially dark parenchyma. × 180.

5. Effect of L*-dopa after combined decarboxylase and MAO inhibition.* The combined enzyme inhibition (IX) followed by administration of L-dopa produced results similar to those from treatment with decarboxylase inhibitor alone and L-dopa (V), that is, the whole brain parenchyma had a diffuse, rather intense fluorescence, and few capillaries, having but a very slight green fluorescence in their walls, could be detected.

6. Effect of L*-dopa after reserpine treatment.* L-Dopa administered to mice previously treated with reserpine (X) resulted in the same vascular fluorescence picture of both central and peripheral tissues as did L-dopa injection alone (II).

7. Effect of D*-dopa.* After a huge systemic dose of D-dopa (IV) a large number of capillaries emitting a green light of high intensity was visible, but the fluorescent material was in this series almost exclusively confined to the capillary lumen, where the erythrocytes stood out as dark spots. Only few capillaries with weak, if any, wall fluorescence could be detected. The parenchyma was essentially nonfluorescent, as in the untreated animals, but a diffuse parenchymal fluorescence of relatively high intensity was seen in certain restricted areas, such as the median eminence, the regions of the arcuate nuclei, and the intercolumnar tubercle. Especially in the region of the arcuate nuclei were found capillaries with fluorescent walls. Further, a strong fluorescence appeared in the lumen, but not in the wall, of larger intracerebral vessels. In contrast to this, the pial vessels were loaded with fluorescent material in their walls. A diffuse intense light was also emitted from the choroid plexus.

As after the L-dopa injections, a pronounced green fluorescence was present diffusely in all tissues of the peripheral tracheal preparation. No accumulation of fluorescent material occurred in the capillaries or in the special cells of the thyroid and tracheal epithelium (*cf.* 42, 43).

8. Effect of dopamine. Groups of mice received dopamine in four dose levels (XI). The results obtained after the administration of the highest dose, 500 mg per kg, agreed with those obtained after D-dopa (IV), *i.e.*, a marked fluorescence appeared in the blood (*cf.* fig. 7) (not in the erythrocytes) but not in the walls of any intracerebral vessels, whereas the pial membrane and the walls of the pial vessels showed a moderate green fluorescence. Essentially the same data were obtained in the 300 mg per kg group, although the fluorescence intensity of the blood was lower. In both groups, a fairly high fluorescence occurred in all tissues of the choroid plexus.

At all dose levels the general background in the brain substance was dark, as it was in untreated animals. A substantial increase in fluorescence, however, was registered in the median eminence, in the region of the arcuate nuclei, and in the intercolumnar tubercle. In the arcuate nuclei, some capillaries fluoresced in their walls. At the two lowest dose levels, no fluorescence was observed in the blood vessel content.

In the tracheal tissue preparation, a diffuse green fluorescence was found everywhere in all groups. The intensity was huge in the two highest dose groups. Only a slight accumulation of fluorescent material was found in parafollicular cells of the thyroid as well as in the special cells in the tracheal epithelium (*cf.* 42, 43).

In one group of animals, dopamine was administered directly into the cerebellar parenchyma, and the cerebellum was analyzed in serial sections. In a rather wide region around the stich channel, the parenchyma showed a great fluorescence intensity of an almost green-yellow to yellow colour, owing to the very high concentration of the amine (*cf.* 31). Because of the intense fluorescence it was not possible to distinguish any details. Farther out in the parenchyma followed a zone with diffuse high green fluorescence which, adjacent to the area around the stich channel, contained capillaries with nonfluorescent lumina and a low to sometimes moderate fluorescence in their endothelium and pericytes. The capillaries in other more peripheral parts of this zone were, like all larger vessels in the whole zone, nonfluorescent and therefore appeared sharply outlined. Outside the zone, the parenchymal fluorescence gradually decreased towards the essentially dark background.

9. Effect of dopamine after MAO inhibition. The whole mouse brain was studied in serial sections after the combined treatment with dopamine and nialamide (XII). The pial vessels were found to be loaded with fluorescent material in their lumen. The walls also fluoresced, but in the thickest arteries only in a narrow zone near the lumen. A green fluorescence was also emitted by the pial membrane. Immediately after entering the brain substance, the radial vessels that issue from the pial vascular system lost their wall fluorescence, although the lumen content was still highly fluorescent. The choroid plexus emitted everywhere a high, diffuse, green fluorescence (fig. 6). Intensely fluorescent material was often present at the surface of the ependymal lining in the ventricular system.

The parenchyma of the central nervous system was, as in untreated mice, essentially nonfluorescent. The capillaries contained intensely fluorescent ma-

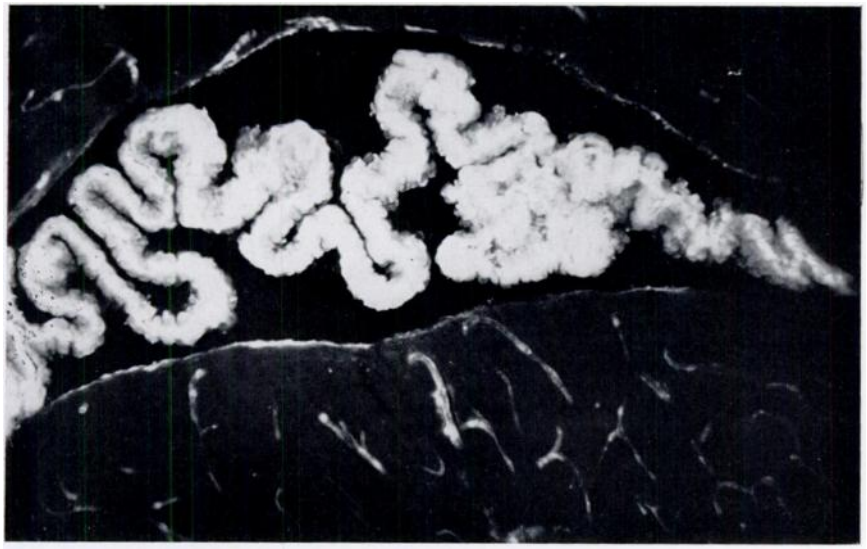

FIG. 6. Nialamide and dopamine. Intense fluorescence in all components of choroid plexus of lateral ventricle. Fluorescence at surface of ventricular lining. Several capillaries with fluorescence in their lumina run in essentially dark parenchyma. × 115.

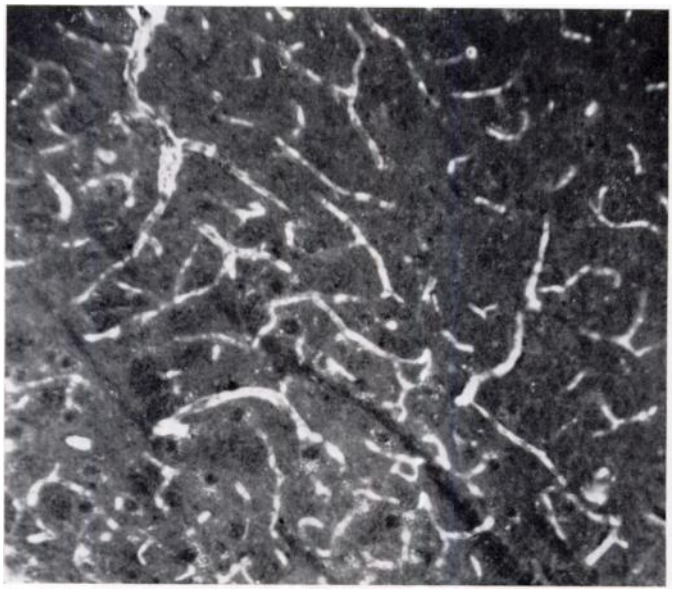

FIG. 7. Nialamide and dopamine. Cerebellum. Numerous capillaries, together with one noncapillary vessel (above, left), emitting an intense fluorescence from their lumen content but little or no fluorescence in their walls (*cf.* fig. 3). Parenchyma essentially nonfluorescent. × 115.

terial in their lumina (fig. 7), but little or no fluorescence was exhibited from the capillary walls (including those of the optic tract); these were completely nonfluorescent in many regions. In certain areas, such as the median eminence, there occurred a clearly increased fluorescence intensity of the adrenergic neurons compared with those found in the rest of the brain. The diffuse parenchymal fluorescence was also higher than in surrounding brain areas. In this region, moreover, were found rather numerous capillaries showing moderate fluorescence in the wall, apart from that of the lumen content. The parenchymal fluorescence was markedly increased also in a peripheral rim at the medial surfaces of the olfactory bulbs (fig. 8). Several moderately green-fluorescent nerve-cell bodies were found in the region, as in experiment IV. As in the median eminence, the capillaries showed an intense luminal fluorescence together with a slight pericyte-endothelial fluorescence.

A considerable fluorescence, without any special accumulation in the capillaries, was registered diffusely in the peripheral tracheal preparation. A high specific accumulation occurred in parafollicular cells of the thyroid, and in the special cells in the tracheal epithelium (42, 43).

DISCUSSION

The concept of a barrier between the blood and the brain parenchyma originates from the observation of Ehrlich (27) that certain aniline dyes pass freely from the circulation into peripheral tissues but are excluded from the central

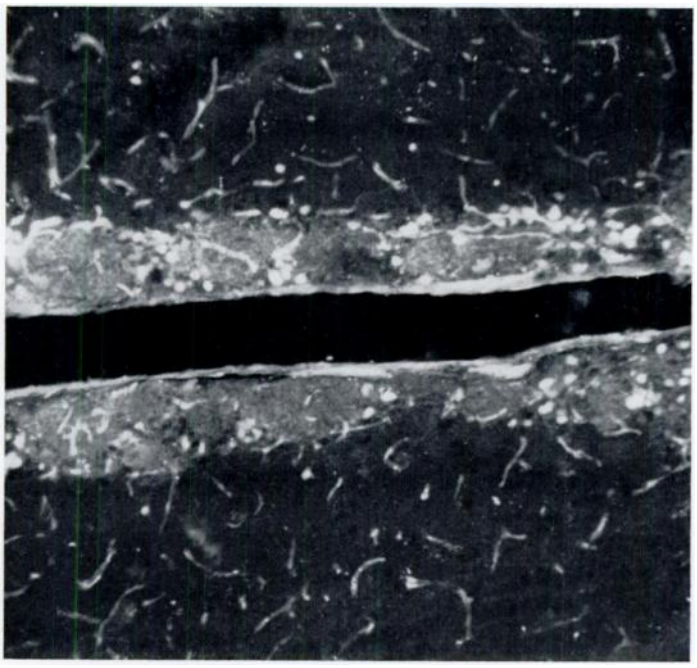

FIG. 8. Nialamide and dopamine. Medial surfaces of the olfactory bulbs (middle of figure), frontal section. Increased parenchymal fluorescence in a narrow zone, containing fluorescent nerve-cell bodies and capillaries. Outside this zone essentially dark parenchyma with numerous capillaries exhibiting lumen fluorescence (*cf.* fig. 5). × 110.

nervous system. Since then, numerous other substances have been found to be more or less prevented from entering the brain. It has been suggested that several tissue structures and biochemical processes constitute the hypothetical blood-brain barrier (*cf.* 25, 39). Although no definite principle has been demonstrated, it can be assumed that there are different mechanisms which impede the passage of various substances into the brain.

It is well established that the catecholamines and 5-HT do not reach the brain parenchyma from the circulation (8, 48, 52). The results of the present investigation strongly indicate that dopamine in blood does not pass the vascular endothelium into the brain, except in certain areas. Thus, in spite of the high sensitivity of the histochemical method (47), no fluorescence could be observed in the walls of the intracerebral vessels or in the contiguous brain parenchyma, even after administration of huge doses (500 mg/kg) of dopamine, at which dose level the luminal content is highly fluorescent. This does not prove that the luminal membrane is more or less impermeable to dopamine; the amine may penetrate this membrane but then rapidly disappear from the vascular walls without ever reaching a concentration there high enough to be demonstrated histochemically.

The facts that large amounts of dopamine are accumulated in the capillary

walls after L-dopa administration in animals pretreated with nialamide (further discussed below), and that 500 mg per kg of dopamine after MAO inhibition produces only a weak fluorescence in a few brain capillaries, show that dopamine penetrates the luminal membrane only to a limited extent. Moreover, in areas known to be located outside the blood-brain barrier, a definite fluorescence in the vascular walls and the parenchyma appears after administration of dopamine with or without previous MAO inhibition. Further, under the same condition, the pial arteries show both a luminal florescence and a wall fluorescence, whereas the intracerebral arteries display only a luminal fluorescence. Thus, in one and the same section of the brain, vessels can be demonstrated which are apparently either permeable or impermeable to dopamine.

The results after systemic administration of L-dopa contrast sharply with those obtained after the dopamine injections: already at dose levels of 20 to 50 mg per kg (depending on the route of administration) L-dopa gave rise to a clearly observable accumulation of fluorescent material in the walls of the intracerebral capillaries, whereas no fluorescence could be detected in their luminal content. As in the dopamine-treated animals, no changes in the fluorescence characteristics were recorded in the adrenergic terminals, parenchyma, or noncapillary vascular walls of the cerebellum, compared with normal animals. Parallel chemical determinations revealed no change in the cerebellar NE, but measurable amounts of dopamine were now demonstrated. Since little dopa appeared to be present, the observed capillary fluorescence can probably be ascribed to dopamine.

Treatment with a MAO inhibitor before the L-dopa administration produced only one marked effect histochemically: a great enhancement of the capillary wall fluorescence, whereas the lumen content remained nonfluorescent. Fluorimetrically, there was a considerable increase in cerebellar dopamine; the NE level was raised only slightly above that after nialamide treatment alone. This implies that the fluorescent material was, at least mainly, derived from dopamine.

Since dopamine obviously passes the luminal membrane of the brain capillaries only with the greatest difficulty, the most reasonable conclusion to draw from these findings is that L-dopa readily passes into the capillary walls, where it is decarboxylated to dopamine. The accuracy of this assumption was confirmed by the fact that after decarboxylase inhibition, and even after simultaneous MAO inhibition, treatment with L-dopa resulted in a diffuse fluorescence throughout the brain parenchyma, whereas the capillary walls were only faintly fluorescent. Concomitantly, a considerable amount of dopa, but only little dopamine, was detected.

These results leave little doubt that both dopa-decarboxylase and MAO are present in the walls of the brain capillaries, where they form an enzymatic barrier mechanism to L-dopa, as well as to 5-hydroxytryptophan (12). The experiments with intracerebellar deposition of L-dopa indicate that the amino acid easily passes also from the parenchymal side into the capillary. The capillary fluorescence after L-dopa injection, seen particularly well after previous MAO inhibition, was located in the thin, continuous, endothelial sheath and also in the pericytes known to be included at various intervals in the capillary walls

(38, 46). The uptake of L-dopa into the capillary lining seems to be specific in the sense that D-dopa in huge doses gives practically no capillary fluorescence. It is known that the D-isomers of many amino acids penetrate certain cell membranes with greater difficulty than the corresponding L-forms (21, 24, 40).

On the other hand, there seems to be no specific storage mechanism for the dopamine formed from L-dopa within the capillary walls. Dopamine is obviously present in both the cytoplasm and the nucleus of the pericytes and endothelial cells, and reserpine does not change its accumulation (*cf.* 42). Further, it disappears rapidly unless MAO is inhibited (12, 35), in which case it accumulates in very high quantities.

After transection of the spinal cord, no change in the formation of dopamine from L-dopa within the capillary walls could be detected below the lesion. Thus, the capillary decarboxylase activity must represent at least part of the extraneuronal activity recently found chemically in the spinal cord (7).

As judged from the histochemical observation, this specific barrier mechanism seems to be fairly efficient since a break-through of L-dopa could first be observed at the dose level of 80 mg per kg. This seems to agree with the observation that when administered in relatively high doses L-dopa reaches central neurons (13, 18).

It should be borne in mind, however, that also very small doses of exogenous L-dopa readily penetrate into certain regions known to be located outside the blood-brain barrier, *e.g.*, the median eminence, the area postrema (*cf.* 36, 48), and the choroid plexus. In the present investigation, also in an extensive area at the medial surfaces of the olfactory bulbs there appeared a considerable, diffuse parenchymal fluorescence after administration of L-dopa as well as dopamine.

While the barrier for L-dopa at the capillary level is an enzymatic trapping mechanism, the barrier for dopamine seems to be of quite another nature. Even though large amounts of dopamine are present in blood or deposited in brain parenchyma in amounts high enough to penetrate all parenchymal cells, the amine does not pass into the capillary walls from either side unless these are exposed to excessive concentrations of the amine. In the noncapillary intracerebral vessels this type of membrane barrier mechanism appears to be operating for both dopa isomers and for dopamine.

In the peripheral tissues, the pial vascular system (including the choroid plexus), and certain circumscribed brain areas beyond the barrier system there occurred a free diffusion of the amino acids and dopamine without any detectable accumulation in the vascular walls.

Thus, it has been demonstrated that the intracerebral vessels are unique, precisely in terms of barrier properties, compared with noncerebral vessels.

SUMMARY

The passage of dopa and dopamine into the mouse brain has been studied with a combination of chemical determination and histochemical localization of certain monoamines and their precursors. L-Dopa, but not D-dopa or dopamine,

readily penetrates into the capillary walls. These contain dopa decarboxylase and MAO, which impede the further passage of L-dopa into the brain. None of the compounds can pass into the endothelial lining of noncapillary intracerebral vessels. A free passage occurs in peripheral tissues as well as in brain areas known to be located beyond the blood-brain barrier.

The results demonstrate the existence of specialized brain barrier mechanisms at the vascular level.

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