MORPHOLOGY AND PATHOMORPHOLOGY

ADRENERGIC STRUCTURES OF PIAL ARTERIES AND THEIR CONNECTION WITH THE CORTEX

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It has been shown in the last few years that the pial arteries constitute the main vascular mechanism controlling the blood supply to the cerebral cortex [3, 4], and that the smaller arteries actually in the cortex take practically no part in this regulatory activity [5]. "Nutritive" dilatation of pial arteries is apparently neurogenic [6, 7].

Nerve structures in the vicinity of pial arteries have been described by many investigators, employing silver impregnation or methylene blue staining methods [1, 2, 18, 21, 24], but it was, of course, impossible to determine the functional nature of these fibers by these methods. A histochemical method [13] has recently been devised for demonstration of catecholamines in nerve fibers, and so for the study of adrenergic structures.

An investigation of adrenergic structures in the vicinity of pial arteries, in the pia mater surrounding them and in the underlying cortex was therefore undertaken.

METHOD

The experiments were carried out on 29 rabbits. From 2 to 6 pieces of brain, measuring 4 × 4 × 3 mm, (and similar pieces of cardiac ventricle as a control) were removed immediately after the animal's death, produced by the injection of air into a vein. The tissue was frozen in liquid propane, cooled with liquid nitrogen. After lyophil drying for 4 days in a vacuum refrigerator, the material was exposed to moisture-free formaldehyde vapor for 1 or 3 h in the incubator at 80°C.

In the process some of the catecholamines were condensed into products which exhibited intense green or greenish-yellow fluorescence. The theoretical basis of this method has been published in the course of the last few years [11, 12, 14]. Formaldehyde has the effect of converting primary catecholamines (such as noradrenaline) into derivatives of 1, 2, 3, 4- tetrahydroisoquinoline, which, in the presence of protein, are then rapidly converted into fluorescing 3,4-dihydroisoquinoline. Only catecholamines with OH groups in the third and fourth positions produce intensely fluorescing derivatives. Secondary catecholamines (such as adrenaline) are also readily condensed into corresponding tetrahydroisoquinolines, but their subsequent conversion into fluorescing products proceeds more slowly, as 3,4-dehydroisoquinoline with a quaternary nitrogen is formed. In the case of tryptamines, the condensation reaction proceeds in the same way, but products fluorescing an intense yellow are produced. The method is highly specific and adequately sensitive for demonstration of catecholamines in nerve elements [13, 15, 23].

The procedure was then as follows. The material was embedded in paraffin in vacuo at 60°C for 10 min. Sections 4 μ thick were placed on dry, heated slides and mounted in Euthellan, a non-flourescing medium (to remove the paraffin). Total preparations of pia mater measuring 4 × 4 mm were also prepared under the binocular microscope; they were dried on the slide at room temperature in vacuo for an hour. The preparations were examined in the fluorescence microscope, fitted with a dark field oil condenser. The light from a high-pressure mercury-quartz lamp first passed through a filter (type VG-12), 4 mm thick, transmitting a maximum wave-length of 400 mμ; there was a second filter (type OG-4), absorbing most light of wave-length less than 500 mμ, between objective and eyepiece.

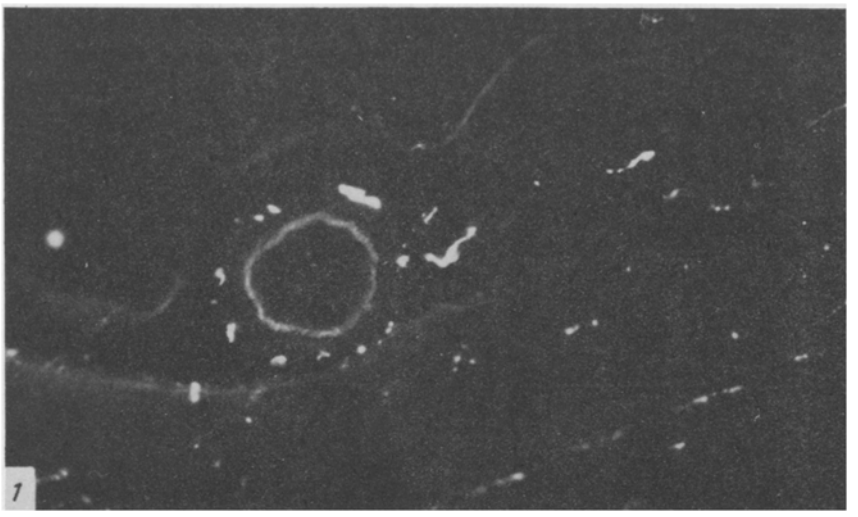

Fig. 1. Brightly fluorescing nerve fibers and bundles in adventitia of pial artery, cut transversely. Similar nerve fibers seen in pia mater to right of artery. Internal elastic membrane of artery exhibits powerful autofluorescence (seen also in tissue not treated with formaldehyde). Arachnoid membrane seen above vessels, and below, cortex, in which fluorescing nerve fibers can also be seen. Normal rabbit. × 210.

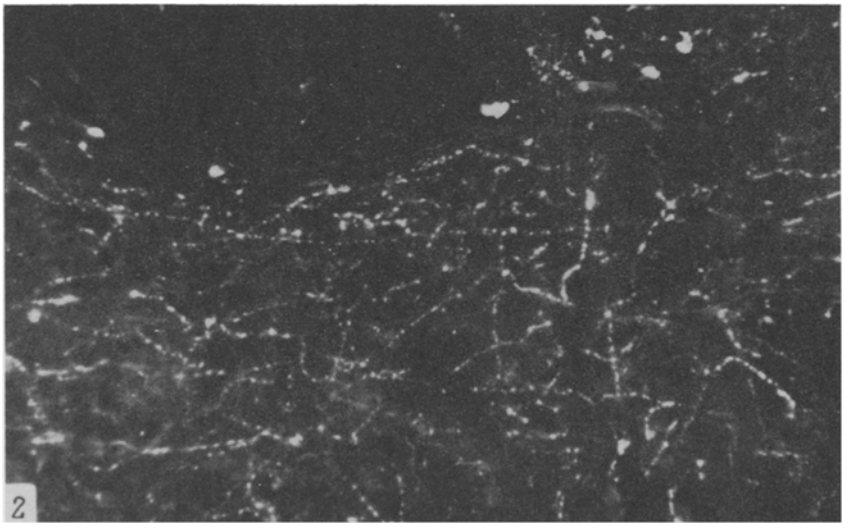

Fig. 2. Numerous fluorescing nerve fibers, most lying parallel to surface of cortex in its outer layers (upper part of photomicrograph). Some autofluorescing pigment cells in pia mater. Sympathectomized rabbit given Nialamide. × 180.

RESULTS

There were varicose nerve fibers, giving intense green fluorescence, characteristic of catecholamines, in the vicinity of the walls of the pial arteries (Fig. 1). They frequently were grouped together into delicate bundles consisting of several fibers, with frequent connections between them. The network of nerve fibers in the adventitia of the pial vessels was somewhat sparser than those round vessels in some other organs [13], and particularly the pineal body [22, 23]. The network of nerve fibers around pial veins was still more open. The nerve fibers of the pial arteries also ran along the radial arteries entering the cortex.

Nerve fibers, giving green fluorescence also ran from the nerve bundles of the pial arteries into the superficial

layer of the cortex. Unlike the fibers accompanying the radial arteries, they frequently entered the cortex at an acute angle.

The preparations of pia mater revealed a network of varicose fibers, giving green fluorescence, which often formed connections between pial vessels, either as single fibers or in the form of thin bundles. Pigmented cells containing granular substance, which gave orange fluorescence, were also seen in the pia.

The nerve fibers of the pial arteries thus gave off two types of collateral, one passing into the cortex and the other, into the surrounding pia mater.

There was a rich network of relatively thin fibers, giving intense green fluorescence in the cerebral cortex (Fig. 2 and 3). More intense fluorescent thickenings could be seen at regular intervals along their entire length. Similar appearances have been observed in other parts of the brain [9]. These fibers were particularly numerous in the outermost layers of the cortex, where most lay parallel to its surface, often in close proximity to pial vessels (Fig. 2). In the deeper layers of the cortex these fibers ran in all directions, forming a more open, three-dimensional reticulum (Fig. 3). The fibers generally ran straight, but at times, particularly in the vicinity of the non-fluorescing bodies of nerve cells, they described curves; in some cases the appearances suggested that the fibers terminated in these areas. The nerve fibers in the cortex ran past radial vessels, without entering their adventitia. A brownish-yellow fluorescence was also seen in the bodies of cells, but this was of the type of autofluorescence, seen in material which had not been treated with formaldehyde.

Bilateral cervical sympathectomy, performed in the case of 9 rabbits four days before the collection of material, led to complete disappearance of the fluorescing nerve fibers in the regions of both the pial vessels and their branches running in the pia and into the cortex. The network of fluorescing fibers in the cortex itself was still present.

It had been shown earlier that disappearance of fluorescence from the distal segments of divided sympathetic fibers was the forerunner of structural changes [13]. It was therefore concluded that the nerve fibers of the vessels in the pia mater, which contained catecholamines, were the postsynaptic axons from the sympathetic nervous system. The cortical fibers, however, or at any rate most of them, were of different origin, although where their cell bodies are is still unknown.

Nialamide, a monoamino-oxidase inhibitor, which is concerned in the activation of catecholamines [25], was injected intraperitoneally (150 mg/kg) to seven rabbits six hours before material was collected; the green fluorescence of the fibers in the cortex was thereby intensified slightly.

Reserpine (5 mg/kg) injected subcutaneously to four rabbits 24 h before the collection of material, resulted in complete disappearance of fluorescence from all fibers, both in the region of pial vessels and in the cortex.

α-Methyl-metatyrosine, given intravenously to three rabbits 24 h before collection of material, led to considerable reduction in the quantity of catecholamines in both vascular and cortical nerve fibers. Only a few, poorly fluorescing fibers could be found in the cortex of some animals. The same was seen in the region of the pial vessels, where catecholamines could only be detected at occasional points, generally in the bundles of nerve fibers.

The effects of reserpine and α-methyl-metatyrosine in doses producing selective exhaustion of catecholamines in nerve tissue [8, 9], viewed in conjunction with the results of this and earlier investigations [11, 12, 14, 16, 23], indicate that the nerve fibers described above are adenergic. The conditions under which the histochemical reactions took place (comparatively short treatment of the material with formaldehyde vapor) suggest that the nerve fibers discovered in the region of pial vessels and in the cortex contain primary (dophamine and noradrenaline) and not secondary (adrenaline) catecholamines. Also, as green fluorescence of the nerve fibers was still very much reduced even 24 h after the injection of α-methyl-metatyrosine, which reduces the noradrenaline content of tissues for long periods and the dophamine content only very temporarily [9], it may be concluded that the nerve structures examined contained mainly noradrenaline.

Microsurgical division of all nerve connections linking the pial arteries with surrounding pia and underlying cortex had the effect of abolishing the dilatation of these vessels associated with intensified cortical activity (from local application of strychnine) or with markedly defective blood supply [7]. As this operation also interrupted the adrenergic nerve fibers branching off from the nerves of the pial arteries and entering the cortex, these must also play some part in the development of this vasodilatation. That this is the case is also indicated by the reduction of vasodilatation produced by ergotamine or ergotoxine. As these adrenergic nerve fibers are branches from axons of

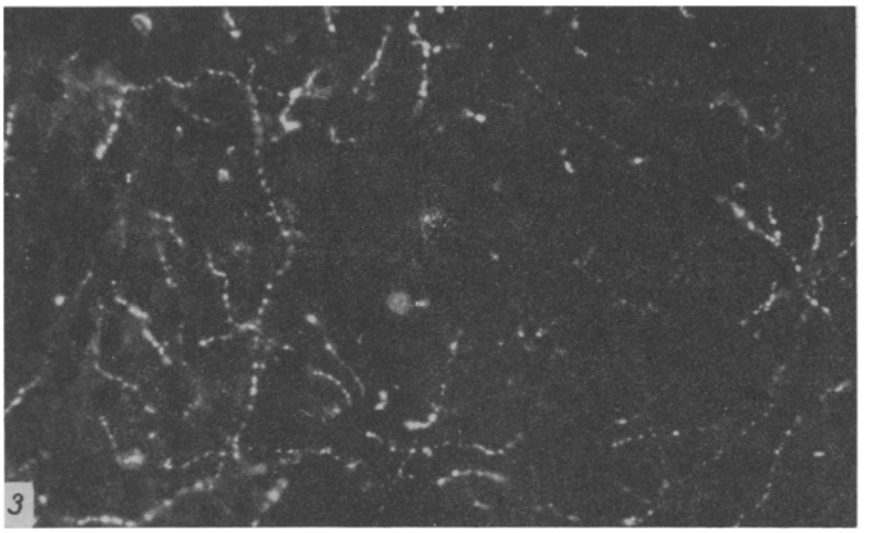

Fig. 3. Fluorescing varicose nerve fibers in deeper layers of cortex, running in all directions between non-fluorescent nerve cells (dark nuclei stand out against clearer background). Sympathectomized rabbit given Nialamide. × 200.

nerve cells in the sympathetic ganglia, it may be concluded that connection between cortex and pial arteries is effected through sympathetic ganglia, but only in the region of the peripheral parts of the axons, on the "axon reflex" principle, as well as through the bulbar vasomotor center. This confirms the results of investigations carried out by Ingvar [19], in which cervical sympathectomy failed, in acute experiments, to abolish the vascular reaction in the cortex associated with increased activity.

All this does not mean, however, that other physiological mechanisms have no part in "nutritive" dilatation of the pial arteries. This may be inferred from the fact that "nutritive" dilatation of the pial arteries associated with intensified cortical activity (as a result of the local application of 0.5% strychnine solution to the cortex) was still not abolished 7-14 days after cervical sympathectomy in rabbits. Vasodilatation after the operation in our experiments averaged 32 ± 32% (M ± σ) of the original size of the vessels, and in control experiments, 31 ± 17%. There was, in fact, no significant difference ($P > 0.5$). Under such conditions, "nutritive" vasodilatation of the pial arteries may be achieved by cholinergic mechanisms, as it has been shown that atropinization reduced vasodilatation by about half, although cholinergic structures in the region of the pial arteries have not yet been demonstrated. Another possibility is the direct effect of metabolites (CO_2 is generally considered to be most important), diffusing from the cortex into the region of the pial arteries [17, 20], although there is still but little evidence on this point, and the idea, though widely prevalent, is still hypothetical.

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