CHOLINERGIC NERVOUS APPARATUS OF THE PIAL AND CEREBRAL BLOOD VESSELS

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Acetylcholinesterase was demonstrated in cats by Gerebtzoff's method in nonmedullated nerve fibers of the pial blood vessels and the initial parts of the intracerebral arteries. After removal of the superior cervical sympathetic ganglion activity of the enzyme was modified, being spread diffusely into the tissues. Its content in degenerating fibers was reduced. The authors consider that the vessels of the brain possess a motor nervous apparatus with vasodilator functions.

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Nervous regulation of the cerebral circulation is a problem of great importance in modern physiology and continues to attract the attention of investigators. During the last 15-20 years a particularly intensive study has been made of vascular sensitivity, and in the meninges this has been investigated in particular detail. Much less attention has been paid to vascular mobility, and in this case much remains unexplained or contradictory. Investigators are unanimous in considering that the blood vessels of the brain react actively by dilatation [5, 11], but its nervous mechanism still remains little understood.

The problem of special vasodilator nerves was once extensively discussed in the specialized literature. Some workers argued that dilatation of the medullary arteries is produced by parasympathetic fibers contained in the trunks of the vagus [10], facila [13], greater superficial petrosal [15], and oculomotor [5] nerves. However, careful tests did not confirm these views, and nowadays there is considerable doubt about the existence of special parasympathetic dilators, and in some cases their existence is completely rejected [12, 15].

Neurohistological experiments have conclusively demonstrated only one source of vasomotor fibers: the lateral sympathetic trunk, its cervical and, to some extent, its thoracic ganglia [1, 8, 9]. On this basis, not only for cerebral vessels, but also for the whole vascular system, the view has been put forward that dilatation and constriction are produced by fibers of sympathetic nature only [2, 6]. The use of pharmacological agents (inhibitors of monoamine oxidase and cholinesterase) has demonstrated the invalidity of the classical subdivision of autonomic nerves into sympathetic, vasoconstrictor and parasympathetic, vasodilator. The majority of nerve trunks, regardless of the division of the autonomic nervous system to which they belong, have been shown to contain both adrenergic and cholinergic fibers [3]. The latter, it must be assumed, are found in the cerebral vessels, for it is unanimously agreed that they respond to injection of acetylcholine by dilatation [4].

Modern histochemical methods have enabled the nature of fibers to be determined selectively, and they have undoubtedly broadened our outlook concerning the nervous mechanisms of vascular mobility.

Acetylcholinesterase activity was studied in the blood vessels of the pia mater and in the initial parts of the intracerebral arteries.

EXPERIMENTAL METHOD

The pia mater of the brain of 24 cats was investigated. The animals were anesthetized with thiopental and exsanguinated. Material was taken immediately after craniotomy. The pia was stretched on a slide

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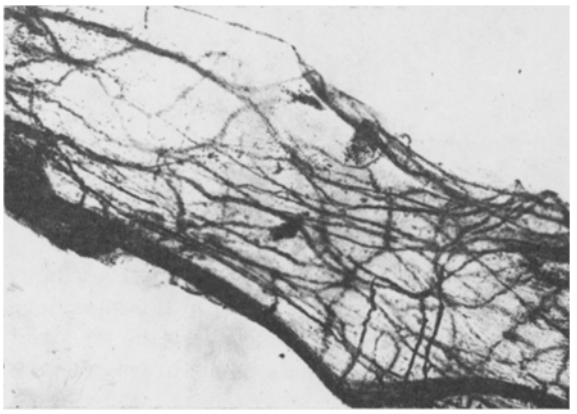

Fig. 1. Pial artery. Reaction for acetylcholinesterase reveals a nervous plexus of cholinergic fibers. Gerebtzoff's method, 200×.

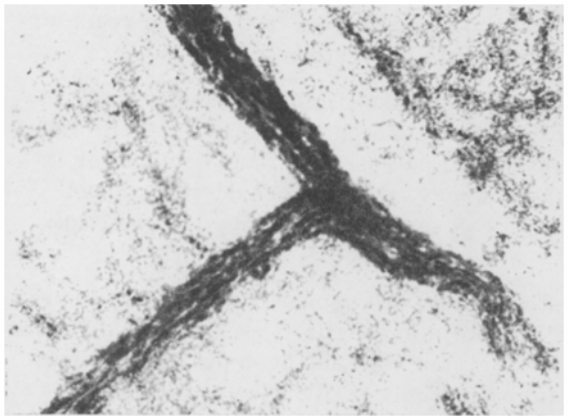

Fig. 2. Cerebral cortex. Intramedullary nerve. High acetylcholinesterase activity in its fibers.

and immersed in the fresh, unfixed state in the incubation medium for 1.5-2.5 h.

The brain of six rabbits also was investigated. Thin pieces were cut from the frontal and parietal lobes of the cortex together with the pia mater, and sections cut from them in a cryostat. Acetylcholinesterase and cholinesterase were detected by Gerebtzoff's method using the iodide of acetylthiocholine and butyrylthiocholine. Control areas of the pia were incubated without detergent. Eserine was used as inhibitor. The enzyme was found by the presence of brown granules of copper sulfide in the sections.

EXPERIMENTAL RESULTS

Acetylcholinesterase was detected in nonmedullated nerve fibers located mainly on the walls of arteries. Brown granules were found along their course, and the dotted line or short continuous line of these granules indicated the position of the fibers.

On arterioles 60-80 μ in diameter well-developed plexuses of cholinergic fibers were found, enclosing the vessel in a dense, looped framework (Fig. 1). Where the vessels divided, the nerve fibers followed each branch. Their number gradually diminished and arterioles 15-18 μ in diameter were the last vessels on which acetylcholinergic fibers were present.

Together with arteries, cholinergic fibers from the pia mater ran into the brain substance.

In other words, the intracerebral vessels receive a cholinergic nerve supply.

Confirmation of this view is given in Fig. 2. This shows a large, dividing intramedullary nerve in the rabbit cerebral cortex. It enters the brain from the side of the pia mater together with a blood vessel. On the photograph the vessel was out of focus. Because of high enzyme activity the nerve can be clearly traced against the background of the brain substance with moderate acetylcholinesterase activity.

In arteries, but not in veins, places with high enzyme activity were frequently found. These were usually approached by a very thin fiber, the position of which could be established from the acetylcholinesterase granules. These fairly localized segments with high enzyme activity unquestionably corresponds to the point of contact of the terminal effector cholinergic apparatus with the vessel wall.

It is considered that acetylcholinesterase is present not only in the glial end-plate of the effector and the Schwann cells of the nerve fiber, but also in the cylinder itself, including its telodendrons. In fact, by the use of Gerebtzoff's method, in some cases fibers and endings have been found with particular morphological demonstrativeness [9]. On some blood vessels intensely black and strictly localized areas with a high acetylcholinesterase content are found. By their clarity and sharp demarcation they resemble the patterns of cholinesterase activity in the motor end-plates of the myon [7]. In other vessels typical endings can be seen which are indistinguishable in their external appearance from the pattern obtained by the use of an impregnation technique. The terminal apparatus is reproduced entirely together with the glial bases [2].

These findings concerning the presence of acetylcholinesterase in the axon and endings are not in conflict with recent observations made with the electron microscope [14].

When determining acetylcholinesterase activity it is always important to know of the possible presence of cholinesterase (pseudocholinesterase in the old nomenclature) in nerve structures. This enzyme is detected in fewer nerve fibers, and these fibers possess very low activity; besides fibers it is also present in the smooth muscle cells of arteries.

We are convinced that acetylcholinesterase was detected in the motor fibers on the basis of the following additional data. Removal of the superior cervical sympathetic ganglion in cats leads to changes in activity of the enzyme and to its more diffuse spread into the tissues. The content of enzyme is degenerating fibers is reduced [16], as was observed in the present experiments.

The motor function of the nervous apparatus is indicated by the complete absence of cholinergic fibers on the capillaries and their very small number in the tissues of the pia mater. Cholinergic fibers in the latter were found only when passing from one vessel to another.

The pial vessels and their continuations into the brain thus possess a well-developed cholinergic apparatus, responsible for the vasodilator effect during stimulation of nerves [5]. The blood supply to the brain from the functional aspect has specific differences from other hemodynamic regions.

While obeying the general rules, the cerebral blood flow is characterized by a well marked adaptation to the energy requirements of the brain.

Without exaggeration it can be said that regulation of the cerebral circulation is directed primarily at supplying the nerve cells with oxygen and glucose in emergency. It is therefore tuned primarily to dilatation of the cerebral vessels [5]. This reaction, of great biological significance, maintains the cerebral blood flow at the optimal level.

The results of the present investigation show that dilatation of the cerebral vessels is produced by means of a special nervous apparatus whose terminal structures liberate acetylcholine. This mediator lowers muscle tone and produces a vasodilator effect. It can be concluded from experiments on cats that sympathetic ganglia are the sources of the cholinergic fibers.

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