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Peptidergic innervation of human cerebral blood vessels and saccular aneurysms

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Abstract Peptidergic innervation of the human cerebral vasculature has not yet been described in detail and its role in the maintenance of cerebral autoregulation still needs to be established. Similarly, few data exist on the innervation of vascular malformations. The aim of this study was to clarify the peptidergic innervation patterns of human cerebral arteries of various sizes, and, for the first time, that of saccular aneurysms. Light microscopic study of whole-mount preparations of human cerebral arteries and aneurysm sacs resected either during tumor removal or after neck-clipping were carried out by means of silver-intensified light microscopic immunocytochemistry visualizing neuropeptide-Y, calcitonin gene-related peptide and substance P immunoreactivity. Systematic morphological investigations confirmed the presence of longitudinal fiber bundles on the adventitia and a network-like deeper peptidergic system at the adventitia-media border, while in smaller pial and intraparenchymal vessles, only sparse longitudinal immunopositive axons could be detected. The innervation pattern was totally absent in the wall of saccular aneurysms with the complete disappearance of peptidergic nerve fibers in some areas. To the best of our knowledge neither the disappearance of this network on small pial and intraparenchymal vessels, nor the absence of an innervation pattern in saccular aneurysms have been described before. Nonhomogeneous peptidergic innervation of the human cerebral vascular tree might be one of the factors responsible for the distinct autoregulatory properties of the capacitance and resistance vessels. Malfunction of this vasoregulatory system might lead to the impairment of autoregulation during pathological conditions such as subarachnoid hemorrhage.

Key words Cerebral aneurysm · Autoregulation · Neuropeptides

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Introduction

Peptidergic innervation of the cerebral vessels has been described in several species including felines [10, 30, 31, 36, 53], mongolian gerbil, guinea pig [33], and rat [44, 50, 52], and in a few human specimens [3, 9, 11, 16, 22, 27, 32]. The origin of these fibers was predicted on the basis of widespread tract-tracing studies [1, 12, 28, 34, 44, 50]. Axons from the sympathetic, parasympathetic and the trigemino-vascular system form the perivascular fiber system (for review see [8]).

The delicate balance between the vasoconstrictor [neuropeptide-Y (NPY), noradrenaline] and the vasodilator [vasoactive intestinal polypeptide, nitric oxide syntethase, calcitonin gene-related peptide (CGRP), substance P (SP)] mediators of these fiber systems has a substantial role in cerebral autoregulation under normal and pathological conditions ([2, 6, 8, 16, 19, 24, 29, 35, 39, 40, 42]; for detailed discussion see [43]). The functional significance of the vessels of the vascular tree in autoregulation varies with their diameter and wall structure [7, 20]. Histopathological studies revealed dramatic structural changes in the muscular elements of the aneurysm [47]. Presumably, the fibers innervating the vessel also display an aberrant arrangement due to fragmentation of the aneurysm wall.

The aim of this study was to describe the normal peptidergic innervation of human cerebral arteries, focusing on signature mediators of the sympathetic and the trigeminovascular system. Special attention was paid to

the different innervation patterns of the larger (capacitance) and smaller pial, intraparenchymal vessels. Analysis of the structural changes in the innervation of saccular aneurysms was also performed, as it was expected to correlate with the disappearance of normal muscular wall structure in this disease. Silver-intensified peroxidase-antiperoxidase immunocytochemistry was employed to describe the course of fibers and fiber bundles in whole-mount preparations and tissue sections.

Materials and methods

Fixation and section preparation

Normal temporal arteries supplying intact brain tissue were dissected during temporal lobectomy for glioma in six cases. Removal of normal temporal lobe tissue had to be performed either for internal decompression or because the temporal tip had become devitalized during tumorectomy. The arteries were cut into smaller segments in a medium containing 0.1 M phosphate-buffered saline (PBS), and immersed in 4% paraformaldehyde in the same buffer overnight.

After clipping the neck of aneurysms in five cases, the domes were excised. The greatest diameter of the aneurysms varied from 5 mm to 2 cm. The specimens were treated in the same way as described above and were studied as whole-mount preparations.

A third group of vessels was visualized in cross-sections of the temporal lobe that had been resected. After 2 days of immersion fixation in the same fixative, the small temporal lobe blocks (each approximately 1 cm^3) were sectioned on a vibratome (50-μm slices into PBS). All the tissue samples subsequently underwent the same immunocytochemical staining procedures.

Immunocytochemistry

Having been washed in PBS, the sections were stained using the unlabelled peroxidase-antiperoxidase (PAP) method described by Sternberger et al. [48]. The penetration of the primary antibodies was facilitated by pretreatment with 0.2% Triton X-100 (Sigma) followed by 10-min rinsing in PBS and 10 min in 2% normal sheep serum to block non-specific binding activity. The sections and the tissue samples were incubated for 2 days with previously tested solutions of primary antisera developed in rabbit against NPY (1:5000), SP (1:1000) and CGRP (1:1000) (kindly donated by Dr. István Merchenthaler, Wyeth Ayerst, Radnor, Pa.) followed by the secondary antibody (sheep anti-rabbit immunoglobulin G, Sigma, 1:200) and PAP complex (Sigma, 1:200) for 1 h each. The immunolabel was amplified by silver-gold intensification of the 3,3′diaminobenzidine (DAB) chromogen as described by Gallyas et al. [18] and Merchenthaler et al. [38]. The samples from vessels and aneurysms were mounted, coverslipped and visualized as whole-mount preparations.

Controls

In spite of the fact that the primary antisera have already been well characterized and tested, we tested them again using a series of increasing dilutions. This approach resulted in a gradual decrease and finally disappearance of the staining. The withdrawal of the primary antisera from the staining procedure resulted in immunonegative control sections. For further validation additional experiments were performed employing pre-absorption with the antigens that also resulted in immunonegative control sections in the case of each neuropeptide.

Results

Immunocytochemistry of cerebral arteries

All the branches of the middle cerebral arteries studied revealed SP-, CGRP- and NPY-like immunoreactivity (LI).

The distribution of immunoreactive fibers was remarkably similar for each neuropeptide; according to their morphological appearance two groups of axons could be distinguished. In the first population of axons the silver-intensified DAB endproduct of the immunocytochemical reaction displayed primarily longitudinally arranged fiber bundles, clearly visible when the photomicroscope was focused on the surface (periadventitia-adventitia) of the whole-mount preparations (Figs. 1 A, 2 A). The other group of immunopositive nerve fibers was detected in a deeper layer, in the adventitia-media border of the vessel wall, displaying a network-like, reticular structure (Figs. 1 B, 2 B).

The fact that all the different antibodies employed revealed similar innervation pattern of the human cerebral vasculature gives further credit to our observations. Sagittal and cross-sectional studies of several arteries in the temporal lobe specimen also confirmed the observations

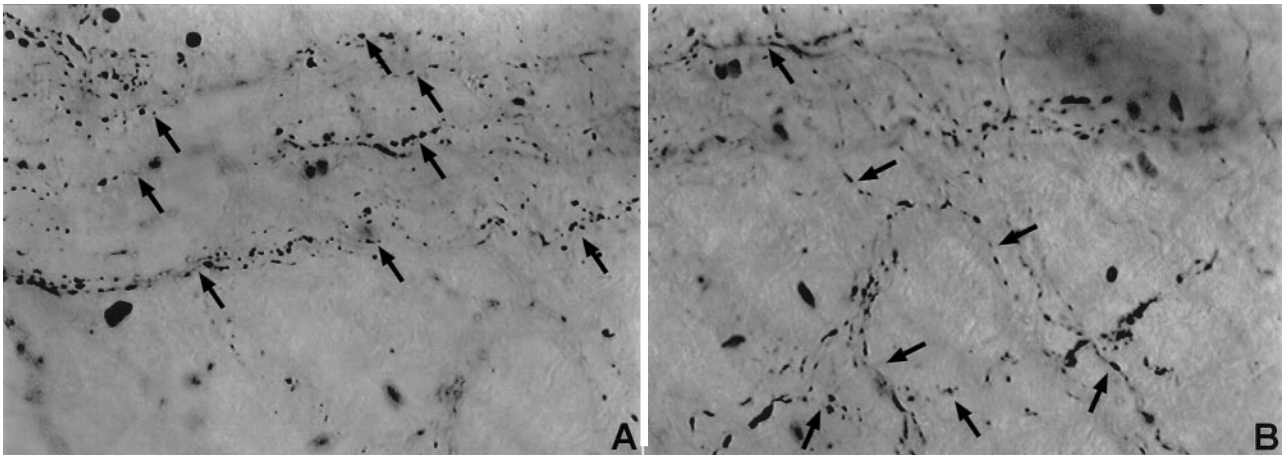

Fig. 1 **A** Longitudinal, mainly parallel SP-immunopositive nerve fibers at the surface of the anterior temporal artery *(arrows)*. **B** The photomicroscope is focused to the deeper layer – media-adventitia border – a network-like SP immunopositive fiber system is visible *(arrows)* (*SP* substance P). **A, B** × 200

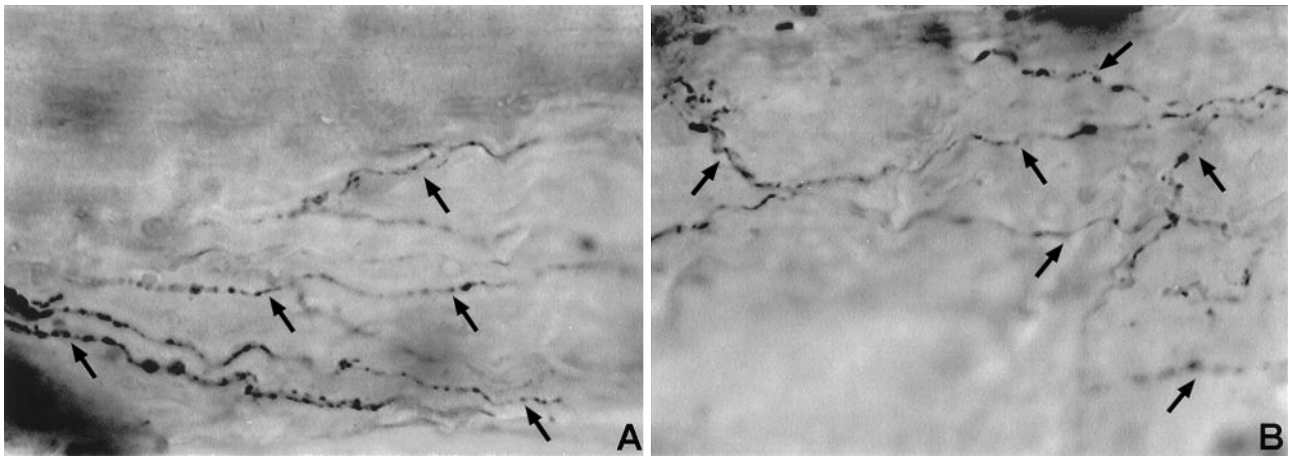

Fig. 2 **A** Longitudinal, NPY-immunoreactive fiber bundles at the surface of the anterior temporal artery *(arrows)*. **B** Focusing the photomicroscope deeper, to the media-adventitia border reveals a network of NPY-immunopositive fibers *(arrows)* (*NPY* neuropeptide-y). **A, B** × 300

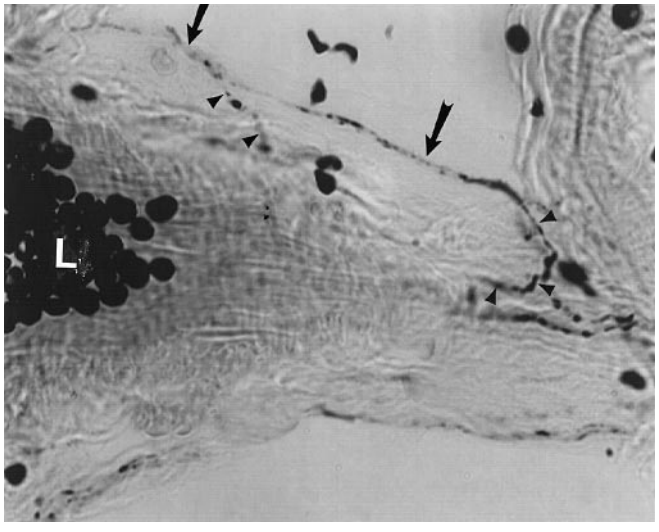

Fig. 3 Sagittal, partially tangential section of an artery from the temporal surface. NPY-immunopositive axons can be seen in longitudinal section *(arrows)* at the adventitia, as they approach the vessel. The tangential part of the section demonstrates, how longitudinal, superficial fiber system forms the origin of the deeper fiber network that can be found in the adventitia-media border *(arrowheads).* The lumen of the vessel is just slightly opened *(L)*. × 700

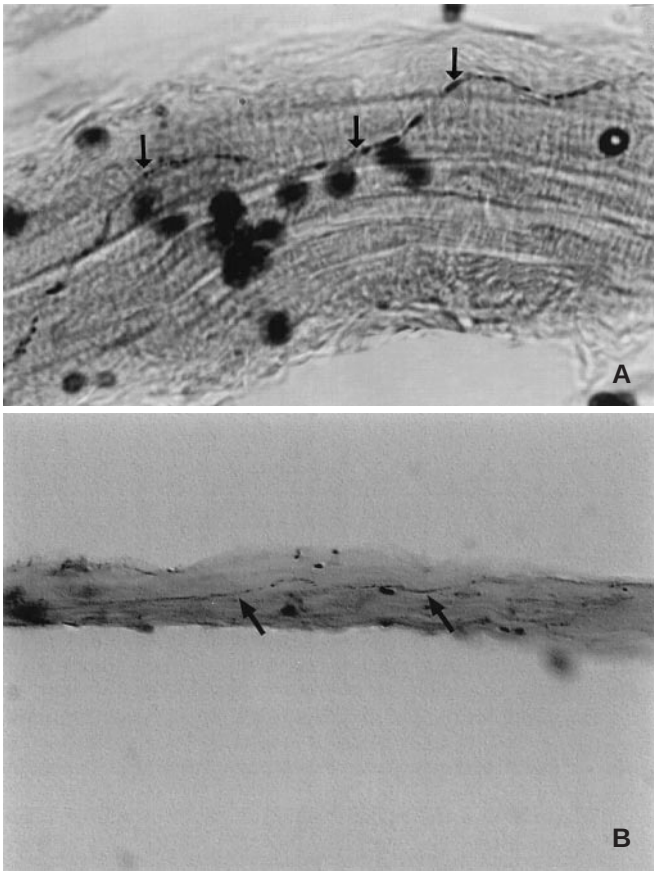

Fig. 4 **A** A small pial blood vessel contains only a single, longitudinal, CGRP-immunoreactive fiber curving on its surface *(arrows)*. **B** A single longitudinal SP-immunopositive fiber *(arrow)* detected at the surface of a smaller branch of the temporal artery (*CGRP* calcitonin gene-related protein). **A** × 800, **B** × 500

described above. Some sections also revealed how the branches of the superficial fiber system turned downwards, forming the fiber network at the adventitia/media border (Fig. 3).

Smaller arteries (pial, intraparenchymal vessels) displayed substantially different morphological arrangements in their innervation patterns: CGRP-, NPY- and SP-immunopositive fibers around the wall were less abundant and coursed only in one direction, longitudinally. The above-mentioned network-like innervation profile was not observed in vessels with a diameter under 50 μm (Fig. 4 A, B).

Immunocytochemistry of saccular aneurysms

Although NPY-, SP- and CGRP-LI fibers were observed in the wall of intracranial saccular aneurysms, these immunoreactive axons did not follow any organized pattern. As did all of the wall components, the fibers displayed major morphological changes. They showed wave- and

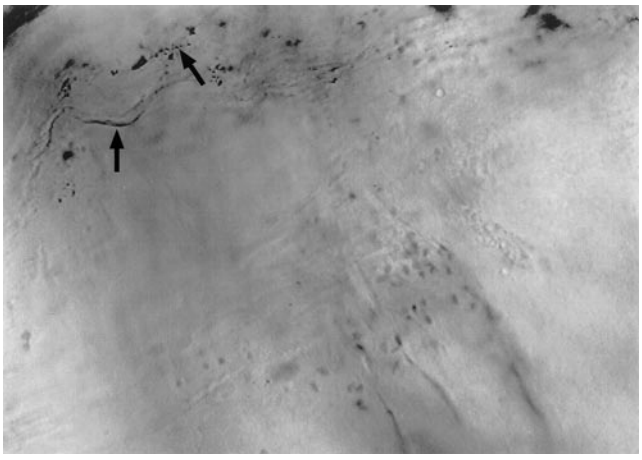

Fig. 5 Whole-mount preparation of a saccular aneurysm. Large areas of the wall do not display any immunoreactivity (immunostaining for SP), only scattered fibers can be detected *(arrow)*. × 300

whirl-like patterns and in circumscribed areas no peptidergic immunoreactivity could be detected (Fig. 5). These morphological changes in innervation were similar in every type of neuropeptide-LI fiber that was investigated.

Discussion

That cerebral vessels are innervated by peptidergic neurons is well established in several species [12, 16, 31, 33, 50, 52, 53]. Functional studies suggest that trigeminal fibers providing sensory innervation for the vast majority of the vascular tree contain vasodilatatory peptides [4, 5, 51]. The vasoactive effect of these neurons is presumably coupled to the action of nitric oxide and endothelin and should be assessed together with other mechanisms of the autoregulatory "machinery", such as the effect of autacoids and other local chemical-parenchymal factors [37, 45, 49, 54]. Although the continuous CGRP innervation could maintain a tonic vasodilatatory effect via a persistent axon reflex mechanism described in the case of sensory ganglia [21], the majority of recent studies indicate that the trigeminovascular peptidergic system does not affect the resting cerebral blood flow, so there is a lack of tonic peptidergic innervation [11, 19, 23]. These articles also suggest that the role of the SP- and CGRP- (and of other tachykinins) containing fibers is similar to a surveillance mechanism. According to this concept these pathways are active in the presence of an aggressive vasoconstrictor agent, a characteristic implicating them in the maintenance of autoregulation in subarachnoid hemorrhage [6, 13–15, 23, 41, 49]. As several clinical investigations suggest, when the equilibrium of the system is disturbed or exhausted, vasoconstriction can develop causing severe tissue damage, with ultimate cerebral infarction at the end of the process [15, 17, 24, 25]. One of the potential targets of pharmacotherapy of vasospasm is restitution of the vasodilator capacity by exogenous supply of the mediators [17, 24].

The morphological structure of this innervation has been widely investigated in animals. Several authors have found a periadventitial, and a deeper system (at the adventitia-media border) [7, 46, 52]. Edvinsson and coworkers [11, 16, 22] describe a similar distribution of peptidergic fibers by means of an immunofluorescence method in human specimens. A similar developmental fiber pattern was also described in human fetal cerebral arteries [3, 26].

In this histological study we demonstrated SP- and CGRP- containing fibers in the wall of human temporopolar and anterior temporal arteries using silver-intensified PAP immunocytochemistry. The above-mentioned fiber arrangements, i.e., mainly longitudinally running, superficial fiber bundles and a network-like deeper innervation pattern, were detected. Axons from the superficial fiber bundles were frequently detected curving into the deeper layers of the wall to give rise to the fiber network at the media-adventitia border. This finding corresponds closely with previous observations that demonstrated a gradual decrease of fiber density parallel with the distance from the circulus arteriosus Willisii [1, 27]. NP-Y-like immunoreactive fibers, purportedly responsible for vasoconstrictor effects, showed the same innervation pattern.

Although our results basically correlate with the pioneering findings described by Edvinsson et al. [16], we failed to find any noteworthy difference in the density or staining intensity between nerve fibers displaying SP/CGRP-LI and NPY-LI. Nevertheless, the present report did not include any quantitative analysis to directly address this issue. Further, according to the previous passages, the immunoreactivity for a neuropeptide in a fiber bundle is supposed to be substantially determined by the actual functional activity of that particular peptidergic system.

Scrutiny of small pial arteries revealed a slightly different morphological appearance: individual longitudinal SP-, NPY- and CGRP-like immunoreactive fibers were detected. We were not able to detect a reticular arrangement of fibers in smaller arteries of diameter less than 50 μm. The different role of the cerebral resistance and capacitance vessels in the maintenance of autoregulation is well known [7, 20]. Blood volume and blood pressure increases result in constriction of the resistance vessels followed by a compensatory dilatation of the smaller pial, intraparenchymal capacitance vessels to maintain the normal cerebral blood flow. It is not yet known whether the morphological differences in the fiber distribution are responsible for such functional differences or whether these mechanisms depend on a delicate combination of neurogenic, metabolic and other factors. Another question arises from the results of the immunocytochemistry studies of human saccular aneurysms. On the basis of these results, we can state that the normal – or common – arrangement of peptidergic fibers described in branches of human middle cerebral arteries are not be found in the wall of a saccular aneurysm. The innervation pattern is almost chaotic, the fibers displaying a whorl-like appearance. A note-

worthy feature is the complete loss of peptidergic innervation in circumscribed areas of the aneurysmal wall. Whether these changes are somehow related to the impaired autoregulation of these parts of vessels or complicate the decreased autoregulatory potential of the fragmented, destroyed muscular and elastic elements of the vessel wall remain questions to be answered in the future. The altered innervation pattern may indicate a different functional efficacy of the trigemino-vascular system in the affected territories that might be of importance in the autoregulation of these sequences. These areas might be more vulnerable to the effects of spasmogenic agents after subarachnoid hemorrhage, leading to serious neurological disability. On the other hand, these short segments can be more prone to the direct spasmogenic effects of surgical manipulations as their capability for post-interventional dilatation might be decreased.

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