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PATHWAYS AND NERVE DENSITIES IN CEREBROVASCULAR INNERVATION

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SUMMARY

• // is gradually becoming clear that cerebrovascular nerves contribute to the control of the cerebral circulation although the knowledge of the functional mechanisms is far from complete. However, many aspects of the morphologic substrate have been identified. The basal cerebral arteries receive sympathetic, parasympathetic and sensory innervation, utilizing the superior cervical and stellate, the pterygopalatine and otic, and the trigeminal ganglia, respectively, as the main peripheral sources. Many of the neural pathways to the cerebral arteries have been elucidated. Those to the supratentorial arterial tree are distributed via the cavernous sinus and surrounding regions. Not only the "classical" neurotransmitters, but also many neuropeptides are found in cerebrovascular nerves. This will lead to new insights since the concepts of cotransmission and neuromodulation have been established now. In the arterial wall, a multilayered organization of nerves has been recognized, consisting ofparavascular nerve bundles of passage, a superficial plexus and a terminal plexus located at the adventitial-medial border. Human basal cerebral arteries display a topographical heterogeneity of densities of terminal nerve plexuses. Highest nerve densities are found in arterial segments forming the circle of Willis, in the efferent part of the posterior cerebral artery and in the anterior choroidal artery. Nerve density appears to be determined by locality rather than vascular diameter. Furthermore, local decreases in nerve density are observed with ageing and disease in animals and humans.

INTRODUCTION

• The existence of nerves around and in the walls of cerebral arteries has been known for a long time. Already in 1664 Sir Thomas Willis described in humans minute nerves on the arteries of the circle now bearing his name (1). However, there has been a controversy about their role (2), which was often considered of little importance. Cerebrovascular innervation has received more attention over the last few decades and the development of techniques such as immunohistochemistry and antero- and retrograde neuronal tracing led to a rapid increase of knowledge in this field. Nerves in the walls of the major cerebral arteries have been demonstrated in many species, including human (3), monkey (4), rabbit (5), rat (6), mouse (5), cat (7), dog (8), bullfrog (9) and bat (10). These perivascular nerves have been classified as sympathetic, parasympathetic and sensory, each group characterized by particular neurotransmitters (11). The aforementioned subpopulations of nerves constitute the so-called extrinsic system of cerebrovascular innervation which utilizes peripheral sources, i.e. sympathetic, parasympathetic and sensory ganglia (Fig. 1). There are also reports on an intrinsic system consisting of direct connections between central origins, like *locus coeruleus* and *nucleus tractus solitarii,* and intraparenchymal arteries (12,13).

Knowledge of the function of cerebrovascular nerves is still far from complete. It is generally assumed that they play some role in the regulation of cerebral blood flow next to other factors as arteriovenous perfusion pressure, chemical factors (ar-

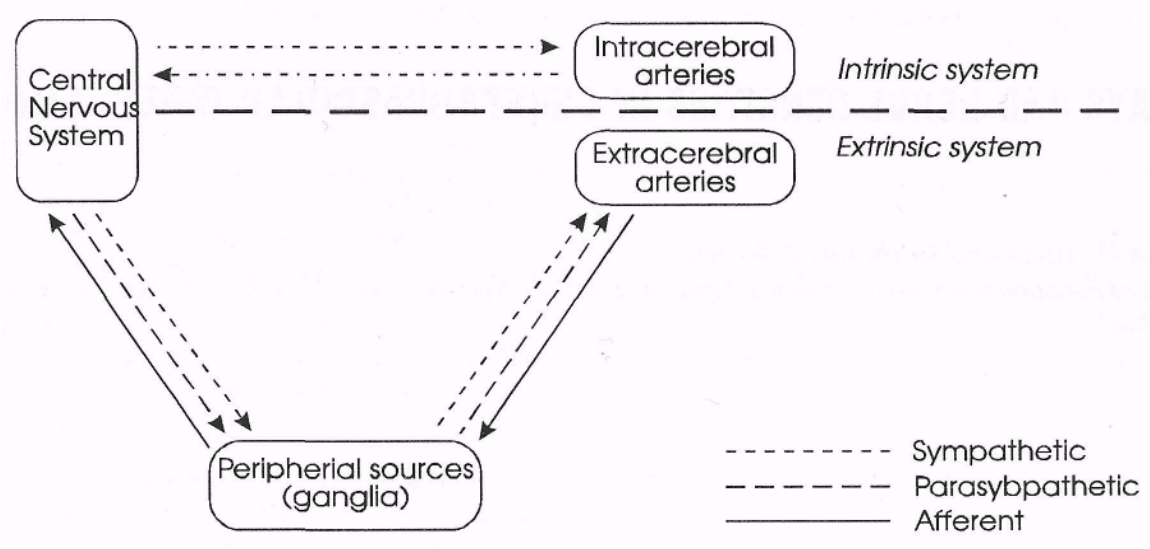

Figure 1. *Classification of cerebrovascular nerves. The intrinsic system consists of direct connections between central origins and intraparenchymal arteries. The extrinsic system utilizes peripheral sources: from the central nervous system (CNS) sympathetic and parasympathetic fibres reach extracerebral arteries after synapsing in an autonomic ganglion, while sensory fibres run from the arteries to the CNS via a sensory ganglion, without synapsing.*

terial pCO, and pO,), metabolic factors (brain tissue metabolites such as FT, K^+, adenosine) and the endothelium (14-16). It seems likely that neural regulation contributes broadly and considerably to the control of the cerebral circulation, although the effects are far from clear. Moreover, it may influence the different parts of the cerebral vasculature to varying extents and under certain conditions. It has become evident that there is a well defined role for sympathetic nerves in shifting the limits of autoregulation to higher pressure levels during hypertension and thereby preventing breakthrough of the blood-brain barrier (17-19). Recent findings suggest a protective role for parasympathetic nerves in focal cerebral ischemia (20-21). Function needs not be limited to flow adjustments, since it has been reported that intrinsic innervation of intraparenchymal microvessels influences the blood-brain barrier (22), and that extrinsic innervation of the choroid plexus influences the production of cerebrospinal fluid (19). There are also functions outside the field of circulation control. For example, cerebrovascular nerves exert a trophic influence on the arteries, especially during development (23). Furthermore, these nerves are ascribed a role in vascular pain syndromes (24,25).

Investigations of the past decade have revealed many aspects of the morphologic substrate of cerebrovascular innervation, i.e. sources of innervation, neural pathways to and nerve densities in the cerebral arteries, both in humans and laboratory animals. An accurate description of these aspects is a prerequisite for further investigations. The identification of a source of innervation. e.g. a sympathetic ganglion, indicates involvement of the particular nerve subpopulation in cerebrovascular innervation. The determination of the complete neural pathway is necessary to perform and interpret denervation experiments accurately, while high densities of terminal nerve plexuses in particular arteries may indicate special significance of the innervation of these vessels. In the present review the recent discoveries in this field are addressed. Only the extrinsic system is considered.

SOURCES AND PATHWAYS OF THE EXTRINSIC SYSTEM

• Most of the peripheral sources and the pathways from the ganglia to the cerebral arteries were obscure for a long time, except for parts of the sympathetic system, where much of the sympathetic input courses from the superior cervical ganglion along the internal carotid artery to the cerebral arteries. Knowledge in this field has increased substantially, especially in rat, for which animal the sources and pathways are briefly summarized as follows. Sympathetic nerves originate in superior cervical and stellate ganglia and reach the cerebral arterial tree along the internal carotid and vertebral arteries, respectively (26,27). Parasympathetic nerves originate in the pterygopalatine and otic ganglia and gain access to the cerebral arteries via the internal ethmoidal artery, located rostrally in the cranial cavity, and the internal carotid artery, respec-

lively (28-30). Small ganglia such as the internal carotid ganglion, situated in the carotid canal, provide parasympathetic fibres as well (28). Additional parasympathetic innervation may derive from ganglia situated in the cavernous sinus, reaching the internal carotid artery retrogradely via the abducens nerve (31). These ganglia include a large cell mass along the Vidian nerve, which is continuous with the "classical" pterygopalatine ganglion and may be considered a cavernous part of the pterygopalatine ganglion, and scattered small groups of ganglion cells (31). Sensory nerves originate mainly in the trigeminal ganglion (32,33) but an additional source is the internal carotid ganglion (33,34) whilst the afferent vessels to the circle of Willis are further supplied by the superior vagus ganglion (34) and the lst-2nd cervical spinal ganglia (32). Nerves from the cerebral arteries reach the trigeminal ganglion via the internal ethmoidal arteries and the ethmoidal nerves (33), and possibly via connections between the internal carotid artery and the abducens nerve as well (31).

Fig.2 summarizes the sources and pathways to the rostral cerebral arteries in rat, demonstrating that there are four sites where nerves can join the cerebral arteries. In cat, monkey and humans, to a large extent the same peripheral sources as in rat are most probably involved, but in each species the pathways may possess their own specific characteristics related to the anatomy of the species.

THE CAVERNOUS SINUS AND PATHWAYS FOR CEREBROVASCULAR NERVES

• Neural pathways for the innervation of intracranial structures, including the supratentorial part of the cerebral arterial tree, mainly converge upon the cavernous sinus and surrounding regions. Here, a close relationship between the internal carotid artery and several cranial nerves is found, while the pterygopalatine ganglion is in the vicinity. Neural connections between these structures can be expected to occur in this region.

Many of the pathways described in the preceding section were discovered by the application of immunohistochemistry and antero- and retrograde neuronal tracing, thereby providing evidence for projections from peripheral sources to cerebral arteries. However, irrefutably morphologic evidence for the topography of the pathways, including the course of nerves that connect cerebral arteries to neighbouring neural structures, was not always provided. Complementary anatomic information, necessary to perform and interpret tracer and denervation experiments precisely, can be obtained by staining whole-mount preparations.

For this reason the extent of neural connections in the cavernous sinus region was investigated recently in rat (31), using a sensitive acetylcholinesterase (AChE) method for staining whole-mount preparations (35). In the peripheral nervous system, AChE is distributed among many subpopulations of nerves, including adrenergic and sensory nerves (36), and can therefore be considered a general neural marker. This was confirmed by double staining for AChE and another general neural marker, protein gene product 9.5 (POP 9.5), of whole-mount preparations which revealed an almost complete overlap in staining (unpublished observation). The cavernous plexus was found to consist of an extensive cavernous plexus proper with small ganglia, mainly in the cavernous sinus lateral wall, and a lateral extension above the trigeminal nerve, related to the oculomotor and trochlear nerves. The cavernous plexus is connected to the pterygopalatine ganglion, the trigeminal ganglion and the abducens nerve. The consequences for pathways to cerebral arteries have been outlined in the preceding section.

Neural connections in the human cavernous sinus are investigated at present. To a large extent a similar organization of neural elements is found as that described in rat but some differences are observed as well (unpublished observation). The connections found strongly suggest a mixed nature of the cavernous sinus plexus: sympathetic fibres can be supplied by the internal carotid nerve, parasympathetic fibres by a connection to the pterygopalatine ganglion and sensory fibres by connections to the trigeminal ganglion, the ophthalmic nerve and possibly the maxillary nerve. An extension of this work combined with immunohistochemical characterization of neural structures in the cavernous sinus in rats and humans will further clarify intracranial neural pathways. This will enable a more precise performance of denervation experiments when investigating functional aspects of cerebrovascular innervation, whilst a proper extrapolation to humans from experiments performed in rats, requiring parallel morphologic data from rats and humans, is feasible. Also, the explanation of the symptoms of clinical syndromes that are linked with the cavernous sinus, e.g. cluster headache (25,37), will have a more solid basis.

HEUROTRAHSMITTERS IN CEREBROVASCULAR NERVES

• The nerves in and around the walls of cerebral arteries constitute a mixed population of sympathetic, parasympathetic and sensory nerves. Nowadays, it has become clear that this classical subdivision has limitations. Originally based on the presence of (nor) adrenaline inpostganglionic sympathetic neurons, and acetylcholine in postganglionic parasympathetic neurons, the discovery of many "new" neurotransmitters, including neuropeptides, requires extension of this classification. However, it is still widely used because it is not only based on pharmacological and physiological differences, but also on anatomical grounds. The cerebrovascular sympathetic nerves fit the classical sympathetic pathway, i.e. originating in the thoracic part of the spinal cord and synapsing in cervi-

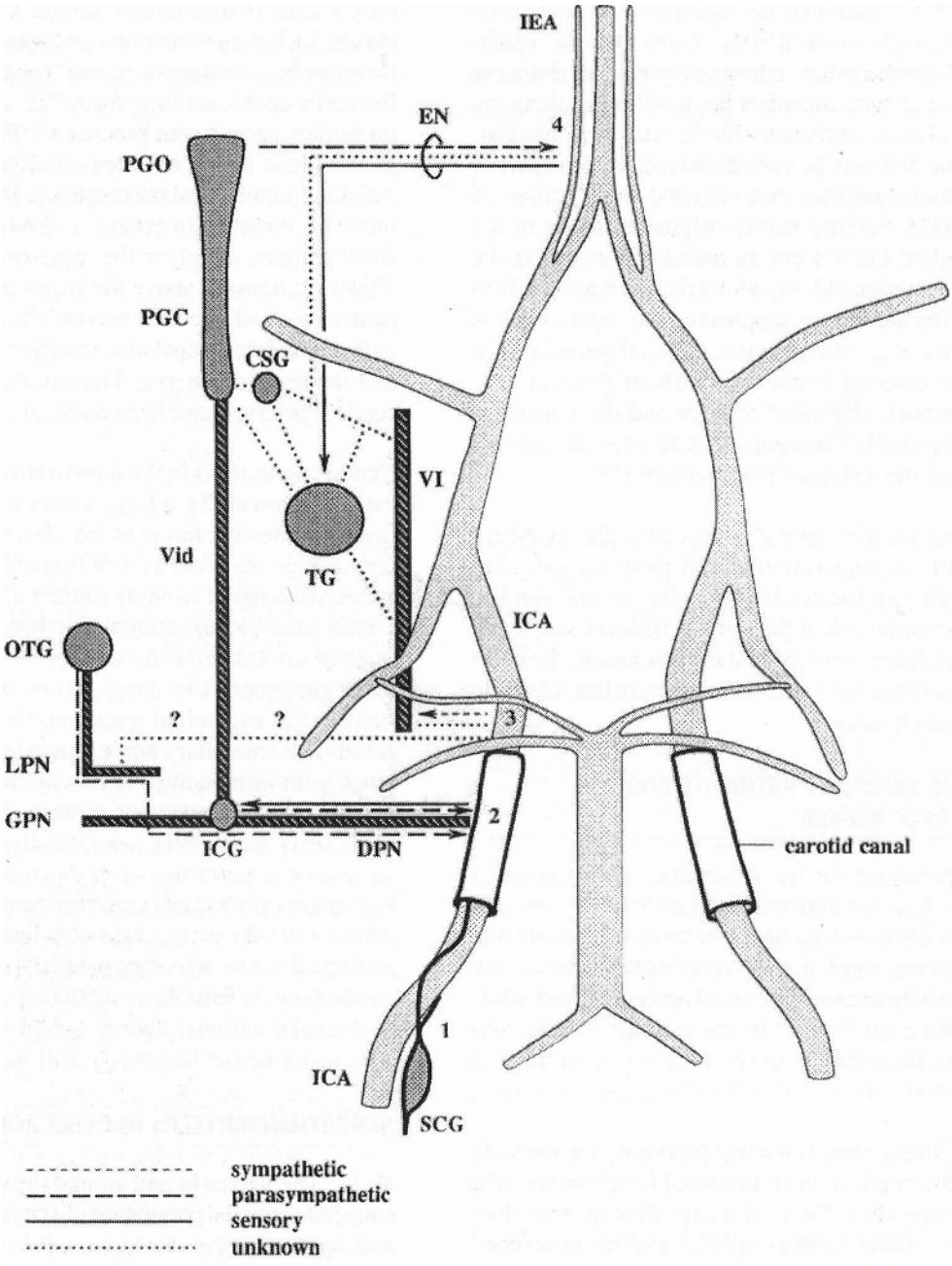

***Figure** 2. Drawing of the rat cerebral arteries, peripheral sources for the innervation of its rostral part, and the pathways to the arteries. The nature of the neural connections is indicated. There are four sites where nerves can join the cerebral arteries: 1. the ICA at the level of the superior cervical ganglion; 2. the ICA at the level of the internal carotid ganglion (in the carotid canal); 3. the ICA where it is crossed by the abducens nerve (intracranial); 4. the internal ethmoidal artery. Abbreviations: CSG - cavernous sinus ganglia (miniganglia), DPN-deep petrosal nerve, EN-ethmoidal nerve, GPN-greater petrosal nerve, ICA - internal carotid artery, ICG - internal carotid ganglion, IEA - internal ethmoidal artery, LPN - lesser petrosal nerve, OTG - otic ganglion, PGC - cavernous part ofpterygopalatine ganglion, PGO - orbital part ofpterygopalatine ganglion, SCG - superior cervical ganglion, TG - trigeminal ganglion , Vid - Vidian nerve, VI - abducens nerve.*

cal sympathetic trunk ganglia, while the parasympathetic nerves originate in the brainstem and synapse in ganglia in the head.

The best known neurotransmitters in cerebrovascular nerves are listed in Table 1. They include the classical neurotransmitters (noradrenaline and acetylcholine), 5-hydroxytryptamine and neuropeptides. The concept of cotransmission has been well established (15) and describes the capacity of nerves to synthesize, store and release more than one transmitter. The release of more than one substance from one neuron may indicate a complex interaction, known as neuromodulation, in which the effects of the neurotransmitters are modified by other released substances (15). It has become evident that (/) noradrenaline and neuropeptide Y (NPY) are colocalized in sympathetic nerves (38,39). (»') acetylcholine and vasoactive intestinal polypeptide (VIP) occur in parasympathetic nerves (29), and *(Hi)* substance P (SP) and calcitonin generelated peptide (CGRP) are present in sensory nerves (40). None of the neuropeptides, however, can be considered an exclusive marker for one of the three main nerve populations. Thus occurrence in another population is possible, as is the absence of the peptide in the principal population. For instance, it has been reported that NPY is not only colocalized with noradrenaline, but also with acetylcholine and VIP in subpopulations of parasympathetic neurons (41-43). It is also known that SP and CGRP do not occur in all trigeminal nerve fibres and cell bodies (44,45). Furthermore, extensive colocalization of acetylcholine and VIP has been questioned (46).

ARRANGEMENT AND DENSITIES OF NERVES IN THE VESSEL WALL

• The arrangement of nerves around and in the vessel wall is schematically represented in Fig. 3. It is based on the description by Nakakita *et at* (47) for rats and was recently confirmed in humans (48). Outside the adventitia are large, longitudinally arranged, paravascular nerve bundles, on their way to more distal parts of the arteries. From these bundles, nerves penetrate the adventitia where they form a superficial plexus, and run to the adventitial-medial border, to form the terminal plexus which is mainly circumferentially oriented. This innermost layer, also named "deep plexus" or "intrinsic plexus", is supposed to provide local functional innervation because of the close neuromuscular relationship (60-500 nm) and the presence of varicosities: beadlike widenings of axons where transmitter substances are stored (15,48-51). Because of the position of the terminal plexus at the surface of the *tunica media,* only the outermost smooth muscle cells are directly innervated. Nexuses between the muscle cells offer low electrical resistance pathways and electrical coupling between adjacent smooth muscle cells (52,53).

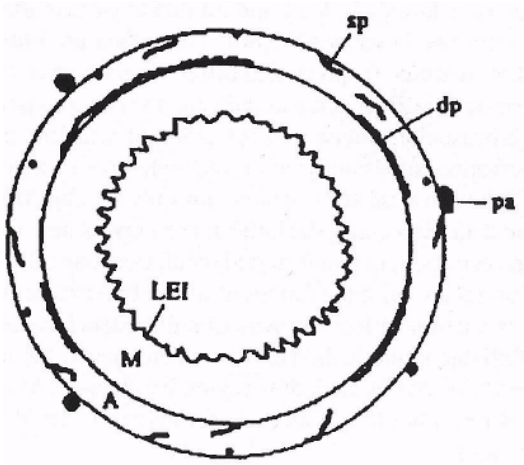

Figure 3. *Drawing of a transverse section of an artery and its nerves, -which are organized in, several layers. From large paravascular nerves (pa), branches penetrate the adventitia, where they form a superficial plexus (sp). From here nerves run to the adventitial-medial border to form the circumferentially oriented deep plexus (dp). A - tunica adventitia, M- tunica media, LEI - lamina elastica interna.*

Table 1. *Neurotransmitters in cerebrovascular nerves and predominating effects on pial arteries*

Neurotransmitter	*Abbreviation*	*Cerebrovascular response*
Noradrenaline	NA	constriction
Acetylcholine	ACh	dilation
5-Hydroxytryptamine (Serotonin)	5-HT	constriction
Neuropeptide Y	NPY	constriction
Vasoactive intestinal polypeptide	VIP	dilation
Substance P	SP	dilation
Calcitonin gene-related peptide	CGRP	dilation

Differences in nerve density between various cerebral arteries have been reported in several species. The most conspicuous one is the denser nerve plexus in the rostral part of the circulation (internal carotid system, including middle and anterior cerebral arteries) in comparison to the caudal circulation (vertebrobasilar system). This has been reported for various species including rat (54-56), guinea pig (57) and cat (58), mainly based on semiquantitative observations. In these investigations the data were obtained after staining for a variety of neural markers. Furthermore, these data are based on the complete nerve population, i.e. paravascular nerve bundles, superficial adventitial nerves and terminal plexuses taken together.

Until recently, quantitative data from humans, based on morphology, were largely lacking and limited to parts of the basal cerebral arteries in fetuses (59,60). These data are important for several reasons: (/) presumed differences in nerve density will become objective, and can indicate a varying importance of cerebrovascular innervation for different arteries, and (») the innervation pattern is dynamic and undergoes changes with age (61) and disease (62), which can only be objectified by numeric data. Especially the latter reason opens new insights and perspectives. A dynamic neural regulation may offer therapeutic strategies, once the functions of cerebrovascular nerves and their relation with cerebrovascular disorders have become clear. Reliable numeric data on nerve densities in the human basal cerebral arteries, including regressive changes, have been obtained recently (48,63) and are addressed in detail in the next sections.

NERVE DENSITIES IN THE HUMAN BASAL CEREBRAL ARTERIES: TOPOGRAPHICAL DISTRIBUTION

• As indicated in the preceding section, a basic step in revealing the mechanisms of neuronal regulation in human basal cerebral arteries is to provide a detailed description of the nerve fibres in these arteries, based on quantitative data. We have investigated the topographical distribution of densities of the terminal nerve plexuses in human basal cerebral arteries as well as the effects of ageing and Alzheimer's disease on nerve densities (48,63). These investigations have shown a regionally specific distribution of nerve densities located at the adventitial-medial border. Using the general neural marker PGP 9.5 for staining whole-mount preparations of various segments of the basal cerebral arteries (Fig.4), and computerized image analysis, high nerve densities were found in segments of the circle of Willis proper (intracircular arteries), especially in the posterior communicating artery and the intracircular part of the posterior cerebral artery (PI segment). Of the efferent arteries the extracircular part of the posterior cerebral artery (P2 segment) and the anterior choroidal artery showed highest nerve densities (Fig.5). There was no signifi-

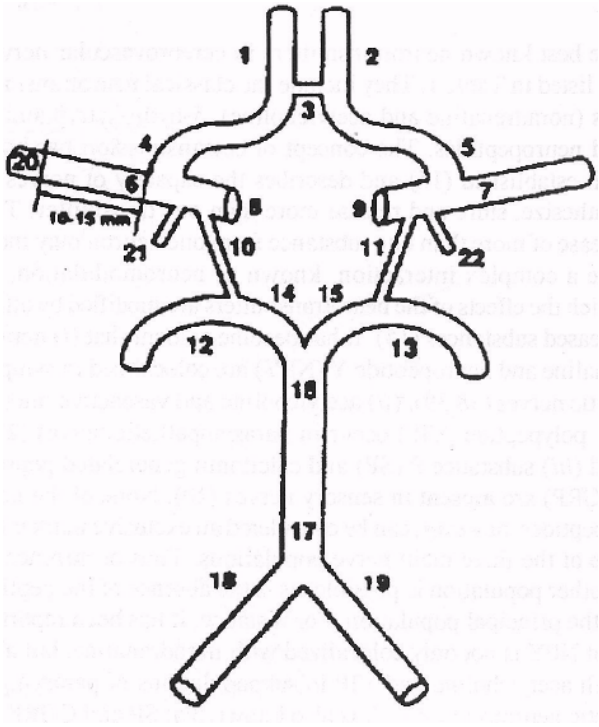

Figure 4. *Drawing of the human circle of Willis with its afferent and major efferent arteries. The investigated segments are indicated. Explanation of the abbreviations used:*

1 . 2	*A2*	*postcommunicating part of anterior cerebral artery*
3	*ACom*	*anterior communicating artery*
4. 5	*Al*	*precommunicating part of anterior cerebral artery*
6 . 7	*MCApr*	*proximal part of middle cerebral artery*
8. 9	*ICA*	*internal carotid artery*
10, 11	*PCom*	*posterior communicating artery*
12, 13	*P2*	*postcommunicating part of posterior cerebral artery*
14, 15	*PI*	*precommunicating part of posterior cerebral artery*
16	*BASdi*	*distal part of basilar artery*
17	*BASpr*	*proximal part of basilar artery*
18, 19	*VERT*	*vertebral artery*
20	*MCAdi*	*distal part of middle cerebral artery (10-15 mm from MCA-A1 bifurcation)*
21, 22	*ChA*	*anterior choroidal artery*

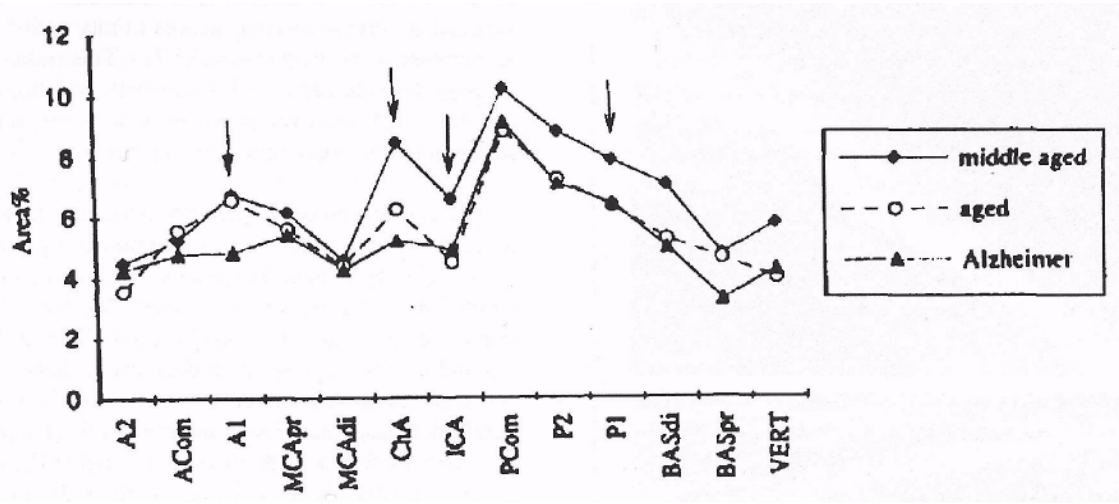

Figure 5. *Nerve densities expressed as area percentage of the vessel -wall in the human basal cerebral arteries. Comparison of the mean values of the three groups investigated: middle aged (32-52 years), aged (62-85 years) andAlzheimer 's disease (62-85 years). From the shapes of the graphs it can be observed that the topographical distribution ofdeepperivascular nerves is to a large extent similar for the three groups. Arrows point to statistically significant differences. Between middle aged and aged/Alzheimer groups (open arrows): significant decrease with ageing ofarea% in ChA, ICA and PL Between aged andAlzheimer groups (black arrow): significant decrease inAlzheimer 's disease ofarea% inAl. For abbreviations, see Fig.4.*

cant correlation between nerve density and vascular diameter: nerve density appeared to be determined by locality rather than vascular diameter.

The topographical heterogeneity that we found in humans is different from that found in rats. Denser innervation of anterior vessels in comparison with posterior vessels, as described in rat, could not be confirmed. Instead, in humans the highest nerve density was found in the posterior communicating artery. Furthermore, the deep plexus in this artery consisted of very thick, dense bundles of nerve fibres with varicosities (Fig.6). This artery is important for the hemodynamic balance between the internal carotid and vertebrobasilar circulations, acting as a regulator, a sensor, or combining both functions. Findings on electron microscopy were highly suggestive of motor function of the nerves, but the presence of vaso-sensory nerves cannot be excluded. High nerve densities were also found in other intracircular arteries (PI segment and Al segment), thereby indicating roles in regulation and/or sensing of flow in these arteries as well. In the efferent arteries, the nerve density in the P2 segment was significantly higher than in the extracircular part of the anterior cerebral artery (A2 segment) and the middle cerebral artery. This may be related to greater fluctuations in the metabolic need of the vascular territory of this segment (e.g. visual cortex) in comparison to other vascular territories. Furthermore, in rat the very dense innervation of the anterior cerebral artery could also implicate an important regulatory function in this artery, supplying a part of the brain that is relatively more important in this species than in humans: the rhinencephalon.

The abovementioned differences in nerve densities between the species provide an indication of locally specific neural regulation of the cerebral circulation. The argument for local function is reinforced by the lack of correlation between density of the deep nerve plexus and vessel diameter that we found in similar arterial segments in humans. Further indications of locally specific neural regulation related to high nerve densities are provided by the literature (64-66).

CHANGES IN AGEING AMD DISEASE

• From studies in laboratory animals it is known that cerebrovascular nerves display changes in a temporally specific manner (67). A decline of the overall nerve population in the ageing rat was found (68), whilst subpopulations of nerves can follow different patterns (69). Furthermore, it has become clear that certain diseases lead to changes in autonomic nerves, including cerebrovascular nerves. This has been demonstrated in spontaneously hypertensive rats (56) and in streptozotocin-diabetic rats (70).

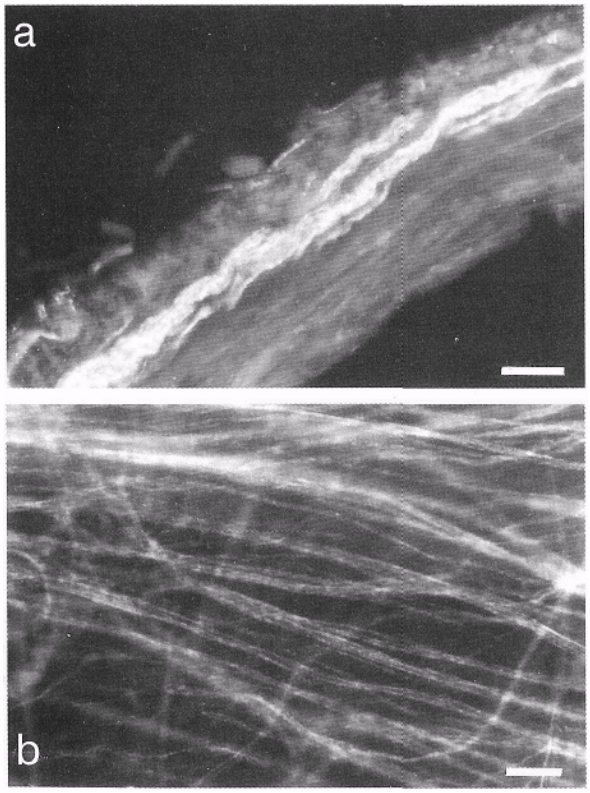

Figure 6. *Human posterior communicating artery and its deep nerve plexus, located at the adventitial-medial border, stained for PGP 9.5. (a) Whole-mount preparation demonstrating the transversely oriented deep plexus, which in this artery is dense and consists of thick nerve bundles, (b) Transverse cryostat section of the same* artery *illustrating thick nerve bundles at the adventitial-medial border with varicosities. Bars, 50 urn.*

We have recently obtained data on densities of the terminal nerve plexuses in the basal cerebral arteries from ageing humans and age-matched patients with Alzheimer's disease (63). Local decreases in nerve density were observed both with ageing and Alzheimer's disease (Fig.5). Ageing resulted in a significant decrease of nerve density in the internal carotid artery, the PI segment and the anterior choroidal artery, whilst in Alzheimer's disease there was a significant decrease in the intracir.cular part of the anterior cerebral artery (Al segment). These results confirm the view that age changes in perivascular and other peripheral nerve plexuses occur characteristically in a regionally-specific way (61).

It has been proposed (61,71) that decreased availability of target-derived neurotrophic factors such as nerve growth factor (NGF) underlies age- and disease-related neurodegeneration. Recent experiments have shown that treatment of ageing, degenerating cerebrovascular nerves in rats with NGF results in an increase of nerve density (72,73). This indicates that these nerves retain the capacity for regrowth and plasticity with old age. It is not known yet, however, whether regrowth of nerves following NGF treatment involves functional recovery.

At present we can only speculate about the factors that relate to local changes in nerve density with ageing and disease, and to the topographical heterogeneity. Hemodynamic factors may be the link to the alterations in nerve density (74). It is known that there are local reductions in cerebral blood flow with ageing and disease. The specific decrease of nerve density in the Al segment in Alzheimer's disease may be related to a severely decreased activity of neurons in the *nucleus basalis* of Meynert, as found in Alzheimer's patients (75) because this artery is involved in the vascular supply of the nucleus. It seems likely that local patterns of innervation can be adapted to altered circumstances. Future research will be directed at the interactions between hemodynamic factors, neurotrophic factors and nerve densities (74).

From a clinical point of view age- and disease-related changes are important, considering the stroke-protective role of sympathetic (76) and parasympathetic (20,21) nerves. This warrants further investigations of densities of nerve subpopulations in human cerebral arteries. If the abovementioned plasticity of cerebrovascular nerves exists in humans as well, neurotrophic factors could become important as therapeutic agents (77). More insight has to be gained first, however, into the function of cerebrovascular nerves and alterations in function in cerebrovascular diseases. Improvement of the understanding of functional and regulatory aspects of cerebrovascular innervation is of importance in relation to ageing and diseases with known neuropathic influences.

CONCLUSIONS

• The development of techniques such as immunohistochemistry and antero- and retrograde neuronal tracing led to a rapid increase of knowledge in the field of cerebrovascular innervation over the past few decades. A wealth of discoveries has made clear that this area is very complex, involving several subpopulations of nerves and many neurotransmitters and neuromodulators. Much progress has been made in the mapping of sources of innervation and neural pathways to the cerebral arteries, enabling more precise experiments in laboratory animals in order to reveal functional aspects. Parallel data on sources and pathways from animals and humans, necessary for the extrapolation of such experimental results, are becoming available. Human basal cerebral arteries display a topographical heterogeneity of densities of the terminal nerve

plexuses, as well as local changes with ageing and Alzheimer's disease. This is an indication of local neuronal control on segments of the basal cerebral arteries. Findings in laboratory animals that indicate: (*i*) stroke-protective roles for sympathetic and parasympathetic nerves, (*ii*) retained plasticity of the autonomic nervous system in old age, and (*iii*) an influence of neurotrophic factors on nerve densities, open interesting fields for further research and may be a basis for future therapeutic strategies.

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