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Neuropeptide Y- and substance P-like immunoreactive nerve fibers in the rat dura mater encephali

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Summary. Density and pattern of nerve fibers with neuropeptide Y-like immunoreactivity (NPY-LI) and substance P-like immunoreactivity (SP-LI) in the rat dura mater encephali were investigated by light and electron microscopy using whole-mount preparations. NPY-LI fibers are observed throughout the encephalic dura mater. A remarkable net of NPY-LI nerve fibers is located in the walls of the sagittal and transverse sinuses. Beyond that NPY-LI network, distinct NPY-LI nerve fibers or plexus occur in the rostral falx, parietal dura mater of the olfactory bulb, supratentorial dura mater, parietal dura mater of the cerebellum, tentorium cerebelli and the ventral dura mater. Electron microscopic studies reveal that NPY-LI is exclusively located in unmyelinated axons of small and large nerve fiber bundles, with or without a perineural sheath. Immunopositive C-fibers are predominantly associated with the vascular bed.

SP-LI nerve fibers have a moderate and more uniform distribution in the encephalic dura mater. A distinct plexus of SP-LI fibers follows the branches of the middle meningeal artery and the adjacent dura mater. SP-LI fibers are most prominent in the parietal dura mater of the cerebellum. Fine beaded SP-LI fibers, arising from larger SP-LI fiber bundles, are observed in close association to the capillary bed. SP-LI axons are all unmyelinated. They are found in larger nerve fiber bundles with a perineural sheath or in Schwann cells lacking any perineural sheath.

The function of NPY-LI and SP-LI nerve fibers in the rat dura mater is discussed in relation to their topography, density and termination.

Key words: Neuropeptide Y – Substance P – Immunocytochemistry – C-fibers – Dura mater – Dural sinus – Meningeal arteries – Electron microscopy

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Introduction

The innervation of the dura mater encephali has attracted particular interest in connection with the pathogenesis of vascular headache (Moskowitz 1984; Moskowitz et al. 1989). In man, high-intensity stimulation of the dura mater along the blood vessels elicited pain, whereas stimulation of the connective tissue of the dura mater did not result in pain (Ray and Wolff 1940; Penfield and McNaughton 1940; Wirth and von Buren 1971). Ultrastructural studies showed mechanoreceptive and most likely nociceptive afferents at different segments of the meningeal vessels and in the connective tissue of the dura mater (Andres et al. 1987a; 1987b).

Modern tracing techniques have revealed that the innervation of the dura mater consists of a variety of nerve fibers arising from different cranial, spinal and autonomic ganglia such as the trigeminal, vagal, second and third dorsal root, sphenopalatine, otic and the superior cervical ganglia (Mayberg et al. 1981, 1984; Steiger et al. 1982; Keller et al. 1985; O'Connor and van der Kooy 1986; Uddman et al. 1989). Adrenergic, cholinergic and peptidergic nerve fibers such as substance P (SP), neurokinin A (NKA), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) were observed in the dura mater of mammals using immunocytochemical methods (Amenta et al. 1980; Edvinsson and Uddman 1981; Furness et al. 1982; Edvinsson et al. 1983, 1987, 1988; Mayberg et al. 1984; Suzuki et al. 1988, 1989; Silverman and Kruger 1989). The occurrence of a remarkable sympathetic nerve fiber plexus was recently documented using dopamine-β-hydroxylase immunocytochemistry (Keller et al. 1989).

A systematic study of the distribution and topography of neuropeptides in the dura mater is still lacking. In the present investigation therefore the topography of neuropeptide Y-like (NPY-LI) and substance P-like (SP-

LI) immunoreactive nerve fibers in the rat dura mater encephali was studied. NPY occurs in postganglionic sympathetic nerve fibers in the dura mater and can be colocalized in neurons of the superior cervical ganglion (Ekblad et al. 1984; Edvinsson et al. 1987; Tamamaki and Nojyo 1987). Furthermore a colocalization with vasointestinal polypeptide (VIP) and cholinacetyltransferase in neurons of the sphenopalatine ganglion has been reported (Leblanc et al. 1987; Gibbins and Morris 1988; Suzuki et al. 1989). Nerve fibers with substance P immunoreactivity are possibly sensory in function and have their origin in cells of the trigeminal ganglion (Moskowitz et al. 1983; Uddman et al. 1985; Yamamoto et al. 1983). Apart from these results SP fibers also emerge from nerve cells of the internal carotid miniganglia (Suzuki et al. 1989). Further there is much experimental evidence that substance P is involved in mechanisms of neurogenic inflammation (Lembeck et al. 1982; Saria et al. 1983; Lundberg et al. 1984; Moskowitz et al. 1984; Markowitz et al. 1987; McDonald 1988).

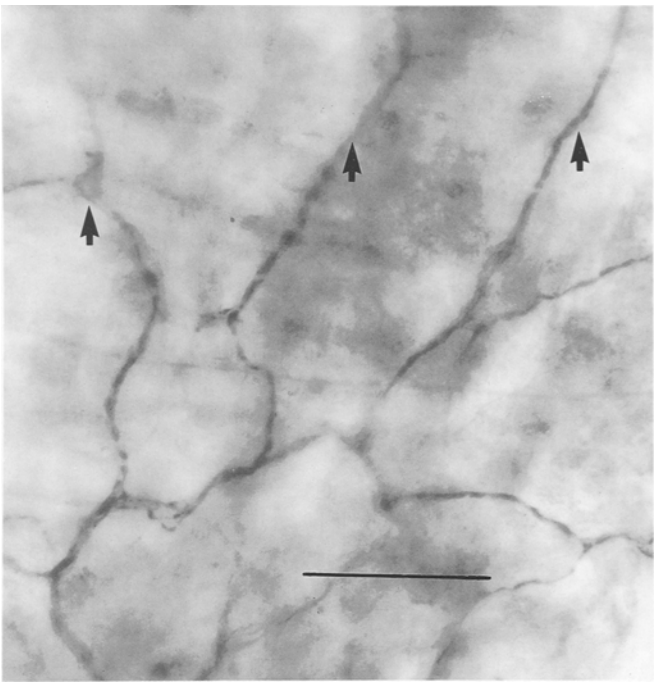

Fig. 2. Detail of the parietal dura mater covering the olfactory bulb in the area of the transitional zone where the NPY-LI nerve fiber net (some fibers of the net are out of focus) passes over to straight running fibers (*arrows*). Whole-mount preparation, × 500; bar 50 μm

Materials and methods

Four male Sprague Dawley rats between 140 and 400 g were anaesthetized with sodium pentobarbital (120 mg/kg body weight intraperitoneally). Prior to fixation 0.5 ml heparin was injected intracardially to prevent intravasal coagulation. Cardiac perfusion started with a 15-s flush of Tyrode solution (pH 7.4, 38° C) followed by 2000 ml of 4% paraform-aldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4, 22° C) for 30 min. Without delay the dura mater was carefully dissected from the skull and rinsed in a 0.1 M phosphate buffer, pH 7.4 containing 7.5% sucrose.

For immunocytochemistry, free floating specimens were smoothly agitated on a horizontally rotating table in all incubation steps. The specimens were treated with 1% solution of sodium borohydride in PBS (phosphate buffered saline) for 30 min; followed by washing in PBS and preincubation in a solution containing 20% normal goat serum (NGS), 0.3% Triton X-100 and 0.05% phenylhydrazine in PBS for 30 min at room temperature. As prima-

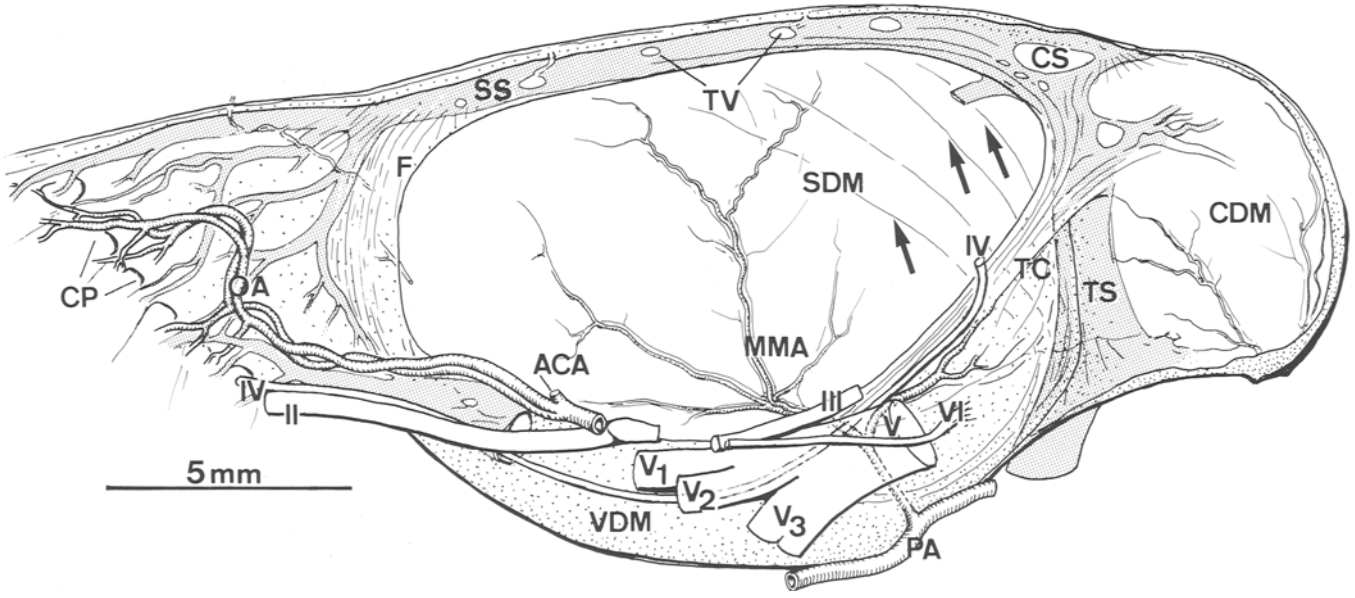

Fig. 1. Ventromedial view of the dura mater encephali with rostral falx (*F*), olfactory artery (*OA*), cribriform plate (*CP*), anterior cerebral artery (*ACA*), sagittal sinus (*SS*), tributary vein (*TV*), confluens sinuum (*CS*), transverse sinus (*TS*), supratentorial dura mater (*SDM*), middle meningeal artery (*MMA*), pterygopalatine artery (*PA*), dura mater of the cerebellum (*CDM*), ventral dura mater (*VDM*), tentorium cerebelli (*TC*), optic nerve (*II*), oculomotor nerve (*III*), trochlear nerve (*IV*), ophthalmic nerve (*V1*), maxillary nerve (*V2*), mandibular nerve (*V3*), abducent nerve (*VI*), bundles of dural nerves running to the sagittal sinus (*arrows*)

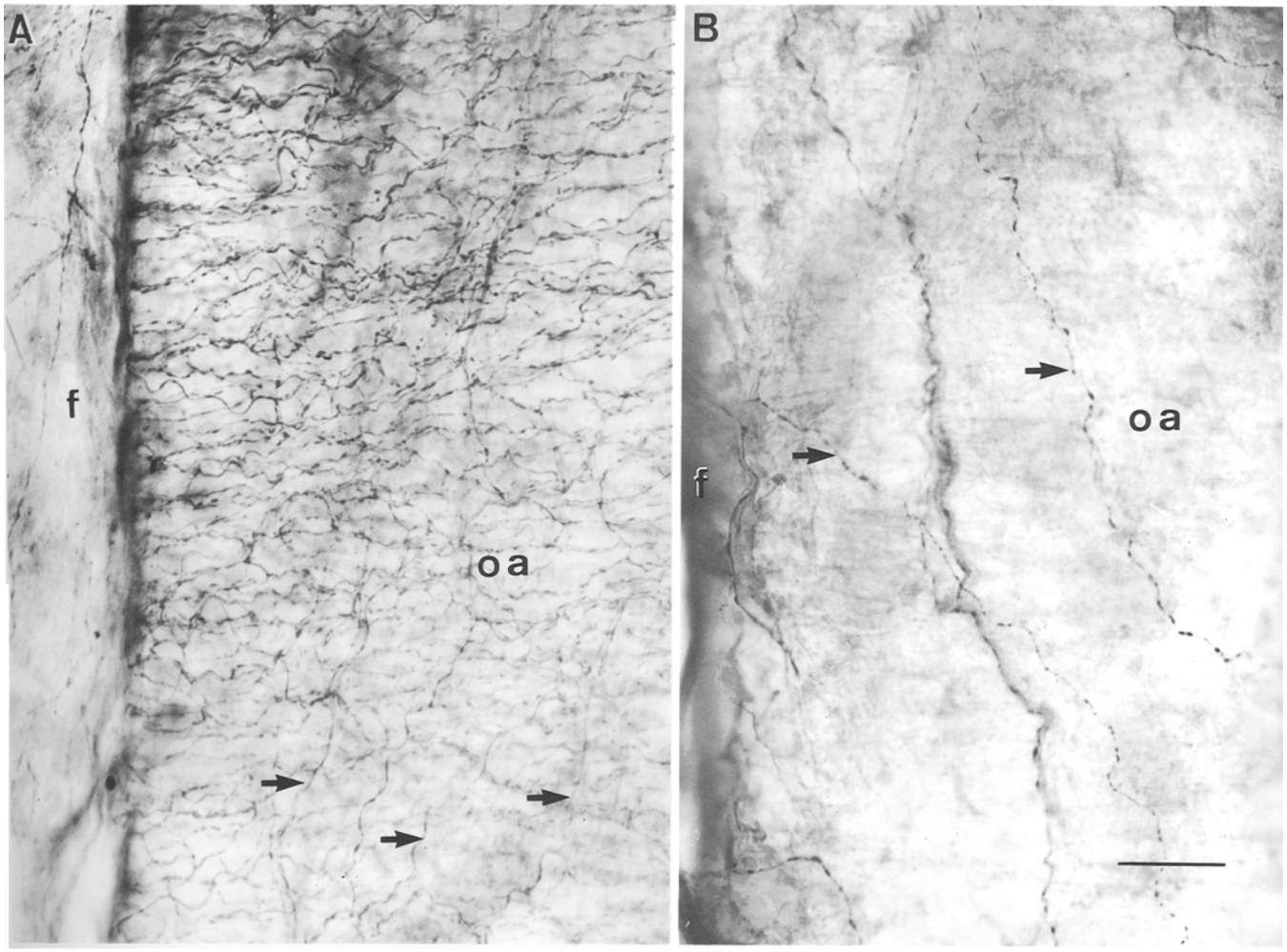

Fig. 3A, B. Olfactory arteries (*oa*) in the rostral part of the falx (*f*). **A** Note the prominent circular orientated NPY-LI nerve fibers. Some thin fibers run parallel to the long axis of the artery (*arrows*). **B** Moderate number of substance P-LI nerve fibers longitudinally orientated (*arrows*). × 300; bar 50 µm

ry antibodies either rabbit anti-SP (Cambridge Research Biochemicals, Cambridge, UK) diluted 1:100 in a solution consisting of 20% NGS, 0.3% Triton X-100, 0.01% Thimerosal and 0.1% sodium azide in PBS or rabbit anti-NPY (Amersham International, Buckinghamshire, UK) diluted 1:1000 in the above solution, was applied for 36 h in the cold room (4° C). After three washings in PBS the specimens were exposed to biotinylated goat anti-rabbit IgG (Medac, Hamburg, FRG), diluted 1:200 in PBS-A (2 mg/ml bovine serum albumin in PBS) for 24 h at 4° C. Three washings in PBS were followed by treatment with ABC-reagent (avidin/biotinylated peroxidase complex; Camon, Wiesbaden, FRG) diluted 1:1000 in PBS-A for 6 h at 25° C. After rinsing in PBS, immunoreactivity was visualised with a solution consisting of 5 mg 3,3′diaminobenzidine and 68 mg imidazole in 10 ml of 50 mM tris buffer pH 7.6, supplemented with 5 µl 30% H_2O_2 for 15 min at room temperature. After another washing in PBS the specimens were postfixed with 0.4% aqueous OsO_4 for 30 min at room temperature.

For the control, the primary antibody was omitted or replaced by irrelevant antibody. No specific staining of nerve fibers was observed. Nevertheless cross-reactivity with peptides or proteins sharing the same amino acid sequences cannot be excluded. Therefore the immunoreactive material is referred to as SP-like and NPY-like. For light microscopy two whole specimens were mounted on gelatin coated slides dehydrated in graded ethanol and coverslipped with Entellan (Merck, Darmstadt, FRG).

Two other specimens for electron microscopy were dehydrated and in toto embedded in araldite to get the correct topography of the labelled nerve fibers. Immunoreactive nerve fibers are selected by visual inspection. Alternating series of semithin and ultrathin sections were cut on a Reichert-Jung Ultracut E. Photodocumentation was performed with a Zeiss Photomicroscope II. Stained and unstained ultrathin sections were examined with the Philips 300 electron microscope.

Results

NPY-like immunoreactive nerve fibers

Light microscopy

NPY-LI nerve fibers are observed in the entire dura mater encephali with local differences in density, pattern and relationship to the vascular bed. They are characterized by varicosities at irregular intervals. Due to the vascular perfusion technique all vessels, including the microvessels, can be identified lightmicroscopically in the whole-mount preparations. Pattern and distribution

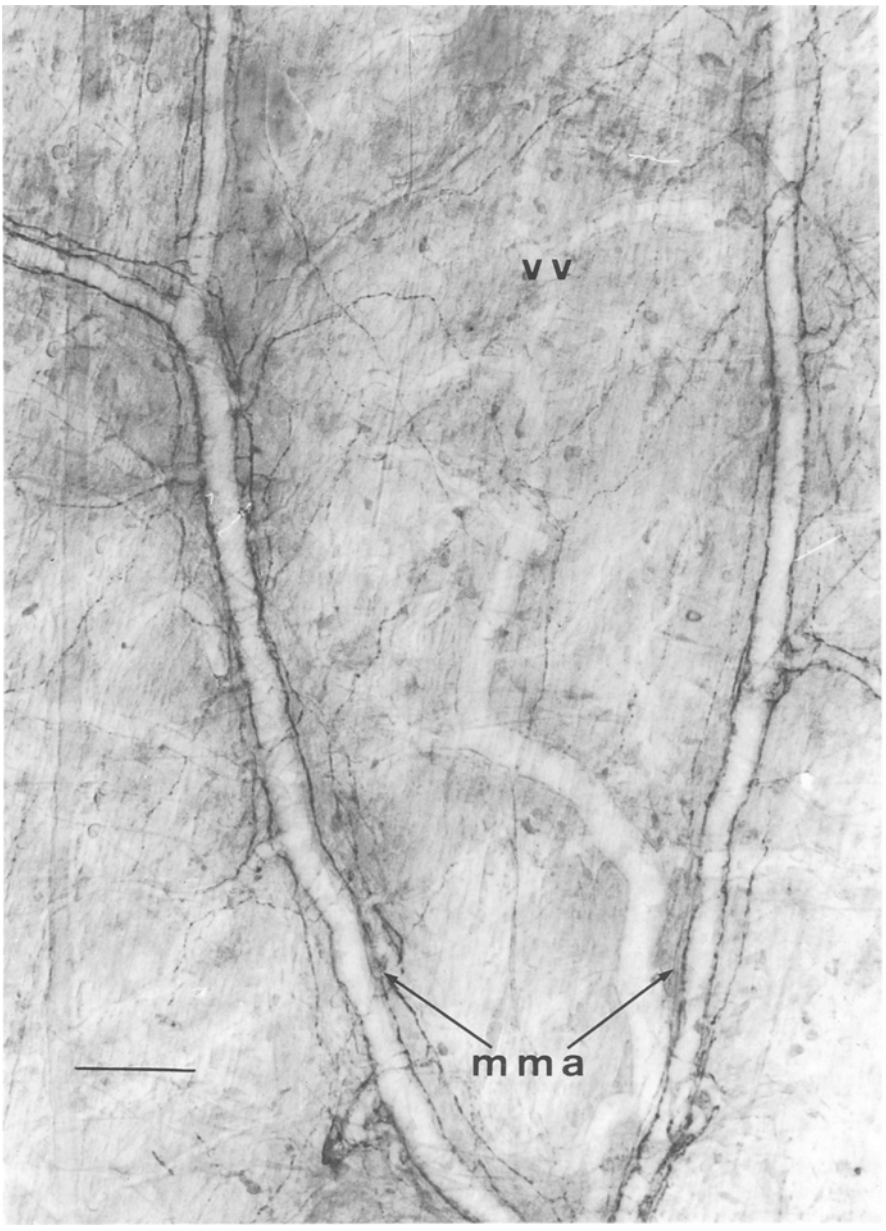

Fig. 4. NPY-LI nerve fiber plexus in close association with the distal branches of the middle meningeal artery (*mma*) and within the adjacent parietal dura mater. Venous vessel (*vv*) of the dura mater. Whole-mount preparation, ×160; bar 100 µm

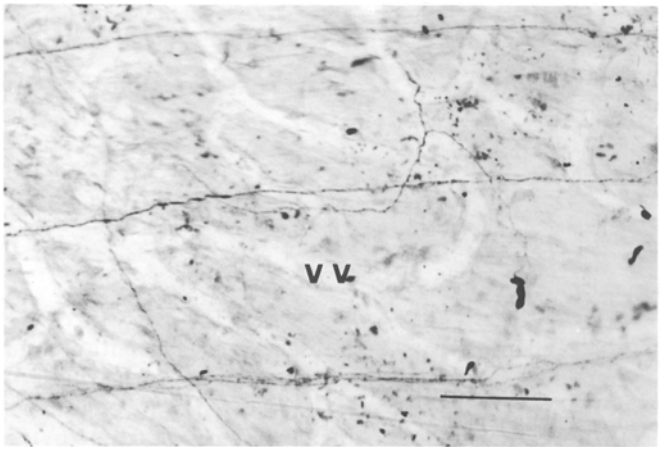

Fig. 5. NPY-LI nerve fibers in the dorsal part of the supratentorial dura mater. Note the parallel course of the nerve fibers. Whole-mount preparation, ×160; bar 100 µm

of NPY-LI nerve fibers are similar on both sides of the encephalic dura mater.

The parietal dura mater covering the olfactory bulb and the rostral segment of the falx separating the two bulbs (Fig. 1) exhibit in their ventral region a prominent immunoreactive nerve fiber net close to the blood vessels. The fibers course parallel to the long axis of the vessels. More dorsally the labeled fiber net passes over in thick fibers running straight and independent from vascular structures to the sagittal sinus (Fig. 2). The olfactory artery, a branch of the anterior cerebral artery, passes through the ventrorostral falx to the nasal cavity (Fig. 1) and exhibits a dense and predominantly circular NPY-LI nerve fiber plexus (Fig. 3A). There are nerve fiber connections between this plexus and the plexus of the septal dura.

In the supratentorial dura mater NPY-LI fibers follow the branching of the meningeal vessels or run apart

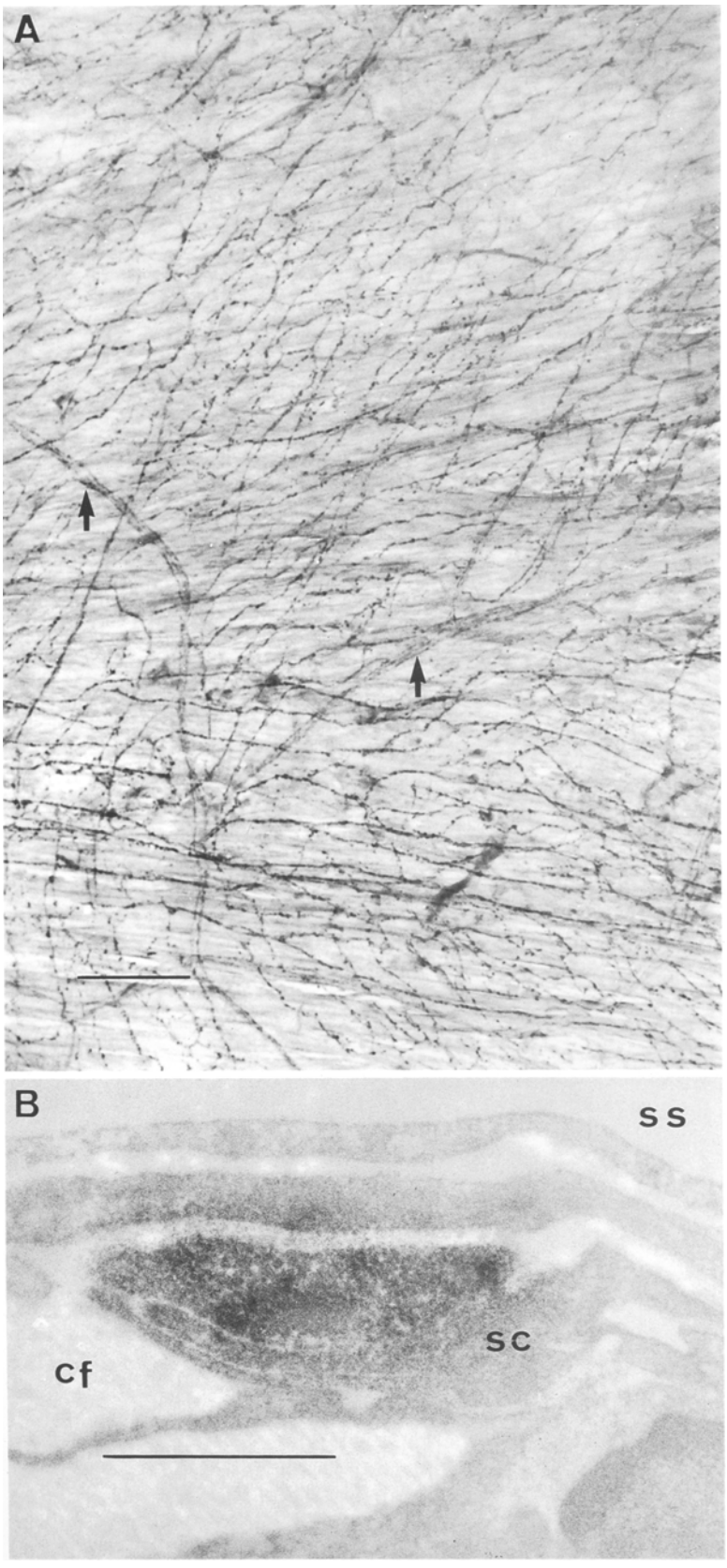

Fig. 6. **A** Segment of a whole-mount preparation of the sagittal sinus after removal of the dorsal wall. NPY-LI nerve fibers with a predominant longitudinal orientation. Note the thicker nerve fiber bundles (*arrows*). **B** Electron micrograph (unstained) showing a nerve fiber profile with NPY reaction product in the lateral wall of the sagittal sinus (*ss*) of the rat. Schwann cell (*sc*), collagen fiber bundle (*cf*) **A** ×160; bar 100 μm; **B** ×33000; bar 1 μm

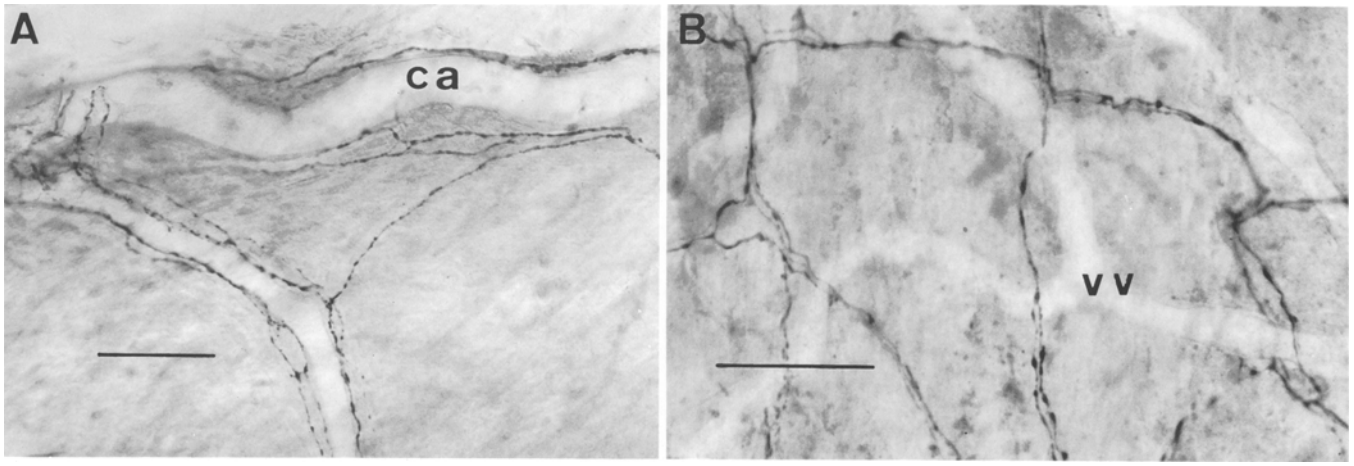

Fig. 7 A, B. NPY-LI nerve fiber plexus in the parietal dura of the cerebellum. **A** Beaded nerve fibers close to a cerebellar meningeal artery (*ca*). **B** NPY-LI nerve fibers independent from vascular structures, venous vessel (*vv*), whole-mount preparation, **A** ×280; bar 50 μm; **B** ×400; bar 50 μm

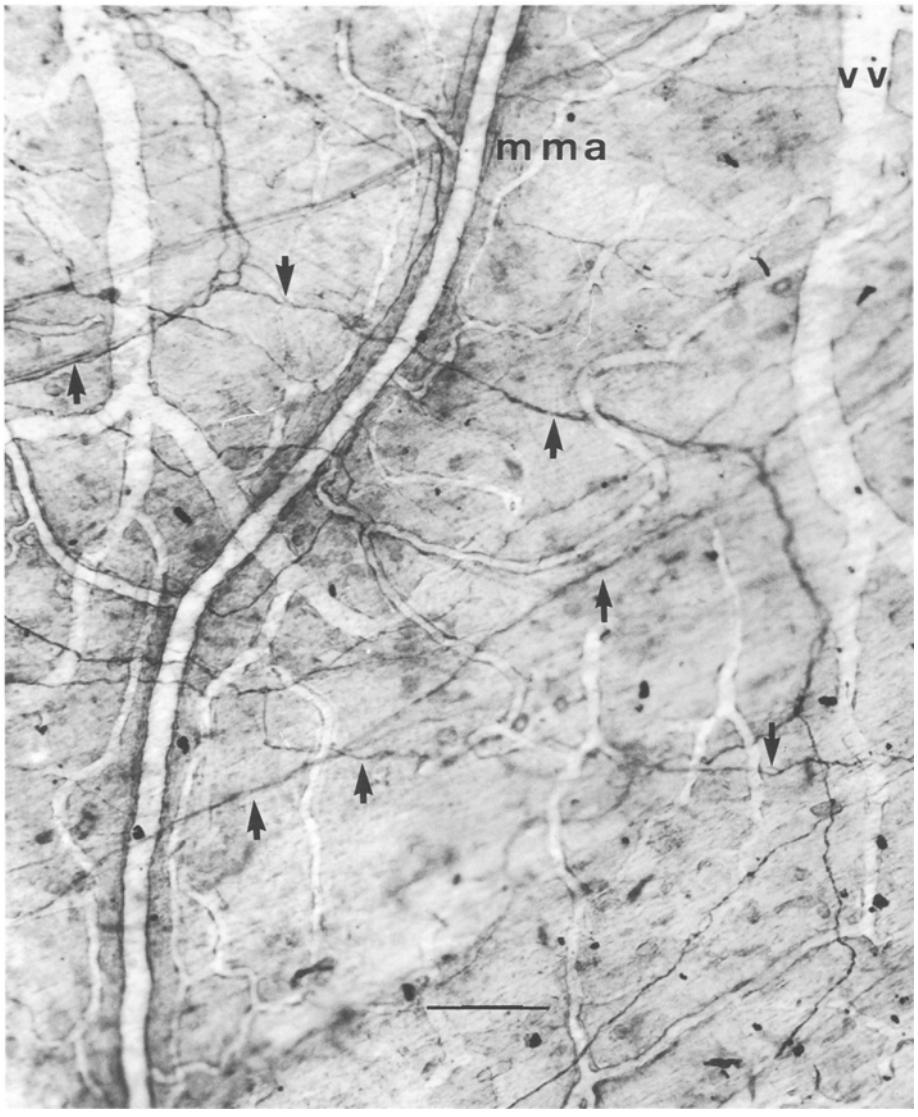

Fig. 8. SP-LI nerve fiber plexus in the parietal dura mater adjacent to the posterior branches of the middle meningeal artery (*mma*). *Arrows* mark a prominent fiber net independent from the vessels. Venous vessel (*vv*). Whole-mount preparation, ×160; bar 100 μm

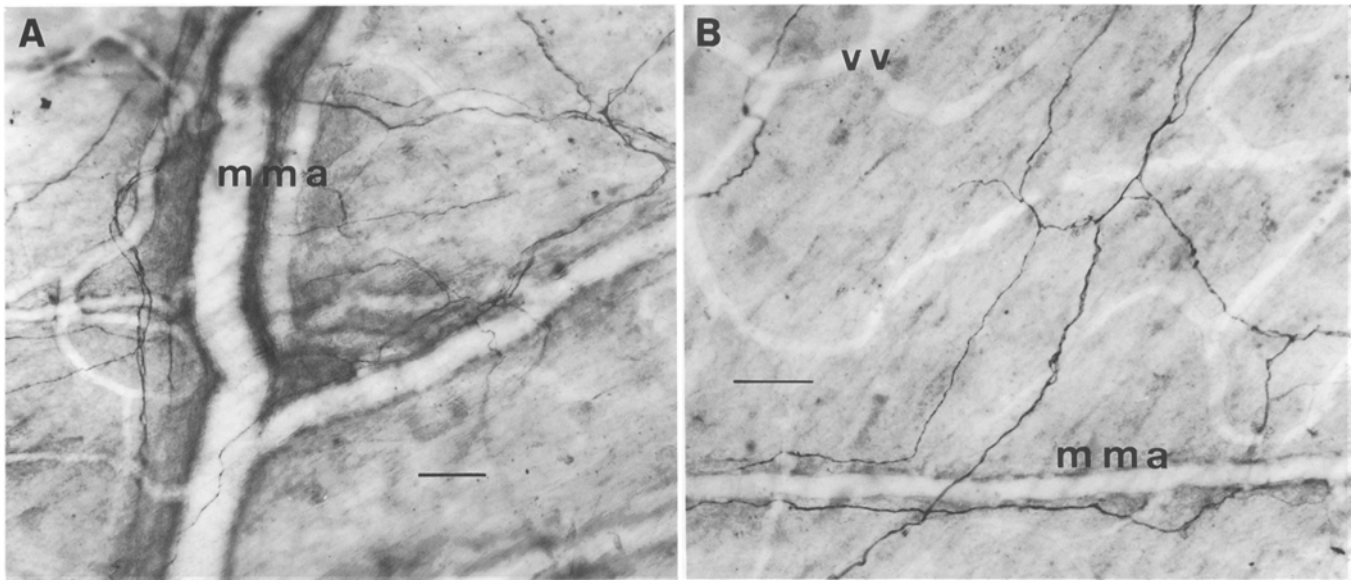

Fig. 9. A, B. Delicate SP-LI nerve fiber plexus at the posterior branch of the middle meningeal artery (*mma*), venous vessel (*vv*). Whole-mount preparations, **A** ×180; bar 50 μm; **B** ×210; bar 50 μm

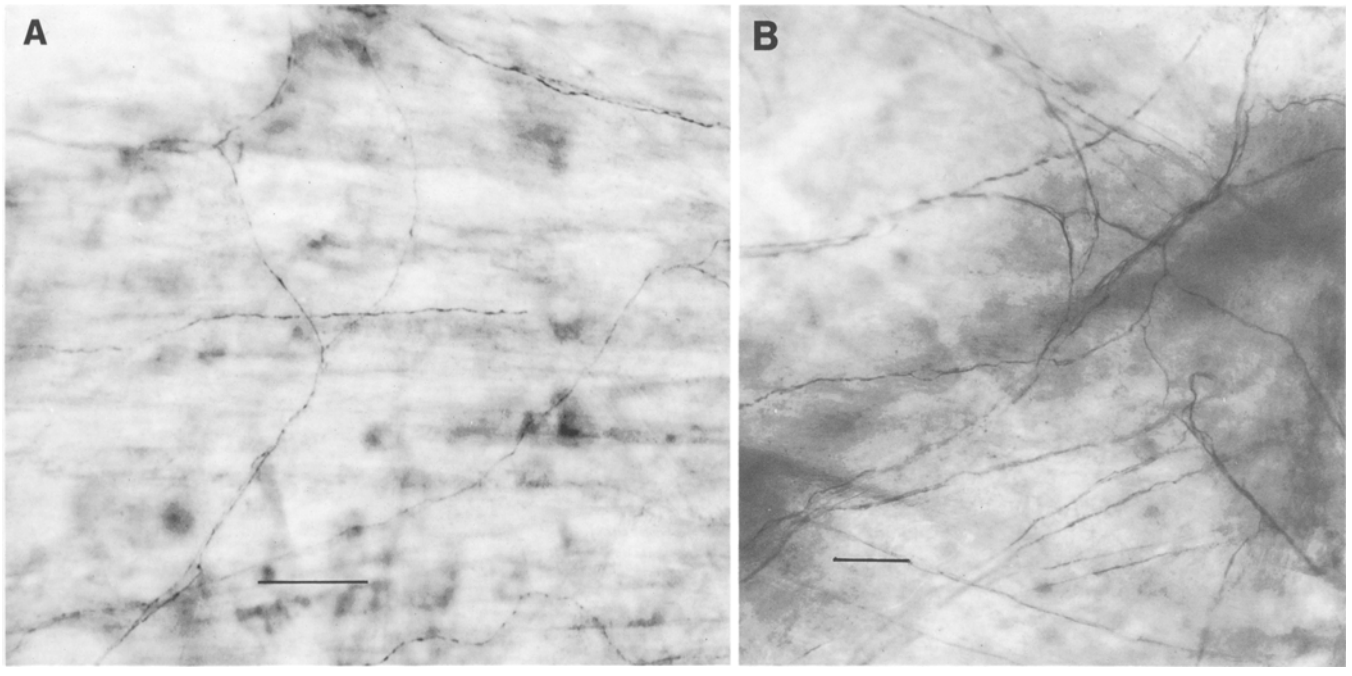

Fig. 10. A Widely meshed SP-LI nerve fiber plexus of the middle region of the sagittal sinus after removal of the dorsal wall. **B** SP-LI fibers at the confluens sinuum. Whole-mount preparation, **A** ×280; bar 50 μm; **B** ×200; bar 50 μm

from any vascular structure. The middle meningeal artery and its branches exhibit vessel associated nerve fiber plexus which become most prominent along the distal branches (Fig. 4). At the site of the dural exit of the maxillary nerve a moderate number of NPY-LI nerve fibers follows a ventrodorsal direction to the sagittal sinus. A widely meshed net of beaded and thin fibers is restricted to the adjacent ventrolateral part of the supratentorial dura mater. Single and straight running NPY-LI fibers are observed in the dorsolateral part between the transverse and sagittal sinus (Fig. 5).

A prominent NPY-LI nerve fiber plexus extends from the middle region of the sagittal sinus to the confluens sinuum. Some thick fiber bundles with a more transverse course supply this plexus, which consists of 40 to 50 immunolabeled nerve fibers. The fibers are predominantly parallel to the long axis of the sinus and are mainly located in its lateral walls (Fig. 6A). The

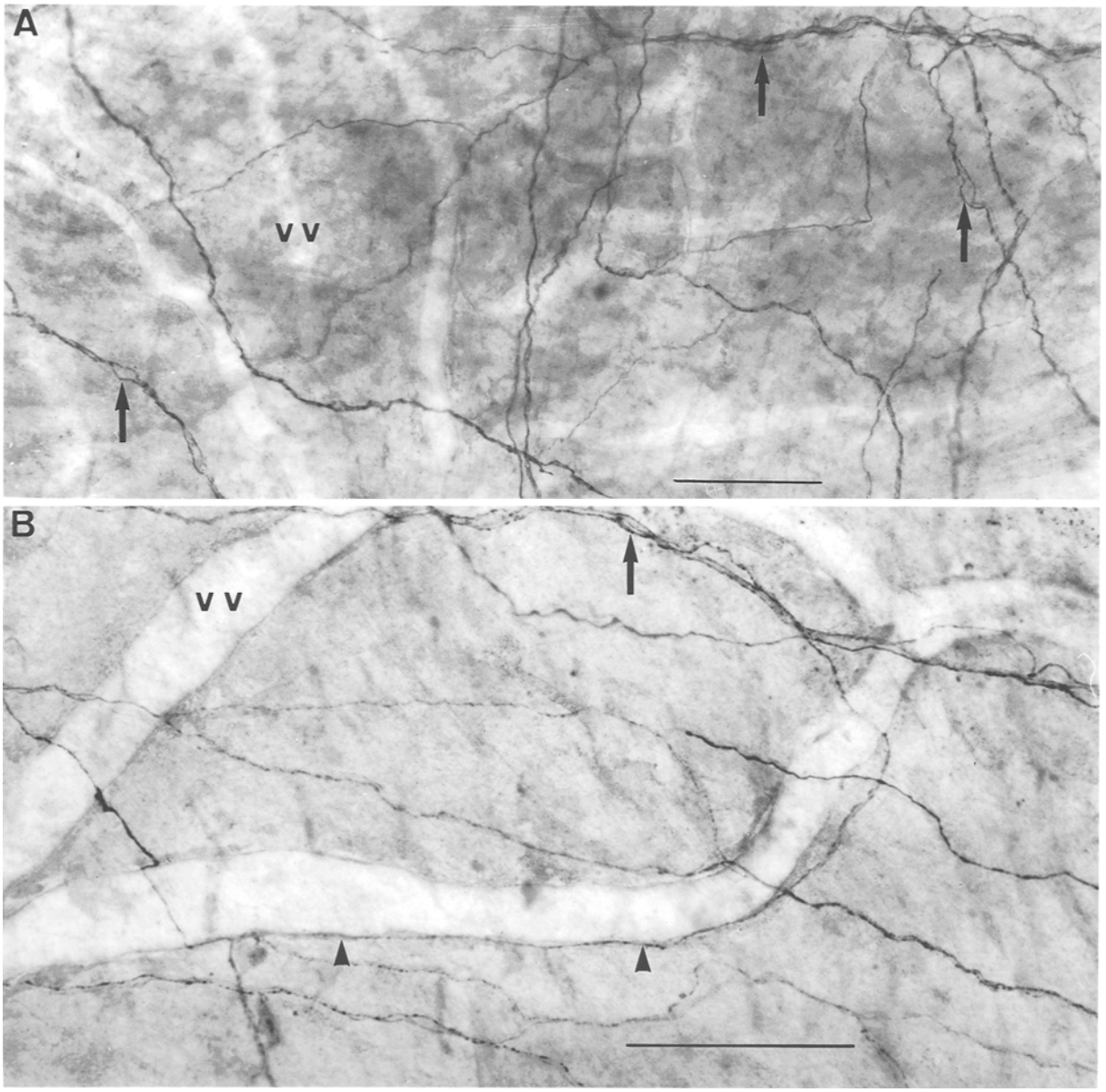

Fig. 11 A, B. Prominent SP-LI nerve fiber plexus in the parietal dura covering the cerebellum. Thick fiber bundles (*arrows*), venous vessel (*vv*). **B** SP-LI nerve fiber close to the microvessel (*arrowheads*) arising from the thick fiber bundle (*arrow*) **A** ×200; bar 100 µm; **B** ×330; bar 100 µm

NPY-LI plexus in the adjacent rostral region is less dense, with about 20 immunoreactive fibers following the long axis of the sinus. In addition to this, other NPY-LI nerve fibers take a distinct circular course. Distribution and density of NPY-LI fibers around the transverse sinus are similar to the rostral part of the sagittal sinus. Only few and delicate labeled fibers are observed in the dura mater covering the sagittal and transverse sinuses. Tributary veins (Fig. 1) have a moderate net of NPY-LI fibers surrounding their circumference.

The parietal dura mater covering the cerebellum contains distinct labeled nerve fibers with a predominantly straight course from ventrocaudal to dorsorostral to the confluens sinuum. Adjacent to the sinus the pattern changes into a distinct net of fine fibers (Fig. 7A, B). NPY-LI nerve fibers form a dense net within the tentorium cerebelli. Some of the nerve fibers follow a ventrodorsal direction to the transverse sinus wall. Thinner immunoreactive fibers seem to link the thicker fibers. A net-like pattern of varicose NPY-LI axons characterizes the ventral dura mater except the capsule of the hypophysis where no labeled axons are found.

Electron microscopy

Only unmyelinated nerve axons contain the reaction product. Labeled axons are found in nerve fiber bundles with or without a thin perineural sheath. A single Schwann cell ensheaths both NPY positive and negative profiles. The majority of the labeled axons are associated with Schwann cell processes and close to arterial and venous blood vessels of different diameter, with at least 0.1 µm distance to the smooth muscle cells (Fig. 6B). Quantitative studies of selected areas of the sagittal sinus

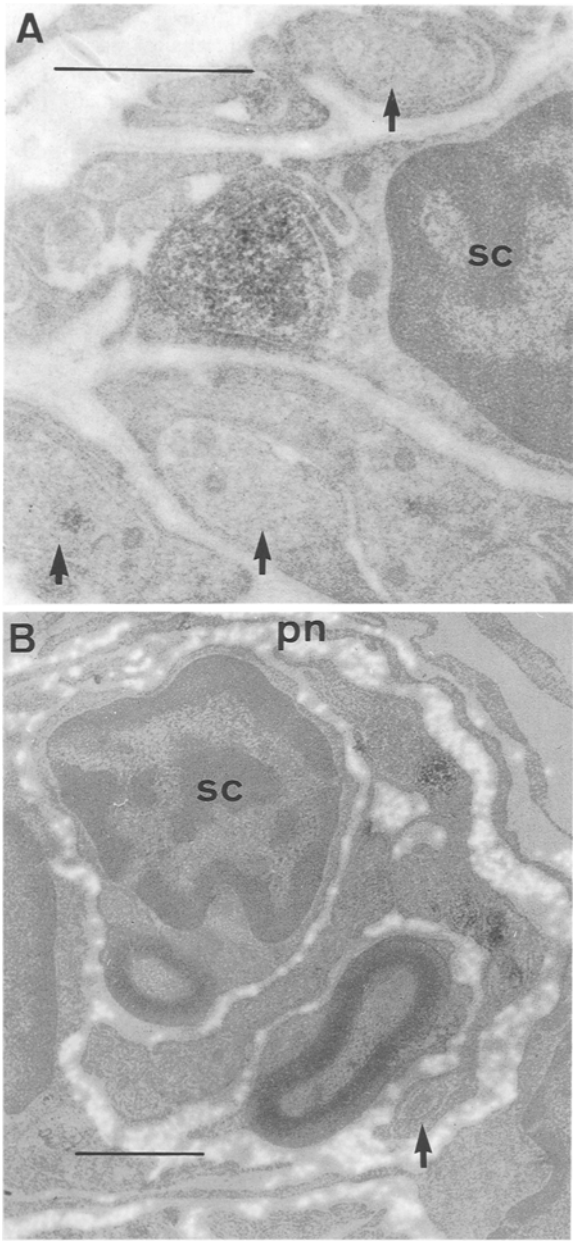

Fig. 12. **A** Electron micrograph of immunoreactive unmyelinated axons in a C-fiber bundle. **B** SP-LI axons in a nerve fiber bundle with perineural sheath. *Arrows* indicate unlabeled axons. Perineural sheath (*pn*), Schwann cell (*sc*). **A** ×26500; bar 1 μm; **B** ×17500; bar 1 μm

near to the confluens sinuum show up to six immunolabelled axons in one C-fiber bundle. About 150–180 immunoreactive axons are counted in one cross section of the sinus wall.

Substance P-like immunoreactive nerve fibers

Light microscopy

The parietal dura mater covering the olfactory bulb is supplied by a moderate number of SP-LI nerve fibers. They run towards the superior sagittal sinus independent from vascular structures, whereas in the rostral falx SP-LI fibers are closely associated to the microvessels. In the ventral region of the rostral falx few labeled fibers follow a rostrocaudal direction. The olfactory artery is accompanied by a moderate number of SP-immunoreactive nerve fibers (Fig. 3B).

The ventrolateral region of the supratentorial dura mater exhibits few immunolabeled nerve fibers. In the dorsolateral region of the dura mater, where the distal branches of the middle meningeal artery and the accompanying venous vessels ramify, the immunoreactive nerve fibers form a moderate plexus along the vascular tree and a vessel-independent widely meshed plexus in the adjacent dura (Figs. 8; 9A, B). The proximal segment of the middle meningeal artery exhibits very few isolated thin SP-LI fibers. SP-immunostained fibers form a uniform, moderate and widely meshed network of fine varicosed fibers along the sagittal and transverse sinuses (Fig. 10A). Only at the entrance of tributary veins and around the confluens sinuum does it become more prominent (Fig. 10B).

The parietal dura covering the cerebellum has a rich supply of SP-LI nerve fibers (Fig. 11). Bundles containing several SP-LI fibers show a predominantly vessel-independent course, whereas thin beaded SP-LI fibers which leave the bundles run in close association to the microvessel wall. In the tentorium cerebelli the majority of SP-LI fibers run parallel to the course of the trochlear nerve to the transverse sinus. In the ventral dura mater a moderate SP-LI nerve fiber net is observed. Thicker nerve fiber bundles give off fine immunoreactive fibers which run in the dura mater apart from vessels.

Density and distribution of SP-LI nerve fibers are the same on both sides of the encephalic dura mater.

Electron microscopy

All SP-immunoreactive profiles are identified as unmyelinated axons. Most of them are observed in C-fiber bundles lacking a perineural sheath, but they also occur in nerve fiber bundles with a perineural sheath (Fig. 12A, B). Axons are often found adjacent to immunonegative nerve profiles which usually outnumber the positive stained axons. The density of the immunoreaction product obscurs in most cases the ultrastructure of the axonal elements. SP-LI axons are observed in C-fiber bundles in the dural connective tissue and in C-fiber bundles accompanying arteries, capillaries, venous vessels and the sinus walls.

Discussion

The present study shows the topography and distribution of NPY- and SP-LI nerve fibers in the rat dura mater encephali. Local differences in density and pattern of NPY- and SP-LI fibers are obvious in the entire dura mater. Both neuropeptides only occur in C-fibers.

The pattern and topography of NPY-LI nerve fibers in the supratentorial dura mater resemble the occurrence of dopamine-β-hydroxylase fibers (Keller et al. 1989). In this context a colocalization of NPY and dopamine-β-

hydroxylase which has been described for perivascular nerves (Ekblad et al. 1984; Edvinsson et al. 1987), may be discussed for the dural nerves, too. Due to our whole-mount preparations we cannot define the origin of NPY-LI nerve fibers to the dura mater, but the course and the topography suggest another source in addition to the superior cervical ganglion. In accordance with our observation, experimental studies have shown that removal of the superior cervical ganglion did not abolish the total amount of NPY-LI fibers to the dura mater (Edvinsson et al. 1987). Further results show that NPY-LI fibers to the cerebral vessels have their origin additionally in parasympathetic ganglia such as the sphenopalatine, otic and internal carotid ganglia (Suzuki et al. 1989). The observed NPY-LI nerve fiber plexus accompanying the internal carotid, ethmoidal and olfactory arteries may support this observation. These arteries traverse the ventral dura mater and there is some evidence that NPY-LI nerve fibers may enter the dura mater via these vessels.

The function of NPY-LI nerve fibers in the encephalic dura mater remains speculative. The close association of the NPY-LI fibers to smooth muscle cells and pericytes of the vessels suggests a vasomotor effect. This is in good agreement with the physiological experiments provided by Edvinsson et al. (1983). The high density of NPY-LI fibers in the sinus wall in comparison to other venous vessels correlates with the high amount of smooth muscle cells in the sinus wall (Andres et al. 1987).

In contrast to NPY, the distribution of SP-LI nerve fibers in the dura mater with particular respect to the sinus wall is more homogeneous and less dense. Two characteristics of substance P occurrence are remarkable, the local differences concerning density and pattern, and the association of SP-LI nerve fibers to the different segments of the vascular bed. SP-LI nerve fibers close to the microvessels are observed in the entire dura mater, but are quite obvious in the rostral falx. This observation may be related to the hypothesis that antidromic activation of sensory neurons induces neurogenic vasodilatation and extravasation mediated by substance P (Jancso et al. 1967; Lembeck and Holzer 1979; Pernow 1983; Lembeck 1985). The SP innervation of the falx may be involved in local defence mechanisms necessary to prevent infections of the dura mater exposed to the nasal cavity.

There is finally the question whether SP-LI nerve fibers characterize nociceptive afferents, or whether they are involved in other sensory functions such as mechanoreception. Visceral afferents with SP-LI in the urethral wall have been discussed in relation to mechanoreceptive and nociceptive function (Iwanaga et al. 1985). Further serial ultrathin section of immunolabeled fibers and reconstructions are necessary to characterize the SP nerve fiber terminals and the microenvironment involved in order to elucidate the relationship of function to localization.

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References

Amenta F, Sancesario G, Ferrante F, Cavallotti C (1980) Acetylcholinesterase-containing nerve fibers in the dura mater of guinea pig, mouse and rat. J Neural Transm 47:237–242

Andres KH, v Düring M, Muszynski K, Schmidt RF (1987a) Nerve fibers and their terminals of the dura mater encephali of the rat. Anat Embryol 175:289–301

Andres KH, v Düring M, Muszynski K, Schmidt RF (1987b) The innervation of the dura mater encephali of the rat. In: Schmidt RF, Schaible HG, Vahle-Hinz C (eds) Fine afferent nerve fibers and pain. VHC Verlagsgesellschaft mbH Weinheim, pp 17–25

Edvinsson L, Uddman R (1981) Adrenergic, cholinergic and peptidergic nerve fibers in dura mater – involvement in headache? Cephalalgia 1:175–179

Edvinsson L, Rosendal-Helgesen S, Uddman R (1983a) Substance P: Localization, concentration and release in cerebral arteries, choroid plexus and dura mater. Cell Tissue Res 234:1–7

Edvinsson L, Emson, P, McCulloch J, Tatemoto K, Uddman R (1983b) Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. Neurosci Lett 43:79–84

Edvinsson L, Copeland JR, Emson PC, McCulloch J, Uddman R (1987) Nerve fibers containing neuropeptide Y in the cerebrovascular bed: immunocytochemistry, radioimmunoassay and vasomotor effects. J Cereb Blood Flow Metab 7:45–57

Edvinsson L, Brodin E, Jansen I, Uddman R (1988) Neurokinin A in cerebral vessels: characterization, localization and effects in vitro. Regul Pept 20:181–197

Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Håkanson R, Sundler F (1984) Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. Regul Pept 8:225–235

Furness JB, Papka RE, Della NG, Costa M, Eskay RL (1982) Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs. Neuroscience 7:447–459

Gibbins IL, Morris JL (1988) Co-existence of immunoreactivity to neuropeptide Y and vasoactive intestinal peptide in non-noradrenergic axons innervating guinea pig cerebral arteries after sympathectomy. Brain Res 444:402–406

Iwanaga T, Fujita T, Yanaihara N (1985) Nerve fibers in the urethral mucosa of canine penis are immunoreactive for both substance P and calcitonin gene-related peptide. Arch Histol Jpn 48:547–554

Jancso N, Jancso-Gabor A, Szolcsanyi J (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother 31:138–151

Keller JT, Saunders MC, Beduk A, Jollis JG (1985) Innervation of the posterior fossa dura of the cat. Brain Res Bull 14:97–102

Keller JT, Marfurt CF, Dimlich RVW, Tierney BE (1989) Sympathetic innervation of the supratentorial dura mater of the rat. J Comp Neurol 290:310–321

Kimmel DL (1961) Innervation of spinal dura mater and dura mater of the posterior cranial fossa. Neurology 11:800–809

Leblanc GG, Trimmer BA, Landis SC (1987) Neuropeptide Y-like immunoreactivity in rat cranial parasympathetic neurons: Coexistence with vasoactive intestinal peptide and choline acetyl transferase. Proc Natl Acad Sci USA 84:3511–3515

Lembeck F (1985) Substance P and sensory neurones. In: Jordan CC, Oehme P (eds) Substance P. metabolism and biological actions, Taylor & Francis, London, pp 137–151

Lembeck F, Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedebergs Arch Pharmacol 310:175–183

Lembeck F, Donnerer J, Barthó L (1982) Inhibition of neurogenic vasodilatation and plasma extravasation by substance P antag-

onists, somatostatin and (D-meth^2, pro^5) enkephalinamide. Eur J Pharmacol 85:171–176

McDonald DM (1988) Neurogenic inflammation in the rat trachea. I. Changes in venules, leucocytes and epithelial cells. J Neurocytol 17:583–603

Markowitz S, Saito K, Moskowitz MA (1987) Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. J Neurosci 7:4129–4136

Mayberg M, Langer RS, Zervas NT, Moskowitz MA (1981) Perivascular meningeal projections from cat trigeminal ganglia: Possible pathway for vascular headaches in man. Science 213:228–230

Mayberg M, Zervas NT, Moskowitz MA (1984) Trigeminal projections to supratentorial pial and dural blood vessels in cats demonstrated by horseradish peroxidase histochemistry. J Comp Neurol 233:46–56

Moskowitz M, Liu-Chen LY, Mayberg MR, Zervas NT, Han DH, Brody MR (1983) Cat pio-arachnoid contains immunoreactive substance P within neurons projecting from the fifth cranial nerve. In: Reivich M, Hurtig HI (eds) Cereb Dis 363–371 Raven Press, New York

Moskowitz MY (1984) The neurobiology of vascular head pain. Ann Neurol 16:157–168

Moskowitz MY, Buzzi MG, Sakas DE, Linnik ME (1989) Pain mechanisms underlying vascular headaches. Rev Neurol (Paris) 145:181–193

O'Connor TP, van der Kooy D (1986) Pattern of intracranial and extracranial projections of trigeminal ganglion cells. J Neurosci 6:2200–2207

Penfield W, McNaughton F (1940) Dural headache and innervation of the dura mater. Arch Neurol Psychiatry 44:43–75

Pernow B (1983) Substance P. Pharmacol Rev 35:85–141

Ray BS, Wolff HG (1940) Experimental studies on headache. Pain sensitive structures of the head and their significance in headache. Arch Surg 41:813–856

Silverman J, Kruger L (1989) Calcitonin-gene-related-peptide-immunoreactive innervation of the rat head with emphasis on specialized sensory structures. J Comp Neurol 280:303–330

Steiger HJ, Tew JM, Keller JT (1982) The sensory representation of the trigeminal ganglion of the cat. Neurosci Lett 31:231–236

Suzuki N, Hardebo JE, Owman Ch (1988) Origin and pathways of cerebrovascular vasoactie intestinal polypeptide-positive nerves in rat. J Cereb Blood Flow Metab 8:697–712

Suzuki N, Hardebo JE, Owman Ch (1989a) Origins and pathways of cerebrovascular nerves storing substance P and calcitonin gene-related peptide in rat. Neuroscience 2:427–438

Suzuki N, Hardebo JE, Kahrström J, Owman Ch (1989b) Neuropeptide Y coexists with vasoactive intestinal polypeptide in parasympathetic cerebrovascular nerves originating in the sphenopalatine, otic and internal carotid ganglia of the rat. Neurscience (submitted)

Uddman R, Edvinsson L, Ekman R, Kingman R, McCulloch J (1985) Innervation of the feline cerebral vasculature by nerve fibers containing calcitonin-related peptide: trigeminal origin and co-existence with substance P. Neurosci Lett 62:131–136

Uddman R, Hara H, Edvinsson L (1989) Neuronal pathways to the rat middle meningeal artery revealed by retrograde tracing and immunocytochemistry. J Auton Nerv Syst 26:69–75

Wirth FP, Van Buren JM (1971) Referral of pain from dural stimulation in man. J Neurosurg 34:630–642

Yamamoto K, Matsuyama T, Shiosaka S, Inagaki S, Senba E, Shimizu Y, Ishimoto I, Hayakawa T, Matsumoto M, Tohyama M (1983) Overall distribution of substance P-containing nerves in the wall of the cerebral arteries of the guinea-pig and its origin. J Comp Neurol 215:421–426