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Effect of a Serotonin Agonist (Sumatriptan) on the Peptidergic Innervation of the Rat Cerebral Dura Mater and on the Expression of *c-fos* in the Caudal Trigeminal Nucleus in an Experimental Migraine Model

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The supratentorial cerebral dura of the albino rat is equipped with a rich sensory innervation including nociceptive axons and their terminals, which display intense calcitonin gene-related peptide (CGRP) immunoreactivity both in the connective tissue and around blood vessels. Stereotactic electrical stimulation of the trigeminal (Gasserian) ganglion, regarded as an experimental migraine model, induces marked increase and disintegration of club-like perivascular CGRP-immunopositive nerve endings in the dura. Intravenous administration of sumatriptan, prior to electrical stimulation, prevents disintegration of perivascular terminals and induces accumulation of CGRP in terminal and preterminal portions of peripheral sensory axons. Consequently, immunopositive terminals and varicosities increase in size; accumulation of axoplasmic organelles results in a "hollow" appearance of many varicosities. Since sumatriptan exerts its anti-migraine effect by virtue of its agonist action on 5-HT_{1D} receptors, we suggest that sumatriptan prevents the release of CGRP from dural perivascular terminals by an action at 5-HT_{1D} receptors. In the caudal trigeminal nucleus electrical stimulation of the trigeminal ganglion induces, in interneurons, increased expression of the oncoprotein *c-fos* which is not prevented by intravenous application of sumatriptan. Disparate findings regarding this effect are partly due to the fact that sumatriptan very poorly passes the blood–brain barrier and partly to different experimental paradigms used by different authors. J. Neurosci. Res. 48:449–464, 1997. © 1997 Wiley-Liss, Inc.

Key words: CGRP; stimulation; trigeminal (Gasserian) ganglion; sumatriptan; dura mater

INTRODUCTION

Innervation and vascular supply of the cerebral dura is known to play an important role in the pathogenesis of migraine headache. While the mass of collagenous fibers which build up the cerebral dura are widely regarded as an inert tissue, microstructural studies have revealed that it is richly innervated. Andres et al. (1987) and Keller and Marfurt (1991) have disclosed that the vascular supply of the dura is intimately correlated to vasomotor axons as well as to most of the sensory fibers, which derive mainly from the ophthalmic (and, to a smaller extent, from the maxillary and mandibular) branches of the trigeminal nerve, with several sensory axons of glossopharyngeal and vagal origin. Sensory axons display calcitonin gene-related peptide (CGRP) and substance P (SP) immunoreactivity. Vasomotor nerve fibers originate mainly from the pterygopalatine, the otic, and the superior cervical ganglion. Nerve fibers coming from the pterygopalatine and otic ganglia establish parasympathetic innervation, characterized by cholinergic transmission mechanism, and displaying acetylcholinesterase (AChE) staining and choline acetyltransferase (ChAT) immunoreactivity, coexistent with vasoactive intestinal polypeptide (VIP). Adrenergic nerve fibers, coming from the superior cervical sympathetic ganglion, display formaldehyde-induced fluorescence and neuropeptide Y (NPY) immunoreactiv-

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ity (Edvinsson and Uddman, 1981; O'Connor and van der Kooy, 1988; Keller and Marfurt, 1991). Sensory, parasympathetic, and sympathetic axons proceed together in common strands ensheathed by the cytoplasm of Schwann cells (Andres et al., 1987).

According to a current view, migraine headache is initiated by spreading depression (Olesen et al., 1990) resulting in lowered pH and [K+] and increased intracranial blood pressure. This may result in sensitization and activation of meningeal afferents, originating mainly from the trigeminal ganglia, and consequently to the release of neuropeptides from the peripheral, mainly perivascular, nerve terminals. Neuropeptides released from these terminals induce neurogenic inflammation in the cerebral dura (Buzzi and Moskowitz, 1992).

Sumatriptan (Imigran, Glaxo: 3-[2-dimethylaminoethyl]-N-methilindole-5-methanesulfonamide), a serotonin agonist, is a highly selective ligand for 5-HT_{1D} receptors (Buzzi and Moskowitz, 1992); it has little affinity for other 5-HT and non-5-HT receptors (Beattie et al., 1994). Sumatriptan is a widely used, efficacious anti-migraine drug (Dechant and Clissold, 1992); if subcutaneously applied for the acute treatment of migraine attacks, it resulted in an improvement in 70–86% of migraine patients (Welch, 1993). Sumatriptan causes marked contraction of isolated basilar and middle cerebral arteries via activation of vascular 5-HT_1 receptors (Connor et al., 1989); it has also a vasoconstrictive effect on human dural vessels in vivo (Jansen et al., 1992; Henkes et al., 1996) but produces only a modest vasoconstriction of human isolated coronary arteries, via 5-HT_2 receptors (Wilkinson et al., 1995). Sumatriptan blocks neurogenic inflammation and the release of nociceptive neuropeptides via activation of 5-$HT_{1B/D}$ receptors (Huang et al., 1993). It is a well-tolerated drug: Most of its side effects, including chest symptoms and tachycardia, are mild to moderate in intensity, are short-lived, and resolve spontaneously (Russell et al., 1994).

The objective of the present investigations was to show, by means of light and electron microscopic immunohistochemical methods, the cytological effects of sumatriptan in an experimental migraine model, both in the periphery (in the supratentorial cerebral dura mater) and in the central nervous system (in the caudal trigeminal nucleus of the medulla). Using morphometrical analysis of the light microscopic immunohistochemical specimens, we attempted to establish those cytological alterations which lead to neuropeptide release and to decipher those light and electron microscopic immunocytochemical changes induced by sumatriptan which might be responsible for the alleviation of the migraine attack. At the same time, we sought to reveal whether sumatriptan, known to pass the blood–brain barrier very poorly, has any central effect at all in this migraine model.

MATERIALS AND METHODS

Investigations were performed on 24 young adult albino rats of both sexes, R-Amsterdam strain, 200–250 g body weight. Care of the animals complied with the guidelines of the Hungarian Ministry of Welfare; experiments were carried out in accordance with the European Communities Council Directive (November 24, 1986; 86/609/EEC), the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 85-23, revised 1985), and the Albert Szent-Györgyi University Medical School Guidelines for Ethics in Animal Experiments. The animals were divided into six groups, each group consisting of four rats. Group I, treated by isotonic saline, served as absolute control; group II was treated by isotonic saline and subjected to 30-min electrical stimulation of the trigeminal ganglion; group III was treated with 6 mg/kg sumatriptan i.v.; group IV was treated with therapeutic doses of sumatriptan (0.12 m/kg i.v.); group V was treated with 6 mg/kg sumatriptan, followed by 30-min electrical stimulation of the trigeminal ganglion; group VI received 0.12 mg/kg sumatriptan, followed by 30-min stimulation. Rats were subjected to deep anesthesia with i.p. injection of chloral hydrate, 0.4 g/kg body weight. Stimulation of the trigeminal ganglion was performed by means of concentric bipolar electrodes (FHC, Brunswick, ME, cat. No: 17-75-2, center pole connected to cathode) which were placed by means of a stereotactic apparatus into the left trigeminal ganglion, by insertion through a hole bored in the skull 3.2 to 3.4 mm posteriorly from the bregma and 2.8 to 3.2 mm laterally. Depth of the tip of the stimulating electrode was 9.3 mm from the dural surface. These values correspond to those described for the trigeminal ganglion in the stereotactic atlas of Schneider et al. (1981). Stimulation was performed with square pulses of 5 msec duration, 5 Hz frequency, 0.5 mA for 30 min. Group II, V, and VI animals were subjected immediately after stimulation to transcardial fixation with 500 ml cold picric acid–formaldehyde fixative (Zamboni and DeMartino, 1967), containing 0.1% glutaraldehyde, preceded by a brief flush of 125 ml 0.1 M phosphate-buffered saline, pH = 7.4 at room temperature; rats from groups I, III, and IV were subjected to the same kind of fixation 15 min after the i.v. injection. Following perfusion, the cerebral dura, the trigeminal ganglion, and the medulla were dissected, immersed in the same fixative at 4°C for 30 min, and postfixed in glutaraldehyde-free Zamboni's solution at 4°C for 12 hr. The location of the tip of the electrode in the trigeminal ganglion was controlled by autopsy using frozen sections stained with methylene blue. The cerebral dura was removed in toto and cut in two at the midline. Left and right sides were marked and used as whole-mount preparations. For the demonstration of CGRP, samples pretreated with 2% H_2O_2 were processed either

according to the classic pre-embedding peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970) or the streptavidin-biotin system (ABC, Vectastain Elite, Vector Laboratories). Incubation in the primary antibody (anti-CGRP, Amersham, UK) in a dilution of 1:2,000, containing 0.3% Triton X-100, was performed at 4°C for 36 hr, or at room temperature for 12 hr. The anti-CGRP serum used in these studies recognizes both α and β CGRP but does not cross-react with calcitonin. The reaction was visualized with diaminobenzidine (DAB) to which hydrogen peroxide was added (3 µl of 30% H_2O_2 to 10 ml of DAB) or with nickel-DAB. Whole mounts of the cerebral dura were either mounted on gelatine-pretreated slides, dehydrated in a graded series of ethanol, and processed through carbol-xylene, or treated according to the free-floating technique. Slides were coverslipped with Canada balsam or Permount. Specificity of the immunohistochemical reaction was assessed by incubating slides in normal rabbit serum lacking the primary antibody, or by pre-absorption of the CGRP antiserum with the commercially available synthetic rat CGRP, or by omitting steps to visualize the antigen-antibody reaction. Absence of immunohistochemical staining in any of the above control experiments proves the validity of the CGRP immunoreaction in the experimental material. For electron microscopic immunohistochemistry, CGRP was visualized in whole-mount dura preparations which were incubated in the same manner as the light microscopic specimens, except that Triton X-100 was omitted from the incubation solution. The sections were flat-embedded on liquid release pretreated slides in Durcupan ACM. Relevant areas were excised with a razor blade and remounted to prepolymerized blocks and sectioned with a diamond knife on a Reichert Ultrotome. Serial sections, silver interference color, were collected on slot grids and stained with lead citrate and uranyl acetate. Sections were photographed on a JEOL 1010 electron microscope.

Immunohistochemical localization of *c-fos* was performed in the medulla, using 40-µm cryostat sections obtained from the Zamboni-fixed brains of the same animals. Serial sections were incubated with primary antibody for *c-fos* protein, obtained from Genosys Biotechnologies (Cambridge, UK) in 1:1,000 dilution in PBS with 0.3% Triton X-100 and 2% rabbit serum at 4°C overnight. The further procedure followed the avidin-biotin technique; visualization of the reaction product in the *c-fos*-expressing cells was performed with nickel-DAB.

Morphometry

Immunostained sections were viewed on a Nikon Microphot FX1 microscope. Microscopic fields were converted into black-and-white digital images by means of an MTI CCD 72 video camera and amplifier, and transferred to a Macintosh Quadra 700 computer running the NIHImage 1.55b image analysis software. Sizes of the terminals and the individual varicosities were estimated by determining their longest and shortest diameters. The ''size'' of the terminal or the varicosity was calculated on the basis of its projection, likening it to an ellipse (profile area = $a/2 \cdot b/2 \cdot .3.14$). Cells exhibiting *c-fos* immunoreactivity were counted by direct visual inspection. Uninterrupted series of 40-µm-thick cryostat sections were obtained from four rats; the numbers of immunoreactive cells were counted in 21-21 consecutive sections in each animal, starting 1 mm caudally from the level of the obex and proceeding caudalward. Statistical analyses of several populations were performed using analysis of variance (ANOVA) for repeated measurements (in which the repeated measure was time). When comparing two groups of data, Student's t-test (two-sample for means assuming unequal variances) and the Mann-Whitney U-test (z-test: two sample for means) were performed, using the QuattroPro-5.0 software. All of the treatment groups depicted in Figure 3 exhibited significant effects by ANOVA with repeated measures.

RESULTS

Supratentorial cerebral dura mater

Under normal conditions, the cerebral dura displays numerous CGRP-immunopositive nerve fibers which proceed together with blood vessels, partly in larger or smaller nerve trunks or as single fibers, terminating either perivascularly or in the connective tissue. These fibers, originating mainly from the trigeminal ganglion (and, to a lesser extent, from the glossopharyngeal and vagal sensory ganglia), establish a dense network throughout the dura; total length of CGRP-positive, light microscopically visible axons is 52 ± 8 mm per square mm of the supratentorial cerebral dura mater in the vicinity of the superior sagittal sinus. The immunoreactive nerve fibers proceed in nerve fascicles, together with several similar axons. Enclosed by the Schwann envelope, CGRP-positive preterminal axons are surrounded by other, nonreactive fibers. These may include NPY-, SP-, and VIP-positive axons. Only terminal parts of these fibers are, at least partly, devoid of a Schwann envelope; such nerve endings terminate either as simple ''free'' nerve endings, or, more often, in the form of club-shaped terminals, either in the connective tissue of the dura, or in close microtopographical relation to blood vessels, including precapillary arterioles, capillaries, and postcapillary venules (Fig. 1). Under normal conditions, most of the CGRP-positive axons are smooth, and only some of the preterminal nerve fibers are characterized by bead-like varicosities (Fig. 2a,b).

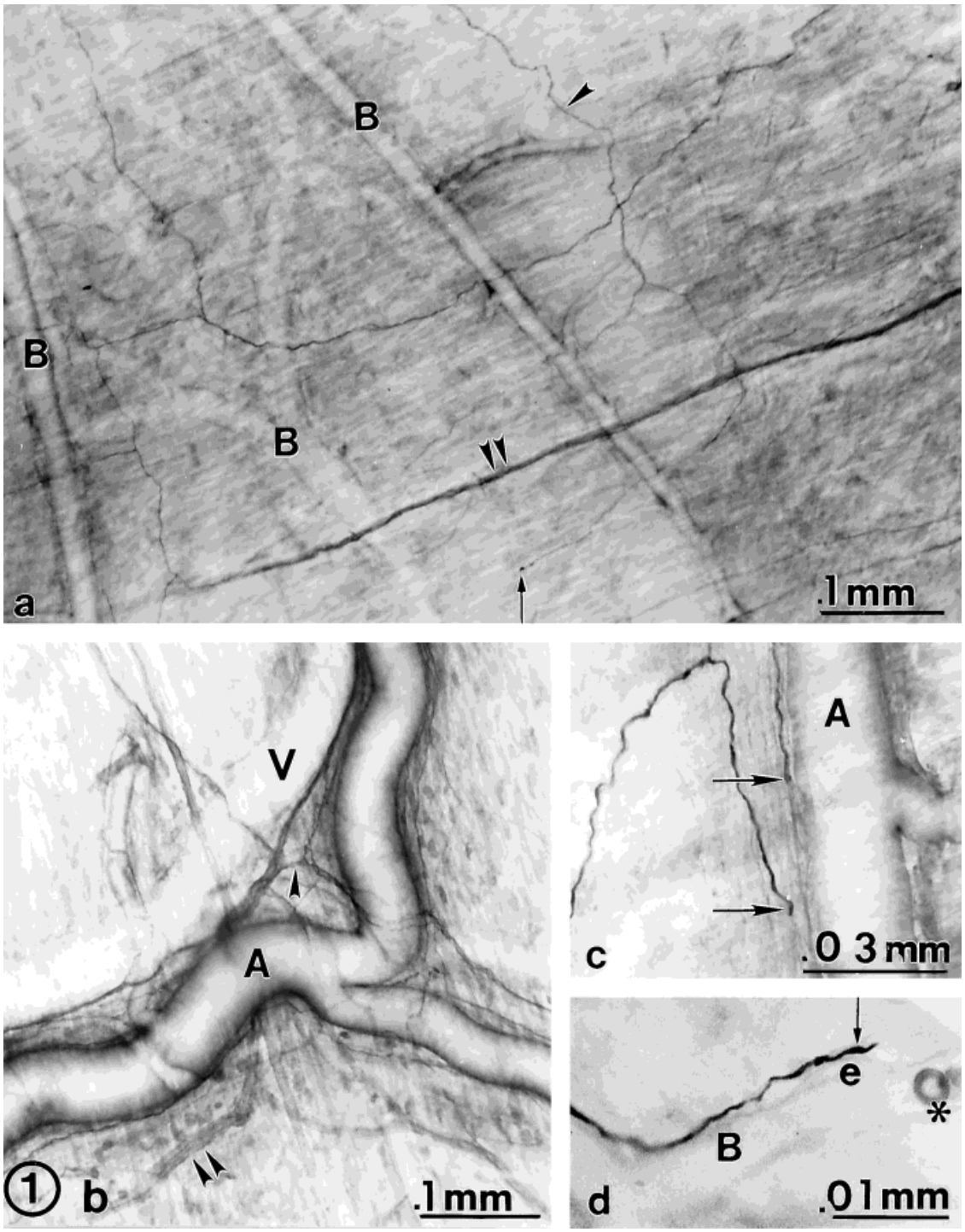

Fig. 1. Innervation of the supratentorial cerebral dura mater of the rat by CGRP-immunoreactive sensory nerve fibers, under normal conditions. **a:** Overall view of a part of the dura adjacent to the superior sagittal sinus. The network of CGRP-positive preterminal axons (arrowhead) among blood vessels (B) derives from a nerve fascicle (double arrowhead) ensheathed by the Schwann envelope. Arrow points to a nerve terminal. **b:** CGRP-immunoreactive nerve fibers proceeding individually (arrowhead) or in fascicles (double arrowhead) innervate arterioles (A) and venules (V). **c:** CGRP-immunoreactive axon terminals (arrows) alongside an arteriole (A). **d:** CGRP-immunoreactive axon following course of small blood vessel (B); its enlarged ending (arrow) is attached to the surface of an endothelial cell (e). Red blood cell in the lumen is marked by asterisk.

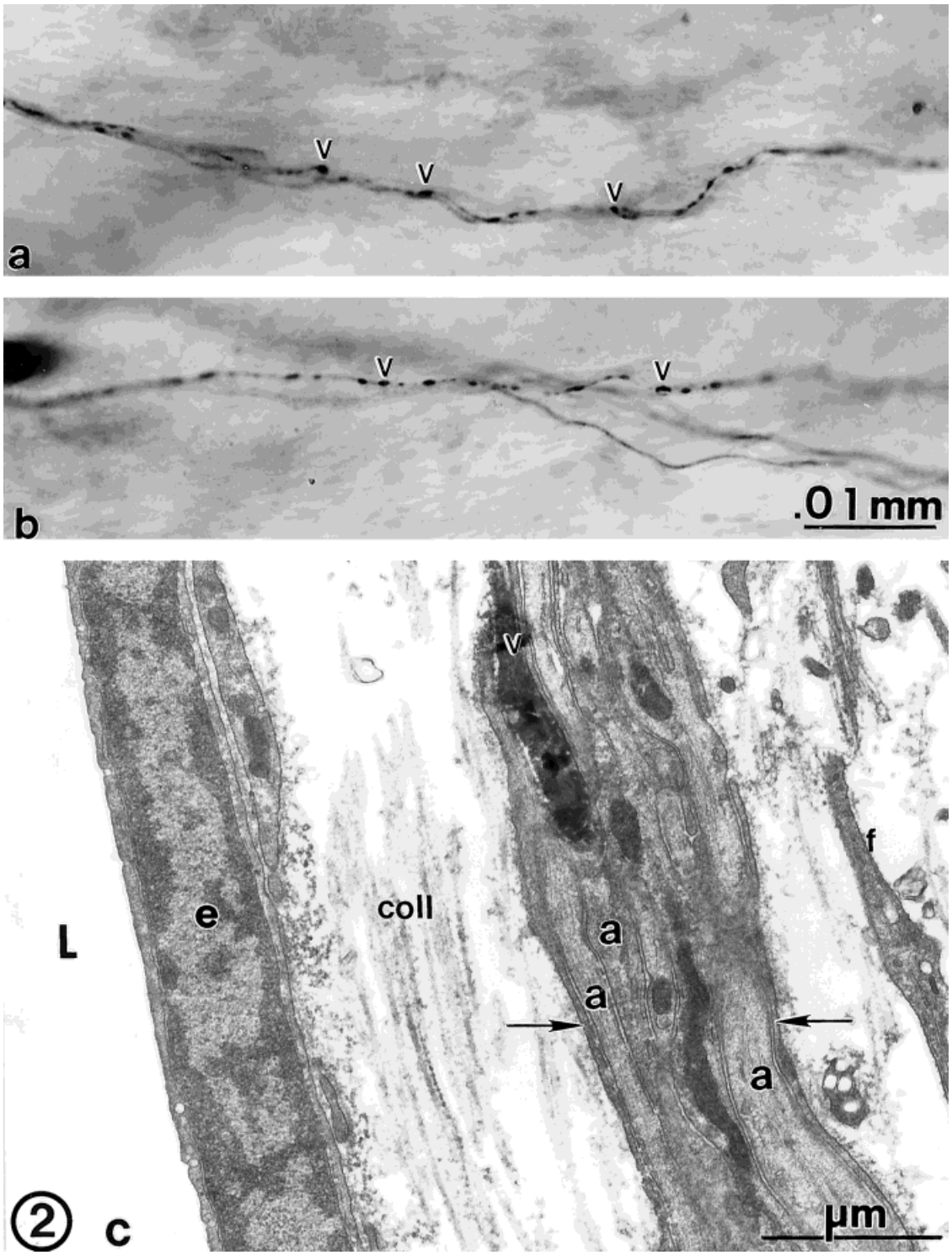

Fig. 2. Varicosities (v) of preterminal axons under normal conditions. **a,b:** Light microscopy. **c:** Electron microscopy. Note CGRP-immunoreactive varicosity of a single nerve fiber among other, nonreactive axons (a) in the same fascicle. Arrow points to the Schwann envelope. L, lumen of blood vessel; e, endothelial cell nucleus; coll, collageneous fibers; f, fibrocyte processes.

Administration of sumatriptan, especially when followed by electrical stimulation of the trigeminal ganglion, induces alterations in sizes of axon terminals and varicosities (Figs. 3, 4). The profile area of CGRP-immunoreactive club-like perivascular axon terminals, which is 2.6 ± 1.4 μm^2 under normal (resting) conditions, increases to 11.4 ± 5.9 μm^2 after 5-min stimulation and to 11.6 ± 1.9 μm^2 after 30-min stimulation, characterized by corroded outlines of the terminals. After i.v. administration of a clinical dose (0.12 mg/kg) of sumatriptan, the profile area of the terminals is increased to 7.7 ± 2 μm^2; if this is followed by electrical stimulation of the trigeminal ganglion, the profile area increases to 12.3 ± 2.2 μm^2. Large doses of sumatriptan (6 mg/kg) result in terminals which are increased to the same size as those seen after administration of the clinical dose, i.e., 7.7 ± 2.5 μm^2; however, if this is followed by electrical stimulation, the terminals exhibit a tremendous increase, to 20.7 ± 2.4 μm^2. Differences between control and sumatriptan-treated and/or electrically stimulated terminals are significant ($P < .01$); the difference between terminals observed in control samples and those seen after large doses of sumatriptan and stimulation is highly significant ($P < .001$, Fig. 3, upper histograms).

Also varicosities show similar alterations. Average size of a varicosity is 0.8 ± 0.1 μm^2 in the normal dura; this is increased to 2.3 ± 0.1 μm^2 after administration of the clinical dose (0.12 mg/kg) of sumatriptan and to 3.8 ± 0.2 μm^2 if this is followed by electrical stimulation of the trigeminal ganglion. Large doses of sumatriptan (6 mg/kg i.v.) result in the increase of varicosities to 2.9 ± 0.3 μm^2; following electrical stimulation of the ganglion, the resulting gigantic varicosities, most of which appear hollow-cored, measure 7.8 ± 0.4 μm^2. Here again, the alterations are statistically significant ($P < .01$) as compared to the control ones, and, if a large dose of sumatriptan is followed by electrical stimulation of the trigeminal ganglion, the difference is highly significant ($P < .001$; Fig. 3, lower row of histograms).

Distributions of the sizes of varicosities are illustrated in Figure 4. In the normal control dura mater, the distribution is nearly homogeneous: the peak of the curve is between 0.1 and 1 μm^2. In contrast, after administration of sumatriptan, and especially if this is followed by electrical stimulation of the trigeminal ganglion, the curves representing sizes of the varicosities are protracted with repeated elevations and declines. This is especially conspicuous in the graph representing sizes of the varicosities after 6 mg/kg sumatriptan followed by electrical stimulation: At the extreme right end of the curve, three elevations can be seen at 8 μm^2, 8.5 μm^2, and 9 μm^2, respectively.

Structural alterations of axon terminals and varicosities are illustrated by photomicrograms Figures 5–9.

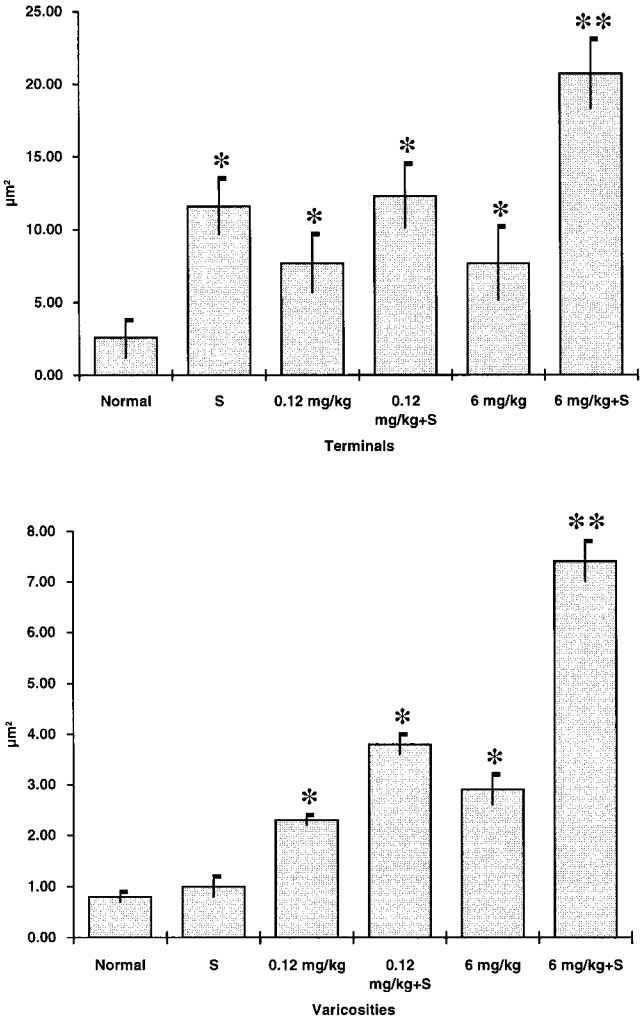

Fig. 3. Sizes of axon terminals (N = 200, above) and varicosities (N = 500, below) in the rat dura mater in normal control material versus experimental conditions (mean ± SEM in μm^2). **Upper row:** Sizes of terminals in normal (control), after 30-min electrical stimulation of the trigeminal ganglion (S), after administration of clinical dose of sumatriptan i.v. (0.12 mg/kg), the same followed by 30-min electrical stimulation of the trigeminal ganglion (0.12 mg/kg + S), after high dose of i.v. sumatriptan (6 mg/kg), and the same followed by 30-min electrical stimulation of the trigeminal ganglion (6 mg/kg + S). **Lower row:** Sizes of varicosities in normal (control), after 30-min electrical stimulation of the trigeminal ganglion (S), after administration of clinical dose of sumatriptan i.v. (0.12 mg/kg), the same followed by 30-min electrical stimulation of the trigeminal ganglion (0.12 mg/kg + S), after high dose of i.v. sumatriptan (6 mg/kg), and the same followed by 30-min electrical stimulation of the trigeminal ganglion (6 mg/kg + S). *, $P < .01$; **, $P < .001$ as related to the control.

Sumatriptan (6 mg/kg i.v.) results in enlargement of club-shaped perivascular CGRP-positive nerve endings and varicosities (Fig. 5), as quantified above. After application of clinical doses of sumatriptan (0.12 mg/kg)

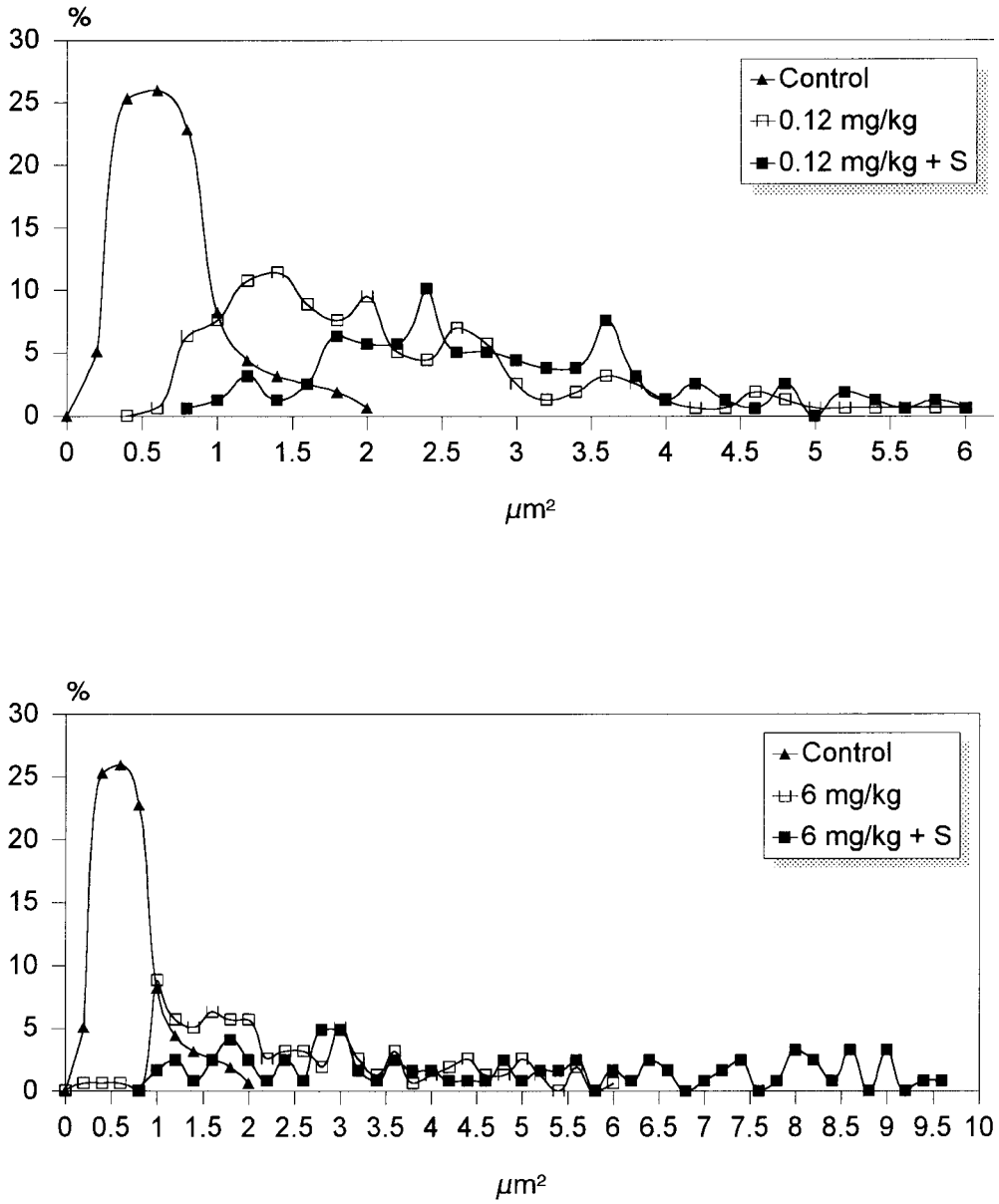

Fig. 4. **Upper graph:** Statistical distribution of the sizes of varicosities (N = 1,200) in the rat dura mater in normal control material (triangles) versus i.v. administration of clinical doses (0.12 mg/kg) of sumatriptan (open squares) and sumatriptan followed by 30 min electrical stimulation of the ipsilateral trigeminal ganglion (solid squares). Note that the mean profile of the varicosity is 0.1–2 μm^2 in the control sample with a peak of the curve at 0.6 μm^2, while the distribution is very extended in electrically stimulated and/or sumatriptan-treated samples, with two minor elevations of the curves at 2.5 μm^2 and 3.5 μm^2, respectively. **Lower graph:** Statistical distribution of the sizes of varicosities after i.v. administration of high doses (6 mg/kg) of sumatriptan. Note oscillation of the extended curves representing varicosities in electrically stimulated and/or sumatriptan-treated samples.

the enlargement of terminals and varicosities (see above) is moderate (Fig. 6).

Electrical stimulation of the trigeminal ganglion induces swelling of CGRP-immunopositive axon terminals, as we have shown earlier (Knyihár-Csillik et al., 1995). In addition to enlargement, 30-min stimulation results in a corroded appearance of the perivascular terminals, accompanied by similar changes of the varicosities throughout the preterminal portion of CGRP-immunopositive axons (Fig. 7a).

Sumatriptan, if administered in high doses (6 mg/kg) 15 min prior to 30-min electrical stimulation, induces a

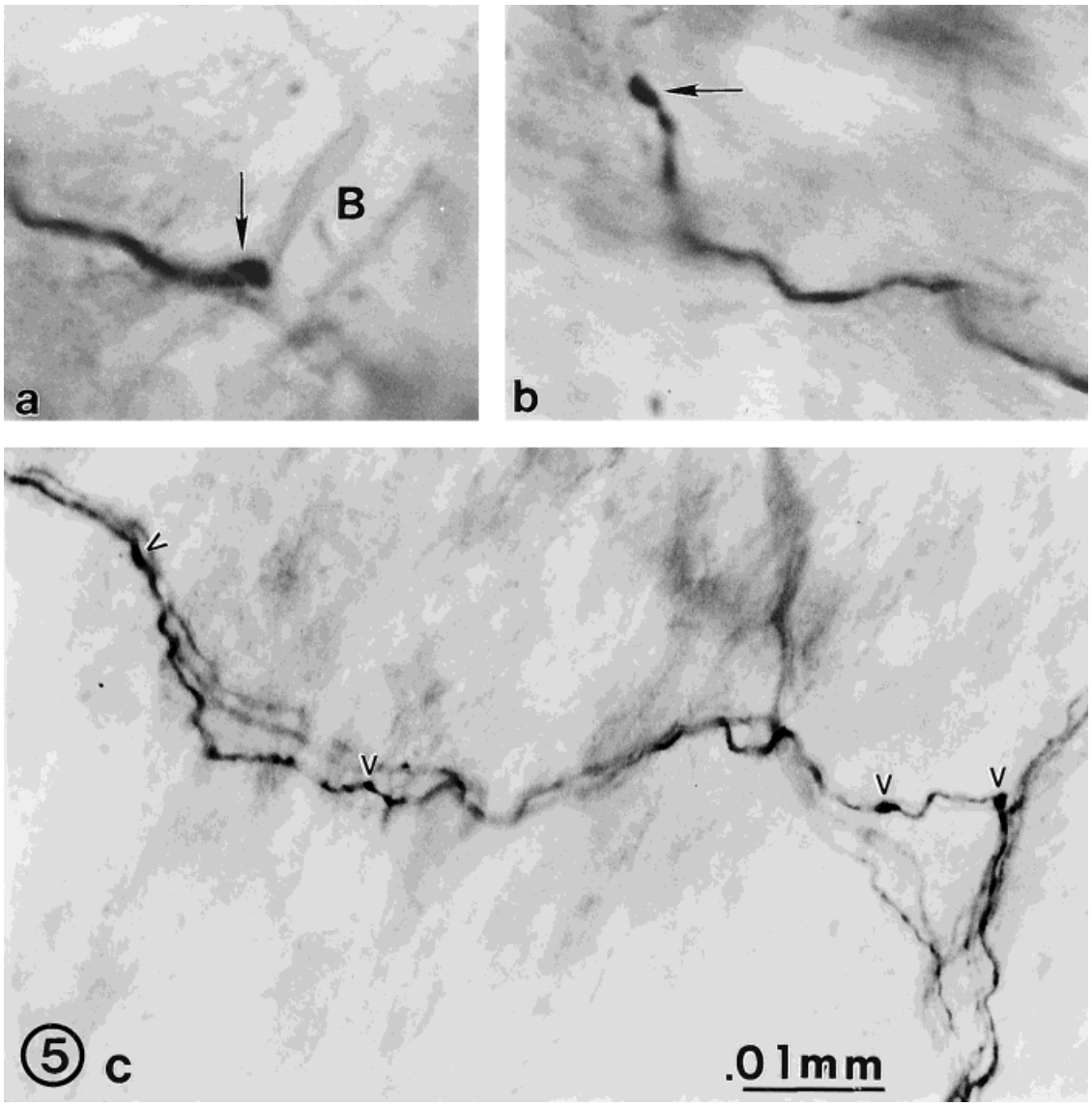

Fig. 5. Effect of 6 mg/kg sumatriptan on the morphology of CGRP-immunoreactive axons in the rat dura mater. **a,b:** Axon terminals (arrows); B, blood vessel. **c:** Varicosities (v). Both the terminals and the varicosities are enlarged as compared to the control (see Figs. 1c,d and 2a,b).

series of structural alterations. Axon terminals appear extremely swollen and develop exophytes; at the same time, all of the preterminal axons display varicosities (Fig. 7b). Due to accumulation of CGRP, also preterminal enlargements can be observed which are similar to Herring bodies (Fig. 7c). Varicosities of the preterminal portion increase in number and size to such an extent that they become six to ten times larger than normal. Some of the extremely large varicosities are characterized by an empty central ''core,'' surrounded by an immunoreactive rim (Fig. 8a,b). Sumatriptan, administered in clinical doses (0.12 mg/kg) 15 min prior to 30-min electrical stimulation of the ipsilateral trigeminal ganglion, induces moderate swelling of terminals and varicosities; (Fig. 9); empty-looking varicosities are absent.

At the level of electron microscopic immunohistochemistry, numerous nerve bundles enclosed by Schwann envelopes can be found embedded in the collageneous tissue (Fig. 2c). A smaller number of these bundles are located close to the blood vessels following their course. In the bundles, both in the connective tissue and near the blood vessels, only a few of the nerve fibers exert CGRP immunoreactivity. In the vicinity of blood vessels, several CGRP-immunopositive axons are not ensheathed by the Schwann envelope; these approach the basement membrane of the vessel to 200 to 800 Angstrom units. In the

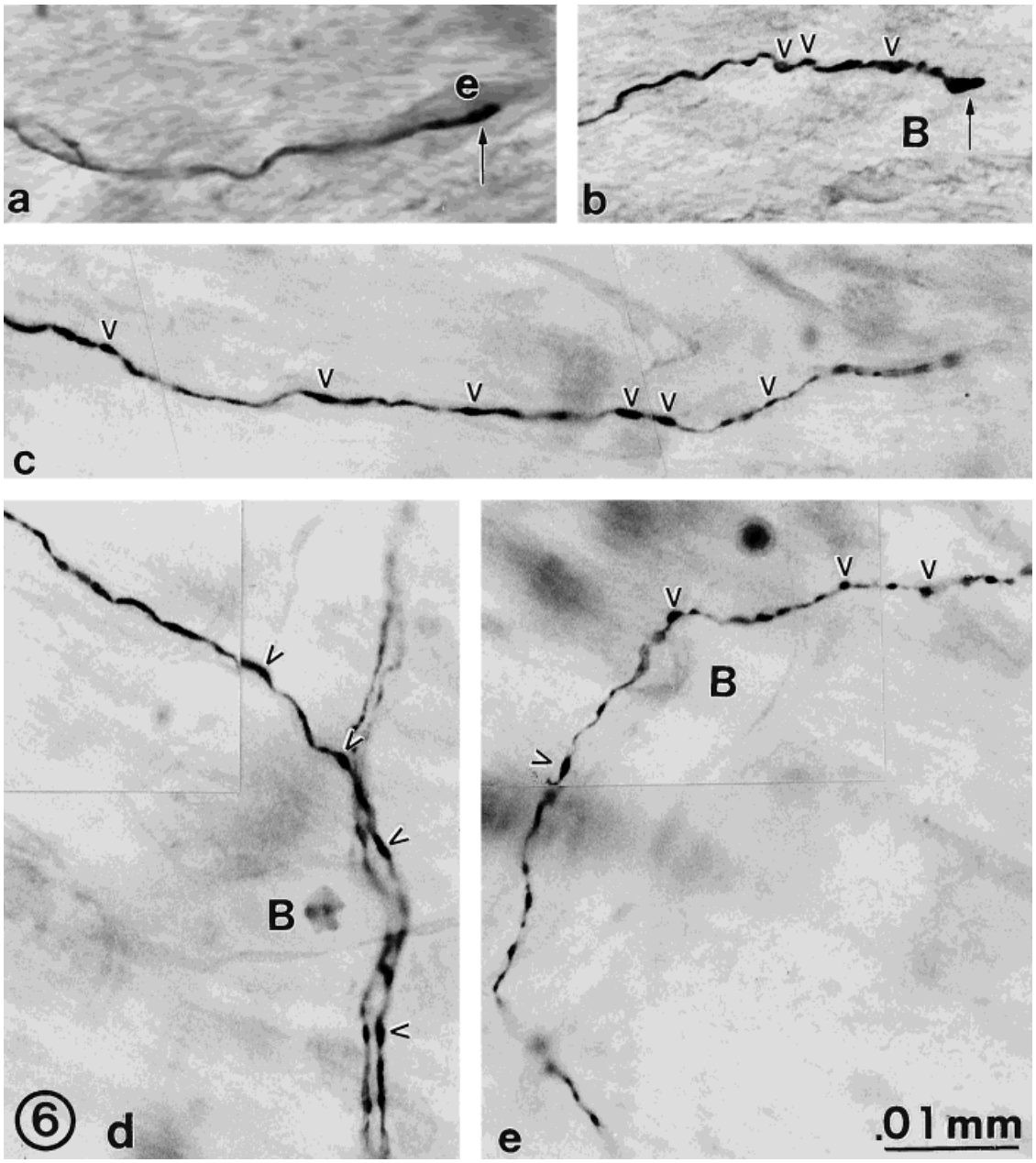

Fig. 6. Effect of 0.12 mg/kg sumatriptan on the morphology of CGRP-immunoreactive axons in the rat dura mater. **a,b:** Axon terminals (arrows); v, varicosities; B, small blood vessel. **c–e:** Varicosities (v) in preterminal portions of the axons.

dura of Sumatriptan-treated and ganglion-stimulated animals, mitochondria, dense-core vesicles, and vacuoles are aggregated in the nonreacting ''core'' of enlarged varicosities, surrounded by a CGRP-immunoreactive ''rim'' (Fig. 8c).

Medulla

Under normal conditions, i.e., in saline-treated and in sham-operated animals or contralaterally to the electrically stimulated trigeminal ganglion, *c-fos* immunoreactivity is present in 5.4 $\pm$ 4 interneurons per section; these are scattered over the entire extent of the caudal trigeminal nucleus.

Electrical stimulation of the trigeminal ganglion (30 min) greatly enhanced expression of the oncoprotein *c-fos* at the side of stimulation. While in the normal (contralateral, nonstimulated) side of the caudal trigeminal nucleus, the number of cells expressing *c-fos* was

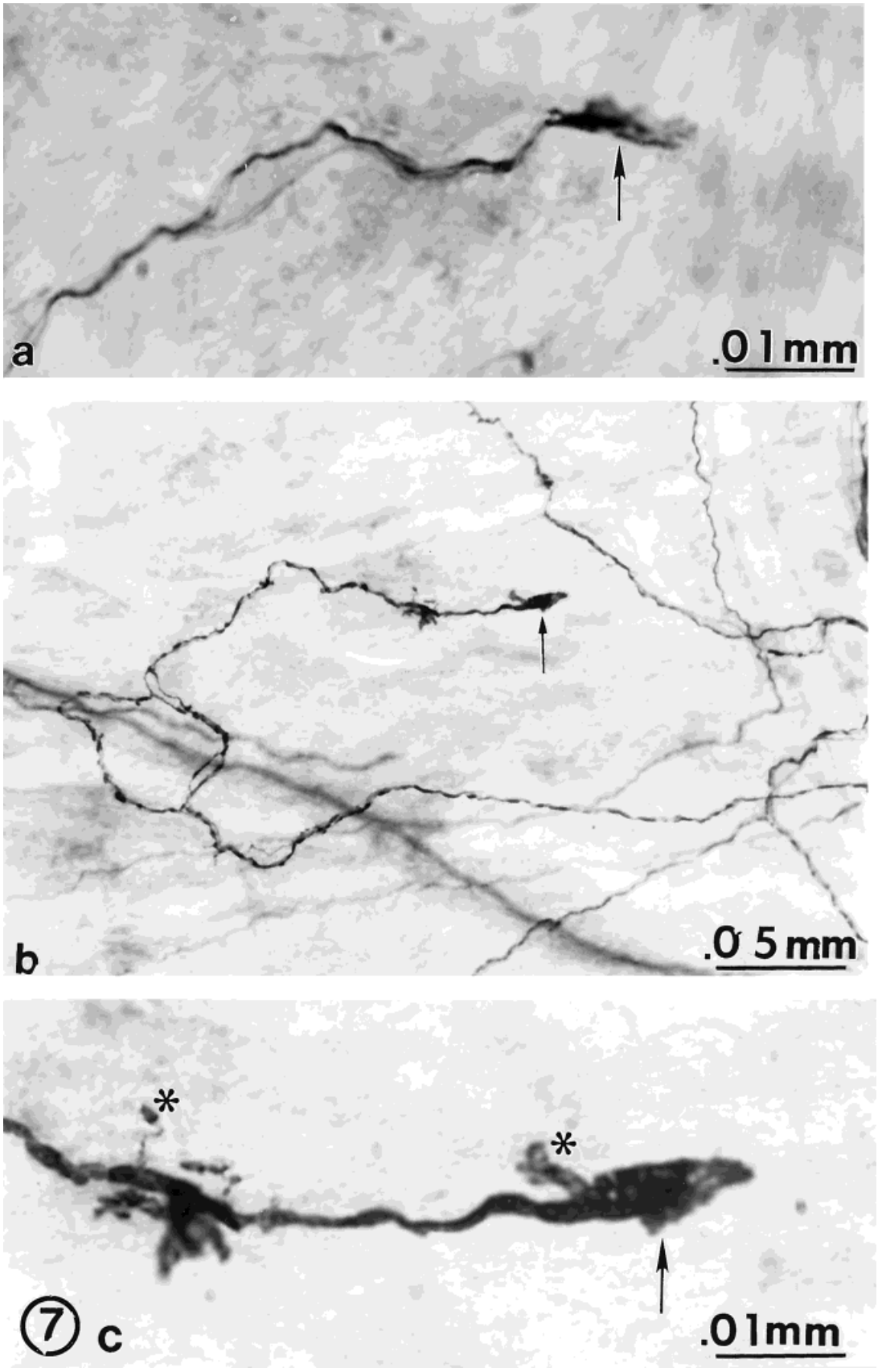

Fig. 7. **a:** Effect of 30-min electrical stimulation of the trigeminal ganglion upon terminals (arrow) of nerve fibers. **b:** Effect of 6 mg/kg sumatriptan + stimulation. Note increased size of terminal (arrow) and that all of the preterminal axons are beaded. **c:** The same under high power. Note peculiar exophytes (asterisks) characterizing terminal and preterminal portions of axons in the sumatriptan-treated and stimulated sample.

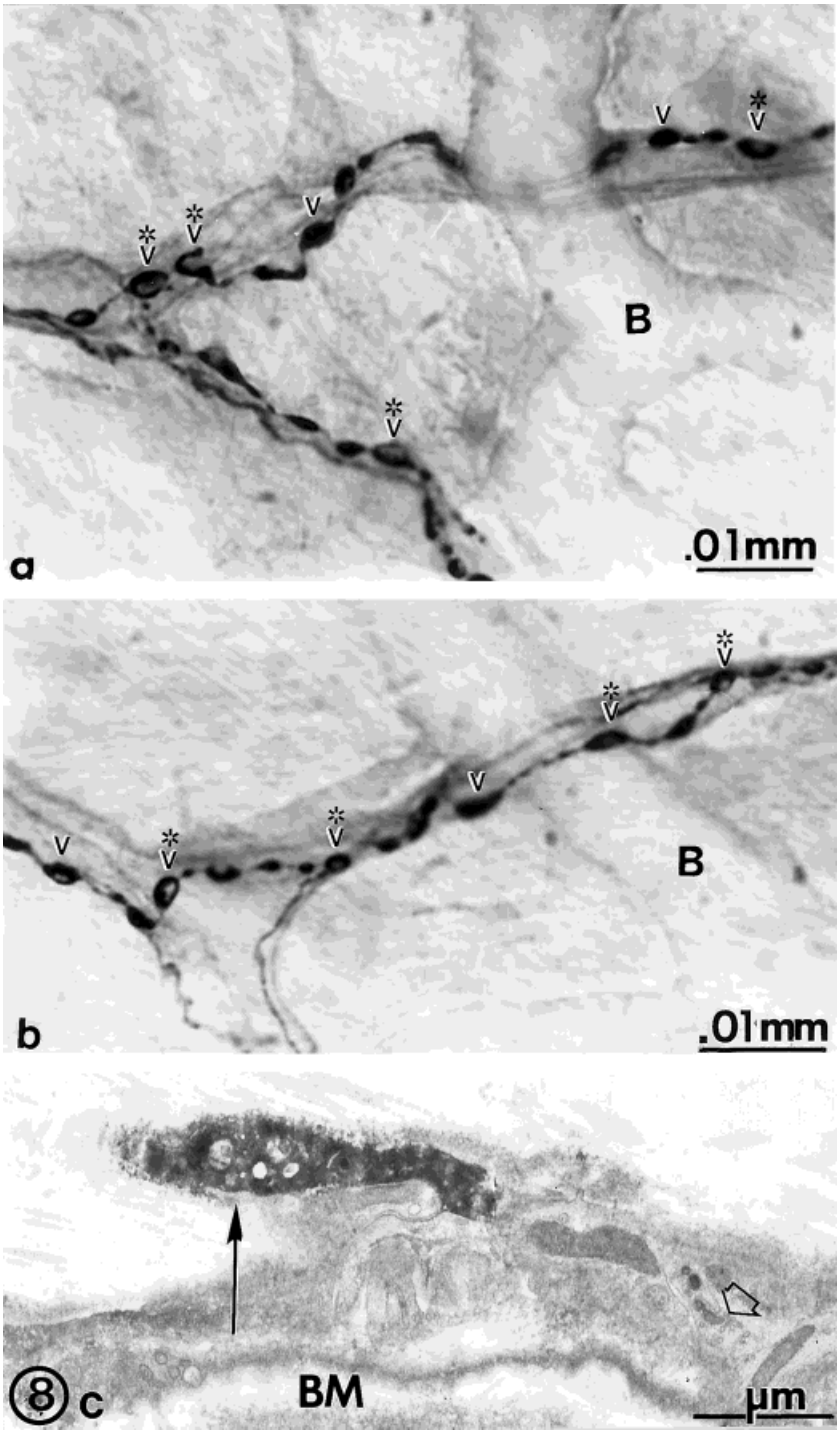

Fig. 8. Effect of 6 mg/kg sumatriptan +30-min electrical stimulation of the trigeminal ganglion on the morphology of varicosities (v) of CGRP-immunoreactive axons in the rat dura mater. In addition to extreme enlargement of the varicosities, many of them display hollow ''cores'' (v*). **a,b:** Light microscopy. B, small blood vessel. **c:** electron microscopy. Hollowness of the ''core'' of the enlarged varicosity (arrow) is due to accumulation of intra-axonal organelles. BM, basement membrane of the capillary. Hollow arrow points to a small nonreacting axon profile containing synaptic vesicles.

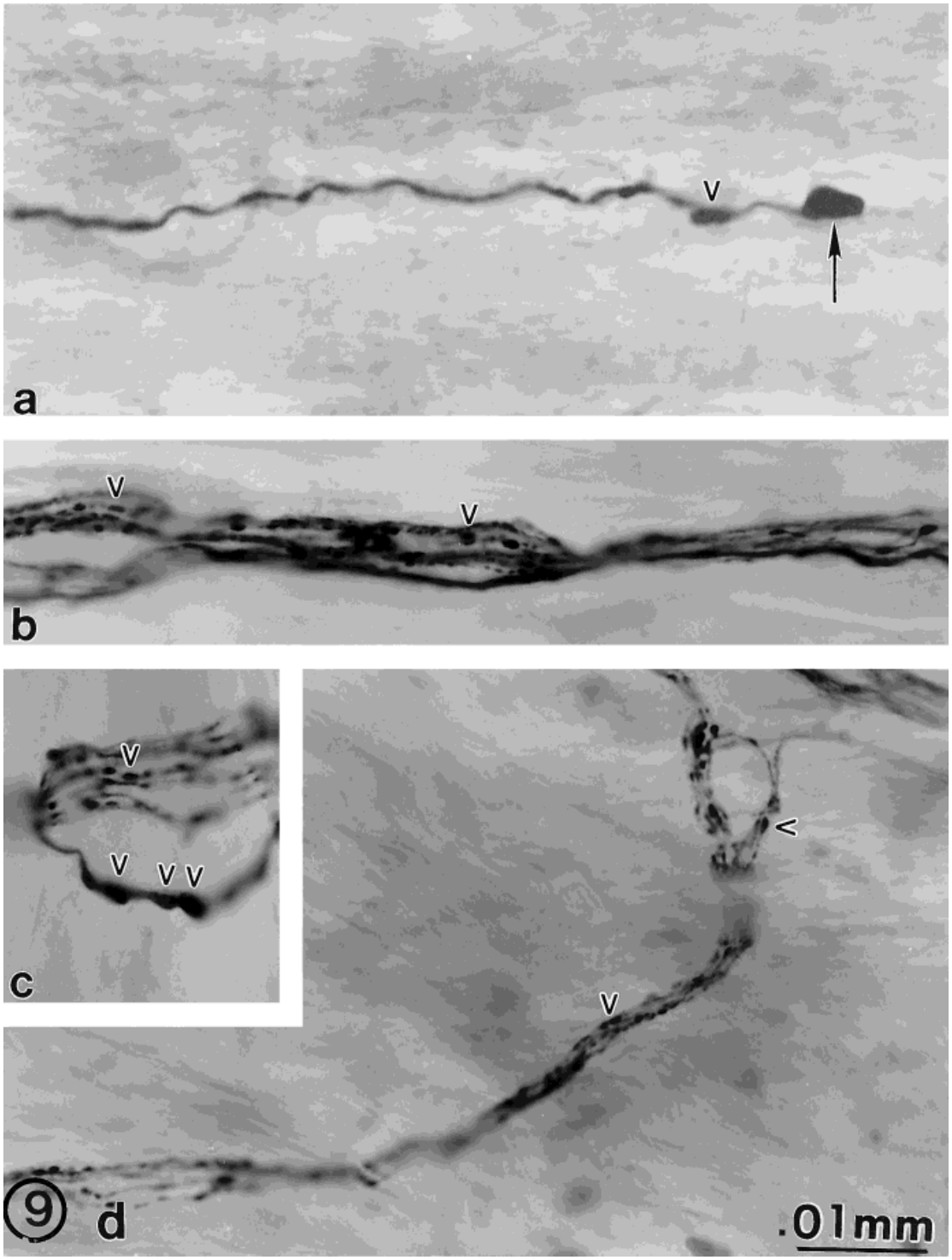

Fig. 9. Effect of 0.12 mg/kg sumatriptan + 30-min electrical stimulation of the trigeminal ganglion on the morphology of terminals (arrow) and varicosities (v) of CGRP-immunoreactive axons in the rat dura mater. Note absence of hollow-cored varicosities after administration of this relatively small (clinical) dose of sumatriptan.

5.4± 4 per section (48% in lamina I, 30% in lamina II, and 22% in lamina III), the number of cells expressing *c-fos* was increased to 38.8 ± 10.5 per section (42% in lamina I, 34% in lamina II, and 24% in lamina III) after 30 min stimulation of the trigeminal ganglion. The difference between the two sets of values is significant ($P < .01$).

Sumatriptan treatment prior to electrical stimulation of the trigeminal ganglion (30 min) resulted in immunohistochemical patterns entirely similar to those seen in animals subjected to electrical stimulation without sumatriptan treatment (Fig. 10a,b). The number of cells expressing *c-fos* was virtually unchanged, being in the range of 5.2 ± 4.1 (42% in lamina I, 34% in lamina II, and 24% in lamina III) on the control side and 36.4 ± 9.5 (47% in lamina I, 27% in lamina II, and 26% in lamina III) on the side of electrical stimulation. The difference between the numbers of *c-fos*-immunopositive cells in stimulated versus nonstimulated sides of the rostral medulla is significant ($P < .01$). On the other hand, there is no significant difference between the numbers of *c-fos*-immunopositive cells in electrically stimulated versus sumatriptan-treated and stimulated samples.

DISCUSSION

According to the present investigations, sumatriptan, a serotonin agonist acting on 5-HT_{1D} receptors, induces marked alterations in the microscopic pattern of CGRP-immunoreactive elements of the supratentorial dura mater, when injected intravenously prior to electrical stimulation of the trigeminal ganglion. In contrast, sumatriptan does not prevent increased *c-fos* expression in the interneurons of the caudal trigeminal nucleus which follows electrical stimulation of the trigeminal ganglion.

Electrical stimulation of the trigeminal ganglion has been shown to induce release of CGRP from nerve fibers of the cerebral dura mater (Buzzi et al., 1991). Since this is analoguous to the alterations observed during migraine headache (Moskowitz, 1984, 1992; Goadsby et al., 1990; Kovács et al., 1991; Goadsby and Edvinsson, 1993, 1994), stimulation of the trigeminal ganglion is regarded as an experimental migraine model (Goadsby et al., 1990; Zagami et al., 1990; Buzzi et al., 1991; Knyihár-Csillik et al., 1995, 1996). Accordingly, the immunohistochemical patterns seen in animals treated with sumatriptan prior to stimulation of the trigeminal ganglion might explain, at the microstructural level, the process of how sumatriptan alleviates the migraine attack.

In our present experiments, the trigeminal ganglion was stimulated for 30 min rather than for 5 min as in the original experiments of the Moskowitz group (Buzzi et al., 1991). The reason to do so was that, as we have shown earlier, only a relatively long (30-min) stimulation results in dramatic microstructural signs as bursting and disintegration of club-like perivascular terminals in the dura mater.

The immunohistochemical alterations observed after administration of sumatriptan and electrical stimulation of the trigeminal ganglion include extreme swelling of CGRP-immunopositive axon terminals, both in the connective tissue and perivascularly, increasing sizes of bead-like varicosities in preterminal portions of CGRP-immunopositive axons, and the transformation of some of the compact beads into gigantic hollow-cored varicosities. These alterations can be regarded as immunohistochemical consequences of the accumulation and stagnation of the CGRP-positive axoplasmic material in peripheral branches of pseudounipolar primary sensory ganglion cells. This would suggest that release of CGRP is blocked. Sumatriptan alone (without stimulation of the trigeminal ganglion) induces moderate swelling of the terminals, probably due to a block of the tonic release of CGRP. Therefore, we assume that sumatriptan acts at the physiological release sites of CGRP. Since sumatriptan is known to bind to 5-HT_{1D} receptors, we suggest that sumatriptan prevents the release of CGRP from dural perivascular axon terminals by an action at 5-HT_{1D} receptors.

Subtypes of the serotonin receptor were analyzed since the early 1980s. Of the 5-HT_1 receptor, subtypes A, B, C, and D have been distinguished (Peroutka, 1993). According to recent investigations (Boess and Martin, 1994) the 5-HT_1 receptor has A, B, D, E, and F subtypes; of these, sumatriptan is selective for the 5-HT_{1D} receptor (Humphrey et al., 1991). The beneficial effect of sumatriptan in migraine headache is well documented (Saxena, 1994; Beattie et al., 1994). Two major theories have been proposed to explain the beneficial effect of sumatriptan in migraine headache: selective vasoconstriction and/or presynaptic inhibition of neurotransmitter release in the domain of the trigemino-vascular system. The present investigations point at the importance of presynaptic effects.

Immunohistochemical identification of *c-fos* protein, product of the proto-oncogene *c-fos*, has been used as a marker for neuronal activity after peripheral stimulation throughout the central nervous system (Hunt et al., 1987; Morgan et al., 1988; Menetrey et al., 1989; Mugniani et al., 1989; Bullitt, 1990). The rationale of this approach is that *c-fos* is expressed within several types of neurons following voltage-gated calcium entry into the cell, i.e., during depolarization (Morgan and Curran, 1986). A changing pattern of *c-fos* expression was noted in the spinal cord (Molander et al., 1992) and in the gracile nucleus (Persson et al., 1993) after electrical stimulation of the chronically injured sciatic nerve. In this respect it is noteworthy that electrical stimulation of the superior sagittal sinus (Kaube et al., 1993) or mechanical

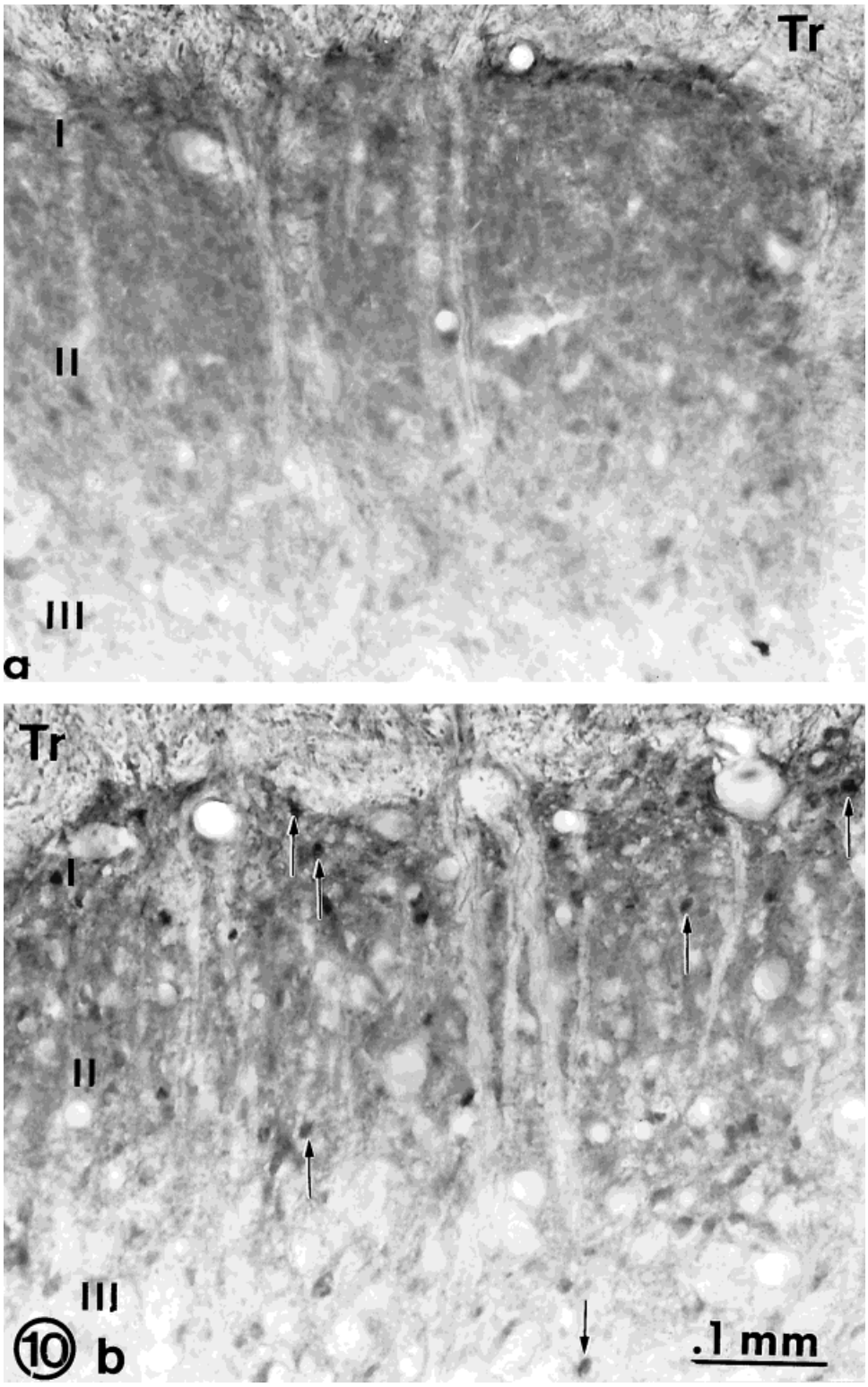

Fig. 10. *C-fos* immunoreactivity in the caudal trigeminal nucleus after i.v. administration of 6 mg/kg sumatriptan, followed by 30-min electrical stimulation of the left trigeminal ganglion. **a:** Control (right) side. **b:** Stimulated (left) side. Arrows point to some of the nuclei expressing *c-fos*. Tr, spinal trigeminal tract. Roman numerals indicate laminae of the spinal trigeminal nucleus.

stimulation of dural blood vessels (Strassman et al., 1994) induced increase in the number of *c-fos*-expressing neurons in the upper cervical dorsal horn and in the medulla. It appears that *c-fos* expression induced by mechanical noxious stimulation is mediated by nitric oxide (Lee et al., 1992); indeed, expression of nitric oxide synthase in spinal cord neurons, following noxious stimulation, is co-localized with the transcription factors *Jun, Fos,* and *Krox* (Herdegen et al., 1994), providing evidence for the role of nitric oxide synthase in nociception (Lee et al., 1993; Csillik, 1996).

One of the characteristic features of sumatriptan is that it passes the blood–brain barrier very poorly (Shepheard et al., 1995). According to our results, intravenous sumatriptan pretreatment of the animals did not prevent activation of *c-fos* expression in interneurons of the caudal trigeminal nucleus. These observations are in accord with those published by Shepheard et al. (1995). It is noteworthy, however, that, according to Nozaki et al. (1992), i.v. administered sumatriptan was effective in preventing enhanced expression of *c-fos* in the medulla, when stimulating the meninges, rather than the trigeminal ganglion. It should be added that the new drug CP-93,129, which penetrated the blood–brain barrier, was more effective in this respect that sumatriptan (Nozaki et al., 1992). Although the parameters of the experiments, as applied by the two above-mentioned experimental groups, were only slightly different, it seems that the results are essentially contradictory.

What is the reason that our results, like those of Shepheard et al. (1995), are at variance with those of Nozaki et al. (1992)? We assume that the salient point is how the trigeminal system was stimulated. Both in our experiments and in those of Shepheard et al. (1995), the trigeminal ganglion was directly stimulated, which, in due course, resulted in excitation of both peripheral and central axon terminals. Consequently, i.v., sumatriptan prevented release of CGRP from peripheral axon terminals (where it interfered with 5-HT_{1D} receptors) but had no effect at the central axon terminals which were virtually inaccessible for the drug. In the experiments published by Nozaki et al. (1992), however, the trigeminal ganglion was not directly stimulated; it was excited only indirectly by the orthodromic impulses arising from the peripheral terminals, located in the cerebral dura, which was subjected to chemical stimulation. Accordingly, blockade of the release of CGRP from the peripheral terminals by i.v. administration of sumatriptan resulted in a smaller inflammatory response and thus less sensitization and activation of trigeminal peripheral sensory terminals. Therefore, neither the ganglion cells nor their central axon terminals were excited under these circumstances.

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