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CO-LOCALIZATION OF THE VANILLOID CAPSAICIN RECEPTOR AND SUBSTANCE P IN SENSORY NERVE FIBERS INNERVATING COCHLEAR AND VERTEBRO-BASILAR ARTERIES

Z. VASS^{a,b}, C. F. DAI^{b,c}, P. S. STEYGER^b, G. JANCsó^d, D. R. TRUNE^b, and A. L. NUTTALL^{b,e,*}

^aDepartment of Otolaryngology, Albert Szent-Györgyi Medical University, Szeged, Hungary H-6725

^bOregon Hearing Research Center, Oregon Health & Science University, Portland, OR 97239-3098, USA

^cDepartment of Otolaryngology, Eye Ear Nose and Throat Hospital, Fudan University, Shanghai 200031, People's Republic of China

^dDepartment of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary H-6720

^eKresge Hearing Research Institute, Department of Otolaryngology, University of Michigan, Ann Arbor, MI 48109-0506, USA

Abstract

Evidence suggests that capsaicin-sensitive substance P (SP)-containing trigeminal ganglion neurons innervate the spiral modiolar artery (SMA), radiating arterioles, and the stria vascularis of the cochlea. Antidromic electrical or chemical stimulation of trigeminal sensory nerves results in neurogenic plasma extravasation in inner ear tissues. The primary aim of this study was to reveal the possible morphological basis of cochlear vascular changes mediated by capsaicin-sensitive sensory nerves. Therefore, the distribution of SP and capsaicin receptor (transient receptor potential vanilloid type 1—TRPV1) was investigated by double immunolabeling to demonstrate the anatomical relationships between the cochlear and vertebro-basilar blood vessels and the trigeminal sensory fiber system.

Extensive TRPV1 and SP expression and co-localization were observed in axons within the adventitial layer of the basilar artery, the anterior inferior cerebellar artery, the SMA, and the radiating arterioles of the cochlea. There appears to be a functional relationship between the trigeminal ganglion and the cochlear blood vessels since electrical stimulation of the trigeminal ganglion induced significant plasma extravasation from the SMA and the radiating arterioles.

The findings suggest that stimulation of paravascular afferent nerves may result in permeability changes in the basilar and cochlear vascular bed and may contribute to the mechanisms of vertebro-basilar type of headache through the release of SP and stimulation of TPVR1, respectively. We propose that vertigo, tinnitus, and hearing deficits associated with migraine may arise from perturbations of capsaicin-sensitive trigeminal sensory ganglion neurons projecting to the cochlea.

Keywords

capsaicin; C-fibers; plasma extravasation; trigeminus; headache; guinea-pig

C-type sensory nerve fibers are small unmyelinated neurons that transmit nociceptive and heat information to the central nervous system (CNS) (afferent function). Their peripheral terminals also release a variety of pro-inflammatory neuropeptides (local regulatory or sensory efferent function), such as substance P (SP) or calcitonin gene-related peptide (CGRP), that cause vasodilatation and extravasation (Jancsó, 1968, 1992; Jancsó et al., 1977; Szállasi and Blumberg, 1999). However, the precise mechanisms of these inflammatory agents are not known.

Carlisle et al. (1990) have demonstrated expression of SP, CGRP, vasoactive intestinal polypeptide, and neuropeptide Y in the guinea-pig spiral modiolar artery (SMA). The expression of these neuropeptides in the SMA (as in cerebral blood vessels; Wahl and Schilling, 1993) indicates that sensory vasodilator mechanisms may be present in the cochlea. Consistent with this, capsaicin induces a significant increase in cochlear blood flow (CBF) under both normal (Vass et al., 1994) and pathological conditions (Vass et al., 1995a). Moreover, SP mediates the capsaicin-induced CBF response in the normal animal (Vass et al., 1995b) and retrograde tracer evidence indicated that the trigeminal ganglion innervates cochlear blood vessels (Vass et al., 1997). Biocytin anterograde labeling showed fibers originating from the trigeminal ganglion innervating the SMA and stria vascularis of the cochlea and the vestibular dark cell region (Vass et al., 1998; Riemann and Reuss, 1999).

At low doses, capsaicin activates a subpopulation of C-type primary sensory neurons of the trigeminal ganglion (Holzer, 1988) which express the transient receptor potential vanilloid type (TRPV1) or capsaicin receptor, a non-selective cation channel that is gated by a variety of stimuli, including capsaicin, extracellular acidic pH (<6.3) and noxious heat >42 °C (Tominaga et al., 1998; Caterina et al., 1997; Dedov and Roufogalis, 2000). In mature neurons, co-expression of the TRPV1 receptor with the TrkA receptor and vasoactive peptides characterizes a subpopulation of sensory fibers that respond to noxious heat, pH and pressure (Caterina et al., 1997; Otten et al., 1982; Ogun-Muyiwa et al., 1999; Neumann et al., 1996).

Currently it is not known whether TRPV1 receptors are expressed on blood vessels in the cochlea, or whether the nerve fibers containing SP (Carlisle et al. 1990) may co-express TRPV1. Therefore, in the present study the possible co-expression of SP and TRPV1 was investigated by double immunolabeling. In addition, the effect of activation of trigemino-cochlear sensory fibers by electric stimulation of the trigeminal ganglion was also determined by the demonstration of plasma extravasation of colloidal silver or Evans Blue from vessels of the cochlea and the vertebro-basilar system.

EXPERIMENTAL PROCEDURES

Immunolocalization of TRPV1 and SP: animal preparation

Healthy male and female albino guinea-pigs ($n=33$; Hartley) with normal Preyer's reflex (300–350 g) were used in these studies. Albino guinea-pigs were chosen to avoid melanin pigments that may obscure the fluorescence signal. Animals were anesthetized with ketamine hydrochloride (100 mg/kg i.m.; Abbot Laboratories, North Chicago, IL, USA) and xylazine (2 mg/kg i.m.; Phoenix Scientific, Inc. St. Joseph, MO, USA). Anesthetized animals were perfused through the left heart ventricle with 200 ml 0.9% saline, and subsequently with 4% paraformaldehyde in 0.02 M phosphate-buffered saline (PBS; 300 ml)

(Sigma–Aldrich Chemical Corporation, St. Louis, MO, USA). Both inner ears, the basilar artery (BA) with its branches, and cervical dorsal root ganglia were dissected and placed in fresh fixative (4% paraformaldehyde in 0.02 M phosphate buffer, pH 7.2–7.4) for 2 h. After fixation BA and anterior inferior cerebellar arteries (AICA) were dissected free and placed into 0.02 M PBS (pH 7.4). The fixed SMA, radiating arterioles, and the stria vascularis were also isolated for immunocytochemistry (Fig. 1).

The tissues were washed in fresh 0.02 M PBS (pH 7.4), permeabilized in 0.5% Triton-X 100 (Sigma, St. Louis, MO, USA) in 0.02 M PBS (pH 7.3) for 30 min, and immunoblocked in 10% goat serum in 1% bovine serum albumin (BSA) in 0.02 M PBS (1% BSA-PBS) for 60 min.

For immunocytochemistry, tissues were incubated with rabbit anti-TRPV1 antiserum (diluted 1:1000 in 1% BSA-PBS; gift from Dr. Michael J Caterina, John Hopkins University) for 48 h. After washing in 1% BSA-PBS, tissues were subsequently incubated in Alexa-488-conjugated goat anti-rabbit IgG for 3 h (Molecular Probes; 1:100 in BSA-PBS). Alternatively, tissues were incubated in rat anti-SP antibody (diluted in 1:100 in 1% BSA-PBS; Sigma) for 48 h. After washing in 1% BSA-PBS, tissues were subsequently incubated in Alexa-568-conjugated goat anti-rat IgG for 3 h (Molecular Probes; 1:100 in BSA-PBS).

For double-labeling immunocytochemistry, tissues were incubated in 1% BSA-PBS with 1:1000 rabbit anti-TRPV1 antibody and 1:100 rat anti-SP antibody (Harlan-Sera Labs, Limited, Loughborough, UK) for 48 h. After washing in 1% BSA-PBS, tissues were subsequently incubated in Alexa-488-conjugated goat anti-rabbit IgG and Alexa-568-conjugated goat anti-rat IgG for 3 h (both diluted 1:100 in BSA-PBS).

Immunocytochemical controls: For positive controls, TRPV1-rich and SP-positive DRG (Caterina et al., 1999; Gibbins et al., 1987) tissues were incubated with primary antibodies. For negative controls, TRPV1 blocking peptide and TRPV1 antisera were mixed for 30 min, then all tissues were incubated in mixture solution for 48 h, and followed by secondary antibodies. Alternatively, the primary antibody was replaced by 1% BSA-PBS for 48 h prior to incubation with the secondary antibodies.

The labeled tissues were mounted with VectaShield (Vector Laboratories, Inc. Burlingame, CA, USA), coverslipped, and observed on a Nikon Eclipse TE 300 inverted microscope fitted with a Bio-Rad MRC1024 confocal laser microscopy system. Samples were illuminated with a krypton-argon laser (American Laser, UT, USA), at 488 nm and 568 nm. FITC fluorescence emissions (515 nm) represented the location of TRPV1 (green in color micrographs), and Alexa 568 fluorescence emission (603 nm) represented the location of SP receptors (red in color micrographs). Confocal images were acquired using the Lasersharp acquisition program and digitally prepared for publication using Adobe Photoshop.

Electric stimulation of the trigeminal ganglion

Twenty-six male and female albino guinea-pigs (Hartley) with normal Preyer's reflex (300–350 g) were used. To reduce sympathetic vasoconstriction, guinea-pigs were pretreated with guanethidine (30 mg/kg; Sigma) 24 h before the following procedure (Salar et al., 1992). Anesthesia was induced by ketamine hydrochloride (100 mg/kg i.m.) and xylazine (2 mg/kg i.m.) and the body temperature of the animal was maintained at 38 ± 1 °C with a thermoregulated heating blanket. The trachea was cannulated and animals were artificially respired with room air (40 respirations/min) after being paralyzed with tubocurarine (Sigma–Aldrich Chemical Corporation) (0.1 mg/kg i.m.). The right carotid artery was cannulated (Intradermic PE 50; Becton Dickinson) to monitor systemic blood pressure (BP) and heart rate (HR). The right jugular vein was cannulated for drug delivery.

The guinea-pigs were secured in a heated headholder, to reduce cochlear heat loss. The left tympanic bulla was opened by a ventrolateral surgical approach to preserve the function of the tympanic membrane and ossicles. Trigeminal ganglion was visualized by drilling a hole (diameter: 0.5 mm) on the dorsal wall of the bulla, just behind the apex of the cochlea (Vass et al., 1999) and making a small incision in the dura to expose the extradural surface of the ganglion. A bipolar stimulating electrode was lowered using a micromanipulator onto the surface of the trigeminal ganglion close to the ophthalmic division.

Thirty minutes were allowed to lapse to stabilize BP resting levels before proceeding. A computerized pulse generator produced the electric stimulus. Capacity-coupled, charge-balanced, bipolar constant current electric stimulation at 6 Hz (rectangular pulses with a 50% duty cycle, charge balanced) was used with current levels of 0.1 or 1 mA. The duration of the stimulation was 5 min.

BP (mm Hg) and HR (beats/min) were digitally sampled at 2 Hz and data stored throughout each experiment. The percentage change relative to the initial baseline was then calculated. Comparisons of results between electrically stimulated and control animals were made with a Student's *t*-test for paired samples. A probability value less than 0.05 was used to indicate a significant difference between treatments.

Histological preparation after electric stimulation

One hour after dye injection (see below) and electric stimulation, the animals were further anesthetized with ketamine hydrochloride (100 mg/kg i.m.) and by xylazine (2 mg/kg i.m.) and transcardially perfused with 200 ml of 0.05% sodium nitrite in normal saline, followed by 750 ml of 3% paraformaldehyde in phosphate buffer (in 0.1 M PB, pH 7.4). The flow of fixative to the cochleae by the common carotid arteries was maximized by occlusion of the brachial arteries and the descending aorta. Following perfusion–fixation, the ipsilateral and contralateral cochleae were quickly dissected and placed in fresh fixative. The apical end of the cochlea was opened and the same cold fixative was gently perfused through openings at the apex and the oval window. After fixation, the stria vascularis was dissected from the bony cochlea and placed on slides.

Evaluation of permeability changes in the cochlea

Visualization of vascular permeability with the colloidal silver method—In the first group ($n=12$), permeability changes in the SMA were rendered visible in histological preparations by the colloidal silver method (Jancsó, 1960; Jancsó et al., 1980). Immediately before 0.1 mA ($n=6$) or 1 mA ($n=6$) electric stimulation of the trigeminal ganglion, a 1% colloidal silver solution (100 mg/kg at body temperature; Aldrich Chemical Co. Inc.) was slowly injected (total injection time: 1 min) into the jugular vein. One hour after electric stimulation and colloidal silver infusion, the animals were transcardially perfused with 200 ml of 0.05% sodium nitrite in normal saline, followed by 750 ml of 3% paraformaldehyde in PB (in 0.1 M PB, pH 7.4). The ipsi- and contralateral inner ears were quickly dissected and placed in fresh fixative (3% paraformaldehyde in 0.1 M PB, pH 7.2–7.4). The apical end of the cochlea was opened, and the same cold fixative was gently perfused through the apex and the oval window. The cochleae were fixed overnight and decalcified for 2 weeks in buffer containing 10% EDTA. The cochleae were infiltrated under vacuum sequentially in 25%, and 50% O.C.D. diluted with distilled water and 100% optimal cutting temperature (O.C.T.) 4583 Compound (Tissue-Tek; Sakura Finetek U.S.A., Inc., Torrance, CA, USA) for 1 h each. Tissues were then frozen in O.C.T. compound and serially sectioned at 10 μ m (Zeiss, Microm Cryostat, HM 506E; Microm Laborgerate GmbH, Germany). The sections were mounted onto glass slides and coverslipped with Pro-Texx mounting medium (American Scientific Product, Pittsburgh, PA, USA).

Sections and whole mounts were examined on a Zeiss microscope (Zeiss, Universal, Oberkochen, Germany) fitted with darkfield optics and a Wild MPS48 Photoautomat and Leica camera (Leica AG, CH-9435; Heerbrugg, Switzerland). Darkfield images were documented using Ektachrome color film (160 ASA; Kodak). Photographs of cochleae from the ipsilateral (stimulated) and contralateral (control) sides were exposed for the same time at the same magnification for comparison of colloidal silver extravasation.

Two animals served as surgery controls where the electrode was placed onto the surface of the trigeminal ganglion without electric stimulation.

Visualization of plasma extravasation with the fluorescent method—A second group ($n=10$) of animals was used to visualize Evans Blue extravasation. Five mg/kg Evans Blue dye (Sigma) was injected i.v. 30 min after surgical exposure and immediately before the electric stimulation of the trigeminal ganglion. One hour after 0.1 mA ($n=5$) or 1 mA ($n=5$) electric stimulation, subjects were transcardially perfused and cochlear blood vessels were isolated and fixed as above. Surface preparations of the SMA, radiating arteriole, and stria vascularis from both ipsilateral and contralateral sides were mounted with Vectashield (Vector Laboratories, Inc.), coverslipped, and observed using confocal microscopy as described above. Specimens were illuminated at 647 nm and Evans Blue fluorescence (>660 nm) was documented using a Nikon Eclipse TE 300 inverted microscope fitted with a Bio-Rad MRC1024 laser scanning confocal microscopy system.

Surgery control animals received Evans Blue without electric stimulation ($n=2$). To determine the degree of autofluorescence, one animal was killed after the surgery without either electric stimulation or Evans Blue application.

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Oregon Health and Science University and conformed to NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1978). Every attempt was made to minimize the total number of animals used and their suffering.

RESULTS

Immunofluorescence labeling

TRPV1 Immunoreactivity—TRPV1 immunoreactivity was observed within axons in the adventitia of the BA and around the AICA, SMA, and radiating arterioles (Fig. 2A–D). The fibers were mostly longitudinal on the BA (Fig. 2A), but some branches ran circumferentially (e.g. arrow b in Fig. 2A) around the wall of AICA, SMA, and radiating arteriole (arrow b in Fig. 2A–D). TRPV1-expressing nerves were homogeneously labeled with no indication of varicosities. The diameters of the labeled fibers were largest around the BA and became progressively smaller on the AICA, and the radiating arterioles (Fig. 2A–D). Labeled fibers were not detected in the spiral ligament and the stria vascularis.

In control experiments, no labeled fibers could be observed in tissues incubated with the antigen-adsorbed TRPV1 antibody (insets in Fig. 2A–D). When the primary antibodies were replaced with 1% BSA-PBS, negligible non-specific fluorescence was observed in the BA, AICA, SMA or radiating arterioles (data not shown).

SP Immunoreactivity—Many SP-immunoreactive fibers were arranged longitudinally and circumferentially around the BA, AICA, SMA, and radiating arterioles (Fig. 2E–H). Fluorescence intensity of SP labeled fibers around the BA, AICA, SMA, and radiating arterioles was qualitatively similar to the TRPV1 labeling. However, in contrast to the homogeneously labeled TRPV1 fibers (Fig. 2A–D), the SP-labeled fibers showed a punctate

staining (Figs. 2E–H and 3). The SP-labeled fibers could not be followed beyond the radiating arterioles of the cochlea. No SP-labeled fibers could be detected in the spiral ligament, stria vascularis, or spiral prominence.

In control experiments, when primary antibody was replaced with 1% BSA-PBS, negligible non-specific fluorescence was observed in the BA, AICA, SMA, or radiating arterioles (insets in Fig. 2E–H).

Co-localization of TRPV1 and SP—In vessels double-labeled for TRPV1 and SP antigens, extensive co-localization occurred in axons within the adventitial layer of the BA, AICA, SMA, and radiating arterioles (Fig. 3B, E, H, K). TRPV1, and SP colabeling of fibers was seen in the longitudinal fibers and also the smaller circumferential network.

In dorsal root ganglia (DRG), co-expression of TRPV1 and SP could be observed in neuronal cell bodies (Fig. 4F). Some cell bodies appeared to express only TRPV1, while some neuronal fibers could also be observed, with labeling for SP only. In control experiments, no TRPV1-labeled cell bodies were seen in the antigen-adsorbed TRPV1 antibody incubated tissue sections (Fig. 5B). Negligible non-specific fluorescence was observed when the primary antibodies are replaced with 1% BSA-PBS (inset in Fig. 4B).

Permeability changes after trigeminal electric stimulation

All animals tolerated anesthesia and surgery well. Electrical stimulation of the trigeminal ganglion and infusion of either colloidal silver or Evans Blue into the jugular vein resulted no significant changes in BP, HR or body temperature of experimental animals when compared with the experimental control or surgical control groups.

SMA—After antidromic electric stimulation of the trigeminal ganglion, mid-modiolar sections revealed dense aggregates of colloidal silver particles in and around the ipsilateral SMA and radiating arteriolar vessels (Fig. 5A, B, E, F). Colloidal silver extravasation was observed in the SMA from the basal to the apical turns of the cochlea. In mid-modiolar sections of the cochleae colloidal silver label alternated from one side of the modiolus to the other, consistent with the spiral orientation of the vessels. The SMA is situated between the acoustic nerve internally and the spiral ganglion externally (Fig. 5A, arrow b). Labeling the midmodiolar sections was associated not only with SMA, but also with spiral modiolar vein.

At higher magnification, we could distinguish the distribution of the colloidal silver particles external to the SMA (Fig. 5E, F). After 0.1 mA electric stimulation the colloidal silver granules were primarily located in the tunica intima and media. Colloidal silver particles were also observed beyond the external elastic lamina into the adventitia. The densest localization of colloidal silver was in the internal elastic lamina, adjacent to the endothelial layer.

Only sparse colloid silver particles were present in the contralateral SMA after 0.1 mA electric stimulation, located just external to the internal elastic lamina.

The contralateral ear and control ears (colloidal silver infusion without trigeminal stimulation) showed colloidal silver granules only within the lumen of the BA and were easily distinguished from granules within the wall of the artery in experimental animals (Fig. 5C, D).

Trigeminal electric stimulation also caused significant Evans Blue extravasation from the SMA (Fig. 6). Confocal microscopy showed distribution patterns of Evans Blue in the wall of the isolated SMA similar to that described for colloidal silver. The fluorescent dye was

present in the SMA tunica media (arrow labeled b in Fig. 6B) and internal elastic lamina (arrow labeled a in Fig. 6B). Electric stimulation of the trigeminal ganglion (1 mA) caused Evans Blue extravasation into the tunica intima, media, and adventitia of the SMA. Evans Blue revealed the undulating morphology of the longitudinal elastic fibers in the internal elastic lamina and the circumferential elastic fibers of the external elastic lamina (Fig. 6A). At higher magnification, Evans Blue extravasation could also be observed into the tunica media and adventitia (arrow labeled c in Fig. 6B).

Negligible tissue autofluorescence (>660 nm) was detected in the SMA and radiating arterioles when illuminated by the 647 nm laser light used at the same intensities as for experimental animals infused with Evans Blue. Evans Blue infusion in the surgical-control and vehicle-control animals resulted in no dye visible outside the lumen after 30 or 60 min (Fig. 6C). After 90 min of Evans Blue infusion some leakage was evident by increasing the intensity of the laser light, the confocal pinhole size, and gain setting over those used to observe Evans Blue extravasation in the experimental groups. This minimal amount of leakage was restricted to the internal elastic lamina. No fluorescence was detected in the tunica media or peripheral to the internal elastic lamina of the SMA and radiating arterioles at 90 min following Evans Blue infusion (Vass et al., 2001).

Radiating arterioles—We were unable to identify extravasation of colloidal silver in the radiating arterioles in the midmodiolar sections of the cochlea. But extravasation of Evans Blue was detected in radiating arterioles, following both 0.1 and 1 mA electric stimulation of the trigeminal ganglion (Fig. 6A, B). Evans Blue extravasation extended to the internal elastic membrane of the radiating arterioles, but in contrast to the SMA, the radiating arterioles have no external elastic membrane. Extravasation was also evident in radiating arterioles entering the stria vascularis (not shown). It was not possible to identify the individual layers of these vessels, but Evans Blue was visible external to their lumen. Permeability changes also were observed in the lateral wall at stria vascularis. These data are the subject of a separate report (Vass et al., 2001).

DISCUSSION

This study provides evidence for the innervation of the BA, AICA, and cochlear arteries by paravascular nerve fibers, which contain SP and exhibit TRPV1-immunoreactivity. Analysis of confocal images disclosed the colocalization of SP and TRPV1 immunoreactivities in paravascular nerve fibers. Circumstantial evidence indicates that SP- and TRPV1-immunoreactive fibers are of trigeminal origin. Previous studies by Suzuki et al. (1989) showed that SP- and CGRP-immunoreactive fibers around the BA originate from the trigeminal ganglion. Bilateral removal of the trigeminal ganglia decreased the SP levels in brain arteries including the rostral BA (Saito and Moskowitz, 1989). Various neuropeptides, including SP and CGRP, have been observed also in the SMA (Carlisle et al., 1990; Kadota et al., 1996). The demonstration of labeled neurons in the ophthalmic division of the Gasserian ganglion after intracochlear injection of wheat germ agglutinin-horseradish peroxidase provided direct anatomical evidence for the trigeminal innervation of cochlear structures (Vass et al., 1997).

The current study furnished further support for the functional relationship between the trigeminal ganglion and cochlear blood vessels. Electrical stimulation of the trigeminal ganglion caused significant extravasation of colloidal silver and Evans Blue from the SMA and radiating arterioles on the ipsilateral side. Both colloidal silver (Jancsó, 1960; Jancsó et al., 1980) and Evans Blue (Jancsó, 1960; Jancsó et al., 1980; Moos and Mollgard, 1993) have been widely used as markers of (neurogenic) plasma extravasation. Previous studies have also shown that both electrical stimulation (Salar et al., 1992) or chemical activation

with capsaicin (Moskowitz et al., 1990) cause neuropeptide release from trigeminal vascular fibers and vasodilatation and fluid accumulation in extracranial (Lambert et al., 1984) and intracranial tissues (Goadsby and Duckworth, 1987; Dux et al., 2003).

Trigeminal sensory innervation has previously been also implicated in the regulation of cochlear vascular reactions. Recent studies demonstrated that intracochlear administration of capsaicin or electric stimulation of the Gasserian ganglion produced vasodilatation and plasma extravasation in cochlear tissues (Vass et al., 1995b, 1998). The present findings provide firm morphological basis for these findings by showing the localization of the capsaicin receptor, TRPV1, and SP in nerve fibers innervating the BA and their branches, involving the SMA. In addition, the results indicate that antidromic electrical stimulation of these paravascular nerves produces plasma extravasation in these blood vessels presumably via the release of SP. This assumption is supported by the findings, which demonstrated that sensory nerve stimulation induced plasma extravasation is mediated by neurokinin 1 receptors in various tissues (Maggi and Meli, 1988). Stimulation of TRPV1 expressing paravascular capsaicin-sensitive afferent nerves by e.g. acidic pH or tissue born vasoactive agents (Tominaga et al., 1998) may bear importance under pathophysiological conditions by participating in cochlear inflammatory processes. The demonstration of the TRPV1 receptor in the basilar vascular bed may suggest a role of these receptors in the mechanisms of basilar type headache. Indeed, sensory neurogenic plasma extravasation has been suggested to play a significant role in the patho-mechanism of certain types of migraine (Moskowitz et al., 1996).

Conclusion

The present findings demonstrate the colocalization of TRPV1 and SP in paravascular the BA, AICA, and cochlear arteries. The findings suggest that stimulation of paravascular afferent nerves may result in permeability changes in the basilar and cochlear vascular bed and may contribute to the mechanisms of vertebro-basilar type of headache through the release of SP and stimulation of TRPV1, respectively. We propose that vertigo, tinnitus, and hearing deficits associated with migraine may arise from perturbations of capsaicin-sensitive trigeminal sensory ganglion neurons projecting to the cochlea.

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Abbreviations

AICA	anterior inferior cerebellar artery
BA	basilar artery
BP	blood pressure
BSA	bovine serum albumin
CBF	cochlear blood flow
CGRP	calcitonin gene-related peptide
CNS	central nervous system
DRG	dorsal root ganglion

EDTA	ethylenediaminetetraacetic acid
HR	heart rate
O.C.T.	optical cutting temperature compound
PB	phosphate buffer
PBS	phosphate-buffered saline
SMA	spiral modiolar artery
SP	substance P
TRPV1	transient receptor potential vanilloid type 1

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Fig. 1. Schematic of the anatomical relationship of the trigeminal ganglion, cochlea, vestibular labyrinth and BA. The cochlea and vestibular labyrinth receive innervation from the ophthalmic branch (Oph) of the trigeminal ganglion, which may pass along the BA and AICA. Mand, mandibular branch of trigeminal ganglia; Max, maxillary branch of trigeminal ganglia; SM, scala media; ST, scala tympani; SV, scala vestibuli.

Fig. 2. Confocal images of TRPV1 (A–D) and SP immunolabeling (E–H) expression in (A, E) BA, (B, F) AICA, (C, G) SMA, and (D, H) RA. Arrows indicate labeled: longitudinal (a), circumferential (b) fibers. Insets in A–D, show negligible labeling in BA, AICA, SMA, and RA incubated with antigen-adsorbed TRPV1 antibodies and secondary antibodies. Insets in E–H show negligible labeling in the BA, AICA, SMA, and radiating arterioles incubated with 1% BSA-PBS and secondary antibodies. Scale bars in microns.

Fig. 3. Confocal images of the BA (A–C), AICA (D–F), SMA (G–I), and radiating arteriole (RAD; J–L) immunolabeled for TRPV1 (A, D, G, J), and for SP (C, F, I, L). A subset of neuronal fibers co-express TRPV1 and SP (white) in merged images (B, E, H, K). TRPV1 and SP co-expressing fibers occur as both longitudinal (a arrows) and circumferential fibers (b arrows). Scale bars in microns.

Fig. 4.

Confocal images of the DRG double-immunolabeled for TRPV1 (A), and for SP (B). (C) A subset of neuronal cell bodies co-express TRPV1 and SP (white, arrows) in the merged image. Inset in (A) shows negligible labeling in the DRG when incubated with antigen-adsorbed TRPV1 antibodies and secondary antibodies. Inset in B shows negligible labeling in the DRG when incubated with 1% BSA-PBS and secondary antibodies. Scale bar in microns.

Fig. 5. Darkfield micrographs of mid-modiolar sections of cochleae after 0.1 mA trigeminal electrical stimulation and colloidal silver infusion. (A, B) Sixty minutes after ipsi-lateral electrical stimulation, colloidal silver grains occur in the arterial wall of SMA, arterioles, and spiral modiolar vein (arrows: a, radiating arteriolar vessels; b, SMA). There was no colloid silver around the cochlear blood vessels in control cochleae after trigeminal electrical stimulation and colloidal silver infusion (C, D; arrows: a, radiating arteriolar vessels, b, SMA). At higher magnification (E, F) colloidal silver particles are present primarily in the tunica intima, but also external to the SMA (arrows: a, SMA). Scale bars in microns.

Fig. 6. Confocal photomicrograph of the SMA and radiating arteriole (RA), 60 min after 1 mA electrical stimulation of the trigeminal ganglion and i.v. Evans Blue infusion. (A) Evans Blue revealed the undulating morphology of the longitudinal elastic fibers in the internal elastic lamina. (B) At higher magnification, Evans Blue fluorescent dye was present in the internal elastic lamina (a), tunica media (b), and tunica adventitia (c). (C) In control experiment, the Evans Blue fluorescent dye does not appear in the layers of SMA and radiating arteriole. Scale bars in microns.