Vanilloid Receptor 1 Expression in Human Tooth Pulp in Relation to Caries and Pain

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Aims: *To investigate the presence of vanilloid receptor 1 (TRPV1) in human dental pulp and to correlate any expression with caries and pain.* ***Methods:*** *Permanent mandibular first molars were collected and categorized as intact or grossly carious. Grossly carious teeth were further categorized as carious asymptomatic or carious painful samples. Coronal pulps were removed and processed for indirect immunofluorescence using antibodies raised against TRPV1 and a neuronal marker, either protein gene product 9.5 or alpha-smooth muscle actin, in conjunction with* Ulex europaeus *agglutinin 1 lectin to fully label the pulp vasculature.* ***Results:*** *Analysis revealed that TRPV1 labeling was not confined to pulpal nerve fibers. TRPV1 was also consistently expressed within pulp microvasculature. Expression of neuronal TRPV1 was significantly increased throughout the pulp in grossly carious samples (*$P < .05$*). No significant differences were found between carious asymptomatic and carious painful samples. A significant increase in vascular TRPV1 expression was observed in arterioles present in the midcoronal pulp in carious painful compared with carious asymptomatic samples (mean area ± SEM [%] of TRPV1 to vascular labeling; 6.48% ± 4.5% for carious asymptomatic teeth, n = 9; 31.21% ± 9.6% for carious painful teeth, n = 9;* $P = .02$*).* ***Conclusion:*** *Expression of TRPV1 in pulpal nerve fibers undergoes marked changes with caries. This may be of relevance in the development of pulpal inflammation, but its relationship to dental pain is still unclear. However, vascular TRPV1 expression does appear to be positively correlated with dental pain, thus providing new insights into symptomatic pulpitis.*

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Key words: blood vessels, inflammation, pain, tooth pulp, vanilloid receptor 1

The vanilloid receptor 1 (TRPV1) is thought to play an integral role in the development of inflammation and nociception. It belongs to the transient release potential (TRP) superfamily[1] and acts as an excitatory, nonselective ion channel. TRPV1 is thought to be expressed predominantly by polymodal nociceptors[1] and Aδ fibers[2,3]; however, more recent evidence suggests that it is also present in several brain nuclei[4] and some nonneuronal tissues.[5,6] The receptor is known to be the target for vanilloids, including capsaicin, although, physiologically, it is now thought to function mainly as a noxious heat sensor.[7,8] Mild, local acidification can facilitate a decrease in the temperature threshold of TRPV1, leading to increased receptor sensitivity.[9] TRPV1 is also activated by inflammatory mediators such as bradykinin and prostaglandins.[10,11] Activation of TRPV1 has been shown to promote release of substance P (SP)[12,13] and calcitonin gene-related peptide (CGRP).[14]

Human immunocytochemical studies using tissue samples from patients with rectal hypersensitivity and inflammatory bowel disease have shown that both the sensitivity of TRPV1 and the density of receptor expression are altered during inflammatory conditions.[15,16] In these studies, neuronal expression of TRPV1 was significantly increased in inflammatory conditions. To date, no studies have found a direct correlation between TRPV1 expression and spontaneous pain. However, there is some anecdotal evidence to suggest that TRPV1 upregulation is greater in patients with symptomatic inflammatory bowel disease.[16] In addition, it has been demonstrated that patients with rectal hypersensitivity show significantly lowered rectal heat thresholds than controls, and this is directly associated with increased TRPV1 expression.[15]

Despite evidence that TRPV1 may be involved in inflammation and pain, little attention has been given to the presence, distribution, and function of this receptor in other models of inflammation, including the inflamed tooth pulp. Initial studies of TRPV1 expression in the trigeminal ganglion described TRPV1 mRNA in rat dental sensory neurons,[3] and subsequent immunocytochemical studies revealed that rat pulpal nerves express both TRPV1 and the vanilloid receptor-like protein VRL-1.[17–19] Interestingly, only 1 study to date has examined the presence of TRPV1 in human tooth pulp and quantified TRPV1 expression in healthy and carious teeth.[20] However, no correlation was seen between TRPV1 and pulpitic pain. The possible involvement of TRPV1 in pulpal pain mechanisms is of interest, as clinically, inflamed teeth often show increased sensitivity to heat. Therefore, the aim of this study was to further investigate the presence and distribution of TRPV1 in human tooth pulp and to correlate its expression with the presence of caries and clinical pain history.

Materials and Methods

The experimental material consisted of 43 permanent mandibular first molars obtained from children requiring dental extractions under general anesthesia(n = 36; 18 girls and 18 boys). Ethical approval was obtained from South Sheffield Ethics Committee. Prior to extraction, a simple pain history was sought from the patient. Pain history was considered positive if the child personally reported spontaneous toothache in the previous few days or if the parent stated that the child had suffered disturbed eating or sleeping attributable to pulpitic pain. Teeth were excluded from the study if any other associated pathology was apparent.

Immediately following a simple forceps extraction, a groove was cut on the buccal side of each tooth, and the tooth was split longitudinally using an osteotome and surgical mallet. Once split, the tooth halves were placed in Zamboni's fixative (4% paraformaldehyde and 0.2% picric acid in 0.1 mol/L phosphate buffer, pH 7.4) for 24 hours at 4°C. In all cases, the time between application of the forceps and placement in fixative was less than 60 seconds. The coronal pulp was then carefully dissected from the pulp chamber and placed in 0.2 mol/L phosphate-buffered saline (PBS). The remaining enamel and dentin were examined to assess the degree of caries at ×20 magnification. The extent of occlusal carious lesions was assessed on the basis of color changes. Those teeth classed as intact showed no color changes within the dentin, whereas grossly carious samples showed yellow/brown pigmentation of the dentine extending more than halfway through the dentine to the pulp chamber.

The coronal pulps remained in PBS for a further 24 hours at 4°C. They were then placed in 0.1 mol/L PBS containing a 30% sucrose solution for cryoprotection (5 hours at 4°C). Pulps were embedded in Tissue-Tek OCT compound (Bayer Diagnostics), and sixty 10-μm-thick longitudinal sections were cut from each pulp and thaw-mounted in 20 sets, with 3 sections on each poly-D-lysine coated slide (Sigma-Aldrich), so that sections 1, 21, and 41 (each 200 μm apart) were mounted on the first slide, and so on. Slides were left to air-dry for 60 minutes at room temperature and stored at –70°C until ready for use.

All tissues from intact, carious asymptomatic and carious painful samples were processed in parallel. Three sections from each pulp were processed for indirect immunofluorescence. Slides were removed from storage and left to air-dry for 60 minutes. Slides were then washed in PBS containing 0.2% Triton X-100 (PBST) in 2 10-minute sessions. In order to minimize nonspecific background staining, sections were first incubated in PBST containing 10% normal donkey serum (Jackson ImmunoResearch Laboratories) for 30 minutes at room temperature. Following this, they were incubated with a mixture of a polyclonal antibody human TRPV1 raised in rabbit (1:2000; GlaxoSmithKline) and either *(1)* a monoclonal antibody to the general neuronal marker, human protein gene product 9.5 (PGP 9.5)[21] raised in mouse (1:1000; Ultraclone) or *(2)* a mixture of

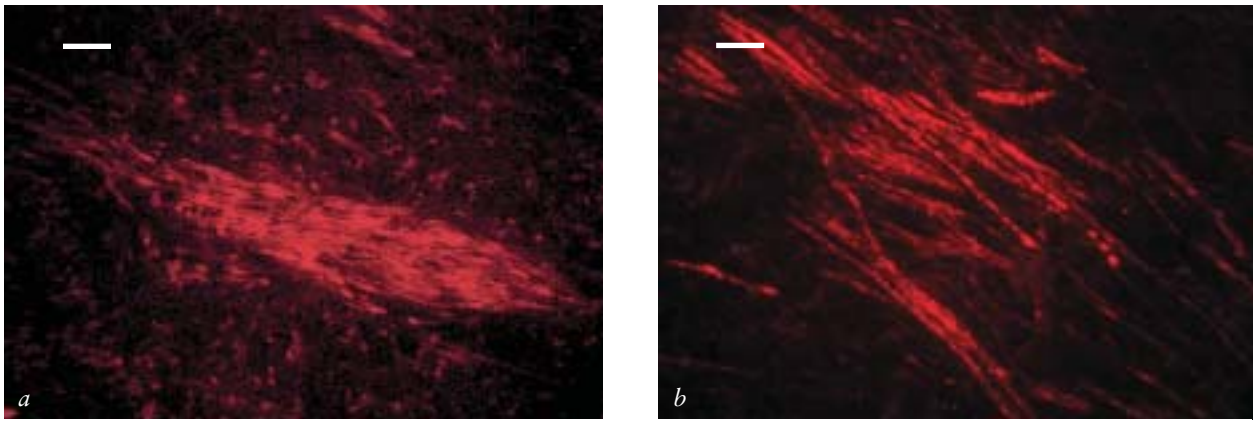

Fig 1 Photomicrographs demonstrating TRPV1 specificity. *(a)* shows TRPV1 control within midcoronal pulp. No positive labeling is evident compared to (*b*), where TRPV1-IR in the subodontoblastic nerve plexus clearly shows the varicose nature of TRPV1-expressing nerve fibers. Scale bar = 30 μm.

biotinylated *Ulex europaeus* agglutinin 1 lectin (UEIL) (1:100; Vector Laboratories) and an antibody raised in mouse against human alpha smooth muscle actin (αSMA) (1:50; Novocastra). UEIL is a lectin derived from the gorse plant and is a well-established marker of human vascular endothelium.[22] αSMA is reactive with the smooth muscle cells found in the walls of blood vessels such as arterioles.[23] The combination of UEIL and αSMA would thus allow the full extent of the pulpal vascular structure present to be labeled. The antisera and UEIL were diluted in PBST containing 5% normal donkey serum, and sections were left to incubate for 24 hours at 4°C.

Slides were again washed (2 × 10 minutes) in PBS before incubation for a further 90 minutes at room temperature with either *(1)* a mixture of donkey anti-rabbit IgG conjugated to Cy3 (1:400) and donkey anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC) (1:40) to visualize TRPV1 and PGP labeling or *(2)* a mixture of donkey anti-rabbit IgG conjugated to Cy3 and donkey anti-mouse FITC plus an FITC-conjugated streptavidin (1:25) to visualize TRPV1 and vascular labeling. Secondary antisera and the streptavidin were diluted in PBST containing 2% normal donkey serum. Finally, slides were washed (2 × 10 minutes, PBS), and sections were carefully dried and mounted in Vectashield (Vector).

Immunohistochemical controls were performed on all primary antisera. Specificity of the TRPV1 antibody was optimized by choosing a peptide immunogen specific for TRPV1 (CGSLKPEDAEVFKSPAASGEK) in a search across the GenBank and EMBL genomic databases.[24] The control procedure involved incubating sections with the primary antibody that had previously been pre-absorbed over a 24-hour period at 4°C with an excess (10 nmol mL^{-1}) of the blocking peptide. Furthermore, the ability of the antibody to specifically recognize TRPV1, but not closely related gene family members, was confirmed using immunocytochemical observation of HEK293 cells transfected with recombinant human TRPV1, TRPV3, and TRPV4. The antibody failed to detect TRPV3 or TRPV4 (personal communication, G.D. Smith, August 2004). The control procedure employed to verify specificity of lectin binding was accomplished by pre-incubating the lectin conjugate with 0.2 mol/L α-L-fucose (Vector) dissolved in PBS containing 0.2% PBST for 60 minutes at room temperature prior to application to the sections.[25] Controls for PGP 9.5 and αSMA were performed simply by omitting the primary antibody. All positive labeling was abolished in each of the controls (Fig 1).

Analysis of Immunolabeling

Tissue sections were viewed using a Zeiss Axioplan fluorescent microscope. All analyses were performed by researchers blind to caries status and clinical pain history. Descriptive analysis of TRPV1 distribution was performed on each of the 3 sections. For quantitative analysis, only 1 section from each specimen was used. Three different areas of the pulp were analyzed: the mesiobuccal pulp horn, the central subodontoblastic nerve plexus between the 2 pulp horns, and the midcoronal pulp region (Fig 2). Each field was viewed using the ×20 objective, which represented 0.22 mm^2 of the pulp tissue. Computer-assisted image

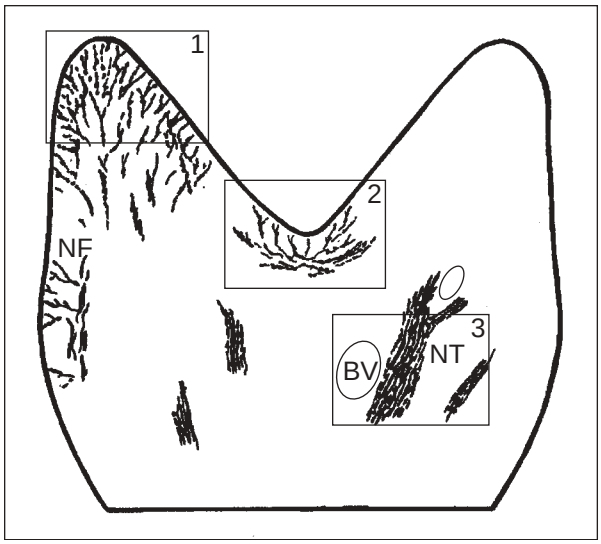

Fig 2 Schematic diagram of the coronal pulp showing the 3 areas of the pulp examined in present study: *(1)* the mesiobuccal pulp horn, *(2)* the subodontoblastic nerve plexus, and *(3)* the midcoronal region of pulp. Each rectangle represents 0.22 mm^2 of pulp. NF = peripheral nerve fibers, BV = blood vessels, NT = nerve trunk.

analysis software (Image-Pro Plus v 3.0; Media Cybernetics) was used to create a digital image from the microscopic image. The total areas of TRPV1 and PGP 9.5 and the total area of blood vessel-labeling were automatically calculated. Neuronal and vascular TRPV1 labeling were measured separately. Previous studies from the authors' laboratory have shown that neural density within the tooth pulp is significantly increased with caries.[26] Similar studies have examined pulpal vasculature, and although a significant increase in vascularity has been found in the pulp horn region with the progression of caries, this was not accompanied by an increase in the number of vessels.[27] Therefore in the present study, the percentage area of neuronal tissue labeled for TRPV1 and the percentage area of vascular tissue labeled for TRPV1 were calculated from the aforementioned measurements. In addition, the total number of αSMA/UEIL–labeled blood vessels was noted in each region, as was the number of vessels that displayed TRPV1-immunoreactivity (IR).

Statistical Analysis

Independent sample *t* tests were used to test for any significant differences in TRPV1 expression in relation to the degree of caries and expression of pain. These tests were performed on data representing both neuronal and vascular TRPV1 expression. All statistical analysis was performed on normalized data (square root). All graphs present data in their raw form. Significance levels were set at $P < .05$.

Results

In total, 43 permanent first molar pulps were analyzed. These consisted of 20 intact, 10 carious asymptomatic, and 13 carious painful samples. The mean age of patients from whom experimental tissue was collected was 9.89 ± 2.10 years.

Preliminary examination of TRPV1 distribution clearly revealed the presence of this receptor in nerve fibers throughout the pulp. Furthermore, TRPV1 expression was also seen consistently within pulp vasculature.

Neuronal TRPV1 Expression

Qualitative Analysis. TRPV1-IR nerve fibers were apparent in the majority of both intact and carious specimens. Both varicose and smooth TRPV1-IR fibers were observed. Fibers showing a more varicose morphology appeared to be more common in the pulp horn and subodontoblastic nerve plexus regions than in the larger nerve trunks. Single varicose TRPV1-IR fibers were also often found to be associated with some of the larger blood vessels present in the pulp. In intact samples, only a moderate number of fibers appeared to express TRPV1-IR, whereas in carious teeth, TRPV1-IR fibers appeared much more abundant and showed a thicker morphology. This was apparent throughout the whole of the pulp (Figs 3a to 3f).

Quantitative Analysis. Figs 4a to 4c show the mean percentage area of TRPV1 labeling present in pulpal nerve fibers in relation to the degree of caries. TRPV1 expression was increased in all areas of the pulp in grossly carious samples compared to intact teeth. Statistical analysis confirmed that caries progression caused a significant increase in the expression of neuronal TRPV1 throughout the entire pulp ($P < .05$, independent sample *t* test). The proportion of neuronal TRPV1-IR also appeared to be increased in relation to pain. However, no significant difference was found between carious asymptomatic teeth compared to carious painful teeth in all 3 areas of the pulp ($P > .05$, independent samples *t* test) (Figs 4d to 4f). Some specimens were omitted from quantitative analysis because of poor staining or damage to sections.

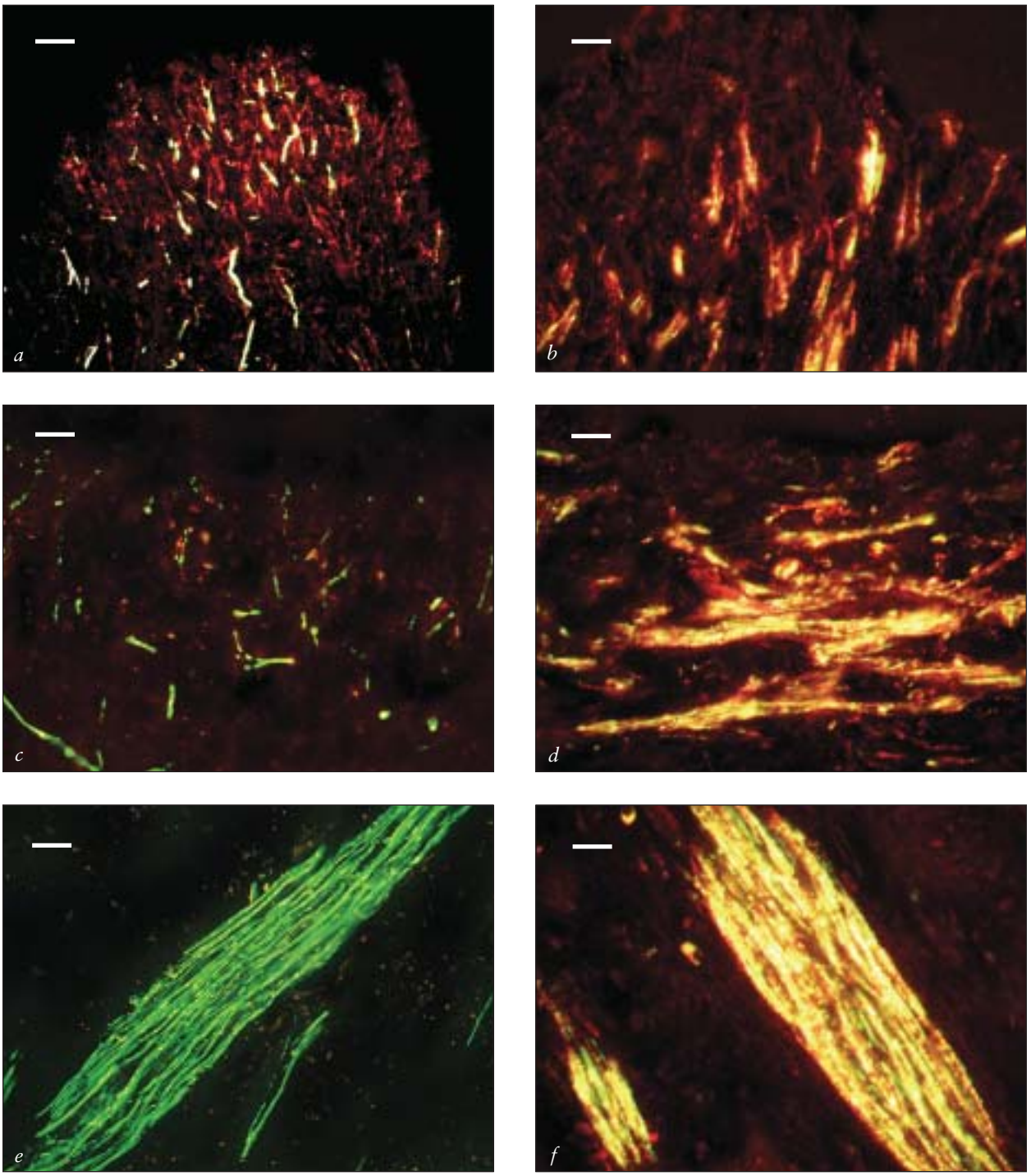

Fig 3 Double-exposed photomicrographs demonstrating differences in distribution of TRPV1-IR fibers between healthy intact and grossly carious teeth. Green stain indicates PGP 9.5, red indicates TRPV1, and yellow indicates colocalization of PGP 9.5 and TRPV1. *(a)* Pulp horn of intact tooth shows double labeling for PGP 9.5 and TRPV1. Few fibers expressing TRPV1 are observed compared to *(b)* the grossly carious pulp horn, which shows a marked increase in TRPV1-IR fibers. *(c)* The subodontoblastic nerve plexus of an intact tooth pulp, showing few TRPV1-IR fibers. *(d)* A marked increase in both PGP 9.5- and TRPV1-IR fibers in subodontoblastic nerve plexus of a grossly carious tooth. *(e)* Nerve trunk in midcoronal pulp of an intact tooth showing very little expression of TRPV1. *(f)* A massive increase in number of TRPV1-IR fibers (represented by yellow stain) in a nerve trunk from the midcoronal pulp of a grossly carious tooth. Scale bar = 30 μm.

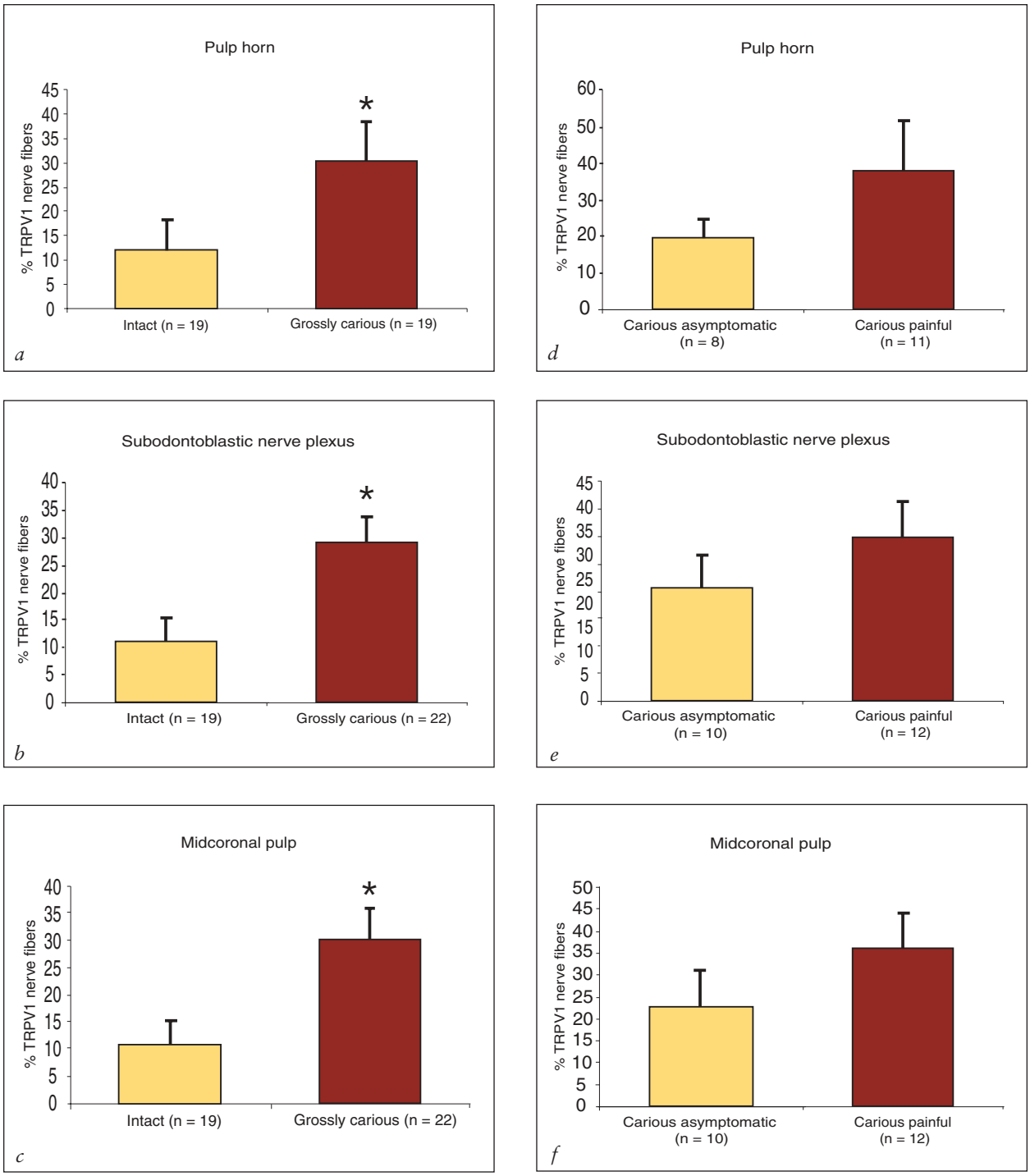

Fig 4 Bar charts showing mean (± SEM) percentage area of PGP 9.5–labeled tissue also labeled for TRPV1 in intact and grossly carious teeth *(a to c)* and carious asymptomatic and carious painful teeth *(d to f)*. Some specimens in each region of the pulp could not be analyzed because of damage to them. **P* < .05 for carious versus intact teeth (independent sample *t* test).

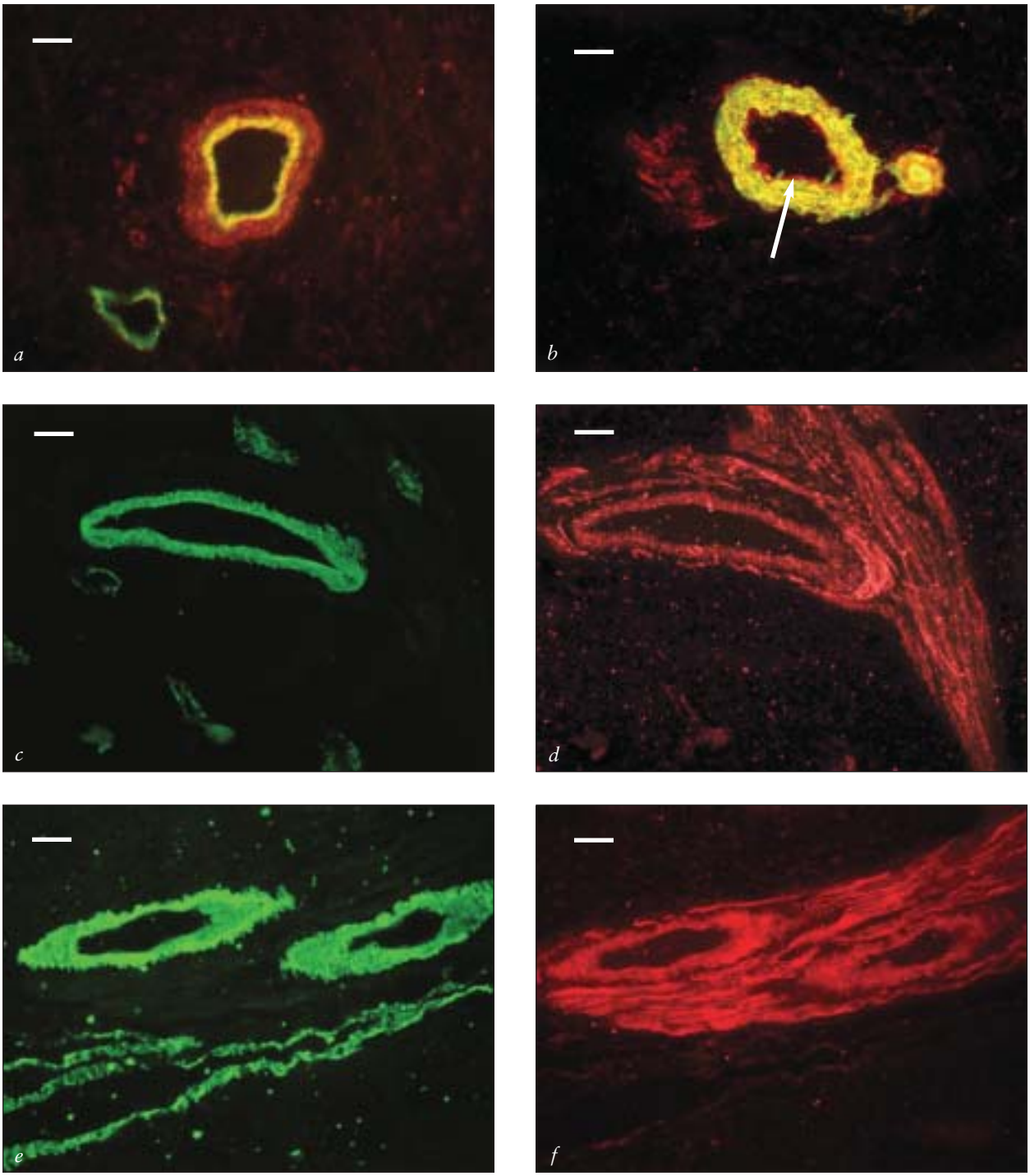

Fig 5 Photomicrographs to demonstrate presence of TRPV1 in blood vessels. *(a)* Double-exposed photomicrograph labeled for vascular endothelium *(green)* and TRPV1 *(red)*. Yellow immunofluoresence indicates presence of TRPV1 in endothelium (UEIL). *(b)* Double-exposed photomicrograph labeled for smooth muscle and TRPV1 *(red)*. TRPV1 is not confined to the endothelial tissue but is also present in smooth muscle *(yellow)*. The inner edge of the blood vessel shows single labeling for TRPV1 *(arrow)*, which indicates the thin layer of endothelium. TRPV1-IR fibers can also be seen close to the blood vessel (αSMA). *(c)* UEIL and αSMA double labeling of a blood vessel *(green)* in the pulp from the midcoronal pulp of a carious asymptomatic tooth. *(d)* TRPV1 single labeling of the same blood vessel surrounded by TRPV1-IR fibers. *(e)* UEIL and αSMA double labeling of a blood vessel in the pulp from the midcoronal pulp of a carious painful tooth. *(f)* TRPV1 labeling of the same blood vessel and surrounding TRPV1-IR fibers. A marked increase in vascular TRPV1 expression can be seen in the carious painful sample *(f)* compared to the carious asymptomatic sample *(d)*. Scale bar = 15 μm for 5a and 5b and 30 μm for 5c to 5f.

Vascular TRPV1 Expression

Qualitative Analysis. In addition to its presence in pulp nerve fibers, TRPV1-IR was consistently found within the pulp vasculature, including capillaries, venules, and arterioles. Furthermore, TRPV1-IR was seen in both endothelial (UEIL-labeled tissue, Fig 5a) and smooth muscle (αSMA-labeled tissue, Fig 5b) layers.

In the pulp horn and subodontoblastic nerve plexus regions, only capillaries were present. In the midcoronal region of the pulp, the majority of blood vessels present were large arterioles, although some venules were also observed (Figs 5c to 5e). The larger arterioles often showed abundant expression of TRPV1-IR compared to venules and capillaries, where TRPV1-IR was much weaker. There also appeared to be a close spatial relationship between some arterioles and nerve trunks expressing TRPV1-IR (Figs 5d and 5f). Interestingly, in a number of painful samples, arterioles showed intense TRPV1-IR (Fig 5f).

Quantitative Analysis. Figs 6a to 6c show the mean percentage of TRPV1 expression in the 3 areas of the pulp according to caries status. Statistical analysis confirmed that there was no significant difference in vascular TRPV1 expression between intact and grossly carious samples ($P > .05$, independent samples t test). Further analysis between carious asymptomatic and carious painful samples (Figs 6d to 6f) showed that TRPV1 expression was significantly increased in carious painful samples in the midcoronal area of the pulp ($P < .05$, independent samples t test) (Fig 6f). However, neither the overall number of blood vessels nor the proportion of blood vessels expressing TRPV1-IR were altered in any area of the pulp with either caries or painful pulpitis. This finding would suggest that the percentage area of TRPV1 expression was not increased through an increase in the number of blood vessels expressing TRPV1-IR, but that the overall increase was due to greater TRPV1 expression within the same vascular population. These data are presented in Fig 7.

No significant differences were found for vascular TRPV1 expression in either the pulp horn or subodontoblastic nerve plexus according to the presence or absence of pain ($P > .05$, independent sample t test) (Figs 6d and 6e).

Discussion

This study has confirmed that TRPV1 is present in human dental pulp nerves and has demonstrated that TRPV1 is not confined to these fibers but is also expressed in both vascular endothelial and arteriole smooth muscle cells. In addition, it was shown that in pulpal nerve fibers, TRPV1 expression increases with caries, and that in blood vessels in the midcoronal pulp, TRPV1 expression is significantly greater in carious painful samples than in carious asymptomatic samples.

Neuronal TRPV1

To date, there has only been 1 publication describing TRPV1 expression in human tooth pulp,[20] in addition to a few studies describing TRPV1 in pulpal neuron cell bodies.[3,17–19] Therefore, little data exist with which to corroborate the findings of the present study. Nonetheless, the observations made in the present study are consistent with immunocytochemical studies of other human tissue in which TRPV1 expression in nerve fibers was greatly upregulated under inflammatory conditions.[15,16] However, a number of discrepancies exist between the present study and the recent publication by Renton et al.[20] In their study, no significant differences in TRPV1 expression were found between healthy and painful pulp samples. This is of interest, as a significant increase in neuronal TRPV1 expression in grossly carious samples compared to healthy pulps was observed in the present study. These discrepancies may be accounted for by methodological differences. These include our use of pulps from permanent mandibular first molars in all cases compared to the use of pulps from third molars extracted because of coronal caries (painful samples) and healthy third molars removed because of surrounding diseased tissue (pericoronitis) in the Renton et al study.[20] In addition, analysis of TRPV1 expression in the present study was performed in 3 different areas of interest, and so TRPV1 expression in these areas could be directly compared between intact and grossly carious teeth. In contrast, Renton and colleagues analyzed 5 random areas of each pulp.[20]

Previous studies have shown that neural density in both primary and permanent molars is significantly increased with caries.[26] The present data show an increase in the area of TRPV1-IR as a proportion of the total area of nerve fiber labeling. This may be because of aborization of existing TRPV1-IR fibers possibly associated with increased axonal transport or expression of TRPV1 in fibers that do not express this receptor in the intact tooth. During the inflammatory process, peripheral nerve growth factor (NGF) is known to be greatly increased in many tissues,[28]

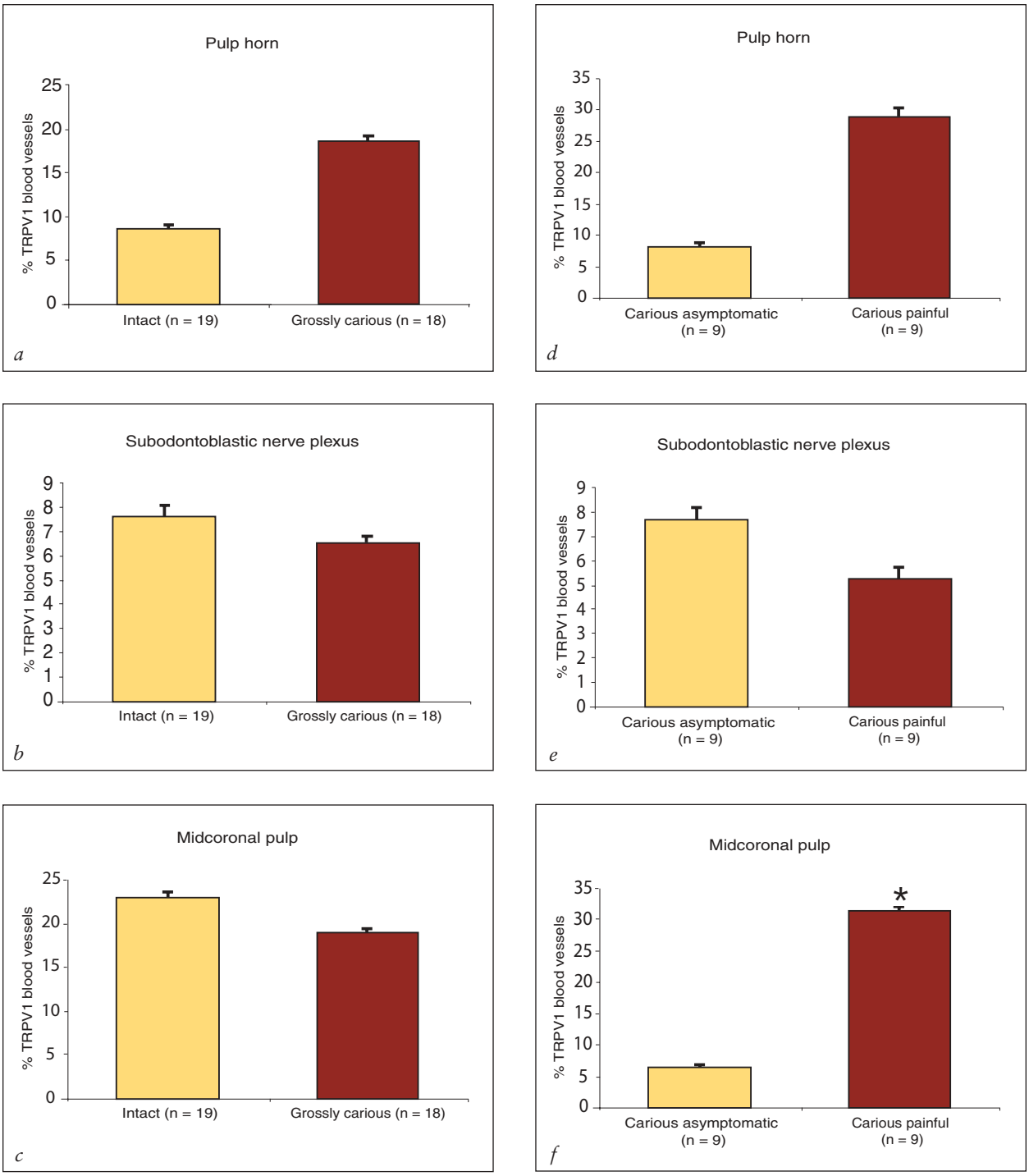

Fig 6 Bar charts showing mean (± SEM) percentage area of UEIL/αSMA–labeled vascular tissue also labeled for TRPV1 expression according to degree of caries *(a to c)* and onset of pain *(d to f)*. Vascular TRPV1 expression was not significantly altered with development of caries, although carious painful teeth showed a significant increase in vascular TRPV1 expression in blood vessels of midcoronal pulp *(f)* compared to carious asymptomatic pulps. No significant changes in vascular TRPV1 expression were observed in the pulp horn or subodontoblastic nerve plexus in painful teeth *(d, e)*. Some specimens in each region of the pulp could not be analyzed because of damage to them.*$P < .05$ carious painful versus carious asymptomatic (independent sample *t* test).

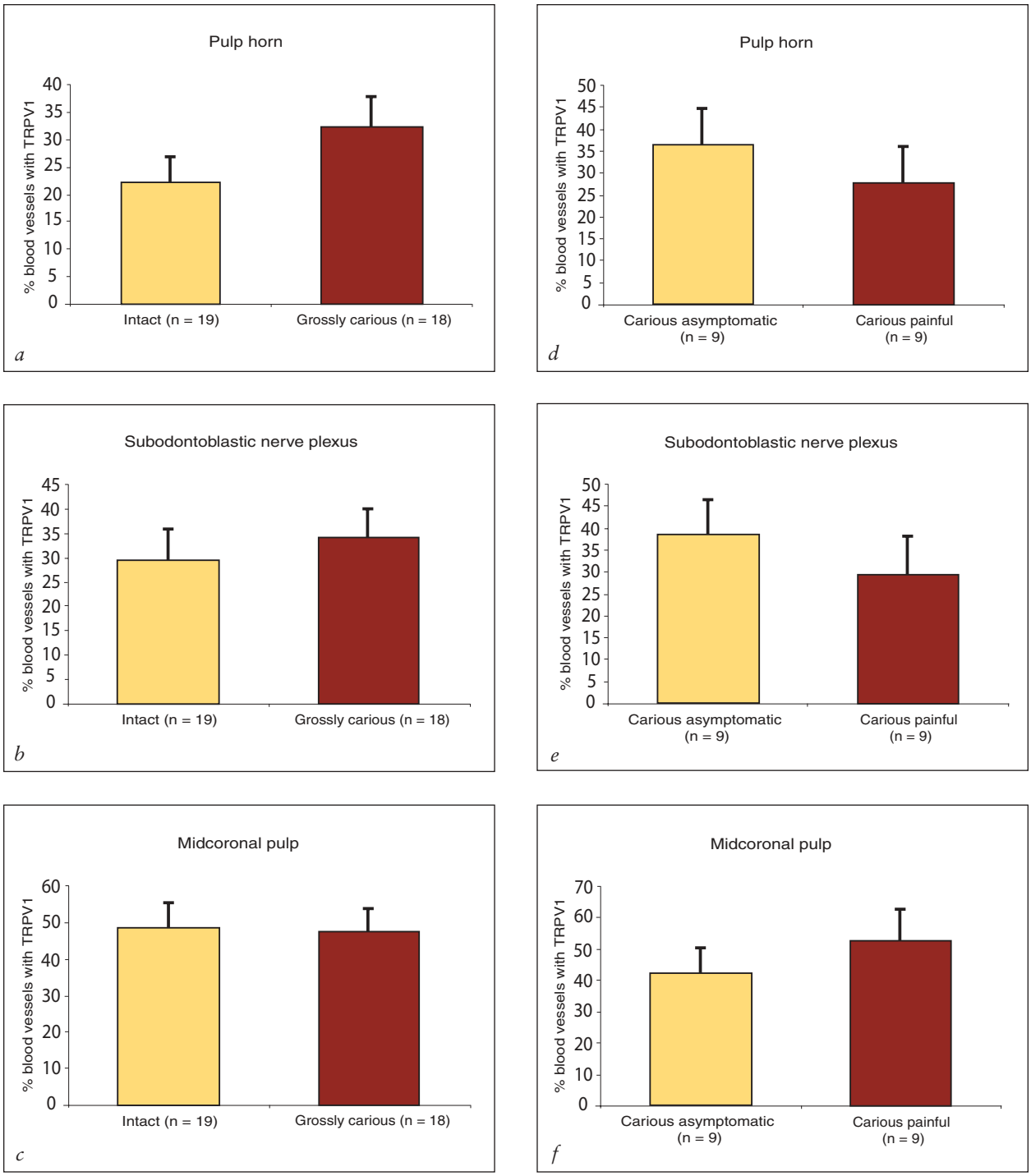

Fig 7 Bar charts showing mean (± SEM) percentage of blood vessels expressing TRPV1 in the 3 areas of the pulp according to degree of caries *(a to c)* and occurrence of pain *(d to f)*. Some specimens in each region of the pulp could not be analyzed because of damage to them.

including the tooth pulp,[29] and recent studies in cultured adult rat dorsal root ganglion (DRG) neurons have demonstrated that NGF treatment actively increases TRPV1 mRNA expression.[30] This may provide a mechanism for the increased expression of neuronal TRPV1 observed. There is also experimental evidence that carrageenan-induced inflammation can cause axonal transport of TRPV1 mRNA from the somata to nerve terminals in rat DRG neurons.[31]

In contrast to increases in neuronal TRPV1 expression in inflammation, no significant correlations between neuronal expression of TRPV1 and spontaneous dental pain were found. In 1 study, TRPV1-IR fibers were found to express weaker immunoreactivity in a patient with asymptomatic inflammatory bowel disease when compared to patients with painful symptoms.[16] This evidence is, however, largely anecdotal, and no other immunocytochemical studies to date have found a consistent correlation between neuronal TRPV1-IR and pain.[15,20]

Vascular TRPV1

This research has shown for the first time that TRPV1 is expressed by both vascular endothelial and smooth muscle cells within human tooth pulp. These data are consistent with previous findings that TRPV1 is expressed by bladder epithelial cells and smooth muscle cells.[5] It is interesting, therefore, that the recent study on TRPV1 expression in human third molars did not reveal any extraneuronal expression of this receptor.[20] TRPV1 expression was not found to be altered with gross caries; however, there were significant differences according to pain status. There was a profound increase in vascular TRPV1 in blood vessels found in the midcoronal area of the pulp of symptomatic as compared to asymptomatic samples. This increase in vascular TRPV1 expression was most evident in the larger arterioles. Vascular TRPV1 expression was calculated as a proportion of the total vascular tissue present in the pulp. Previous studies have found that, in the midcoronal pulp, the pulp microvasculature is not altered with the progression of caries.[27]

In painful pulpitis, it would appear that TRPV1 expression undergoes a dynamic increase within midcoronal pulp arterioles. In both the pulp horn and subodontoblastic nerve plexus there is a complex network of capillaries that are mostly composed of endothelium, in contrast to the arterioles found in the midcoronal pulp, which are composed of both vascular endothelium and a smooth muscle layer (Figs 5a and 5b). Therefore, it is possible that the increase in vascular TRPV1 expression observed occurs in the smooth muscle layer of the blood vessel, which may affect vasodilation. Previous studies have shown that anandamide, an endogenous agonist of TRPV1 synthesized from arachidonic acid by macrophages, vascular endothelium, and primary afferents, binds to the same site of TRPV1 as capsaicin[32] and can induce vasodilation,[33] vas deferens relaxation,[32] and excitation of sensory neurons.[34] Thus, it is possible that increased TRPV1 expression enhances vasodilation directly, increasing pulpal blood flow and infiltration of immune cells, and this may cause increased pain sensation because of the confined nature of the pulp chamber.[35]

TRPV1 in Pain

Studies of TRPV1-knockout mice appear to support the role of TRPV1 in pain development, as these mice showed impaired inflammatory thermal hyperalgesia.[36,37] However, it appeared that TRPV1 was not acting alone, as TRPV1-deficient mice retained some normal sensation of noxious heat.[36,37] TRPV1 antagonist studies also provide further evidence for the role of TRPV1 in pain perception. Capsazepine, a competitive TRPV1 antagonist, has been used in a number of studies and has been shown to inhibit capsaicin-mediated responses in isolated DRG neurons[38] and dental afferent neurons.[3] In addition, capsazepine also inhibits low pH-evoked depolarization of sensory neurons in the guinea pig[39,40] and human DRG neurons.[41] More recent studies have found that capsazepine inhibits responses to both noxious heat and low pH in cloned human TRPV1 expressed in Chinese hamster ovary cells[42] and produces significant reversal in carrageenan-induced thermal hyperalgesia and neuropathic pain in a guinea pig model.[43] These data suggest that TRPV1 antagonists can have analgesic effects in animal models of chronic inflammatory and neuropathic pain, and so it seems likely that TRPV1 has a role in dental pain.

In the present study, a clear trend that neuronal TRPV1 expression is increased (although not significantly) in all areas of the pulp in symptomatic cases was found. Thus, an increase in sample number may give a clearer picture as to the role of TRPV1 in the development of dental pain. Further studies using samples with a more detailed pain history, and pre-extraction testing of teeth for increased sensitivity to noxious heat, may provide clearer evidence for the role of TRPV1 in dental

pain. Further study is planned with teeth that have been tested for heat sensitivity prior to extraction in order to elucidate the role of vascular TRPV1 as well as neuronal TRPV1 in the development of dental pain and inflammation. These studies may lead to a better understanding of the distribution and functions of TRPV1, notably in the human tooth pulp. The positive correlations shown in this study between TRPV1 expression and caries and pain indicate the potential of TRPV1 as a therapeutic target for inflammation and pain within the dental pulp and at other sites.

Acknowledgments

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