The effect of capsaicin on substance P expression in pulp tissue inflammation

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Abstract

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Aim To evaluate the effect of capsaicin on substance P (SP) expression during induced inflammation in rat pulp tissue.

Methodology Radioimmunoanalysis was used to measure SP levels in 36 mandibular molar pulps taken from six Wistar rats. Twelve samples were obtained from healthy pulps and used as negative control group. Another 12 samples were obtained after inducing inflammation with mechanical pulp exposure; these were used as the positive control group. Capsaicin was infiltrated into the inferior dental nerve in the experimental group and 12 samples were obtained after mechanical pulp exposure.

Results The lowest SP expression was found in mechanically exposed pulps where capsaicin pretreatment had been carried out (0.028 ng mL^{-1}), followed by healthy pulps (0.302 ng mL^{-1}). The highest SP expression was found in mechanically exposed pulps with no capsaicin pretreatment (124 ng mL^{-1}). The Kruskal–Wallis test showed statistically significant differences between the groups ($P < 0.001$).

Conclusion Inferior dental nerve infiltration with capsaicin reduces SP expression in dental pulp tissue in rats.

Keywords: capsaicin, pulpitis, radioimmunoanalysis, substance P.

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Introduction

Dental pulp inflammation is a complex process involving a great variety of nervous and vascular reactions, which are key components of the neurogenic phenomenon leading to pulp necrosis (Kim 1990).

Two types of nerve fibres (Aδ and C) mainly provide pulp innervation. The latter are closely related to pulp microcirculation (Awawdeh *et al.* 2002). When stimulated, terminal portions of C-fibres release neuropeptides such as calcitonin gene-related peptide (CGRP), neurokinin A (NKA) and substance P (SP) (Olgart & Kerezoudis 1994).

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Substance P is an undecapeptide synthesized in C-fibre neuronal soma. It reaches terminal portions of fibres via axonal transport where it is stored or released by exocytosis to carry out its functions by binding to high-affinity receptors located on most inflammatory cells, such as mast cells and macrophages. It induces the release of inflammatory mediators such as histamine, cytokines, prostaglandines and thromboxanes, thus increasing vascular permeability and magnifying the inflammatory process (Pernow 1983, Payan 1989, Chancellor-Freeland *et al.* 1995).

The inflammation process leads to a rise in intrapulpal tissue pressure, having a direct effect on pulp tissue, worsened by dental pulp's low compliance environment, reducing blood flow and compromising pulp vitality (Kim 1990). However, recent studies indicate that the pulp has physiological feedback mechanisms that act to oppose increases in tissue pressure, such as increased lymph flow and absorption of interstitial fluid into capillaries in noninflamed areas,

which is why inflammation of the pulp is usually long-standing within a confined area and healing is possible if appropriate treatment measures are taken (Heyeraas & Berggreen 1999).

It has been demonstrated that pulpal irritants (such as deep cavity preparation and high intensity thermal/chemical stimuli) excite C-fibres, causing the release of neuropeptides, including SP (Hargreaves *et al.* 1994). SP expression has been controlled *in vivo* with capsaicin, which is a neurotoxin capable of reducing SP release from C-fibre terminal nerve endings (Buck & Burks 1986) by binding to specific cell membrane receptors on neurones (Ichikawa & Sugimoto 2000).

The purpose of this study was to evaluate the effect of capsaicin when applied locally to the inferior dental nerve on SP expression after inducing inflammation in rat lower molar pulps.

Materials and methods

This study was conducted in accordance with the Universidad Javeriana's requirements for animal experiments.

Experimental animals

Six adult (6-month-old) female Wistar rats from the same litter were used. Their weight was between 240 and 290 g. Six mandibular molars were taken from each rat for a total of 36 dental pulp samples.

Samples preparation

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamida) was purchased from Sigma (Ref. M-2028; Sigma, St Louis, MO, USA) and diluted with ethanol (Sigma Ref. E-2385) and Tween 80 (Sigma Ref. P-4074) to obtain a 1% Capsaicin solution.

Rats were anaesthetized with a mixture of ketamine chlorhydrate (90 mg kg^{-1}) and xylazine (12 mg kg^{-1}) applied intra-peritoneally using disposable insulin syringes. Two rats were assigned to each group, meaning 12 pulp samples per group.

In the experimental group, the inferior dental nerves of the rats were infiltrated with 1% capsaicin solution (25 mg kg^{-1}). Fifteen minutes later, mechanical pulp exposures were carried out in every mandibular molar with a 1/4 round bur in a high-speed hand-piece without irrigation.

In the positive control group, mechanical pulp exposures were carried out in the mandibular molars of rats with a 1/4 round bur in a high-speed hand-piece without irrigation.

No capsaicin pretreatment or mechanical exposure was carried out on the negative control group for quantifying normal pulp SP levels.

The animals were killed 15 min post-treatment by transcardial perfusion of 4% paraformaldehyde solution. The mandibles were block-dissected and pulp tissue was obtained by sectioning molars.

Radioimmunoanalysis

Pulp samples were ultrasonically disaggregated (Ultrasonic Processor S-2028-130; ISC BioExpress, Kaysville, UT, USA) for their homogenization and double-boiled for 10 min at 37 °C. The disaggregated tissue was spun at 2000 g for 45 min (GS-6KR Centrifuge; Beckman, Fullerton, CA, USA) and supernatants were transferred to another tube.

One hundred microlitres of each sample supernatant were submitted to competition binding assays with 50 μL ^{125} I-SP (Ref. IM57; Amersham, Piscataway, NJ, USA), 50 μL 1 : 100 anti-SP (Sigma S-1542) solution, 50 μL different unlabelled SP (Sigma S-6883) concentrations and 500 μL polyethyleneglycol (Sigma P-2139).

Following 2 h incubation, the suspensions were spun at 4000 g for 1 h (Beckman) to precipitate the bound fractions. The supernatants were decanted and pellet radioactivity was read on a Gamma Counter (Gamma Assay LS 5500; Beckman). Scatchard analysis of the binding data assessed the amount of SP present in every sample.

Statistical analysis

Values are presented as SP amount in ng mL^{-1} of dental pulp suspension. Median and maximum/minimum values were obtained for each group. The Kruskal–Wallis test was performed to establish statistically significant differences ($P < 0.05$) between the groups. Mann–Whitney's *post hoc* comparisons between groups were also performed.

Results

Substance P was found to be expressed in all pulp samples (Table 1). Expression for the experimental group (capsaicin pretreated) was between 0.001 and 0.109 ng mL^{-1}. Positive control group expression was between 92 and 185 ng mL^{-1}. Normal SP levels

Table 1 SP expression in rat dental pulp

Sample	Normal SP levels (negative control)[a]	SP expression after inducing inflammation (positive control)[a]	SP expression after capsaicin treatment and induced inflammation[a]
1	0.102	92	0.001
2	0.112	97	0.002
3	0.145	101	0.010
4	0.164	105	0.011
5	0.243	107	0.013
6	0.301	111	0.025
7	0.304	137	0.031
8	0.325	140	0.062
9	0.352	142	0.062
10	0.456	180	0.075
11	0.512	183	0.082
12	0.715	185	0.109
Median	0.302[b]	124[b]	0.028[b]

[a]Values are given in ng substance P per mL dental pulp suspension.
[b]Differences between groups were statistically significant ($P < 0.001$).

(negative control group) were between 0.102 and 0.715 ng mL^{-1}. Medians were 0.028, 124 and 0.302 ng mL^{-1}, respectively.

The Kruskal–Wallis test showed statistically significant differences between the groups ($P < 0.001$). Mann–Whitney's *post hoc* tests showed statistically significant differences between experimental and both control groups. Differences between negative and positive control groups were also significant ($P < 0.001$).

Discussion

Capsaicin is a neurotoxin derived from *Capsicum* peppers. It has been widely used as a topical analgesic in inflammatory conditions, such as rheumatoid arthritis, muscular pain and in a number of neuropathies, including painful diabetic neuropathy and postherpetic neuralgia (Buck & Burks 1986, Yoshimura *et al.* 2000).

Capsaicin acts as an activator of small-diameter unmyelinated and thinly myelinated nociceptive afferent fibres (Kyrkanides *et al.* 2002). The C-fibres of dental pulp are polymodal and responsive to capsaicin and to inflammatory mediators such as histamine and bradykinin. There are also a few slow-conducting thin A-fibres that have capsaicin sensitivity and that may end primarily in pulp tissue (Ikeda *et al.* 1997). The blockage of axonal transport and reduction in intra-axonal SP levels explains ability of capsaicin to desensitize afferent C-fibre nociceptive terminals (Yoshimura *et al.* 2000). It was thus expected to find lower SP expression in the pulp of teeth previously treated with capsaicin. The results of this study agree with such statements, showing statistically significant differences between capsaicin-treated teeth and the group of teeth with induced inflammation.

Results from this study also show a low base level of SP activity in clinically normal pulps. This may be due to basal physiological activity in regulating pulpal blood flow and other homeostatic functions (Grutzner *et al.* 1992).

The inflammatory process was induced by mechanical exposure of pulp tissue. A time period of 5–10 min appears to be sufficient to allow the release of the neuropeptide from terminal fibres (Hargreaves *et al.* 2003). Higher SP expression in the positive control group corroborated the effectiveness of the inflammation inducing procedure, showing a 400-fold increase in SP expression when compared with normal SP levels. The values for the positive control group differed significantly from the other two groups.

It has been reported that capsaicin injections may cause a consistent reduction in CGRP- and SP-immunoreactive fibre numbers in the pulp and a somewhat smaller reduction in periodontal tissues (Jacobsen & Heyeraas 1996). It also produces selective destruction of most small, unmyelinated primary afferent axons (Hylden *et al.* 1992). It is thus important to emphasize the limitations of these findings. As capsaicin is a neurotoxin (and in the absence of microscopic evaluation for the present study) it could not be determined whether capsaicin infiltration of inferior dental nerve caused any structural damage to dental pulps. However, these results could be useful for future research in

assessing adequate doses to obtain benefits from capsaicin without producing collateral damage to dental pulps.

Conclusion

Inferior dental nerve infiltration with 1% capsaicin reduces SP expression in rat dental pulp tissue. This may provide a possible mechanism for controlling pulpal neurogenic inflammation to maintain pulp vitality when harmed by external irritants.

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COMMENTARY

The unit of analysis, and measurement of effect size

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Statistical Advisor for the *International Endodontic Journal*

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In refereeing the preceding article, two statistical issues arose, which are worth drawing to the attention of journal readers. The first relates to a flaw in the study design, which in my experience may be common in