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Effect of the sensory neuropeptide antagonists h-CGRP$_{(8–37)}$ and SR 140.33 on pulpal and gingival blood flow in ferrets

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Abstract

In a previous study, it was concluded that the neuropeptides calcitonin gene-related peptide (CGRP) and substance P are released during resting conditions in the (exposed) ferret dental pulp, contributing to a basal vasodilator tone in the pulpal vessels. In order to exclude the possibility that the method used elicited axon reflexes, which might be responsible for neuropeptide release, the present study was designed without pulp exposure. Non-invasive laser–Doppler flowmetry was used to measure the effects of intra-arterial infusions of the antagonists h-CGRP$_{(8–37)}$ and SR 140.33 (neurokinin 1-receptor antagonist) on pulpal and gingival blood flow before, during and after electrical tooth stimulation. Infusions of h-CGRP$_{(8–37)}$ reduced the basal blood flow in the pulp by $31.4 \pm 5.2\%$ ($p < 0.001$) and in the gingiva by $22.6 \pm 4.8\%$ ($p < 0.05$). A further significant decrease in basal blood flow was measured in both pulp and gingiva following SR 140.33 administration. The reduction in blood flow was $16.9 \pm 1.9\%$ ($p < 0.005$) in the pulp and $19.3 \pm 5.6\%$ ($p < 0.05$) in the gingiva. The systemic arterial pressure remained unchanged both during and after the periods of infusion. Tooth stimulation before the antagonist infusion significantly increased the pulpal blood flow by $71.9 \pm 15.3\%$ ($p < 0.005$). Infusion of h-CGRP$_{(8–37)}$ greatly reduced this electrically induced vasodilatation, indicating that CGRP is the principal factor responsible for the vasodilatation observed after tooth stimulation. This study confirms the previous finding that a resting vasodilator tone due to the release of CGRP and SP exists in the ferret dental pulp. It is concluded that spontaneous, basal release of the neuropeptides CGRP and substance P exists both in dental pulp and gingiva in the ferret.

Keywords: Dental pulp; Gingiva; Circulation; Laser Doppler; Neuropeptides; Vascular tone

1. Introduction

The gingiva and the dental pulp are well-perfused tissues with a relatively low compliance. The regulation of their blood flow has been studied extensively (Edwall and Scott, 1971; Olgart et al., 1989; Kerezoudis et al., 1994; Sasano et al., 1995). Sympathetic nerves appear to induce mainly vasoconstriction and produce little vasoconstrictor tone on pulpal vessels during resting conditions (Tønder and Næss, 1978; Jacobsen and Heyeraas, 1997). However, our previous finding that papaverine infusion almost doubled pulpal

Abbreviations: CGRP, calcitonin gene-related peptide.

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blood flow indicates that the vessels in the pulp have a resting smooth-muscle tone (Tønder, 1975). We have earlier reported a reduced resting blood flow and lowered interstitial fluid pressure in the pulp of sensory denervated teeth in the ferret when compared with innervated and sympathectomized teeth (Jacobsen and Heyeraas, 1997). In a recent study (Berggreen and Heyeraas, 1999) we observed that the CGRP receptor antagonist h-$CGRP_{(8–37)}$ and the neurokinin 1-receptor antagonist SR 140.33 reduce pulpal blood flow and interstitial fluid pressure. Our results also showed that h-$CGRP_{(8–37)}$ greatly reduces but SR 140.33 only partially eliminates the increased blood flow and interstitial fluid pressure measured after tooth stimulation.

In both those studies, we measured simultaneously pulpal blood flow by laser–Doppler flowmetry and interstitial fluid pressure by a micropuncture technique. Micropuncture required direct access of a micropipette into the tissue and the pulp had to be exposed before the pressure could be measured. Accordingly, the pulpal exposure in the previous studies created the possibility that the neuropeptides are released because of an inflammatory reaction and not by a physiological basal action.

The present study therefore sought to (1) exclude the possibility that the release of the neuropeptides was due to an inflammatory reaction created by the trauma of pulpal exposure; and (2) investigate if basal release also occurs in oral tissues other than the dental pulp. For these purposes, we simultaneously measured pulpal and gingival blood flow with non-invasive laser–Doppler flowmetry before and after close intra-arterial infusions of the CGRP antagonist h-$CGRP_{(8–37)}$ and the substance P antagonist SR 140.33.

2. Materials and methods

Seven young ferrets of either sex, aged 5–7 months and weighing 0.9–1.6 kg, were used. The animal experiments were approved by the University of Bergen under the supervision of the Norwegian Experimental Animal Board. The animals were anaesthetized with 1 ml/kg ketamine hydrochloride (1 mg/ml) mixed with 0.1 ml/kg medetomidin hydrochloride (50 mg/ml) given intramuscularly. The ferrets were placed on a heating pad to maintain normal body temperature. A femoral vein was catheterized for supplemental anaesthesia and a femoral artery for continous systemic blood-pressure recordings with a Gould pressure transducer and recorder. The posterior auricular artery (a branch of the maxillary artery) was exposed and cannulated in retrograde direction with a PE 50 polyethylene tube for regional drug infusion. The head was immobilized and simultaneous measurements of systemic arterial pressure, pulpal and gingival blood flow were made. Measurements were recorded from the left maxillary canine pulp and in the gingiva between the canine and the left first premolar during control conditions alternating with periods of electrical stimulation of the canine crown before and after antagonist infusion.

2.1. Blood-flow recordings

A Periflux Model 4001 Master laser–Doppler flowmeter (Perimed KB; Jarfalla, Sweden) with wavelength of 780 nm, equipped with a needleprobe PF 415:10 (125 μm fibre dia. with separation of 500 μm), was used to measure pulpal blood flow in the left maxillary canine tooth. One laser probe was positioned at the coronal part of the tooth and the other 4 mm above the gingival margin buccally between the left canine and first left premolar. Both probes were rotated to the position that gave the largest resting blood-flow signals. The signals were recorded in arbitrary perfusion units (PU). Motility standard calibration of the instruments and fibreoptic probes were carried out according to the manufacturers' specifications. Zero blood flow was determined as the values recorded with the probes positioned at the tooth and at the gingiva after cardiac arrest. The flowmeter time constant was 0.03 sec with an upper bandwidth at 20 kHz and lower bandwidth at 20 Hz. As the laser flowmeter monitors only relative changes in the flux of blood cells multiplied by their velocity, changes obtained during electrical stimulation and after drug infusion were calculated as percentages of the baseline values obtained during control conditions.

2.2. Electrical tooth stimulation

A negative electrode was placed in a shallow cavity at the canine tip. After acid etching with Scotchbond etching gel (3 M Dental Products, St. Paul MN, USA) for 30 sec followed by a water rinse and air drying, the electrode was fixed into position with the composite material Herculite (Dental Material Center, Santa Ana CA, USA). The positive electrode was placed in the upper lip and electrical stimulation was provided from a constant-current Grass stimulator (Quincy, MA, USA), which gave square-wave pulses of 2 msec at 10 Hz, 200–250 μA for periods of 10 sec.

2.3. Experimental protocol and administration of drugs

All drugs were administered by close intra-arterial infusion via the posterior auricular artery. The drugs were diluted in 0.9% saline and the animals received slow infusions over 2 min (1 ml/kg). Simultaneous measurements of pulpal blood flow, gingival blood flow and systemic arterial pressure were made during

control conditions, infusions and stimulations. The animals received one infusion of saline (1 ml/kg) after measurements made during control conditions and one stimulation period. The tooth was restimulated and when the prestimulation flow levels was reached, the animals received an injection of h-CGRP$_{(8–37)}$ (150 μg/kg body wt; Sigma, St. Louis, MO, USA). About 20 min post-infusion, the tooth was stimulated for another 10 sec. When the prestimulation flow was re-established, four animals received another injection with the non-peptide NK_1-receptor antagonist SR 140.333 (300 μg/kg body wt; Sanofi Recherche, France).

2.4. Statistical analyses

Data are presented as means ± SEM. Differences were tested with the Wilcoxon ranked test or paired *t*-test and a *p* value of less than 0.05 was considered as statistically significant.

3. Results

Pulpal and gingival blood flows, and arterial pressure measured under control conditions were compared with measurements made during and after drug infusions. Saline infusions, which were given intra-arterially at the same rate and volume as drug infusions, had no measurable effect on the recorded variables. Control measurements of pulpal blood flow made before drug infusions averaged 24.3 ± 8.1 PU and of gingival blood flow, 39.4 ± 10.8 PU. The mean arterial pressure was 104.7 ± 7.4 mmHg (Table 1). A significant increase in pulpal blood flow of 71.9 ± 15.3% ($p < 0.005$, paired *t*-test) was observed immediately after tooth stimulation for 10 sec, while systemic blood pressure remained unaffected (Table 1; Fig. 1). Close intra-arterial infusion of h-CGRP$_{(8–37)}$ consistently reduced the resting blood flow in both the pulp and the gingiva, whereas the systemic arterial pressure remained unchanged (Figs. 1, 2). The average decrease in pulpal blood flow was from 21.5 ± 7.6 to 15.2 ± 5.7 PU (31.4 ± 5.2%, $p < 0.001$, paired *t*-test), whereas gingival blood flow decreased from 56.1 ± 20 to 43.4 ± 18.2 PU (22.6 ± 4.8% ($p < 0.05$,Wilcoxon ranked test)). The fall in flow started approx. 3–5 min after the end of the infusions and the maximum effect was observed about 10–15 min later. The h-CGRP$_{(8–37)}$ blocking effect lasted between 40 min to 1 h, at which time the blood-flow recordings had returned to the control values detected before the infusions (Fig. 1). Tooth stimulation after h-CGRP$_{(8–37)}$ infusions gave no significant increase in pulpal blood flow, indicating that CGRP is the main factor responsible for the vasodilation observed following tooth stimulation (Table 1).

In four of the animals studied, the substance P antagonist SR 140.333 was administered after h-CGRP$_{(8–37)}$ infusion; this caused a further significant fall in blood flow in both the gingiva and the pulp. The reduction in pulpal blood flow was 16.9 ± 1.9% ($p < 0.005$, paired *t*-test) and 19.3 ± 5.6% ($p < 0.05$, paired *t*-test) in the gingiva (Fig. 2). The mean arterial pressure remained unchanged before, during and after drug administration.

4. Discussion

This study provides evidence that a spontaneous, continuous release of CGRP and substance P from sensory fibres is responsible for the resting vasodilator tone observed in vessels in the dental pulp and in attached gingiva. Furthermore, we have ruled out the possibility that the release of neuropeptides is caused by a method-induced inflammatory reaction. In contrast with our previous recordings (Berggreen and Heyeraas, 1999), the present measurements were made non-invasively, without any exposure of the dental pulp.

Both the attached gingiva and the dental pulp have a pulsatile, relatively high interstitial fluid pressure and probably also a low compliance (Johannessen et al., 1987). The ferret pulpal interstitial fluid pressure is

Table 1
Simultaneous measurements of pulpal blood flow (PBF %) and mean arterial pressure (MAP), before and after electrical stimulation (stim.) of the tooth during control conditions (CC) and after infusion of h-CGRP$_{(8–37)}$[a]

	PBF (%) before stim.	PBF (%) after stim.	MAP (mmHg) before stim.	MAP (mmHg) after stim.
CC	100	171.9 ± 15.3*	104.7 ± 7.4	102.8 ± 8.8
h-CGRP$_{(8–37)}$	100	95.6 ± 17.2**	95 ± 5.3	95 ± 5.3

[a] Number of animals is seven. Values are means ± SEM.
* $p < 0.005$ when compared with measurements before stimulation; ** $p < 0.05$ when compared with measurements before infusion of h-CGRP$_{(8–37)}$.

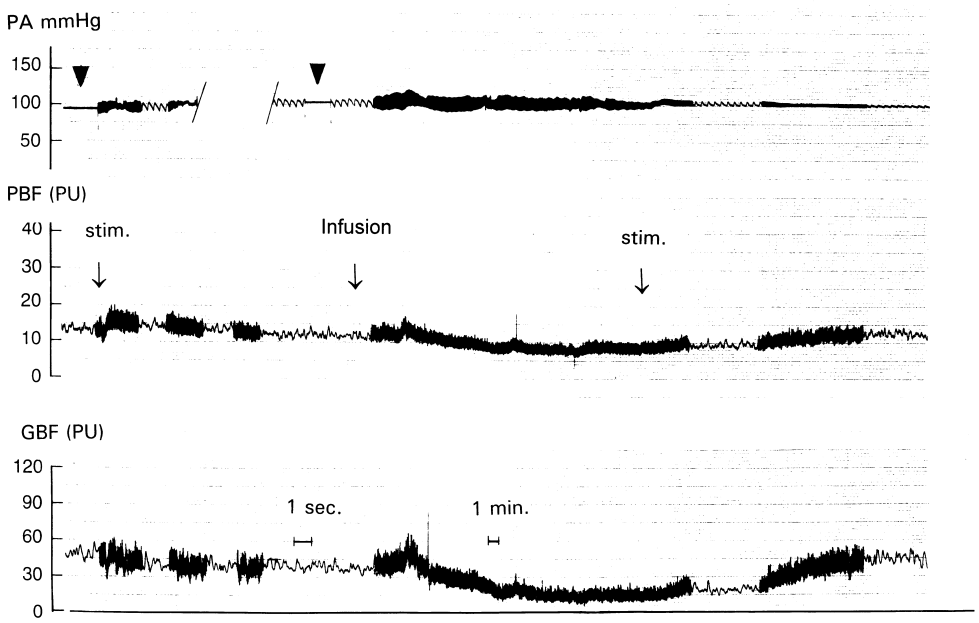

Fig. 1. Original simultaneous recordings of phasic and mean (arrowheads) systemic arterial pressure (PA), pulpal blood flow (PBF) and gingival blood flow (GBF) before, during and after infusion of h-CGRP$_{(8–37)}$. Electrical tooth stimulation (stim.) for 10 sec (2 msec, 10 Hz, 200–250 μA) was given before and after drug administration. A part of the PA curve is omitted because of severe artefacts.

nearly 10 mmHg (Jacobsen and Heyeraas, 1997; Berggreen and Heyeraas, 1999) whereas in the rat the interstitial fluid pressure of attached gingiva averages 7.4 mmHg (Fjærtoft et al., 1992).

It can be speculated that the relatively high and pulsatile interstitial fluid pressure measured during resting conditions both in the pulp and the gingiva may promote the release of neuropeptides from the sensory nerve endings and consequently be responsible for this basal release (Berggreen and Heyeraas, 1999). Our findings complement measurements from the rat knee joint, another low-compliance tissue. In skin-covered knee joints, intra-articular and intra-arterial injections of antagonists to CGRP and substance P, respectively, elicited a significant fall in resting synovial blood flow (McMurdo et al., 1997; Ferrell et al., 1997).

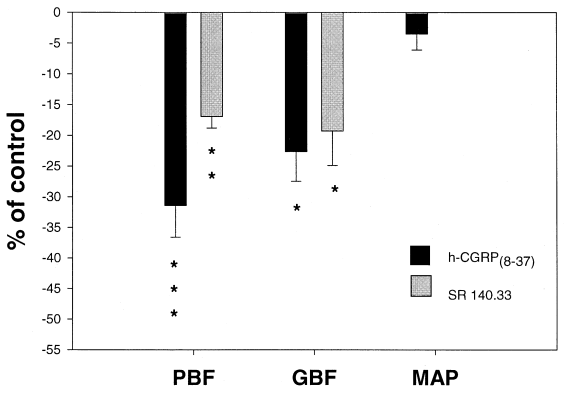

Fig. 2. Pulpal blood flow (PBF), gingival blood flow (GBF) and arterial mean systemic pressure (MAP) after h-CGRP$_{(8–37)}$ (n = 7) and SR 140.33 (n = 4) administrations as per cent of control measurements. Values are mean ± SEM. $^{***}p < 0.001$, $^{**}p < 0.005$, $^{*}p < 0.05$ as compared with control recordings before antagonist infusion.

Our results also agree fairly well with measurements of cutaneous blood flow in the rat hind paw (Yonehara et al., 1992). That study showed that cutaneous blood flow was decreased after rats were pretreated with capsaicin and also after intra-arterial infusion of spantide, a substance P antagonist, and suggest that small-diameter afferent fibres containing substance P tonically regulate vascular tone in cutaneous microvessels.

It has been assumed that the release of neuropeptides from peripheral nerve terminals occurs via a so-called axon reflex. However, several studies have demonstrated that capsaicin induces a tetrodotoxin-resistant release of substance P and CGRP (Maggi et al., 1988, 1989; Hua et al., 1986). Tetradotoxin inhibits the propagation of impulses along axons, and the tetrodo-

toxin resistance of capsaicin-induced neuropeptide release therefore suggests that chemical mediators evoke release acting locally to depolarize the nerve terminal. Tetrodotoxin-resistant release of substance P may also result from a chemically mediated action, independent of depolarization. White and Zimmermann (1988) used bradykinin and showed that they could induce the release of substance P from saphenous nerve neuromas without exciting C-fibre nerve endings. Whether the basal release of neuropeptides in the dental pulp and gingiva is due to a depolarization of nerves or a chemical interaction independent of electrophysiological excitation of the terminal is still unknown.

In the present study, h-$CGRP_{(8–37)}$ greatly reduced the electrically induced vasodilatation observed before antagonist infusion. This observation supports our previous results from the exposed dental pulp where we found that CGRP is the principal factor for the vasodilatation seen after tooth stimulation (Berggreen and Heyeraas, 1999). Our results agree with those of Edvinsson et al. (1998), who found that the cerebral vasodilatation from trigeminal nerve stimulation involves mainly CGRP. They observed a 50% reduction in cerebral vasodilatation after local administration of the CGRP blocker h-$CGRP_{(8–37)}$, compared to a 96% reduction in pulpal blood flow reported in our study.

In conclusion, we provide evidence that a basal vasodilator tone, due to the release of CGRP and substance P, exists both in the dental pulp and the gingiva. We also confirm that the increase in blood flow measured after tooth stimulation is mainly as a result of CGRP release. Whether the basal release of sensory neuropeptides is limited to tissues with relatively high interstitial pressures and low compliance needs further investigation.

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References

Berggreen, E., Heyeraas, K.J., 1999. The role of sensory neuropeptides and nitric oxide on pulpal blood flow and tissue pressure in the ferret. J. Dent. Res. 78, 1535–1543.

Edvinsson, L., Mulder, H., Goadsby, P.J., Uddman, R., 1998. Calcitonin gene-related peptide and nitric oxide in the trigeminal ganglion: cerebral vasodilation from trigeminal nerve stimulation involves mainly calcitonin gene-related peptide. J. Aut. Nerv. Syst. 70, 15–22.

Edwall, L., Scott Jr, D., 1971. Influence of changes in microcirculation on the excitability of the sensory unit in the tooth of the cat. Acta Physiol. Scand. 82, 555–566.

Ferrell, W.R., Lockhart, J.C., Karimian, S.M., 1997. Tachykinin regulation of basal synovial blood flow. Br. J. Pharmacol. 121, 29–34.

Fjærtoft, M., Johannesen, A.C., Heyeraas, K.J., 1992. Micropuncture measurements of interstitial fluid pressure in normal and inflamed gingiva in rats. J. Periodont. Res. 27, 534–538.

Hua, X.-Y., Saria, A., Gamse, R., Theodorsson-Norheim, E., Brodin, E., Lundberg, J.M., 1986. Capsaicin induced release of multiple tachykinins (substance P, neurokinin A and eledoisin-like material) from guinea-pig spinal cord and ureter. Neuroscience 19, 313–319.

Jacobsen, E.B., Heyeraas, K.J., 1997. Pulp interstitial fluid pressure and blood flow after denervation and electrical tooth stimulation in the ferret. Archs. Oral Biol. 42, 407–415.

Johannessen, A.C., Fjærtoft, M., Heyeraas, K.J., 1987. Tissue pressure in rat oral mucosa, measured by micropuncture technique. J. Periodont. Res. 22, 139–143.

Kerezoudis, N.P., Olgart, L., Edwall, L., 1994. CGRP (8–37) reduces the duration but not the maximal increase of antidromic vasodilation in dental pulp and lip of the rat. Acta Physiol. Scand. 151, 73–81.

Maggi, C.A., Santicioli, P., Geppetti, P., Patacchini, R., Frilli, S., Astolfi, M., Fusco, B., Meli, A., 1988. Simultaneous release of substance P- and calcitonin gene-related peptide (CGRP)-like immunoreactivity from isolated muscle of the guinea pig urinary bladder. Neurosci. Lett. 87, 163–167.

Maggi, C.A., Santicioli, P., Geppetti, P., Parlini, M., Astolfi, M., Del Bianco, E., Patacchini, R., Giuliani, S., Meli, A., 1989. The effect of calcium free medium and nifedipine on the release of substance P-like immunoreactivity and contractions induced by capsaicin in the isolated guinea pig and rat bladder. Gen. Pharmacol. 20, 445–456.

McMurdo, L., Lockhart, J.C., Ferrell, W.R., 1997. Modulation of synovial blood flow by the calcitonin gene-related peptide (CGRP) receptor antagonist, $CGRP_{(8–37)}$. Br. J. Pharmacol. 121, 1075–1108.

Olgart, L., Edwall, B., Gazelius, B., 1989. Neurogenic mediators in control of pulpal blood flow. J. Endodon. 15, 409–412.

Sasano, T., Shoji, N., Kuriwada, S., Sanjo, D., Izumi, H., Karita, K., 1995. Absence of parasympathetic vasodilatation in cat dental pulp. J. Dent. Res. 74, 1665–1670.

Tønder, K.H., 1975. The effect of variations in arterial blood pressure and baroreceptor reflexes on pulpal blood flow in dogs. Archs. Oral Biol. 20, 345–349.

Tønder, K.H., Næss, G., 1978. Nervous control of blood flow in the dental pulp in dogs. Acta Physiol. Scand. 104, 13–23.

White, D.M., Zimmermann, M., 1988. Bradykinin-induced release of substance P from nerve fibre endings in the rat

saphenous nerve neuroma is not related to electrophysiological excitation. Neurosci. Lett. 92, 108–113.
Yonehara, N., Chen, J.-Q., Imai, Y., Inoki, R., 1992. Involvement of substance P present in primary afferent neurones in modulation of cutaneous blood flow in the instep of rat hind paw. Br. J. Pharmacol. 106, 256–262.