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Temperature related changes in pulpal microcirculation

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During dental treatment temperatures can be reached which might possibly damage the tooth pulp. To determine the effect of both thermal stimulation on pulpal microcirculation and local anesthesia on thermoregulation we measured the pulpal blood flow by using laser Doppler flowmetry. Experiments were carried out on lower incisors of Wistar rats anaesthetized with thiopental. The rats were divided into three groups, with one remaining untreated, and the others being either desensitized with capsaicin or sympathectomized with guanethidine. In a range between 33 °C and 42 °C there was no substantial change in blood flow, which, however, was the case below 33 °C. Up to 49 °C an increase could be recorded in both untreated and guanethidine pretreated animals, whereas the capsaicin group showed almost no reaction. This increase in blood flow can be blocked reversibly by local anaesthesia. For this purpose we tested articain 5% and mepivacain 3%, both without constrictor. Intravital microscopic studies show that the temperature related increase in blood flow is also associated with plasma extravasation. From these results we draw the conclusion that pulpal thermoregulation is linked to nociceptive sensory neurons and can be described as "neurogenic inflammation". *Proc Finn Dent Soc 1992, 88 (Suppl I): 469—79*

Key words: Pulpal microcirculation, neurogenic inflammation, temperature, plasma extravasation

Surrounding hard tissues can protect the dental pulp against noxious stimuli including thermal injury. With dental treatment, however, it is possible that noxious temperatures are reached as a consequence of the curing of temporary restorations or of insufficient water-cooling during tooth preparation (Graijower et al. 1975).

The tooth pulp is innervated by a large number of sensory neurones, mainly C- but also A-fibres (Byers 1984). They terminate as free nerve endings mostly in the pulpodentinal junction area and mediate pain. Afferent C- and A-delta fibres in the dental pulp contain a high concentration of neuropeptides which are involved in sensory as well as in vasoactive mechanisms. Up to now CGRP, neuropeptide Y, vasoactive intestinal peptide (VIP) and enkephalins have been identified in the dental pulp besides the wellkown substance P (Olgart et al. 1977, Silverman et al. 1987).

Both sensory C- and A-delta fibres can be activated by noxious stimulation. The excitation of nociceptors is accompanied by a release of neuropeptides which in turn cause neurogenic inflammation. This phenomenon has been the subject of extensive research in skin (Gamse et al. 1980, Chahl 1988, Lisney and Bharali 1989). The question to be answered is if the pulp reacts with neurogenic inflammation to thermal stimuli being potentially noxious. This peripheral reaction is linked to the perception of pain. In dental practice pain is usually blocked by means of local anaesthetics. The present examinations

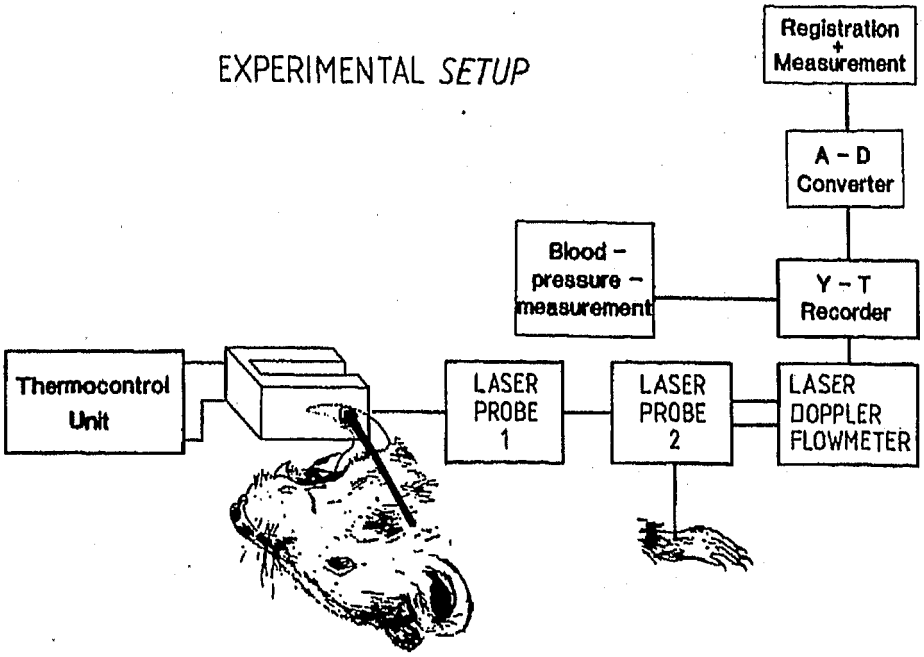

Fig. 2. Experimental setup for the LDF recording.

to be provided. These requirements were met by a thermo-coupled device, which in addition served as fixation for the laser probe. A receptacle of PVC was adapted to the tooth. One wall consits of a Peltier device for thermoregulation (PKE 12A 0020, Peltron). The regulation of the Peletier device is controlled by a thermoelement (Raab 1988, Raab et al. 1988) (Figs. 1 and 2).

The position of the prepared tooth is controlled and corrected if necessary. Final fixation and insulation was accomplished with dental resin. In a last step the thermo-coupled device is charged with synthetic interstitual fluid (SIF) (Bretag 1969).

Pulpal bloodflow was recorded with a He-Ne laser-Doppler flowmeter (LDF) of 2 mW (Periflux PF2B, Perimed, Stockholm). An additional laserprobe on the hind paw served to monitor systemic vascular reactions. The voltage signal of LDF was digitized at a frequency of 10 Hz and recorded on a PC (Tandon AT). The LDF data were analysed offline (Tenland 1982).

The temperature of the SIF-solution was regulated by means of the built-in Peletier device. In addition, it is controlled directly at the exposed dentine surface. Local anaesthesia was obtained by ex changing the SIF-solution for Mepivacain 3% or Articain 5% , both without vasoconstrictor. The local anaesthetic takes effect by diffusion through the prepared cavity. The change in pulpal blood flow after a rise in temperature was measured before application and again at 15 minutes and 60 minutes after discontinuation of the local anaesthetic (Müller and Raab 1990).

Intravital microscopy

The animals were prepared in the same way as for the LDF investigations. In addition, a jugular vein catheter for the application of Evans blue dye was inserted. We prepared the incisor at its mesial and distal surface until the pulpal microcirculation can be observed in the light microscope (Fig. 3). Then the tooth is fixed by means of a manipulator. In order to maintain physiological conditions the tooth is irrigated by SIF of 37°C. Subsequent to the preparation a rest of three hours is kept so that changes in microcirculation due to preparation can regress. (Pohto and Scheinin 1958).

were meant to investigate the following questions:

1. Does the tooth pulp respond to rising temperatures with an increase in blood flow?
2. What is the mechanism of this reaction? Can it be described as neurogenic inflammation, including plasma extravasation?
3. Can this reaction be blocked by local anaesthesia?

Thermal stimulation probably effects both efferent and afferent neurones in the tooth pulp. In order to be able to determine the mechanism and the neurones involved more precisely it is useful to block functionally the nociceptive or postganglionic sympathetic fibres. This aim can be reached by means of certain pharmacological substances.

Capsaicin (8-methyl-N-vanillyl 6-noneamide) is found in plants of the type capsicum, e.g. "hot" pepper. Besides the well-known short and long term effects of local application of this substance (Szolcsanyi 1976, Olgart 1990, Gamse et al. 1980, Jancso and Jancso-Gabor 1980, Carpenter and Lynn 1981, Handwerker et al. 1984) there are also systemic reactions. Using a cumulative dose of 950 mg/kg body weight (BW) subcutaneously JESSEL et al. (Jessell et al. 1978) found a reduction of substance P in the dorsal horn of rats. A significant reduction of substance P in many tissues, however, can be demonstrated with lower doses (50mg/kg BW) (Palermo et al. 1981).

Guanethidine sulfate (Ismelin®) blocks the function of postganglionic sympathetic fibres and is used clinically as an antihypertensive agent. Its mechanism of action is based on an obstruction of the reabsorption of biogenic amines at the synapse. Burnstock et al. (1971, 1979) showed that a dose of 25—100mg/kg BW leads to an almost complete functional sympathectomy. Heath and Burnstock (1977) were able to prove the neuronal degeneration to highly selectively effect the sympathetic nervous system, whereas parasympathetic or sensory nerves are not involved. Investigations of the superior cervical ganglion (Kidd et al. 1986) show a reduction of neurones by 78% after subcutaneous application of guanethidine sulfate (50mg/kg BW) two days prior to the experiments.

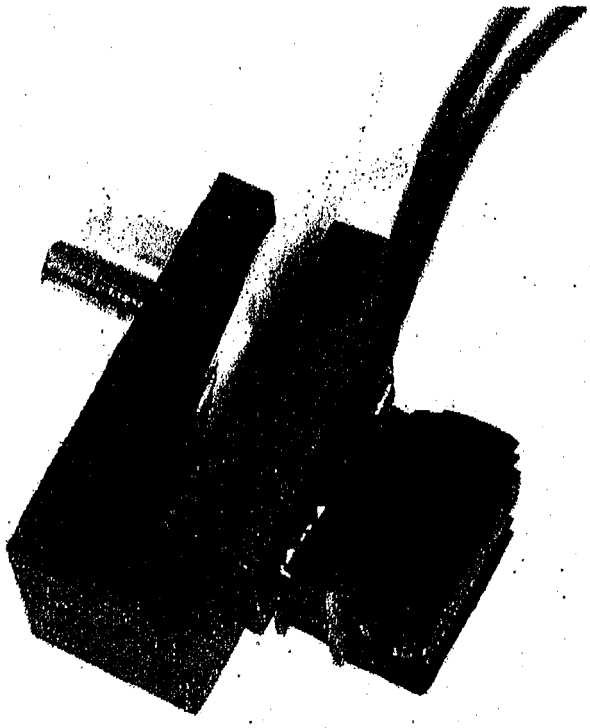

Fig. 1. Thermo-coupled device for LDF recording with prepared lower incisor before fixation. Changes in temperature are induced via a Peltier element on the right side.

Materials and methods

Laser-Doppler flowmetry

The experiments are carried out on 12 Wistar-rats (250—350g bw). After intraperitoneal anaesthesia with thiopental (15mg/100g bw) the animals are tracheotomised and the two hemimandibles were split. The dental hard tissues facing the laser probe were reduced to a 100—150 µm layer covering the pulp. However because of the thinness of the remaining dentinal layer sufficient protection for the pulp against mechanical irritation, temperature variations and loss of fluid has

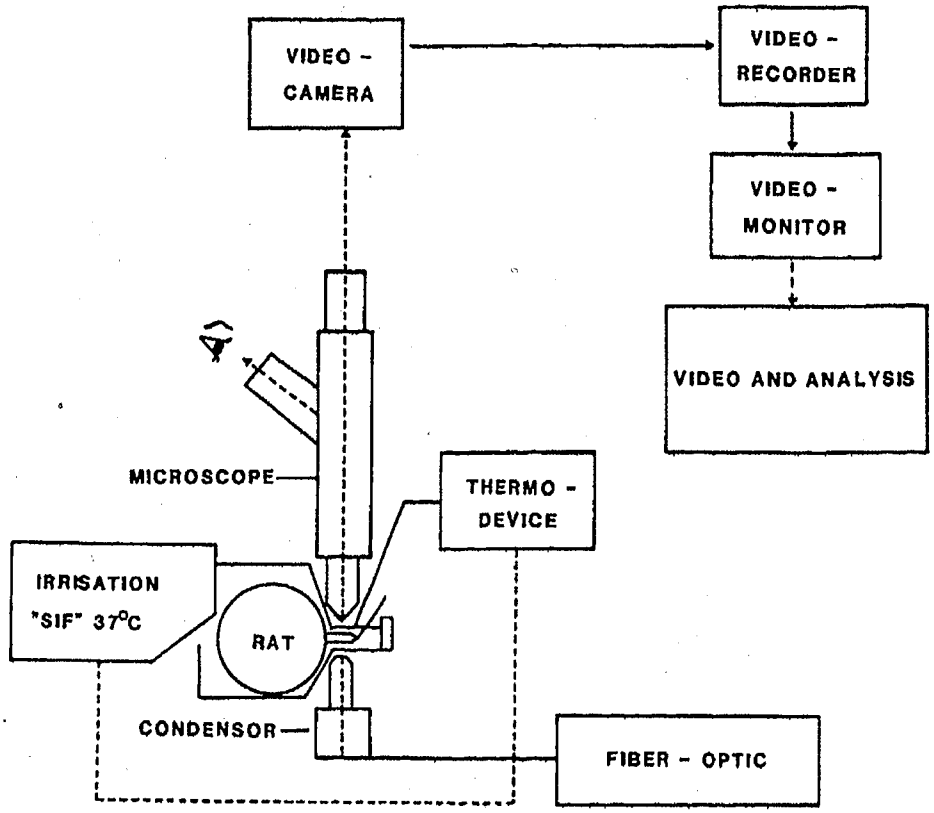

Fig. 3. Experimental setup for intravital microscoy. Changes in temperature are induced by increasing the temperature of the irrigation solution.

At the beginning of the experiments Evans blue dye (50mg/kg BW) was slowly injected into the jugular vein catheter over a period of five minutes. Being bound to albumin evansblue can be taken to demonstrate inflammatory plasma extravasation (Raab 1989).

To observe this process we used an individually adapted microscope with long-focus lenses (Leitz), and a magnification of 100—200. The vascular changes were recorded by a CCD videocamera (F10 Panasonic) and analysed off-line.

The rats were devided into three groups, one remaining untreated the other both being pretreated either with capsaicin or guanethidine.

The rats were desensitized with a 10% capsaicin solution produced according to SZOLCSANYI (personal communication) (1g capsaicin, 10ml ethanol, 10ml TWEEN 80, 80ml physiological saline solution). The animals are pretreated with a cumulative dose of 200mg/kg BW over a period of five successive days, the effect finally being controlled by applying 0.1% capsaicin to the cornea.

The postganglionic sympathetic fibres were funtionally blocked by a subcutaneous application of guanethidine sulfate (50mg/Kg BW) two days prior to the experiments.

Results

Changes in microcirculation

Independent of the type of pretreatment, no remarkable change in pulpal microcirculation was noted between 31°C and 43°C, as shown in the flux-value graphs. Below 31°C a pronounced reduction in blood flow occured in all animals, with a minimum at 17°C of about 50% of the starting level.

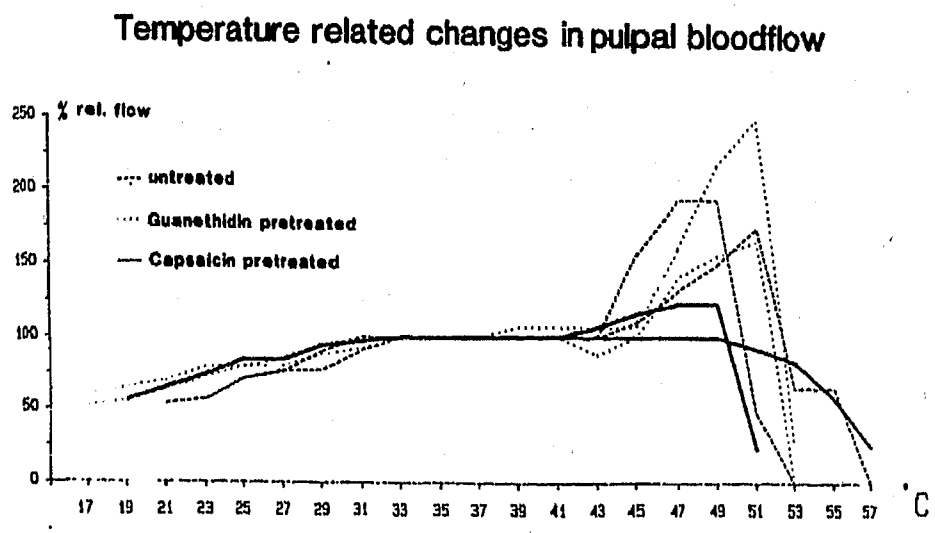

Fig. 4. Temperature related changes in pulpal bloodflow: after desensitivation with capsaicin there is almost no increase in pulpal microcirculation.

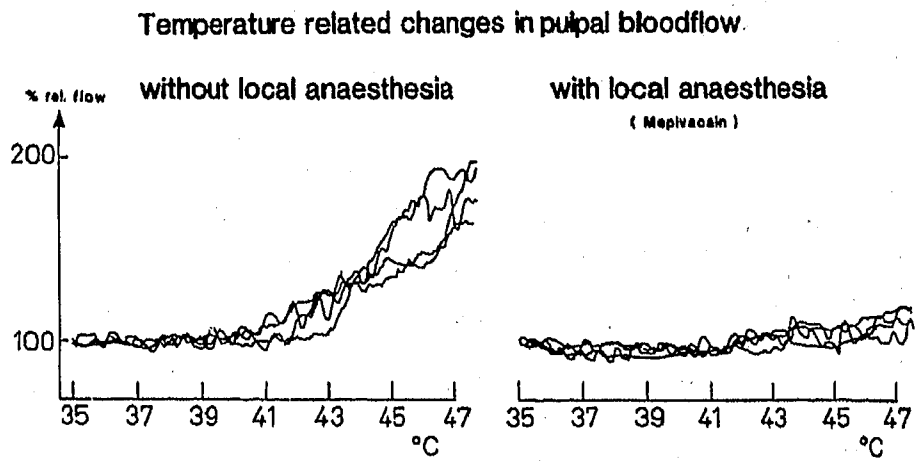

Fig. 5. Influence of Mepivacain 3 p.c. without vasoconstrictor on pulpal microcirculation, presentation of four single measurements before and after local anaesthesia.

Above 43°C a different situation developed, depending on the kind of systemic pretreatment. Similar results were noted for the untreated and guanethidine-pretreated groups. Increasing temperatures lead to an increase in relative blood flow, reaching a maximum (up to 240%) between 49°C and 51°C. Temperatures above these values resulted in a rapid and irreversible breakdown of pulpal microcirculation (Fig. 4).

After pretreatment with capsaicin there were no comparable reactions. There was a slight or no raise in bloodflow up to a temperature of 49°C. Higher temperatures resulted in a rapid breakdown of pulpal microcirculation. All these changes in microcirculation described were restricted to the dental pulp. LDF measurements at the hind paw showed no systemic changes in bloodflow.

Effects of local anaesthesia

To be sure to exclude any irreversible damage of pulpal microcirculation the following experiments were carried out at a temperature range of 35°C to 45°C (Fig. 5). An in-

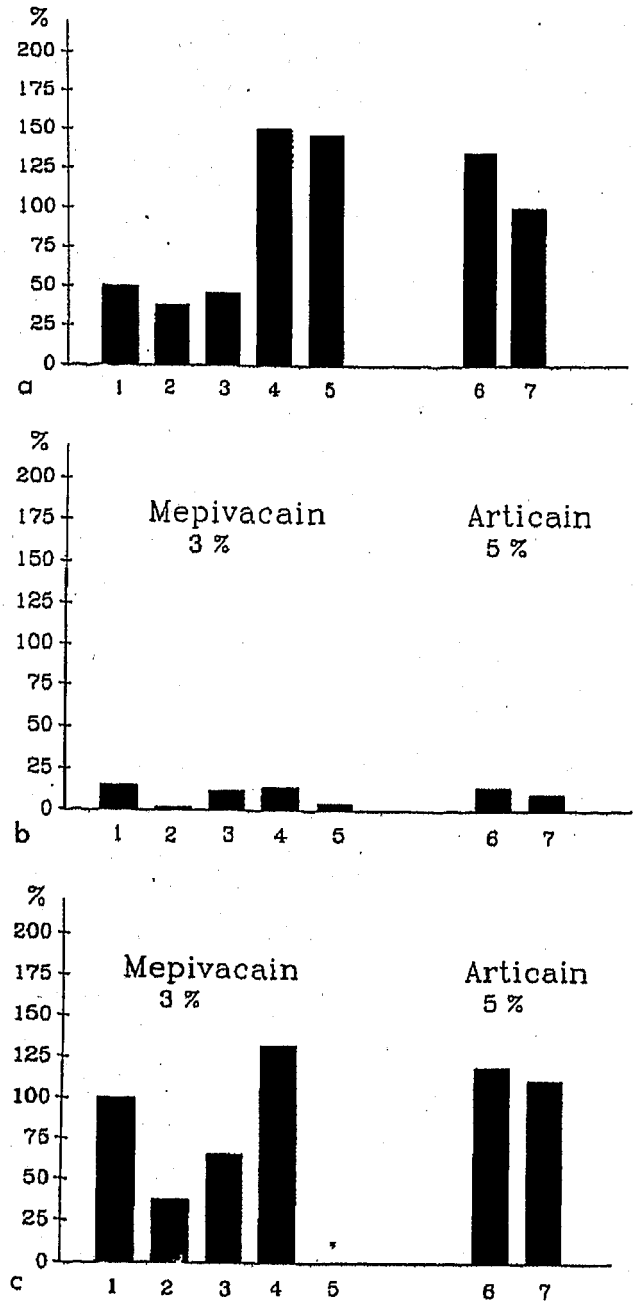

Fig. 6. (a) Relative increase in pulpal microcirculation up to 45°C before local anaesthesia. (b) During local anaesthesia using Mepivacain and Articain. (c) 60 min after discontinuation of the local anaesthesia. * = missing value

crease in temperature from 35°C to 45°C induced an increase in blood flow averaging 100% (95% ± 53% SD) (Fig. 6a). The extent of interindividual reactions differed (a range of 40% to 150%). The intraindividual increase in blood flow, however, was constant and reproducible within physiological variations. Fifteen minutes after application of local anaesthetic identical increases in temperature lead to increases in blood flow of 10% (9.8% ± 5% SD) (Fig.6b). Sixty minutes after discontinuation of the local anaesthesia increases in blood flow returned to their initial value of about 100% (97% ± 40% SD) (Fig. 6c).

There were no detectable differences in blood flow between two anaesthetic agents applied (Mepivacain 3 %, Articain 5 %).

Intravital mircoscopic findings

LDF measurements indicated that increasing temperature beyond 43°C lead to increased pulpal blood flow. Up to a temperature of 43°C to 44°C measured on the dentinal surface little staining of the pulpal interstitium with Evans blue dye was observed (Fig. 7a). With increasing temperature (44°C—45°C) increased staining became visible, starting at the postcapillary venules (Fig 7b,c). At 47°C to 49°C almost complete staining of the interstitial area was observed. At the higher temperatures (47°C—49°C) the extravasation was a rapid process, which needs only a few minutes for the interstitial staining (Fig. 7d). The extravasation involved ultimately peripheral as well as the central parts of the dental pulp. At this point a slowing in pulpal bloodflow was observed, which was more pronounced in the venules. This was accompanied by a spontaneous aggregation of erythrocytes in the venules.

Subsequent cooling by irrigation with SIF at 35°C lead within a few minutes to a reduction of the interstitial staining with evans blue and a regression in erythrocyte aggregation and slow flow. In 10 minutes or less the bulk of the staining had disappeared and the microcirculation had returned to physiological conditions (Fig. 7e).

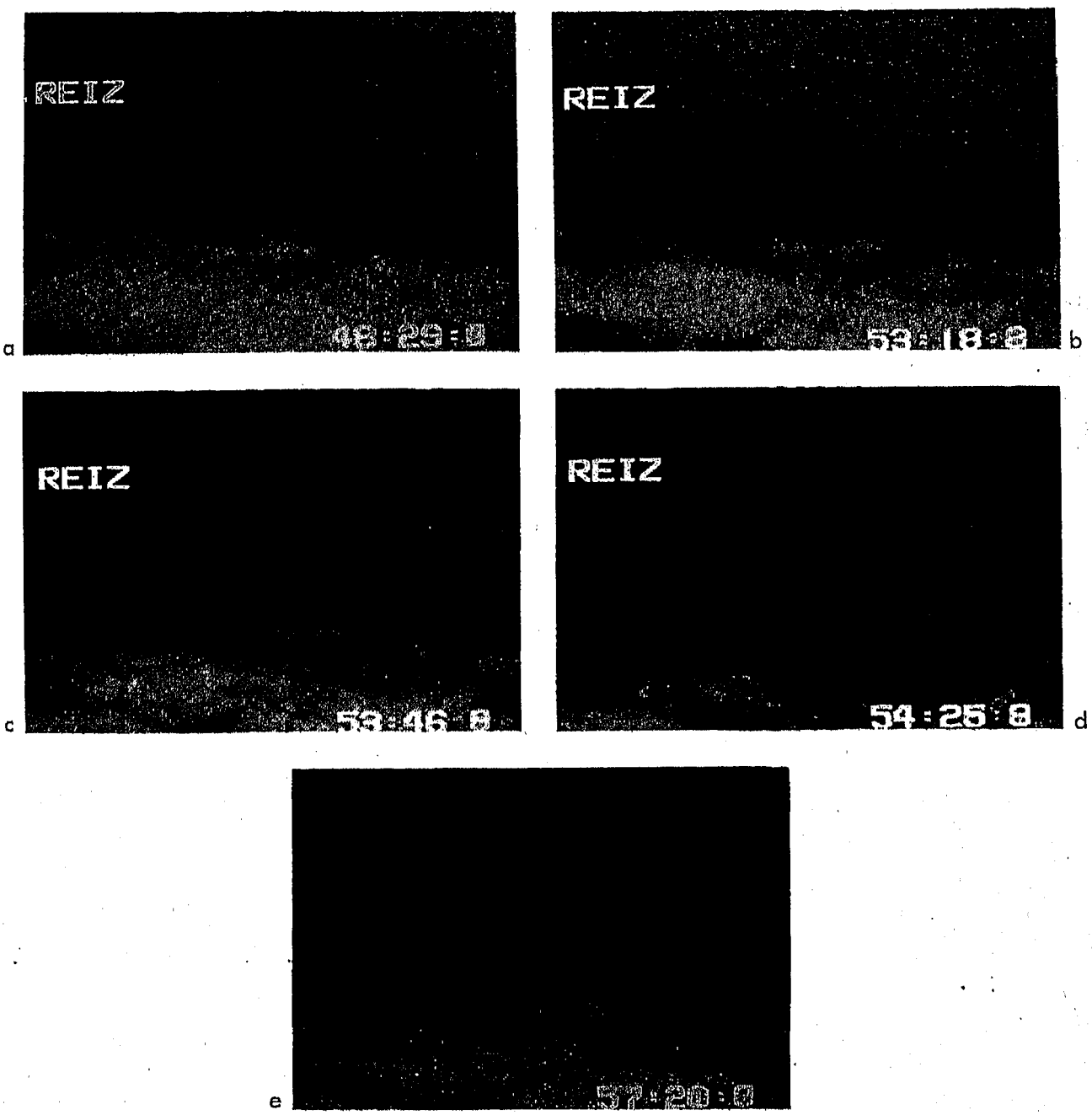

Fig. 7. Intravital microscopic recording of the rat's tooth pulp after injection of Evans bluedye. (a) Temperature about 43°C. (b) Temperature between 44°C and 45°C first staining at the postcapillary venules. (c) Temperature between 46°C and 47°C, increased staining. (d) Temperature at about 48—49°C; almost complete staining of the whole interstitial space. (e) 3min after lowering temperature to 35°C there is a reduction in staining.

Discussion

The results of this study suggest that the dental pulp does not thermoregulate in the traditional sense, e.g., as in the skin. There is no microcirculatory reaction following small temperature changes in the pulpal tissue. Not until potentially noxious temperatures (above 43°C) are reached the pulp reacts by increasing its blood flow. The temperature value of 43°C is about the same as that reported in electrophysiological studies (Matthews 1977,

Närhi et al. 1982) demonstrating an activation of afferent C-fibres at about 44°C, when thermal stimuli were applied to the dental pulp. The assumption that C-fibres are involved in this reaction is supported by the absence of vascular response after petreatment with capsaicin.

Immunohistochemical analyses have shown that the effect of capsaicin administered systemically is promasely on of C-fibres afferent neurons. (Palermo et al. 1981, Virus et al. 1982). Together with the loss of substance P in small nerve fibres there also occurs a reduction of neurogenic hyperaemia and plasma extravasation by 70% (Lembeck and Donnerer 1981). The neuropeptides leuencephaline, neurotensine and VIP are not reduced by adminestration of capsaicin (Priestley 1982).

There is evidence, that pulpal hyperaemia following noxious temperature stimuli is related to the functional integrity of nociceptors, mainly C-fibres. The inhibition of nociceptive neurones by capsaicin results in the absence of a temperature related change in pulpal blood flow. In contrast, the blockade of sympathetic fibers shows no effect. Thus heat — related changes in pulpal blood flow can be explained by the mechanisms of neurogenic inflammation.

The reduction in pulpal blood flow seen at temperatures below 31°C seems not to be connected to the functional integrity of afferent or efferent pulpal C-fibres. Regardless of the type of pretreatment, the reaction is similar in all animals. It seems likely, that this effect is related to the autonomic reaction of the vascular smooth muscle cells.

The stimulation of peptidergic afferent fibres leads to hyperaemia and plasma extravasation at the stimulated area. The extent of this response depends on stimulus duration and intensity (Carpenter and Lynn 1981, Chahl 1988, Lisney and Bharali 1989). Both reactions are initiated via neuropep tides. There

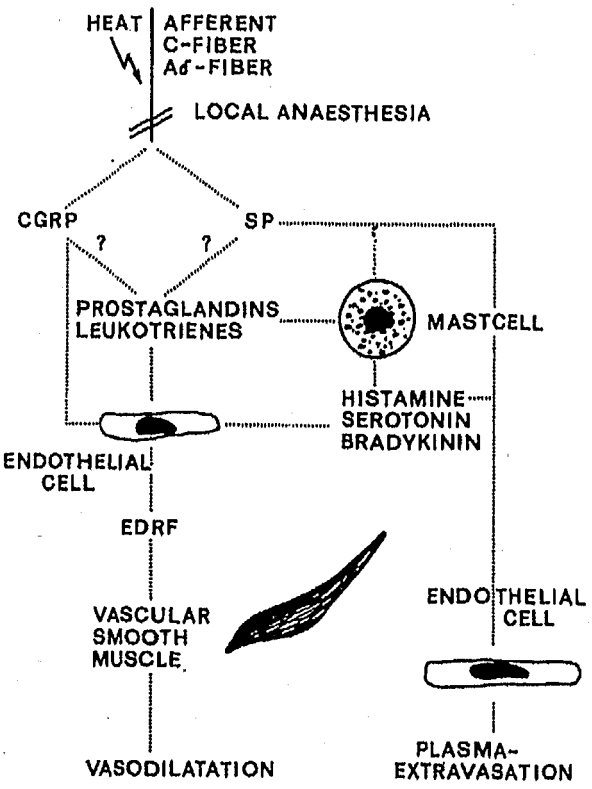

Fig. 8. Schematic drawing showing mechanisms of neurogenic iflammation inducing hyperaemia and plasma extravasation.

is evidence that different neuropeptides potentiate each other effects. This is known for the interactions of SP and CGRP. CGRP is a much more potent vasodilatator than SP but no histamine from mast cells (Chahl 1988). Neurogenic inflammation results not only in hyperaemia but also in plasma extravasation. This dual effect was demonstrated for the dental pulp under the conditions of the present study. The reaction started with hyperaemia at 43°C and was followed by plasma extravasation at about 45°C. The intravital microscopic pictures show a pronounced and generalised plasma extravasation thoughout the dental pulp. The possible role of neuropeptides in both reactions, according to findings in other tissues (Chahl 1988), is shown in Fig.8.

Szolcsanyi (1976) showed that anaesthesia reduces the flare reaction in skin, demonstrat-

ing the involvement of sensory neurones in the triple response. Our results indicate that local anaesthetics eliminate the reaction of the tooth pulp to noxious heat stimuli. The reactive increase in blood flow to heat stimuli under local anaesthesia was about 10 % of that seen without local anesthesia. It seems unlikely that this slight reaction would be able to counteract the effect of noxious temperatures. Therefore, the defense capacity of the tooth pulp has to be regarded as considerably weakened.

Control experiments 60 minutes after the discontinuation of the local anaesthetics demonstrated that this elimination of nocifensive reaction is reversible. After the local anaesthetic effect had worn off, pre-anesthetic increases in blood flow to thermal stimulation could be demonstrated.

Thermal damage during dental treatment probably occurs because the passive protection afforded the insulating hard tissue layer, is reduced during tooth preparation. Under local anaesthesia two important active defense mechanisms are eliminated: pain as a warning and the reactive increase in blood flow.

Hence, dental treatment performed under local anaesthesia requires special care in order to avoid noxious temperatures. Hyperaemia and plasma extravasation as a consequence of neurogenic inflammation do not necessarily lead to irreversible damage of the dental pulp, as the infiltration of the interstitial tissue is transient. This fact can be explained by an increased net reabsorption of fluid and/or lymphatic flow Hegeraas 1977 (Bishop and Malnotra 1990). The biological function of neurogenic inflammation — the reduction of noxious input is also valid for the pulp. The loss of nociceptive innervation in skin leads after some time to trophic damage of the tissue. From these observations neurogenic inflammation has been regarded as a "protective factor", depending on a intact sensory innervation. This protective reaction is not necessarily connected with the sensation of pain. As microneurographic studies from skin demonstrate (Gybels et al. 1979), the peripheral threshold (release of neuropeptides) is lower than the central one (subjective pain sensation). This means that the increase in pulpal microcirculation due to neurogenic inflammation is not necessarily associated with pain. Its innervation with A-delta and C-fibers makes the pulp react not only as a nociceptive but also as a nocifensive system: thus it may be possible to overcome potential thermal damage by the early activation of peripheral defense mechanisms. The fact that local anaesthesia can reduce the extent of neurogenic inflammation of the dental pulp may offer new possibilities in the therapeutic management of pulpal inflammation.

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