Influence of local insults on sympathetic vasoconstrictor control in feline dental pulp

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The present investigation was undertaken to ascertain whether local insults can affect the sympathetic vasoconstrictor regulation of pulpal blood flow.

The rate of disapperance (k-value) of iodide from dentinal cavities was measured in anaesthetized cats. Changes in k-value reflected changes in blood flow. It has previously been shown that stimulation of sympathetic vasoconstrictor nerve fibres generally causes a clearcut decrease in the k-value. Deep cavity preparation was found to inhibit the vasoconstrictor response in a few cases in experiments on mature cats. Heating or cooling the tooth during cavity preparation induced a more frequent inhibition, which proved to be reversible within a few hours. Compound 48/80 applied locally to the cavity was also found to induce inhibition of the vasoconstrictor effect, but in this case no sign of reversibility was observed. Chronic insults were produced by exposing the cavity to oral microorganisms during 1–4 weeks before the experimental procedure. In this case, the sympathetic vasoconstrictor response was not inhibited. A histological study was performed to examine the pulp tissue after cavity preparation and the application of insults. It was found that cavity preparation and acute insults caused little or no disturbance, while chronic insults resulted in severe damage to the pulpal tissue.

Keywords: Blood flow; histology

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INTRODUCTION

Activation of sympathetic vasoconstrictor nerves to the jaws has been shown to reduce pulpal blood flow as observed by vital microscopy (*Scott et al.*, 1972) or measured indirectly by a tracer disappearance technique (*Edwall & Kindlova*, 1971). The vasoconstrictor effect was reported to be absent or reduced in some situations and it was suggested that this could be due to local insults to the pulp, possibly releasing some humoral factor in the pulpal tissue (*Edwall*, 1971).

The purpose of the present study was to investigate whether local insults can inhibit the sympathetic vasoconstrictor response in feline dental pulp. The tracer disappearance method was used as a means of measuring the sympathetic influence on pulpal blood flow. A morphological study of the pulps was performed in order to evaluate the extent of insults produced and also to see if there was a correla-

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tion between disturbances in vasoconstrictor control and morphological changes as observed by conventional histological techniques.

Since compound 48/80 is known to release biologically-active substances in other tissues (*Chakravarty, Högberg & Uvnäs*, 1959) and, furthermore, causes profound excitation of intradental sensory nerves (*Olgart*, 1974), it was considered to be of interest to study whether it influences the sympathetic vasoconstrictor response in the pulp.

MATERIAL AND METHODS

Operative procedures

The experiments were performed on adult cats (2–4 kg). On the basis of radiographs of the canine teeth, the animals were divided into two groups: *young adult*, with incomplete root development, open apices and wide pulps and *mature*, characterized by fully developed roots, closed apices and narrow pulps. The animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.) or chloralose (40 mg/kg) with urethane (50 mg/kg). Blood pressure was recorded in the cannulated femoral artery and the body temperature was kept constant at about 38 °C. The trachea was cannulated.

The techniques used for immobilization, cavity preparation and sympathetic nerve stimulation have been described earlier by *Edwall, Olgart & Haegerstam*, (1973). Maxillary and mandibular canine teeth were used for this study. A cavity was prepared within the middle part of the buccal surface of the tooth. A constant temperature could be maintained by means of a thermode placed in contact with the tooth.

Acute insults

The cavity was either deepened to a moderate depth with the pulp barely visible through the dentin (about 100 μm of dentin remaining in the deepest part of the cavity measured along the dentinal tubules) or extended until the pulp was clearly visible through a very thin layer of dentin (about 50 μm of dentin remaining). In some of the deep cavities a pulp exposure was produced with the tip of a No 2 pulp canal file (Kerr). In a few cases of pulp exposure the whole cavity floor was broken mechanically. After placing Plastibase insulating gel (Squibb) on the enamel around the cavity, the latter was filled with isotonic saline solution and covered with a thin plastic film to prevent evaporation.

To study the influence of heat, the thermode at the tooth surface was kept at 38–43 °C during preparation and the cavity was irrigated with isotonic saline solution at the same temperature. When the effect of cooling was to be studied, the tooth was irrigated with cold saline (6–10 °C), no thermode being used. Irrigation was intermittent. In some experiments compound 48/80 (1 mg/ml) was applied locally to the dentinal cavity.

Chronic insults

When chronic insults were to be produced the teeth were pretreated 1–4 weeks in advance. Cavities were generally prepared at half the crown length and deepened until the pulp was barely visible through the dentin. Some cavities were prepared to a depth corresponding to only half the thickness of the dentin. In some series of experiments the cavities were filled with temporary guttapercha. In other experiments, the cavity bottom was covered with a layer of soft carious dentin obtained from a freshly extracted human tooth. These cavities were filled with amalgam. At the beginning of the experiment the filling was removed and the cavity bottom was freshened up by cautious drilling by hand and irrigated with saline solution. In a few cases the test cavity was deepened considerably or a cavity for registration was prepared in close proximity to the pretreated one.

Disapperance measurements

Changes in pulpal blood flow were determined according to methods described by *Edwall, Olgart & Haegerstam* (1973). ^{125}I, as iodide, was generally used. When two measurements were performed simultaneously, ^{125}I was used in one cavity and ^{131}I in the other. The disapperance of the tracer was monitored by an external scintillation detector. The detector output was fed into one or, in simultaneous measurements, two recording channels of scalers and digital printers. Counting periods of 40 sec were generally used. Three or four tracer disapperance procedures could usually be carried out in sequence in the same cavity. The disappearance rates were calculated as k-values and changes were expressed as percentages of control.

Recording of intradental sensory nerve excitability

Intradental sensory nerve excitability was recorded to ensure that compound 48/80 had passed into the pulpal tissue. The test cavity was prepared within the incisal part of the crown and another cavity within the gingival part. The cavities were filled with isotonic saline solution and Plastibase insulating gel was applied around the cavities. Platinum recording electrodes were placed in the cavities and the potentials obtained from intradental sensory nerves were amplified by a Princeton PAR-113 preamplifier and recorded using the technique described by *Olgart* (1974). Prior to local application of compound 48/80, the saline was removed from the test cavity with a strip of filter paper.

Histological procedures

Test and control teeth in some of the experimental animals were extracted immediately after the physiological measurements had been performed. The apical half of the root was cut off with a diamond wheel under isotonic saline irrigation and the specimen was transferred to 10% buffered formalin solution. The radioactivity in the fixed specimens was allowed to decay for 6–12 months before histological procedures were continued. After fixation, the tooth was decalcified in EDTA or in 5.3% HNO_3 solution, embedded, sectioned and stained, usually with haematoxylin-eosin. Some sections were stained according to the method of *Brown & Brenn* (1931). The slides were examined in a Zeiss Photomicroscope and the thickness of the layer of dentin remaining in the bottom of the cavity was measured along the dentinal tubules using a measuring ocular (Leitz). Photographs were taken of representative areas of the cavity bottom and the adjacent pulpal tissue.

RESULTS

Acute insults – Measurement of sympathetic vasoconstrictor response

Cavity preparation

In order to study the influence of cavity preparation *per se*, a series of experiments were performed without using the thermode, the temperature at the tooth surface thus being 20–22 °C (28 procedures on 4 young adult and 10 mature cats).

In 23 procedures the expected response was obtained, with a clearcut decrease of the k-values during the stimulation period. Following deep cavity preparation on mature cats, the vasoconstrictor effect on sympathetic nerve stimulation was inhibited in 5 procedures. The results of a typical experiment are shown in Fig. 1. Stimulation with 6 Hz did not change the pulpal k-values. Protrusion of the eye bulb and dilation of the iris during the stimulation period indicated that the sympathetic stimulation was adequate and hence that the vasoconstrictor response in the pulp was inhibited by some local factor. When the stimulation was repeated 4 hours later (Fig. 1), a clearcut reduction of the k-values was observed, indicating that the inhibition of the sympathetic effect was reversible.

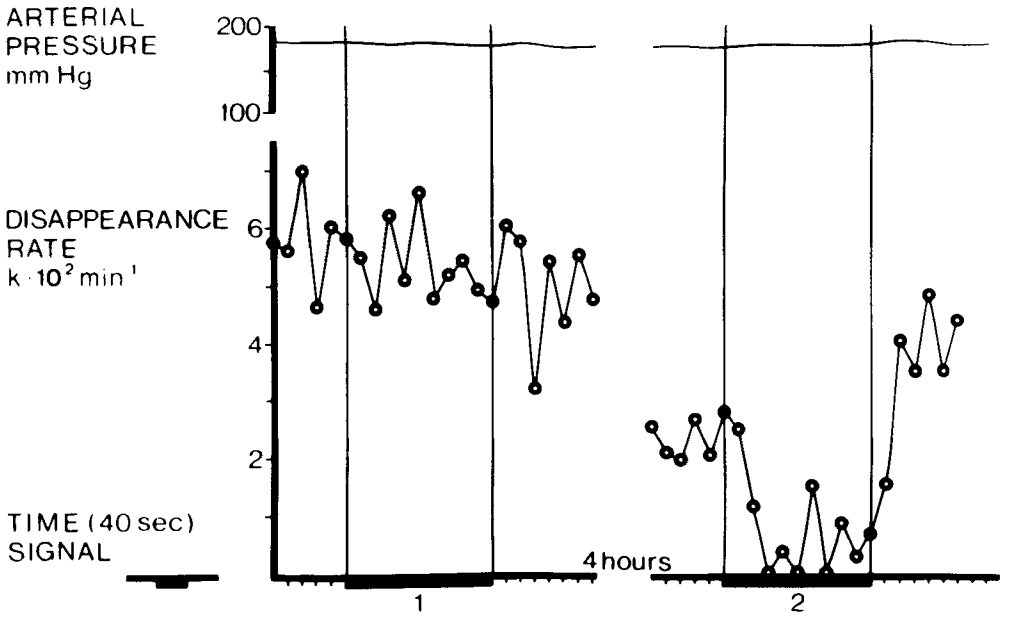

Fig. 1. Influence of deep cavity preparation on tracer disappearance rate and sympathetic vasoconstrictor response in the pulp; initially (1) and after 4 hours (2). Pentobarbital. 1, 2 Sympathetic stimulation with 6 Hz.

Compound 48/80

The effect of compound 48/80 on sympathetic vasoconstrictor regulation in the pulp was studied in 14 procedures (4 young adult and 10 mature). The experiments were started with an initial registration of the disappearance rate and a control stimulation to ensure that the tooth showed the normal pattern of response on sympathetic activation after preparation. A depot of compound 48/80 solution (1 mg/ml) was then placed in the test cavity whilst recording the intradental nerve excitability. After 6–15 min, when the sensory nerves had started to fire and the activity showed a steady state level of 100–200 imp/sec, the depot was washed out with saline solution and replaced by the tracer. In some cases the tracer was added to the compound 48/80 depot. The results of a typical experiment are shown in Fig. 2. The disappearance rate was first registered from the freshly prepared cavity and then after application of compound 48/80 for 6 min. As can be seen in Fig. 2, sympathetic activation with 6 Hz resulted in a marked decrease in the k-values registered from the freshly prepared cavity (47% of control).

In the period directly after stimulation, a rapid rise with an overshoot was seen, followed by a return to control values. The response on stimulation with 2 Hz was less pronounced (19% of control – see Fig. 2). However, after the cavity had been treated with compound 48/80, sympathetic stimulation with 6 and 2 Hz caused only a slight decrease in the k-values, 23 and 8 per cent of control respectively (Fig. 2). The vasoconstrictor response upon sympathetic stimulation was partially or totally inhibited in 8 of 14 procedures, all except one in mature cats. No sign of reversibility was seen. It was a consistent observation that after application of compound 48/80 the resting k-values were depressed.

Acute insults – Morphological observations

Cavity preparation was not found to induce any morphological reaction in the pulpal tissue corresponding to cavities of moderate

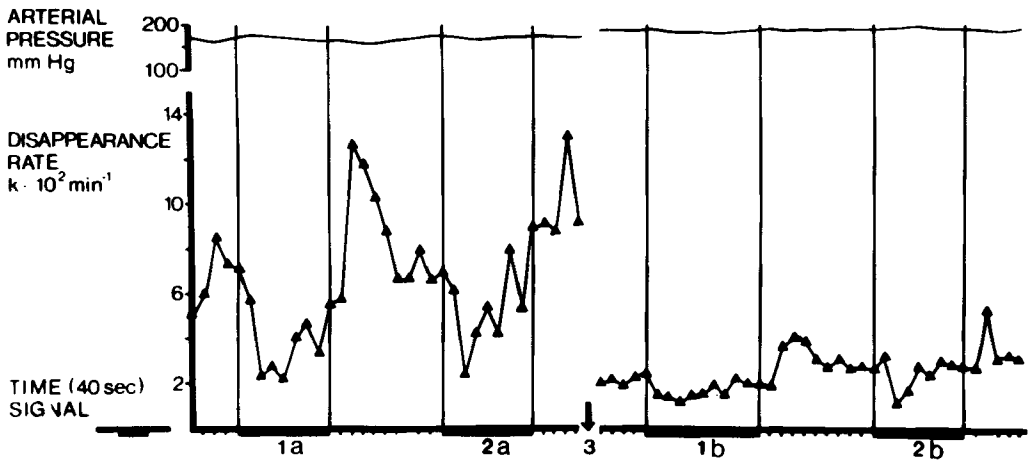

Fig. 2. Influence of compound 48/80 applied locally to the cavity on tracer disappearance rate and sympathetic vasoconstrictor response in the pulp. Pentobarbital. 1. Sympathetic stimulation with 6 Hz. 2. Sympathetic stimulation with 2 Hz. 3. Application of compound 48/80 for 6 min.

depth, that is when 100 μm or more of dentin remained. At greater cavity depth, however, slight disturbances in the odontoblastema could be seen in the region corresponding to the cavity. No inflammatory reaction was seen in the pulpal tissue. If the cavities were deepened to 50 μm or less from the pulp, the odontoblastema was found in some cases to be separated from the dentinal wall; apart from this only minor morphological divergences were observed (Fig. 3).

Acute application of heat, cold or compound 48/80 did not seem to influence the morphology of the pulp tissue *per se*.

Increased or decreased temperature

In order to study the influence of increased or decreased temperature during cavity preparation, experiments were performed on 3 young adult and 11 mature cats (28 procedures). When thermal stimulation was applied during the preparation of cavities of moderate depth, activation of sympathetic nerve fibres produced the normal pattern of decreased k-values in 11 of 13 procedures. However, when thermal insults were applied during deep cavity preparation, the vasoconstrictor response upon sympathetic stimulation was inhibited in 8 of 15 procedures, all except one in mature cats. Heat and cold stimulation were both found to induce the inhibition. When the sympathetic activation was repeated after some hours, the normal response, i.e. a decrease in the k-values, was obtained.

Pulp exposure

The influence of extensive mechanical insults was studied in a series of experiments on 3 young adult and 7 mature cats (15 procedures) with traumatic pulp exposures of varying extents. As the cavities had generally been used previously for other procedures, the exposures were made some hours after cavity preparation. In all cases when the tooth was kept at room temperature (20–22 °C at the tooth surface) – a normal reduction of the k-values was obtained upon sympathetic nerve stimulation (7 procedures, 3 young adult, 4 mature). No difference was found between the effects of small exposures made using a root canal file and a totally broken cavity floor. If the traumatic injury was combined with a temperature of 38–48 °C, the response on sympathetic activation was inhibited in 5 of 8 teeth (2 young adult, 6 mature).

Chronic insults – Measurement of sympathetic vasoconstrictor response

In the chronic experiments where the tooth had been pretreated with guttapercha or carious, human dentin, activation of sympathetic nerve fibres caused a clearcut decrease in the disappearance rate. The results of a representative experiment in which the lower canine tooth had been exposed to carious dentin for 12 days are shown in Fig. 4. A freshly prepared cavity in the upper canine tooth served as control. As can be seen, sympathetic stimulation with 6 Hz resulted in a reduction in the k-values to almost zero in both the test and control teeth. Furthermore, stimulation with 1 Hz caused similar responses in the test and control pulps. This unmodified reaction on sympathetic activation was found in all cases (6 procedures on 5 young adult and 1 mature cat). It was a consistent observation that during the first period of registration (about 30 min) the k-values obtained from a depot in a pretreated cavity were lower than those from a freshly prepared control of about the same depth. If the pretreated cavity was deepened or if a new test cavity was prepared in close proximity to the pretreated one, the resting k-values for the test and control depots were found to be of about the same magnitude.

Chronic insults – Morphological observation

In teeth pretreated with guttapercha as well as with carious human dentin, a layer of bacteria could be seen in the bottom of the cavity. In some cases, dentinal tubules were invaded by microorganisms. The odontoblastema close to the cavity was markedly reduced and in some teeth the predentin zone was also affected, or even absent. A large number of leucocytes were observed at the pulpal wall and/or in the pulpal tissue. In the vicinity of the cavity region the pulp showed dilated, well-filled vessels with leucocytes close to the vessel wall. Aspiration of nuclei into dentinal tubules, oedema in the pulpal tissue and local cell necrosis could also be seen, especially in teeth exposed to carious dentin (Fig. 5).

Fig. 3. Cavity preparation. About 50 μm between cavity bottom and pulp. No inflammatory reaction can be seen. x 120.

DISCUSSION

In the present study we could confirm the finding by *Edwall* (1971) that deep cavity preparation may inhibit the response to sympathetic activation. In contrast to previous observations, however, the inhibited vasoconstrictor response was found only in some cases of deep cavity preparation on mature cats. The divergent result might be explained by the differences in tooth temperature – 35–37 °C at the tooth surface in the former experiments, 20–22 °C in the present study. Support for this suggestion is provided in the present study by the finding that heat stimulation (38–43 °C) during cavity preparation increased the number of cases showing an inhibited vasoconstrictor response. A similar pattern of response was found in cases of pulp exposures.

Vasodilation of pulpal blood vessels can be induced by warming (*Pohto & Scheinin*, 1961; *Edwall, Olgart & Haegerstam*, 1973), which could induce inhibition of the vasoconstrictor response. In the present study, however, thermal stimulation was applied to the tooth only during cavity preparation and, furthermore, heat and cold were both found to induce the inhibition. Hence, it seems more likely that thermal gradients, created by the intermittent irrigation, have increased the preparation insult and thus inhibited the vasoconstrictor response.

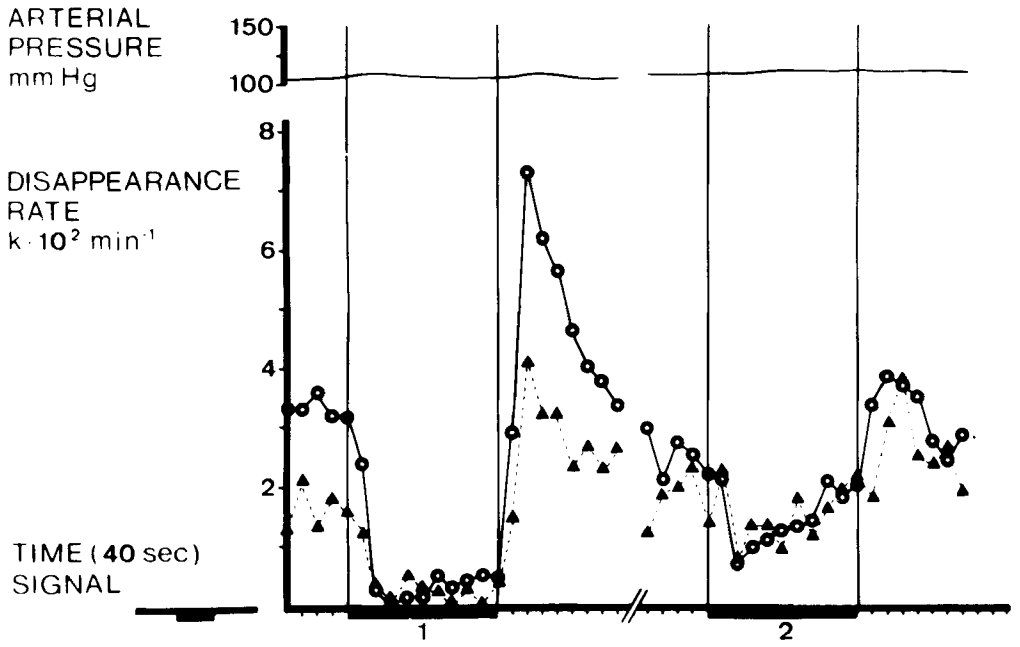

Fig. 4. Influence of chronic insult by carious inlay in the cavity on tracer disappearance rate and sympathetic vasoconstrictor response in the pulp. Pentobarbital. 1. Sympathetic stimulation with 6 Hz. 2. Sympathetic stimulation with 1 Hz. ●– C + control ^{125}I. ▲– C – carious inlay 12 days ^{131}I.

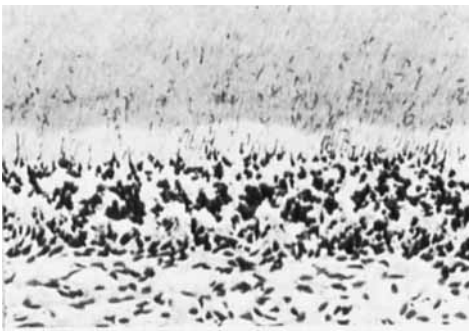

Fig. 5. Moderate cavity depth. Inlay of carious human dentin for 1 week. Numerous leucocytes at the pulpal wall, reduced odontoblastema and aspiration of nuclei into dentinal tubules. x 120.

It has been suggested that the inhibition of the sympathetic response could be due to some humoral factors in the pulp which antagonize the sympathetic effect, for example by causing dilatation of the pulpal vascular bed (*Edwall*, 1971). Support for this hypothesis can be found in a recent study showing that, for example, histamine and bradykinin counteract sympathetic vasoconstrictor tone in the pulp (*Edwall, Olgart & Haegerstam*, 1973). *Kroeger* (1968) reported that a bradykinin-like substance was released into the tooth pulp following deep preparation in dentin. Furthermore, in the present study when the cavity was pretreated with compound 48/80, which is known to induce the release or formation in other tissues of vasodilator substances such as histamine, SRS and prostaglandins, inhibition of the sympathetic vasoconstrictor response was frequently seen.

The depressed k-values consistently found after application of compound 48/80 might be interpreted as an effect of vascular changes in the pulp. It has been shown in guinea-pig skin that a high local concentration of compound 48/80 elicits an increased permeability of the capillaries and an increased plasma outflow, thrombosing the blood vessels (*Miles & Miles*, 1952). If this compound produces similar changes in the dental pulp this might help to explain the considerably reduced transport of

the tracer. However, further investigations using, for example, vital microscopy, are necessary to elucidate the vascular reactions in the pulp which reduce the resting k-values following application of compound 48/80.

In all acute experiments it was a consistent observation that mature cats exhibited a more frequent inhibition of the sympathetic vasoconstrictor response than young adult cats. This difference is presumably due to the different stages of root development and the functional state of the pulp. Teeth with closed apices often show degenerative changes in the pulp tissue (*Reichborn-Kjennerud*, 1959) and there is vast clinical experience showing that human teeth with closed apices are more sensitive to clinical insults than teeth with open apices. Thus it is not surprising that mature pulps in the present study were more sensitive to insults than young pulps.

Chronic insults had no inhibitory influence on the sympathetic vasoconstrictor response, although the morphological study showed severe damage in the pulp area corresponding to the cavity (Fig. 5). These findings seem paradoxical but might be accounted for in the following way. Due to severe local pulp damage the blood supply to these parts is probably considerably reduced or absent. Thus the tracer would diffuse through the injured tissue to adjacent unaffected parts of the vascular bed which has an essentially intact transport function and exhibits a normal sympathetic vasoconstrictor response.

In conclusion, the present results show that inhibition of the vasoconstrictor response in the dental pulp of the cat could be produced by acute local insults and that the inhibition was reversible. Furthermore, we could not observe any correlation between morphological changes in the pulp tissue visualized by conventional histological methods and functional disturbances reflected by an inhibited response on sympathetic nerve activation.

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