Influence of vasodilator substances on pulpal blood flow in the cat

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The objective of the study was to determine the response in the pulpal blood flow to vasodilator substances at different levels of vasomotor tone.
Simultaneous determinations of iodide disappearance rate (k-value) from dentinal cavities and, as control, from the adjacent alveolar submucosa were performed on anesthetized cats. Changes in k-value reflected changes in blood flow. Close i.a. infusion of acetylcholine, histamine and bradykinin did not influence pulpal microcirculation when the sympathetic nervous tone was low. At a higher level of nervous vascular tone, obtained by direct electrical stimulation of the cut sympathetic cervical trunk, the substances were shown to increase the pulpal blood flow. Papaverine and warming had a more pronounced vasodilator influence on pulpal blood flow, suggesting the presence of a local myogenic vascular tone regulating the exchange function, which was unaffected by acetylcholine, histamine and bradykinin. These substances were thus shown to act as inhibitors of sympathetic vasoconstrictor tone in the cat pulp.

Key-words: Dental pulp; blood circulation; radioisotope scanning; vasodilator agents; cats

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Both stimulation of sympathetic vasoconstrictor fibres to the dental pulp and administration of noradrenaline reduce pulpal blood flow (*Edwall & Kindlová*, 1971; *Scott, et al.* 1972; *Olgart, Edwall & Haegerstam*, 1972) and modulate the excitability of intradental sensory units (*Edwall & Scott*, 1971). Findings indicating the presence of several biogenic vasodilator substances, such as bradykinin (*Kroeger*, 1968), acetylcholine (*Pohto & Antila*, 1968) and histamine (*Pohto & Antila*, 1970), in the dental pulp have been reported. It has also been suggested that histamine and bradykinin play a role in the vasomotor regulation in the dental pulp (*Kroeger*, 1968; *Pohto & Antila*, 1970).

In preliminary studies, however, we found that these substances did not have any influence on pulpal blood flow during resting conditions. The present investigation was therefore undertaken to determine the response in pulpal blood flow to vasodilator substances at different levels of vasomotor tone.

MATERIAL AND METHODS

Operative procedure

Experiments were performed on young adult cats (2—4 kg and about 1—2 years

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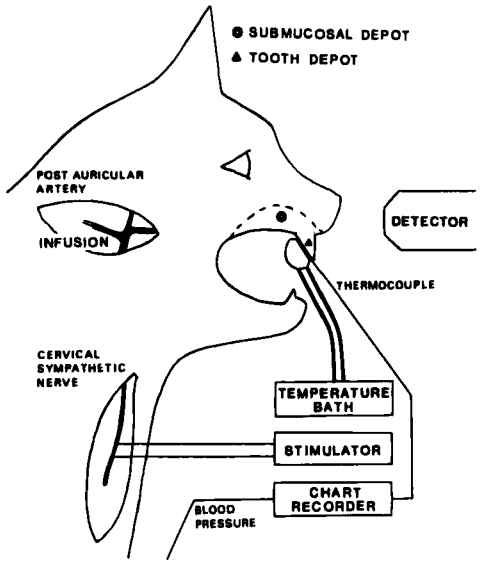

Fig. 1. Drawing showing experimental set up. For explanation see text.

old), anesthetized with sodium pentobarbital (30 mg/kg i.v.) or chloralose (40 mg/kg) with urethane (50 mg/kg). The trachea was cannulated and provision made for recording femoral blood pressure. The rectal temperature was continuously monitored and the body temperature was kept constant at about 38°C by heating lamps. Radiographs were taken of the canine teeth to determine the form of the pulp chamber. The head was immobilized by insertion of a steel rod between the jaws and secured in place by dental acrylic; care was taken to ensure that the canine teeth remained fully exposed for cavity preparation and for insertion of a water circulated thermode to prevent undesirable temperature changes of the tooth (Fig. 1).

Based on the information from the radiograph the cavity position was chosen within the gingival half of the crown in a maxillary canine tooth. The enamel was removed at this location using a diamond instrument operated at slow speed. Isotonic saline solution was used to prevent drying of dentin, and the cavity was deepened by means of a carbide-tipped endcutting bur rotated by a holder held between the fingers and observed through a binocular microscope. The cavity was deepened until the pulp was barely visible through the dentin. After placing Plastibase (Squibb) insulating gel on the enamel around the cavity, the latter was filled with isotonic saline solution and covered with a thin plastic film to prevent evaporation.

The temperature of the tooth was monitored by thermocouples close to the recording cavity. The thermocouples were usually placed on the enamel surface, but in some experiments they were also placed in an adjacent coronal dentinal cavity and in the pulp tissue through a small exposure in the cavity. Using this arrangement it was possible to follow the change in tooth temperature in the different locations when thermal stimuli were applied by running warm or cold water through the thermode. Control level was 32°C.

The cervical sympathetic trunk in the neck region was separated from the vagus. The trunk was cut and the distal stump placed in contact with silver electrodes connected to a Grass Model S4 stimulator. The nerve was covered with Plastibase insulating gel to prevent drying. Square stimulating pulses, having a duration of 1 msec and intensity 6V were used; frequency was varied between 1 and 6 pulses/sec. Each period of stimulation lasted 10—20 min. In some animals the sympathetic trunk was kept intact.

A branch of the external carotid artery (posterior auricular artery) was used for infusions. This artery was reached through an incision behind the ear and was cannulated for retrograde infusion. With this arrangement it was possible to make

a close intra-arterial infusion into the internal maxillary artery supplying the mandibular and the maxillary tissues studied.

Disappearance measurements

Radioactive tracers were obtained in isotonic carrier-free solutions from AB Atomenergi, Studsvik, Nyköping, Sweden. I^{125} and I^{131} (80 μCi/μl) as iodide dissolved in phosphate buffer (pH 7—8) containing sodium thiosulphate were used.

The saline solution in the tooth cavity was replaced by a depot of I^{131} solution (0.1—0.2 μl) which was covered with a thin plastic film. As control, a depot of I^{125} solution (1—2 μl) was injected in the adjacent alveolar submucosa. The submocusal injection was made with a glass capillary with a diameter of 100 μm connected to a Hamilton micro syringe fixed in a stand; injection time was 1—2 min (*Änggård & Edwall*, 1973).

The tracer depots were monitored simultaneously by an external scintillation detector fed into two channels of scalers and digital printers. The experiments were performed 5—15 min after the isotope injection, when the disappearance rate was predominantly monoexponential. Counting periods of 40 sec were generally used. The disappearance rate was calculated as k-values and changes were expressed in per cent of control. For further details concerning the calculations and technique see *Edwall* and *Kindlová* (1971) and *Edwall* and *Scott* (1971).

Acetylcholine 1 μg/ml, bradyklinin 10 μg/ml, histamine 1 μg/ml and papaverine 1 mg/ml were infused via the cannulated posterior auricular artery. The maximal rate of infusion was adjusted to induce a clearcut response in the submucosal k-value.

Infusion was performed under three different conditions:

1) The sympathetic trunk was kept intact.
2) The ipsilateral sympathetic trunk was cut.
3) The ipsilateral sympathetic trunk was cut and the distal stump was stimulated.

Stimulation was started 5—7 min before infusion and was continued during infusion and proceeded 5—7 min after infusion.

RESULTS

Infusion of acetylcholine, bradykinin and histamine

1. Intact sympathetic nerve. Simultaneous measurements of tracer disappearance from the alveolar submucosa and the dental pulp were carried out. A representative experiment is shown in Fig. 2, where two identical infusions of histamine were given. As can be seen, histamine failed to change the disappearance rate (k-value) from the pulp, while the k-value from the alveolar submucosa was increased by about 200%. The results shown in Fig. 2 represent the typical changes observed when histamine (8 procedures, 2 cats), acetylcholine (8 procedures, 3 cats) and bradykinin (2 procedures, 1 cat) were infused.

2. Stimulation of the cut sympathetic trunk. The effects of infusion of acetylcholine during sympathetic nerve stimulation are shown in Fig. 3. Prior to stimulation, k-values from both pulp and submucosa had been constant for about 6 min.

Sympathetic stimulation (Fig. 3:1) depressed both pulpal and submucosal k-values. Infusion of acetylcholine (0.94 μg/min) during the period of depressed

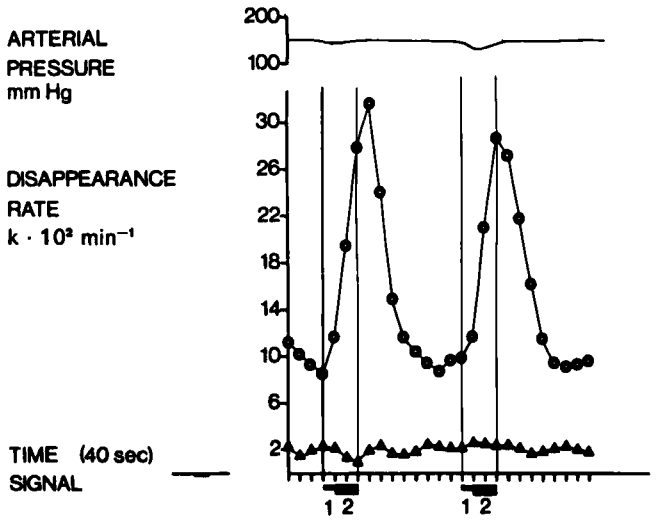

Fig. 2. Influence of i.a. histamine infusions on the disappearance rate of I^{131} from the pulp (△) and I^{125} from the submucosa (○), when the sympathetic trunk was intact.

Chloralose — urethane.

1. Histamine 0.95μg/min.
2. Histamine 1.65μg/min.

k-values increased both k-values (Fig. 3:5) to about the prestimulatory level in both tissues. After the infusion this was followed by a rapid fall of k-values to about the same levels as before the infusion. When the sympathetic stimulation was interrupted the k-values rapidly increased.

Fig. 4 shows the effects of two identical histamine infusions at different levels of vascular tone in the pulp. Sympathetic stimulation (Fig. 4:1) increased the vascular tone and reduced the k-value by up to 80%. The first infusion of histamine (Fig. 4:2 and 4:3) caused an abrupt rise in the k-value. During the following 5 min of sympathetic stimulation the k-value was again reduced. During the period of recovery a rapid rise with an overshoot, followed by return to control values, was seen. The second infusion of histamine was performed without any nervous vasoconstrictor tone. In contrast to the preceding infusion, a slight decrease in the k-value was seen. After the infusion the k-value again returned to control level. A corresponding pattern of response was

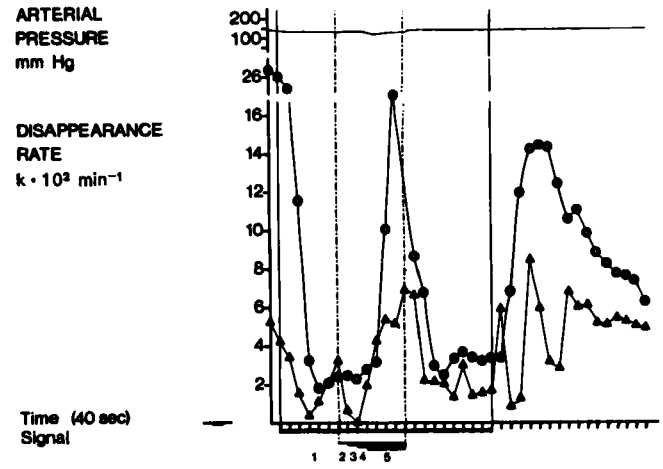

Fig. 3. Influence of i.a. acetylcholine infusion on the disappearance rate of I^{131} from the pulp (△) and I^{125} from the submucosa (○), when the cut sympathetic trunk was stimulated.

Pentobarbital.

1. Stimulation with 6V, 1 msec, 2 imp/sec.
2. Acetylcholine 0.18 μg/min.
3. Acetylcholine 0.30 μg/min.
4. Acetylcholine 0.52 μg/min.
5. Acetylcholine 0.94 μg/min.

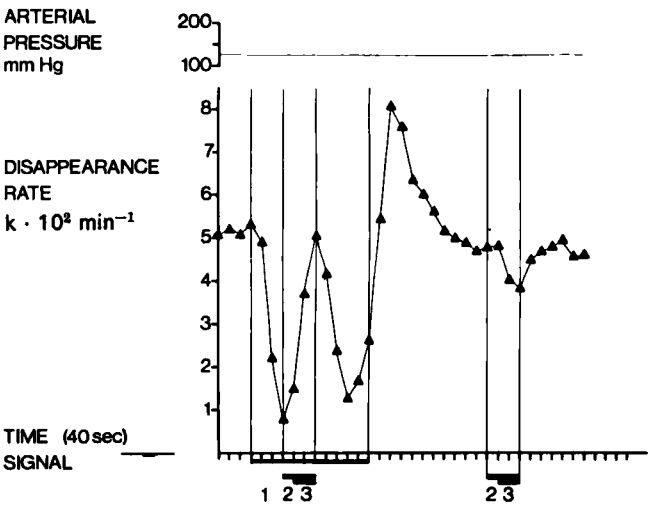

Fig. 4. Influence of i.a. histamine infusions at two different levels of vascular tone on tracer disappearance rate from the pulp.

Pentobarbital.

1. Sympathetic stimulation 6V, 1 msec, 3 imp/sec.
2. Histamine 1.65 μg/min.
3. Histamine 3.00 μg/min.

seen when bradykinin was used (Fig. 5). These results represent the typical changes observed when sympathetic stimulation was combined with infusion of acetylcholine (10 procedures, 5 cats), bradykinin (6 procedures, 2 cats) and histamine (7 procedures, 3 cats) and when the transected sympathetic trunk was not stimulated (10 procedures, 8 cats).

Infusion of papaverine and warming

The inability of histamine, acetylcholine and bradykinin to increase pulpal k-values when the sympathetic nervous tone was absent could be due to diffusion barriers preventing an increased exchange of tracer from the dentinal cavity to the pulpal capillary blood. To evaluate this possibility the following experiments were performed. Papaverine was infused intra-arterially when the sympathetic tone was absent, using the same technique as with the other drugs. At an infusion rate of 0.94 mg/min the pulpal k-value increased by about 60% (Fig. 6:1). This was shown in two

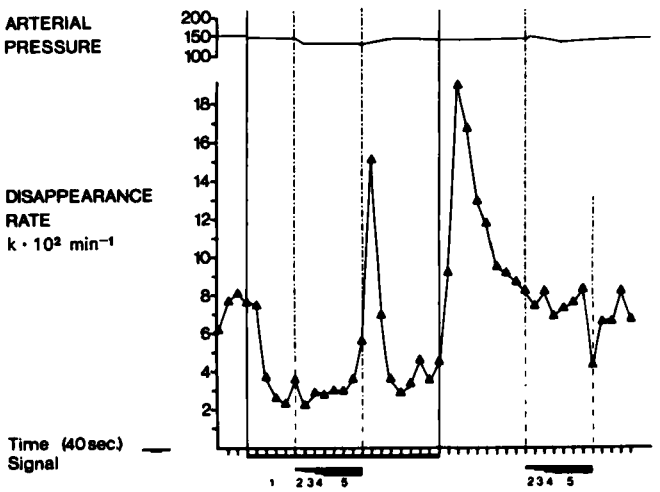

Fig. 5. Influence of i.a. bradykinin infusions at two different levels of vascular tone on tracer disappearance rate from the pulp.

Pentobarbital.

1. Sympathetic stimulation 6V, 1 msec, 2 imp/sec.
2. Bradykinin 0.18 μg/min.
3. Bradykinin 0.30 μg/min.
4. Bradykinin 0.52 μg/min.
5. Bradykinin 0.94 μg/min.

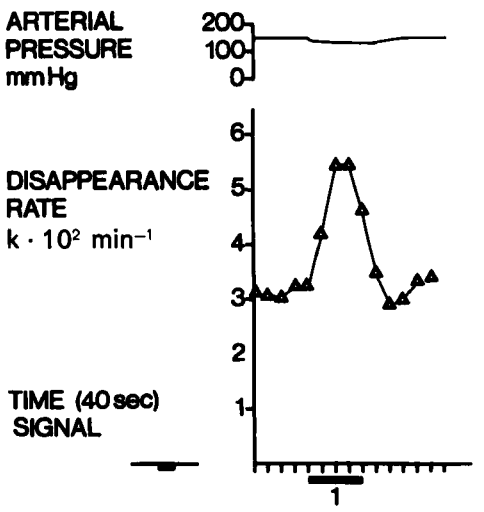

Fig. 6. Influence of i.a. papaverine infusion on the disappearance rate of I^{125} from the pulp when the sympathetic trunk was cut.

Chloralose-urethane.

1. Papaverine 0.94 mg/min.

procedures in one cat. Infusion of acetylcholine (not shown in the figure) in a high dose (5.2—9.4 μg/min), which was instituted after the last papaverine infusion, caused a slight increase (10—20%) in the pulpal k-value. However, this high dose of acetylcholine caused a fall in arterial pressure of about 50 mmHg. During the papaverine infusions the arterial pressure was unaffected or, in the second procedure, reduced by less than 10 mmHg.

Metabolic vasodilatation was induced by increasing the temperature of the thermode. Temperatures of the enamel surface and the dentinal cavity showed parallel increases. It was consistently found that an increase in temperature resulted in a pronounced elevation of pulpal k-values irrespective of whether the sympathetic trunk was intact or cut. In the range between 32° and 42°C the average increase in k-value was 250% or 25% per 1°C; this was shown in 4 procedures in 4 cats.

DISCUSSION

In the present study we have used a tracer disappearance technique (*Kety*, 1949) to measure changes in pulpal and submucosal blood circulation. Recent studies indicate that relative changes in disappearance rate (k-value) are closely related to changes in blood flow in capillaries and are also influenced by local factors affecting the exchange function in the capillary section (*Edwall*, 1971; *Scott Jr. et al.* 1972). Examples of such local factors are the number of perfused capillaries and the presence of barriers to diffusion located between the dentinal cavity and the pulpal capillary blood. In studies dealing with vasodilatation the influence of such local factors may be critical (*Bolme & Edwall*, 1971). Therefore, the experiments using papaverine and warming were conducted to illustrate that in the present experimental situation the pulpal k-value could respond to well-known vasodilator stimuli.

Furthermore, it should be stressed, that the iodide k-values are related to the exchange function of the vascular bed and, hence, are unaffected by increased blood flow through non-nutritional channels such as arterio-venous shunts. In the event of a redistribution of blood flow from shunts to exchange vessels the k-value would increse (cf. *Hultén, Jodal & Lundgren*, 1969). The influence of shunts in the dental pulp is probably of little importance for the k-values, since few shunts have been found histologically (*Provenza*, 1958; *Kramer*, 1960).

The observation that k-values increased above prestimulatory levels after sympathetic stimulation probably reflects a reactive hyperemia in agreement with findings on skeletal muscle (*Bolme & Edwall*, 1971). Direct measurement of venous outflow from isolated muscle

combined with tracer disappearance measurements showed that after sympathetic stimulation the k-values were increased during the transient reactive hyperemia. However, other factors than the blood flow may contribute to the magnitude of this increase of k-value. Such a factor is accumulation of tracer around previously constricted exchange vessels and, hence, facilitated transport of tracer during the reactive hyperemia.

The pattern of response to i.a. infusion of a vasodilator seen in Fig. 2 shows the good reproduceability of the effect in the submucosa, while pulpal blood flow was unaffected when the sympathetic trunk was intact. This lack of effect on pulpal blood flow following infusions of acetylcholine, histamine and bradykinin was consistent as long as the dose had little or no influence on systemic arterial pressure. When the dose of acetylcholine was increased 5—10 times, causing pronounced fall in arterial systemic pressure, a slight increase in pulpal blood flow was seen. This latter observation may support earlier reports that dental pulp pressure was increased following supramaximal doses of acetylcholine given i.v. as bolus injections (*Kawamura, Kato & Kato*, 1967; *Lemay-Laliberte & Simard-Savoie*, 1972).

However, when we increased the sympathetic tone in the oral vascular beds, acetylcholine, histamine and bradykinin had a marked vasodilator influence on pulpal and submucosal blood flow. These results show that pharmacological doses of these vasodilator substances inhibit an artificial pulpal vasoconstrictor tone. These observations, combined with the lack of response when the sympathetic trunk was intact, support earlier suggestions (*Edwall*, 1971) that the pulpal sympathetic nervous tone during resting conditions is low in the anesthetized cat.

Papaverine infusion and warming caused an increased blood flow when the sympathetic tone was low or absent. This indicates that papaverine and warming inhibit a tone which is of a different nature than the neurogenic tone. It seems reasonable to assume that bloodborne catecholamines from the adrenal medulla and other hormonal influences are insignificant for the preservation of this tone (cf. *Celander*, 1954; *Folkow & Neil*, 1971). Hence, this would suggest the presence of a local myogenic tone in the pulp, which can be inhibited by local metabolites produced by warming or by means of papaverine, which is known to completely relax vascular smooth muscle (*Lundgren & Mellander*, 1967).

The results of the present study indicate that acetylcholine, histamine and bradykinin, if present, may influence the vasomotor regulation in the pulp. This influence may also modulate the excitability of pulpal sensory neurons (*Edwall & Scott*, 1971). Their role in the local regulation of blood flow in the dental pulp remains to be elucidated.

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