Sodium acetylsalicylate and the role of prostaglandins in the mechanism of intradental pain

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In order to study the effect of sodium acetylsalicylate and the role of prostaglandins on intradental nerve impulse activity experiments were performed on the teeth of anaesthetized cats. Nerve impulse activity was induced by mechanical and chemical stimuli and recorded by means of electrodes inserted into dentinal cavities. It was shown that such activity could not be blocked by sodium acetylsalicylate or indomethacin given locally or i.v. PGE_2 failed to excite the sensory units when given locally (3.5 μg/ml) or intraarterially (35–140 ng/min) alone or in combination with mechanical and thermal stimuli or combined with local application of histamine (10 mg/ml) or bradykinin (10 mg/ml). Intraarterial infusion or arachidonic acid, a precursor to PGE_2, $PGF_{2\alpha}$, PGG_2 and PGH_2 failed to change the excitability even on applying local stimuli to the pulp or with local application of histamine or bradykinin. These findings seem to indicate that the increased sensitivity of the tooth to thermal stimuli seen during acute pulpitis is not due to formation of prostaglandins.

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It has been suggested that prostaglandins and their unstable intermediates are involved in the production of inflammatory pain by decreasing the threshold for different mechanical or chemical stimuli (*Vane*, 1972). Prostaglandins have been found in inflamed tissue (*Juhlin & Michaelsson*, 1969, *Crunkhorn & Willis*, 1971, *Goldyne et al.*, 1973, *Goodson et al.*, 1974). However, information on whether prostaglandins are present in inflamed dental pulp is still lacking. Likewise, it is not known whether prostaglandins increase the excitability of the intradental sensory neurons to mechanical or chemical stimulation.

Scott Jr. (1968) reported that the prostaglandin synthetase inhibitor acetylsalicylic acid could block intradental sensory nerve impulses induced by heat-stimulation, a finding indicating that prostaglandins might be involved in the production of intradental pain.

The present study was undertaken to determine if the nerve impulse activity induced in the tooth by mechanical or chemical stimuli could be blocked by inhibitors of prostaglandin synthesis. Therefore the influence of prostaglandins on the excitability of these neurons was also investigated.

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METHODS

Experiments were performed on 20 adult cats (2–4 kg and 1–3 years old) anaesthetized with chloralose (40 mg/kg i.v.) and urethane (50 mg/kg i.v.) or in some cases with sodium pentobarbital (30 mg/kg i.v.).

Nerve impulse recording

The experimental procedure used was simular to that described by *Haegerstam, Olgart & Edwall* (1975). Electrical potentials from intradental sensory nerves were recorded by means of platinum wire electrodes inserted in two deep dentinal cavities, one near the pulp horn (test cavity) and one near the gingival margin. Conventional techniques were used to display the signals on a cathode ray tube and to store the signals on analogue magnetic tape (*Edwall & Scott*, 1971). To test the depth of the cavity, hypertonic sodium chloride (90 mg/ml) has been used (*Olgart*, 1974).

Route of drug administration

Drugs were applied either locally to the test cavity or injected systemically. Intravenous (i.v.) injection was performed via a brachial vein. A branch of the external carotid artery (posterior auricular artery) was used for intraarterial (i.a.) infusions.

Temperature stimulation

The temperature of the tooth was continuously monitored using a thermocouple placed on the tooth surface. The normal value was 32 °C. A rapid increase in the temperature of the tooth, which constitutes a subthreshold stimulus (*Olgart, Haegerstam & Edwall*, 1974), was induced by means of a water-circulated thermode in contact with the crown of the tooth.

Pulpal lesion

A small pulpal exposure was made by deepening the test cavity. This treatment has been shown to result in sensory nerve activity, which increases upon a rapid rise in temperature (to 45 °C) (*Ahlberg & Edwall*, personal communication).

Drugs used

Sodium oxalate (65 mg/ml) and compound 48/80 (1 mg/ml) were dissolved in isotonic saline. Sodium acetylsalicylate (9 mg/ml and 150 mg/ml) and indomethacin (2.5 mg/ml) were dissolved in phosphate buffer and the pH was adjusted to 7.4 by means of sodium hydroxide (1N).

RESULTS

Treatment with prostaglandin synthetase inhibitors

A steady state nerve impulse activity of a high frequency was induced by a local application of compound 48/80 at a concentration of 1 mg/ml for one minute. This activity could not be reduced by sodium acetylsalicylate given by intravenous injection at a dose of 200 mg/kg or applied locally at a concentration of 9 mg/ml (10 experiments). The same negative result was obtained with the more potent prostaglandin synthetase inhibitor indomethacin applied locally (2.5 mg/ml) or given i.v. (5 mg/kg) (two experiments).

An early state of inflammation was induced by means of a pulpal exposure. In contrast to the standard preparation, such a tooth responded to heat stimulation with nerve impulse activity or showed a nerve impulse activity of low frequency during resting conditions. Neither nerve impulse activity due to pulpal exposure nor the activity due to a subsequent heat stimulus was affected by the local application of sodium acetylsalicylate (9 mg/ml) (three experiments).

In order to induce a nerve impulse activity similar to that reported by *Scott* (1968), sodium oxalate (65 mg/ml) was applied locally to the tooth. As can be seen in fig. 1, this activity was strongly reduced by local application of isotonic NaCl solution as well as by sodium acetylsalicylate solution (150 mg/ml).

Treatment with PGE_2

PGE_2 was applied locally in the test cavity in order to see if nerve impulse activity was induced. In three experiments it was shown that PGE_2 at a concentration of 3.5 µg/ml did not induce any nerve impulses.

Moreover, it was shown in four experiments that close intraarterial infusion of PGE_2 at a rate of 35 or 140 ng/min to the tooth did not induce any impulse activity.

In order to see if a subthreshold stimulus could induce nerve impulses during infusion of prostaglandins, a rapid increase of the temperature of the tooth surface to 50 °C was brought about (*Haegerstam*, 1976). It was found that a combination of this subthreshold stimulus with an intraarterial infusion of PGE_2 (35 ng/min) failed to induce nerve impulse activity (one experiment). Rapid decreases in the temperature to 20, 15 or 0 °C were also used as stimuli. A rapid decrease of the temperature of the tooth to 15 °C gave rise to some nerve impulses in contrast to stimulation to 20 °C and 0 °C. During PGE_2 infusion (35 ng/min) there was, however, no response to either 20 °C, 15 °C or 0 °C (seven experiments).

Local application of bradykinin at a concentration of 10 mg/ml induced no nerve impulses (one experiment) which is in agreement with earlier studies (*Olgart*, 1974). In order to see if bradykinin in combination with PGE_2 was able to induce any nerve impulses, the former drug was applied locally at a concentration of 10 mg/ml and PGE_2 was given as a close intraarterial infusion at a rate of 140 ng/min. However, no response could be seen (two experiments). Similar negative results were obtained with histamine (10 mg/ml locally) alone or in combination with PGE_2 (140 ng/min i.a.) (one experiment).

Intraarterial infusion of arachidonic acid

Since the lack of effect of PGE_2 might be explained by its inactivation in the blood, the precursor acid was infused at high concentrations. It was shown that intraarterial infusion of arachidonic acid at a rate of 0.1 to 0.5 mg/min induced no nerve impulse activity. Rapid changes in the temperature of the tooth (to either 45 °C or 0 °C) during arachidonic acid fusion (two experiments each) also failed to induce nerve impulses. Furthermore local applications of bradykinin (1 mg/ml) or histamine (1 mg/ml) during arachidonic acid infusion were unable to stimulate intradental sensory units (four experiments).

DISCUSSION

It has previously been shown that impulse activity recorded with the present technique originates from intradental sensory neurons (*Arwill et al.*, 1973). The present experiments show that nerve impulse activity induced in different ways in the tooth of the cat was found to be unaffected by local or systemic application of drugs known to inhibit prostaglandin synthesis. Local application to the tooth cavity of PGE_2 as well as intraarterial infusion of PGE_2 or arachidonic acid failed to induce nerve impulse activity. Intraarterial administration to the tooth of these agents also failed to influence the response to thermal stimulation as well as to local application of histamine or bradykinin.

Scott Jr. (1968), using essentially the same recording technique as in the present study, reported that acetylsalicylic acid blocks the response to heat in a preparation with «spontaneous» activity. To induce such an activity, compounds which reduce the extracellular calcium concentration, such as sodium citrate, were applied. In the present study it could

be shown that nerve impulse activity induced in this way can be reduced not only by sodium acetylsalicylate but also by NaCl (9 mg/ml) (Fig. 1) indicating that the effects seen by Scott Jr. were due to changes in the ionic environment rather than to specific inhibition.

The analgesic effect of the salicylates has been shown to be due to inhibition of prostaglandin synthesis (*Flower & Vane*, 1974). It is unlikely that the pulp should lack prostaglandin synthetase since it has been found that enzymes in the microsomal fraction of most mammalian cells catalyse the formation of prostaglandins (*Flower & Vane*, 1974). The possibility that the prostaglandins do not influence the excitability of the sensory nerves in the tooth seems more likely.

The finding that PGE_2 did not induce any nerve impulses when applied to the tooth is in agreement with results obtained on the blister base (*Horton*, 1963) and following intradermal injections of PGE_2 (*Crunkhorn & Willis*, 1971). On the other hand, the precursor arachidonic acid has been reported to cause «strong» pain following intradermal injection which may be due to formation of active hydroperoxides (*Ferreira*, 1972). In the present study, arachidonic acid injected intraarterially to the tooth, did not induce any nerve impulses. Not only hydroperoxides but also endoperoxided such as PGG_2 and PGH_2 are formed from arachidonic acid as well as prostaglandins such as PGE_2 and $PGF_{2\alpha}$ (*Hamberg et al.*, 1974). However, this formation requires the presence of the necessary enzymes. As mentioned earlier it is not known whether these enzymes are present in the tooth.

Vane (1972) reported that intradermal injection of prostaglandins of E-type in combination with mechanical stimulation or with bradykinin or histamin injection resulted in strong pain. However, in the present study there was no sign of sensitization to either chemical or mechanical stimulation during PGE_2 infusion. However, the possibility remains that the infused PGE_2 had not reached the tooth or that it was inactivated by enzymes in the blood. Since arachidonic acid is the natural precursor to PGE_2, this acid was also infused. No response was seen to any of the stimuli applied during arachidonic acid infusion, suggesting that prostaglandins are either not formed in the dental pulp or do not influence the excitability of the intradental sensory nerves.

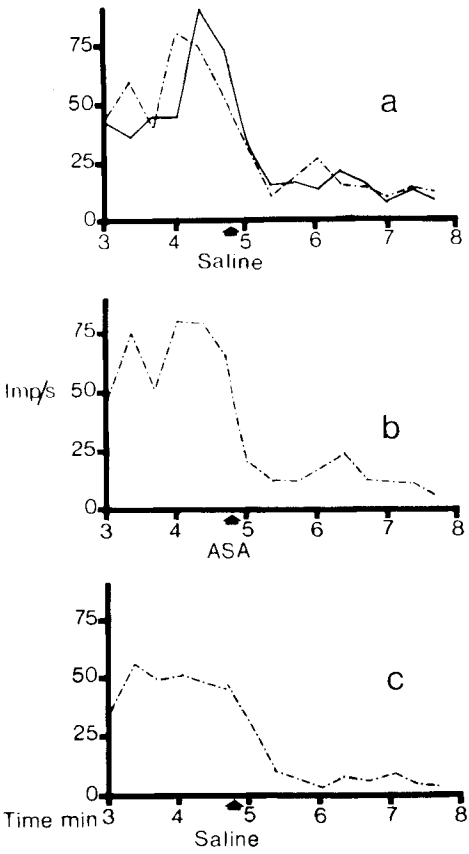

Fig. 1. Nerve impulse activity induced by local application of sodium oxalate (65 mg/ml) in four procedures in the same tooth. In two procedures (1) the activity was reduced by NaCl (9 mg/ml) (saline). In the following procedure (b) the nerve impulse activity was reduced by local application of sodium acetylsalicylate (150 mg/ml) (ASA). In the last procedure (c) the oxalate induced activity was reduced by local application of NaCl (9 mg/ml).

In conclusion, the present results indicate that nerve impulse activity in the tooth induced in different ways cannot be blocked by sodium acetylsalicylate. In addition prostaglandins or arachidonic acid could not increase the excitability of the sensory nerves in the tooth. These data seem to indicate that the increased sensitivity of the tooth to thermal stimuli seen during acute pulpitis is not due to formation of prostaglandins.

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