Responses of feline intradental sensory nerves to hyperosmotic stimulation of dentin

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The responses of intradental sensory nerves to hyperosmotic solutions of sucrose (4 M) and calcium chloride (6 M) applied in dentinal cavities were studied in anesthetized cats. Nerve impulse activity was recorded from canine teeth after application of the test solutions in shallow and deep cavities. In shallow cavities (thickness of remaining dentin, about 500 μm) sucrose and calcium chloride caused an immediate and transient excitation of the nerves in 3 out of 15 teeth and in 8 of 12 teeth, respectively. Treatment of such cavities with lactic acid (1 M) increased the frequency of nerve responses to 100%. When applied in deep cavities (thickness of remaining dentin, 0–50 μm), sucrose induced a burst of impulses followed by continuous nerve activity, whereas calcium chloride decreased the nerve excitability. Our results support the hypothesis that solutions exerting an effective osmotic pressure excite the intradental nerves by an indirect mechanism when applied on the dentin and that they exert a direct effect on nerves when in contact with the pulp. In addition, it is suggested that acids produced in carious dentin may facilitate the induction of pain by hyperosmotic stimuli.
☐ *Cat; calcium chloride; dental pulp; nerve activity; sucrose*

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It is a common clinical observation that sugar solutions in contact with unprotected dentin may give rise to pain. Clinical trials have shown that hyperosmotic solutions of sugar or calcium chloride may induce pain when introduced into freshly prepared dentinal cavities in human subjects (2).

The mechanisms by which such pain is elicited are not completely understood. The most accepted theory, proposed by Brännström (5), states that osmotic stimuli applied on dentin produce pain indirectly by inducing rapid outward movements of the fluid present in dentinal tubules. This may result in a mechanical distortion and excitation of intradental sensory nerves located among the odontoblasts and in the pulp (6). This theory is indirectly supported by histological studies, which have failed to reveal nerve structures in the outer dentin (7, 30), whereas nervous profiles have been found in the innermost dentin and in the pulp (9, 12, 18).

According to another theory, pain-inducing stimuli applied on the dentin directly excite sensory nerves located in the dentinal tubules (for references, see Ref. 19), and there is some histological evidence that the outer layers of the dentin are innervated (28).

In contrast to what has been shown in human studies, Horiuchi & Matthews (15) found, by recording intradental nerve activity from canine teeth in cats, that dextrose solutions did not induce nerve activity unless they were applied in deep cavities or onto the pulp. They suggested that such hyperosmotic solutions do not excite the intradental nerves in animal teeth indirectly by a hydrodynamic mechanism but rather have a direct effect on nerve endings located in the inner dentin or the pulp. However, more recent results (21) indicate that intradental nerves in dogs can be excited indirectly by a hydrodynamic mechanism, since nerve activity was recorded from the inferior alveolar nerve after removal of fluid from the dentin surface by using absorbent paper.

In the present study, using a technique for recording of intradental sensory nerve

activity from feline teeth, we investigated the nerve responses to hyperosmotic solutions of sucrose and calcium chloride applied on dentin at different distances from the pulp. Since it has been shown that the incidence of pain reported after stimulation of human dentin depends to a large extent on the properties of the dentinal tubules (17), we also studied the influence of treatment of the dentin surface with lactic acid, the acid commonly found in carious decay, on the response to the hyperosmotic solutions.

Methods

The experiments were performed on cats (2–4 kg and 1–2 years old) of either sex, anesthetized with chloralose (40 mg/kg) and urethane (50 mg/kg) intravenously, with supplement as necessary. The trachea was cannulated and the blood pressure was recorded from a femoral artery. The body temperature was maintained at 38°C by heating lamps. The jaws were immobilized by means of a steel rod and dental acrylic.

Intradental nerve impulse activity was recorded by inserting platinum electrodes into two dentinal cavities prepared in the canine teeth. The cavities (recording cavities), one prepared over the pulp horn and the other within the cervical part of the crown, were filled with either isotonic saline solution or electrode paste (25). The amplified signals were displayed on an oscilloscope and fed into equipment for frequency analysis (10). The potentials obtained by this method originate from a small group (up to five units) of intradental sensory fibers (13). Such nerve activity recorded from human teeth is associated with the sensation of pain (11).

The test solutions were applied in a third dentinal cavity (stimulation cavity) prepared over the pulp horn on the side opposite the incisal recording cavity (25). The stimulation cavities were divided into two categories—shallow and deep. In shallow cavities, in which the preparation just exceeded the dentino-enamel junction, the thickness of the remaining dentin beneath the cavity was 500–700 μm, as measured after the experiment. In deep cavities the underlying pulp was clearly visible and the remaining dentin 0–50 μm thick. In most of the deep cavities a small pulp exposure was made to ensure a rapid diffusion of the test solutions into the pulp. In such cavities a spontaneous nerve activity of low frequency was always present.

The hyperosmotic solutions tested were 4 M sucrose and 6 M $CaCl_2$. These concentrations were chosen because of their pain-inducing effect in clinical trials (1–3). A solution of NaCl (0.76 M) was always applied in the stimulation cavity before the test solutions were applied. This solution exerts a low effective osmotic pressure across dentin (27) and acts as a stimulus only when contact with nervous elements is established (22). In most cases only one of the test solutions was used in a given tooth. Each application of the hyperosmotic solutions lasted for 2 min. Then the cavity was thoroughly washed with isotonic saline and subsequent applications took place after intervals of 3 or 15 min. All substances were dissolved in isotonic saline. Approximately 0.2 μl of each solution was introduced into the stimulation cavity.

Results

Shallow cavities

The responsiveness of each preparation was tested by briefly applying a cotton pellet soaked in ethyl chloride to the tip of the tooth. Only when this cold stimulation gave rise to a burst of nerve impulses were the recording conditions considered suitable, and testing of the hyperosmotic solutions started. Otherwise the recording cavities were deepened until a response could be obtained. Application of 0.76 M NaCl in shallow cavities consistently failed to induce nerve activity, even if the solution remained in the cavity for 10 min. However, when 4 M sucrose was applied in such cavities in 3 out of 15 teeth (20%), it generated an immediate and transient burst of impulses, which lasted for approximately 30 sec. After this period there was no further response, even if the solution remained in the cavity. On a second

application of the sucrose solution in the same cavity some minutes later the nerve response was attenuated, and after a third application little or no response was obtained (Fig. 1a). In the other 12 teeth sucrose had no effect.

Application of 6 M $CaCl_2$ produced essentially the same effect as sucrose, namely a burst of impulses during the first 30 sec of the 2-min application. However, it was a more effective stimulus than sucrose, and it produced a response in 8 of the 12 teeth tested (67%). The nerve response declined on successive applications at 3-min intervals also in the case of $CaCl_2$ (Fig. 2a).

Acid treatment of shallow cavities. To investigate the influence of acid treatment of dentin on the nerve response to osmotic stimuli the following experiments were performed.

In freshly prepared cavities (seven teeth in five animals) 4 M sucrose was applied twice with a 3-min interval between applications. A nerve response was obtained in one of the teeth. Lactic acid (1 M) was then introduced into the cavity for 2 min, and 5 min after its removal the sucrose applications were repeated. In contrast to the untreated cavities, sucrose application in acid-treated cavities always generated a burst of nerve impulses (Fig. 3). The magnitude of the response was also much greater than that previously obtained in untreated cavities. Similar results were obtained when 6 M $CaCl_2$ was used as test solution (6 teeth in 3 cats) (Fig. 3). The immediate onset, the

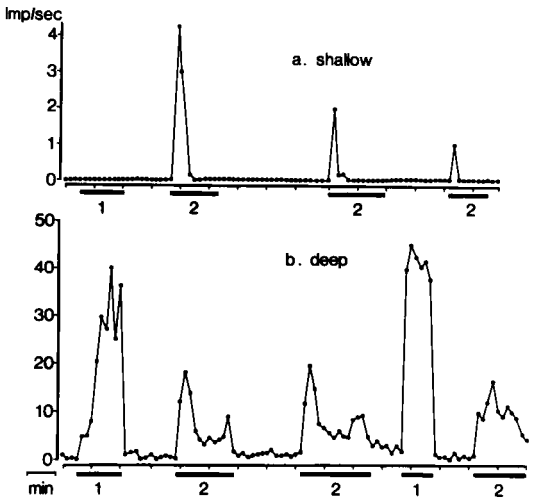

Fig. 1. Effect of 4 M sucrose (2) and 0.76 M NaCl (1) applied in shallow (a) and deep (b) cavities.

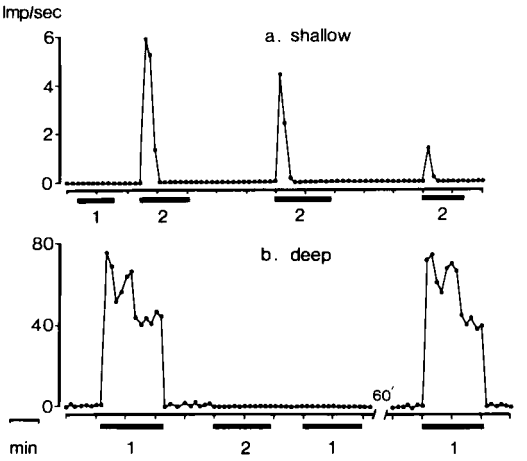

Fig. 2. Effect of 6 M $CaCl_2$ (2) and 0.76 M NaCl (1) applied in shallow (a) and deep (b) cavities.

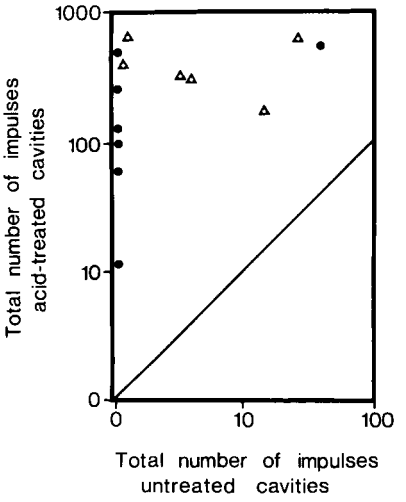

Fig. 3. Relationship between nerve activity (total number of impulses/2 min) obtained before and after acid treatment of the cavities using 4 M sucrose (●) and 6 M $CaCl_2$ (▲) as stimulus. Each point (mean nerve activity during two applications) represents the responses obtained by one solution in one tooth. The scale of both ordinate and abscissa is logarithmic. The straight line indicates the distribution of points if the solution evoked identical responses before and after acid treatment of the cavities.

short duration and the attenuation of the nerve response upon repeated applications remained unaffected by the acid treatment.

Changes in the interval between applications. To try to find an explanation for the observed attenuation in nerve response, the time interval between successive applications of the osmotic solutions was increased. In seven acid-treated cavities 4 M sucrose was applied four times, with a 15-min interval between each application. This experimental design resulted in an almost identical nerve response to each sucrose application (Fig. 4).

A similar series of experiments (six teeth in three cats) was performed with 3-min intervals between each application. The nerve response to consecutive sucrose applications declined, as previously reported (Fig. 4). After a resting period of 1 h, the responsiveness to sucrose had recovered in four of the teeth. When sucrose was reapplied four times at 15-min intervals in these teeth, no further attenuation of the nerve response was observed. Similar findings were obtained in four teeth when $CaCl_2$ was used.

Deep cavities

In all deep cavities application of 0.76 M NaCl induced nerve activity within seconds (generally less than 30 sec) after its introduction into the cavity. The application of 4 M sucrose resulted in an immediate burst of impulses, followed by a period of continuous activity of lower frequency, which lasted as long as the solution remained in the cavity. Successive sucrose applications generated nerve responses of a similar magnitude (six teeth in three cats). The nerve activity induced by 0.76 M NaCl was not influenced by the sucrose application (Fig. 1b).

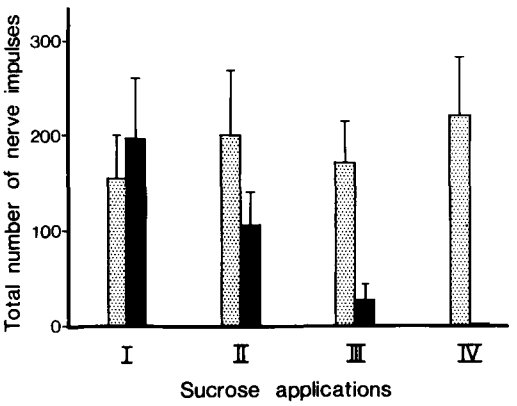

Fig. 4. Nerve activity induced by 4 M sucrose applied in acid-treated shallow cavities. Four applications were used with 3-min (filled bars) (no. = 6) or 15-min (stippled bars) (no. = 7) interval between each trial. Each column represents the total number of nerve impulses (mean ± SEM) induced by the test solution during 2-min application in the cavities.

A different effect was obtained with 6 M $CaCl_2$. When applied in deep cavities, it produced no nerve impulse response. On the contrary, it reduced the nerve excitability, as revealed by the failure of a subsequent application of hypertonic NaCl to induce nerve activity (five teeth in two cats). When 0.76 M NaCl was reapplied 1 h after the removal of $CaCl_2$, it induced a nerve response similar to the initial control (Fig. 2b).

Discussion

The fact that different patterns of nerve activity were induced by sucrose and $CaCl_2$ when applied on dentin and pulp, respectively, implies that they have at least two mechanisms of action on intradental nerves.

When there was a considerable layer of dentin beneath the cavities, both hyperosmotic solutions induced an immediate and transient burst of impulses but no further activity. These results are in agreement with findings obtained in human experiments showing that solutions of sugar and $CaCl_2$ applied on dentin may cause pain felt by the subjects as soon as the solutions are placed in the cavity (1). Our findings and those obtained in human experiments do not support the idea that excitable nerve endings exist close to the dentino-enamel junction. If this were the case, hypertonic NaCl should generate nerve impulses, since in the concentration used it is known to excite the intradental nerves when applied in close contact with them (22, 24). Furthermore, if nerves existed in the outer part of the dentin, $CaCl_2$ would not have induced nerve activity, as it did in our studies. On the contrary, it

would reduce the nerve excitability, as was demonstrated in the present study using deep cavities. Thus, it seems likely that hyperosmotic solutions applied on peripheral dentin excite the intradental nerves by an indirect mechanism. It has been shown that such solutions have the ability to remove fluid from the apertures of the dentinal tubules. Thus, in in vitro experiments conducted on human teeth it was shown that hyperosmotic solutions applied on dentin induce a displacement of fluid from the pulp to the cavity (20). Such a rapid hydrodynamic action may mechanically excite the sensory nerves located in the pulpo-dentinal zone (8). Similarly induced flow rates in vitro were found to be maximal 0.5–2 min after application of the solutions (14). Since both nerve activity recorded in animals and pain in humans start almost immediately after the application of hyperosmotic solutions on dentin, it is probable that in vivo the flow rate in the dentinal tubules reaches its maximum quicker than estimated in in vitro experiments. The short-lasting nerve activity also indicates that the effective fluid flow is very time-limited. However, technical limitations have so far precluded measurements of fluid flow in dentinal tubules in vivo.

According to our results the nerve response can be facilitated by acid treatment of the dentin. In freshly prepared but otherwise untreated cavities the limited ability of hyperosmotic solutions to excite intradental nerves is in accordance with the findings of Anderson & Matthews (2) that sugar solutions induced pain in approximately 50% of the teeth tested. The dramatic elevation of the nerve response frequency to 100% after acid treatment of the dentin as well as the increased magnitude of the response found in the present experiments is consistent with previous findings that similar treatment with acids potentiates the pain response after application of $CaCl_2$ on exposed human dentin (17). It has been shown that acid treatment removes the debris from the tubule apertures (16), increases the hydraulic conductance of dentin (29), and decreases the resistance to fluid flow in human dentin in vitro (26). These results provide evidence that in acid-treated cavities the outward fluid flow brought about by hyperosmotic solutions is more pronounced than in untreated ones, and consequently the resulting nerve activity (and pain) is enhanced.

The attentuated nerve response upon consecutive applications at short intervals (3 min) of either sucrose or $CaCl_2$ is consistent with the findings of Anderson et al. (3) that on successive applications of similar hyperosmotic solutions on human dentin, at 3-min intervals, the experienced pain steadily declined. They concluded that such decreased sensitivity could be due to alterations in either the dentin subjected to stimulation or in the subjacent pulp. Alterations in dentinal fluid composition can be expected to occur as a result of the experimental procedure. Despite repeated washings of the test substance from the cavity, some test solution may still remain in the dentinal tubules. Consequently, in the case of sucrose or $CaCl_2$ the osmotic pressure exerted by these solutions will be less pronounced during the second application than during the first one. Thus, the fluid-withdrawing action of the applied solutions will diminish, and the resulting nerve response (and pain) will also decline on repeated applications. Such an exhaustive mechanism may also account for the brevity of the period of nerve activity induced by the hyperosmotic solutions. This explanation is supported by our findings that the decline in nerve activity on successive applications of hyperosmotic solutions could be prevented by repeated washing and increasing the interval between applications from 3 to 15 min. Evidently, this type of stimulation produces no damage to the excitable structures, since reduced responses recovered when the interval between stimulations was increased.

The pattern of nerve responses observed after application in cavities prepared close to the pulp was different from that observed in studies of shallow cavities. When applied in deep cavities, both hyperosmotic solutions could rapidly reach the sensory nerves, as judged by the immediate nerve response to hypertonic NaCl. The decrease in nerve excitability caused by $CaCl_2$ when applied in such cavities is in agreement with earlier

findings by Olgart et al. (23). It is well documented that Ca^{++} decreases the nerve excitability (4), and this direct effect evidently counteracted the previously demonstrated capability of $CaCl_2$ to induce nerve activity indirectly when applied in shallow cavities.

The pattern of nerve response to sucrose applied in deep cavities was also different from that observed in shallow cavities. However, the first phase of the response, consisting of an immediate and transient burst of impulses, was similar to that seen in shallow cavities and is probably initiated by a fluid movement in the remaining dentin caused by an osmotic pressure gradient. The late part of the response—the period of continuous activity which lasted for as long as the solution remained in the cavity—is probably the result of a direct effect of sucrose on the intradental nerves. Horiuchi & Matthews (15), who found that dextrose applied in deep dentinal cavities in cats occasionally excited the intradental nerves, suggested that sugar solutions may have a direct chemical effect on receptors situated in the inner dentin or pulp. It is possible that sucrose excites the nerves directly by exerting osmotic pressure on the nerve membrane or on the adjacent tissue.

In conclusion, the present results provide functional evidence that hyperosmotic solutions excite the intradental sensory nerves in the cat. This action is different in shallow and deep dentinal cavities. It is suggested that application on a thick layer of remaining dentin produces a response by an indirect excitatory mechanism, thus favoring the hydrodynamic hypothesis proposed by Brännström (5). In deep dentinal cavities they exert, in addition, a direct effect on the nerves. The results obtained with lactic acid suggest that organic acids produced and accumulated in carious dentin may facilitate the indirect excitatory action of hyperosmotic stimuli and may partly explain the hypersensitivity of carious teeth to sugar solutions that frequently precedes symptoms from pulpitis.

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