Effects of ammonia and organic acids on the intradental sensory nerve activity

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Ammonia and organic acids constitute a major part of the bacterial metabolites formed in carious decay. The aim of the present study was to investigate their effect on the intradental sensory nerves. Nerve impulse activity was recorded from canine teeth in cats after application of the test solutions in deep dentinal cavities. Ammonia (17–134 mM) consistently generated nerve impulses, whereas organic acids (0.001–1 M) failed to induce any impulse activity. In contrast, acid application resulted in an inhibition of the ongoing nerve activity induced by various stimuli (hypertonic NaCl solution, mechanical pulp exposure, and compound 48/80). However, acid treatment of the cavities resulted in an enhanced neural response to ammonia stimulation. Thus, the present results demonstrate that these bacterial metabolites can influence intradental sensory nerve activity. It is suggested that they may also modulate the symptoms from decayed teeth. □ *Dental pulp; nerves; dental caries; bacterial metabolites*

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A variety of bacterial metabolites have been found in dental plaque and carious dentin. Ammonia, certain amino acids, several enzymes, and various organic acids are produced and accumulated in carious tissues (13, 24, 25, 27, 30, 33, 40).

In vitro studies indicate substantial penetration of these products through the dentinal tubuli into the pulp (39). The observation that organic acids facilitate this invasion by widening the tubuli (4, 22) strengthens the hypothesis that bacterial metabolites can reach the pulp, thus influencing its functions. Despite the well-known pain-inducing properties of various bacterial metabolites, such as ammonia and lactic acid, on skin and muscles (23), the influence of these metabolites on intradental nerves is still obscure. Ahlberg (1) reported that extracts of human dental plaque or extracts from culture of *Streptococcus sanguis*, a common bacterial strain in dental plaque (7) and in carious dentin (12), induced increased nerve activity when applied in dentinal cavities in feline canine teeth, after a delay of 30 min to 5 h. These findings suggest that various metabolites produced by bacteria commonly found in carious dentin may modulate the intradental sensory nerve activity.

In the present study the effect of ammonia and organic acids on the intradental nerves was examined by recording nerve impulses from the dentin in accordance with the method of Edwall & Scott (10).

Materials and methods

The experiments were carried out on adult cats (2–5 kg) of both sexes, anesthetized with chloralose (40 mg/kg) and urethane (50 mg/kg) intravenously, supplemented as required. The trachea was cannulated, and blood pressure was recorded from the femoral artery. The body temperature was kept constant at about 38°C by heating lamps. The jaws were immobilized by means of a steel rod and dental acrylic.

The experimental set-up is shown in Fig. 1. Intradental nerve impulse activity was recorded from canine teeth by inserting platinum electrodes into two dentinal cavities (recording cavities). One cavity was prepared at the tip of the crown and the other

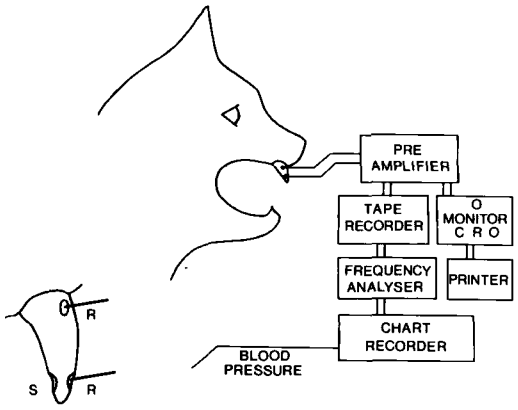

Fig. 1. Diagram of the experimental set-up showing the position of the electrodes in the tooth and their connection to recording arrangement. Enlargement of the canine tooth (lower left side) shows recording cavities and electrodes (R) and stimulation cavity (S). Electrodes are in electrical contact with the pulp via the dentin.

within the gingival part. The cavities, where the remaining dentin was approximately 0.5 mm thick, were filled with electrode paste (Mingograf®, Elema–Schönander, Sweden) or in some cases with isotonic saline. The amplified signals were displayed on a cathode ray tube and fed into equipment for frequency analysis (10). The potentials obtained by using this method have been shown to originate from intradental small myelinated fibers in the cat (3, 17, 18) and to be associated with the sensation of pain in man (11).

The test solutions were introduced in a third dentinal cavity (stimulation cavity) prepared at the pulp horn on the side opposite from the recording cavities (Fig. 1). The pulp was visible through a thin layer (50–200 μm) of dentin, but no attempt was made to standardize the depth of the cavities. Each test solution remained in the stimulation cavity for 2 min, unless otherwise stated. After removal of each test solution, the cavity was washed thoroughly with isotonic saline, and the next solution was applied.

The following substances were tested: ammonia solutions (17–134 mM), lactic acid (0.001, 0.01, 1 M), acetic acid (0.001, 0.01, 1 M), pyruvic acid (0.1, 1 M), butyric acid (1 M), isobutyric acid (1 M), valeric acid (1 M), isovaleric acid (1 M), and propionic acid (1 M). The pH of the acid solutions ranged from 2.0 to 4.0. Acid mixture (1:1, pH 1.9) was prepared from equal parts of lactic (1 M), acetic (1 M), propionic (1 M), and butyric (1 M) acids. Acid mixture (1:10, pH 2.2) was prepared by diluting the acid mixture 1:1 with isotonic saline ten times.

All the agents were dissolved in isotonic saline. A volume of approximately 0.2 μl of each test solution was introduced into the stimulation cavity.

Results

In general, in freshly prepared teeth, with the stimulation cavity filled with isotonic saline, no spontaneous nerve activity was recorded.

Four solutions of ammonia (17, 34, 67, and 134 mM) were applied in the stimulation cavity in ascending order of concentration. As can be seen in Fig. 2, which illustrates results obtained from six teeth (three cats), all ammonia concentrations evoked nerve impulse activity; in each tooth more concentrated solutions generated more impulses than less concentrated ones. These findings were consistently observed in all experiments. In another series of experiments (12 teeth in 4 animals) after the first sequence of applications had been completed, isotonic

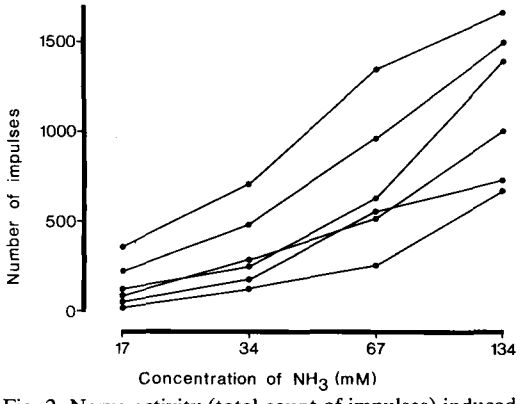

Fig. 2. Nerve activity (total count of impulses) induced by ammonia solutions in different concentrations (17, 34, 67, and 134 mM). Each solution was applied in the stimulation cavity for a period of 2 min. Data obtained from six teeth are shown.

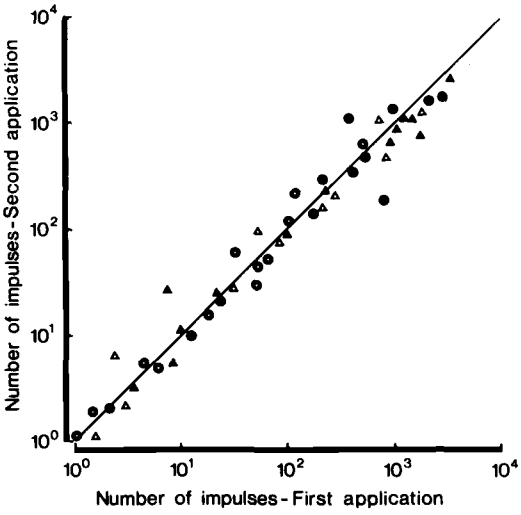

Fig. 3. Relationship of the nerve activity (total number of impulses/2 min) induced by ammonia solutions in different concentrations during two successive applications. Each point represents the response obtained by one concentration of ammonia in one tooth (○ = 17 mM; △ = 34 mM; ● = 67 mM; ▲ = 134 mM). Data obtained from 12 teeth. The scale of both ordinate and abscissa is logarithmic. The straight line indicates the distribution of the points if the solutions had evoked identical responses during the two applications.

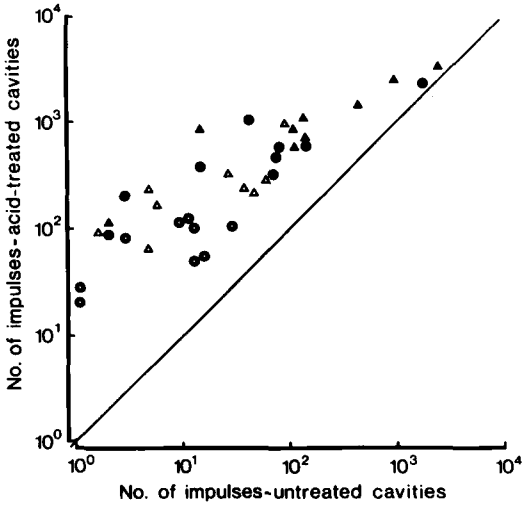

Fig. 4. Relationship of the nerve activity (total count of impulses/2 min) induced by ammonia solutions in different concentrations when applied before and after acid treatment of the stimulation cavities. Each point represents the response obtained by one concentration of ammonia in one tooth (○ = 17 mM; △ = 34 mM; ● = 67 mM; ▲ = 134 mM). Data obtained from nine teeth. The scale of both ordinate and abscissa is logarithmic.

saline was left in the cavity for 4 min, and then the whole series was repeated. Fig. 3 shows the pooled results obtained from these experiments. The number of nerve impulses obtained by each solution during the second round of applications was similar to the corresponding values recorded in the first one. Statistical evaluation of the results did not reveal any significant difference (Wilcoxon rank sum test, $p > 0.1$).

In a similar series of experiments (13 teeth in 6 animals) a solution of acid mixture (1 : 1) was applied in the stimulation cavity for 4 min between the first and the second application of the ammonia solutions. As can be seen in Fig. 4, in nine teeth such a procedure resulted in an enhanced neural response to all solutions of ammonia. The difference in the number of impulses generated by any solution when applied before and after acid treatment of the cavity was statistically significant (Wilcoxon rank sum test, $p < 0.01$). However, in two cavities with pulp exposure and in two cavities treated with acids before the beginning of the experiments, no potentiation was observed (not included in Fig. 4).

In contrast to ammonia, all the organic acids tested failed to induce any nerve impulse activity. This was a consistent finding observed both in teeth exhibiting no spontaneous activity (12 teeth in 4 animals) and in teeth with ongoing activity due to pulp exposure (6 teeth in 2 animals). In contrast, application of acids in the latter case decreased such an activity.

To investigate further this inhibitory effect of organic acids, three types of stimuli with different modes of action were used to induce nerve impulse activity. An acid mixture containing four of the commonest acids in carious tissues—lactic, acetic, butyric, and propionic acids (25)—was used as a test solution.

In one series of experiments (six teeth in five animals) the stimulation cavity was deepened to expose the pulp. This resulted in nerve impulse activity of relatively low

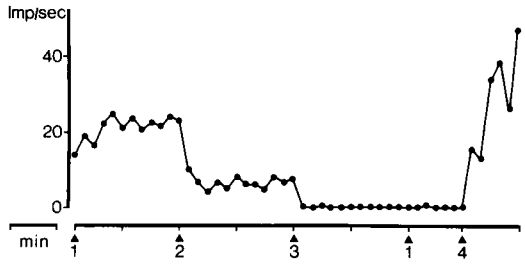

Fig. 5. The effect of organic acids on intradental nerve activity induced by mechanical pulp exposure. Local application in the stimulation cavity. 1 = Isotonic sodium chloride; 2 = 1:10 acid mixture; 3 = 1:1 acid mixture; 4 = sodium chloride (0.76 M).

frequency (less than 20 impulses per second). Such activity, which has been suggested to be due to pulp damage following tooth preparation (34) was always unchanged by subsequent washing with isotonic saline. However, local application of 1:10 acid mixture reduced the activity, and 1:1 acid mixture abolished it (Fig. 5, Table 1). No spontaneous activity could be recorded after replacement of the test solution with saline, but the intradental nerves responded normally to hypertonic 0.76 M NaCl stimulation, indicating that the nerves were still excitable.

Hypertonic sodium chloride solutions excite the intradental nerves by increasing the Na^+ concentration in the vicinity of the nerves (21, 35). Thus, in another group of experiments (12 teeth in 6 animals) attempts were made to block such activity by applying acid mixtures combined with hypertonic solution. The solution containing 0.76 M NaCl with acid mixture 1:10 consistently induced less impulse activity than a solution of 0.76 M NaCl alone (mean decrease, 73%). Furthermore, the mixture consisting of 0.76 M NaCl together with acid mixture 1:1 practically failed to evoke neural responses (Table 1).

In a third series of experiments (nine teeth in five animals) nerve activity was induced by compound 48/80, an agent suggested to excite the intradental nerves by releasing biogenic substances, such as serotonin (5-HT) (36, 37). Compound 48/80 (1 mg/ml) was applied locally in the stimulation cavity, and stable nerve impulse activity was established after 4 min. This long-lasting activity remained unaffected when the agent was replaced by isotonic saline, whereas it was remarkably reduced after local administration of 1:10 acid mixture and abolished when 1:1 acid mixture was placed in the cavity (Table 1). The activity increased again after replacement of the acid solution with isotonic saline.

Discussion

The results of the present study show that the intradental sensory nerves are excited by ammonia, whereas organic acids suppress nerve activity. In addition, acid treatment of dentinal cavities results in an enhanced neural response to ammonia.

The excitatory effect of ammonia on the intradental nerves is in accordance with its algogenic effects on other tissues. Applications of ammonia on mucous membranes, its injections into the skin (29), and swal-

Table 1. Inhibition of nerve activity (in percentage of control) induced by 0.76 M NaCl, compound 48/80, and pulp exposure, by organic acids. The nerve activity induced by each solution was calculated as the sum of nerve impulses generated during the 2-min application of the solution in the cavity (mean ± SEM; no. = number of teeth)

	Reduction in nerve activity (% of control)		
	NaCl, 0.76 M (no. = 9)	Compound 48/80 (no. = 9)	Pulp exposure (no. = 6)
Acid mixt. 1:10	73 ± 8	79 ± 5	85 ± 6
Acid mixt. 1:1	99 ± 0.2	99 ± 0.5	99 ± 0.3

lowing of ammonia solutions (23) are all associated with painful sensations.

The mechanisms by which ammonia excites the nerves are not known. In the present study all ammonia solutions tested were hypertonic, since they were diluted in isotonic saline. It has been proposed that hypertonic or hypotonic solutions induce pain in the skin as a result of changes in the osmotic pressure (29). However, it is unlikely that ammonia excited the intradental nerves because of its hypertonicity, since in our preparation substances like histamine or bradykinin did not excite the nerves (36) even though their hypertonicity was higher than that of ammonia in this study. Keele & Armstrong (23) suggested that ammonia solutions produce pain in the skin because of their high concentration of NH_4^+ and/or OH^- ions. From experiments, hitherto unpublished, in our laboratory it was found that solutions of NaOH did not excite the intradental nerves even when the OH^- ion concentration was 10-fold higher than that of the ammonia solutions used in the present study. Thus, we are inclined to believe that NH_4^+ per se excites the intradental nerves.

In contrast, the inability of organic acids to excite the intradental nerves is not comparable with their effect on other tissues. Thus, lactic acid in concentrations similar to those used in the present study has been shown to cause severe pain when applied to the exposed base of a cantharidin blister (2) or on small experimental wounds on the dorsal aspect of the finger (14). Lactic acid also gives rise to pain after intradermal (16) or intramuscular (31) injections in humans. Furthermore, its intra-arterial infusion in lightly anesthetized dogs (5) or cats (32) results in unmistakable signs of pain. Thus, there seems to be a difference in sensitivity to organic acids between the intradental sensory neurons and the dermal and visceral sensory neurons. The difference may be related to the existence of unmyelinated chemosensitive pain fibers in dermal and visceral tissues (15, 28) which may not be present in the tooth or not recordable by the present technique (17).

The observed ability of the acids to inhibit nerve activity induced by substances with different modes of action favors the hypothesis of an unspecific effect of organic acids on the intradental nerves. It is known that nerves are very sensitive to changes in the ionic composition of their fluid environment (38). Local application of acids of pH 2.0–4.0, as in the present study, increases the H^+ concentration of the intratubular fluid. The local Ca^{++} concentration is probably also increased as a result of dentin decalcification by the acids. It has recently been shown that nerve excitability is reduced after local increase of H^+ concentration (19, 38) or Ca^{++} concentration (34). Therefore, the observed suppressing effect of acids can be explained by decreased nerve excitability as a consequence of an increase in the H^+ and/or Ca^{++} concentration.

The enhanced nerve activity induced by ammonia when applied in cavities pretreated with acids cannot be due to ammonia per se, since successive applications of this substance in untreated cavities resulted in almost identical responses. Thus, the organic acids seem to be responsible for this potentiation by altering the properties of either the pulp or the dentin. The lack of potentiation in cavities with pulp exposure provides evidence against the first hypothesis while it supports the second one. Further evidence of alterations in the dentin is provided by studies conducted in human teeth. It has been shown that the penetration of bacteria into the dentinal tubules is significantly enhanced in acid-treated cavities compared with non-treated ones (41), and that such treatment results in removal of smear plugs and in opening and widening of the dentinal tubules by removing peritubular dentin (4, 22). Thus, the increased nerve activity induced by ammonia in acid-treated cavities can be explained by its facilitated diffusion through the dentin to the nerves in the dentinal pulp border zone. The lack of potentiation observed when acids are applied in cavities pretreated with acid indicates that some hindrance of diffusion through the dentin, for example the smear plugs in the orifices of the tubules, is removed by the first application of acid so that successive trials will apparently have little additional effect.

The present results clearly demonstrate that ammonia and organic acids can influence intradental sensory nerve activity. To propose that these metabolites may be involved in the symptoms from carious teeth it is of importance to know whether they can be produced or accumulated in carious dentin in concentrations similar to those used in the present study. No quantitative studies on the production of such metabolites in situ are available. However, indirect evidence suggests that similar concentrations can be found in carious tissues. For instance, the lowest concentration of ammonia which evoked nerve impulses in the present study is in the range of that found in the human oral cavity. In saliva, for example, concentration of ammonia can be as high as 26 mM (26). Since the density of microorganisms is higher in dental plaque and carious dentin than in saliva, it is reasonable to assume that the production of ammonia is also more pronounced. As far as acid formation and accumulation are concerned, the facts that the pH values of the bottom layers of carious dentin can be as low as 3.2 (mean, pH 3.88) (9) and that phosphate buffer (pH 6.8) is unable to revert the acidic pH in such cavities (6, 8) suggest that the concentrations used in this study are biologically relevant.

In conclusion, the results of the present study suggest that the bacterial metabolites tested may be involved in the symptoms from decayed teeth; ammonia excites the intradental nerves, and organic acids decrease sensory nerve excitability.

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