Neurophysiological and Biophysical Evidence on the Mechanism of Electric Taste

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ABSTRACT The phenomenon of electric taste was investigated by recording from the chorda tympani nerve of the rat in response to both electrical and chemical stimulations of the tongue with electrolytes in order to gain some insight into its mechanism on both a neurophysiological and biophysical basis. The maximum neural response levels were identical for an individual salt (LiCl, NaCl, KCl, or CaCl2), whether it was presented as a chemical solution or as an anodal stimulus through a subthreshold solution. These observations support the idea that stimulation occurs by iontophoresis of ions to the receptors at these current densities (<100 μA/cm²). Electric responses through dilute HCl were smaller than the chemically applied stimulations, but the integrated anodal responses appeared similar to chemical acid responses, as evidenced by an OFF response to both forms of stimuli. Hydrogen may be more permeant to the lingual epithelium and would thus be shunted away from the taste receptors during anodal stimulation. When the anion of electric taste was varied via subthreshold salt solutions, the response magnitude increased as the mobility of the anion decreased. The transport numbers of the salts involved adequately explain these differences. The physical aspects of ion migration occurring within the adapting fluid on the tongue are also discussed. Direct neural stimulation by the current appears to occur only at higher current densities (>300 μA/cm²). If the taste cells of the tongue were inactivated with either iodoacetic acid (IAA) or N-ethyl maleimide (NEM), or removed with collagenase, then responses from the chorda tympani could be obtained only at these higher current densities. Latency measurements before and after IAA or NEM treatment corroborated these findings. The results are discussed in terms of several proposed mechanisms of electric taste and it is concluded that an ion accumulation mechanism can adequately explain the data.

INTRODUCTION Electric taste, produced by passing a small electric current through the tongue, was first reported in the literature over two hundred years ago (Sulzer, 1754), followed by the work of Luigi Galvani (1791) and Alessandro Volta (1792, 1793). However, in spite of its long history, the mechanism of this special form of taste remains elusive.
stimulation has yet to be satisfactorily explained. Consequently, the use of electric taste as a research tool and as a clinical diagnostic tool has been impeded. The research reported here was conducted with the aim of more clearly elucidating this mechanism on a biophysical basis.

Although no formal theories have been proposed concerning the mechanism of electric taste, many suggestions have been made. At present, four theories can be summarized, two of which are as old as the phenomenon of electric taste itself, and two of which are recent in origin. Perhaps the oldest is the direct stimulation mechanism of electric taste, which can be dated back to Volta (1792, 1793). It states that the electric current passing through the tongue directly stimulates the taste nerves. This theory of mechanism has been modified in more recent times to include possible direct effects of the current on the taste cells of the taste bud as well as the afferent nerve fibers (summarized by Bujas, 1971). A direct effect may be interpreted to mean a change in the transmembrane potential of these cells (i.e., the taste nerves and/or taste cells of the bud) in either a hyperpolarizing or depolarizing direction, depending on the current's polarity. It would predict little if any ion dependence of the responses. It seems unlikely that the electric current acts directly on the nerve fibers at low current densities (Nejad, 1960; Warren, 1965); however, the direct (or indirect) effect of the current on the transmembrane potential of taste cells of the bud remains an open question.

The ion accumulation mechanism, which has a more diffuse origin, explains electric taste as an indirect stimulatory mechanism. Ions in the fluid medium bathing the tongue are iontophoresed to the receptors by the current; positive charges in the adapting fluid are delivered to the taste receptors by anodal current and negative charges by a cathodal current. There these charges either accumulate or displace ions already in the receptor vicinity and stimulate the receptors in a manner similar to usual sapid stimuli. Hence, the ion accumulation theory assumes that the transduction process of electric taste is similar to that of sapid stimulation. It predicts that the response to electric stimuli, utilizing different ions as current carriers, will reflect the same sensitivity of the receptors as for sapid stimulation with different ionic solutions. This notion of mechanism has received support from several investigators (Smith and Bealer, 1975; Beidler, 1975; Pfaffmann and Pritchard, 1980; Herness, 1981a, b, 1982; Pritchard and Pfaffmann, 1981; Ninomiya and Funakoshi, 1981a, b).

Recently, two more suggestions have been made concerning electric taste. Kashiwayanagi et al. (1981) and Kobatake and Kamo (1973) have proposed a mechanism for electric taste based on their proposed transduction scheme for taste responses to sapid stimuli in the frog. Sapid stimuli are proposed to induce phase boundary potentials at the taste cell microvillus membrane, which in turn induces current flow through the taste cell synaptic area, resulting in the activation of the afferent nerves. Anodal current is thought to depolarize the synaptic area in a similar manner, whereas cathodal current either hyperpolarizes this region or cancels any current set up by a sapid stimulus. It is interesting that this scheme also proposes that the transduction mechanism is similar for both electrical and chemical stimuli.
Finally, DeSimone et al. (1981, 1984) have reported that there is an ongoing ion transport across dorsal canine and dorsal rat lingual epithelium that can be modified by the concentration of fluid bathing the tongue surface. This ion transport is hypothesized to play a central role in the transduction process of salt stimuli (Heck et al., 1984). Anodal currents would augment the existing currents and thus mimic hyperosmotic salt stimuli, and cathodal currents would antagonize the existing currents, resulting in inhibitions of the ongoing activity. Again, electric and chemical stimuli are thought to converge on a common transduction pathway, in this case involving an active outward transport of sodium ions and an active inward transport of chloride ions.

The research conducted in this communication agrees with the work of most other investigators that electric taste responses are produced by iontophoresis of ions from the adapting fluid to the taste receptors. The transduction mechanism appears to be similar to that for chemical responses, and several possible theories of electric taste mechanism are discussed. The similarity between the responses obtained by chemical and electrical means and the specificity of these responses to the ionic species carrying the current are explained most simply by an ion accumulation mechanism for anodal electric taste in the rat. The possible direct effect of the current on taste nerve fibers is suggested to be minimal. Transference experiments also corroborate the idea of an ion accumulation mechanism and provide some biophysical insight into the molecular mechanism of electric taste.

**MATERIALS AND METHODS**

**Dissection and Recording Procedure**

Male Sprague-Dawley rats weighing 250–350 g were anesthetized with chloropent by an intraperitoneal injection of 2.0 cc/kg body weight. The animal was tracheotomized and secured on an animal board with a stainless steel headholder. A lateral approach was taken for the dissection of the chorda tympani. The zygomatic arch was exposed and removed with bone cutters. Similarly, the condyloid process of the mandible was cleaned and cut. The muscles beneath the mandible were separated, revealing the chorda tympani nerve as it exits the tympanic bulla and courses rostrally and ventrally to join with the lingual nerve. The nerve was carefully cleaned and stripped of connective tissue and its endoneural sheath. It was then placed on a platinum/iridium wire (80:20%), which served as the recording electrode. A similar wire was placed in the adjacent musculature and served as the indifferent electrode. The neural activity was amplified with an AC preamplifier (P-5, Grass Instrument Co., Quincy, MA). The output of the preamplifier was then simultaneously led to an audio monitor, a dual-beam oscilloscope (502, Tektronix, Inc., Beaverton, OR), an integrator, and, for latency measurements, a tape recorder (3960, Hewlett-Packard Co., Palo Alto, CA). The integrator was set with a time constant of 0.25 s. In some preparations, mineral oil (Nujol's) was added to the dissection cavity to prevent the nerve from drying. No changes in response magnitude were observed in the neural responses after addition of the mineral oil.

**Electrical and Chemical Stimulations**

Electrical stimulation of the tongue was achieved with the use of a Grass SD5 stimulator and a constant-current isolation unit (CCU). The output of the CCU was fed to two Ag/
AgCl electrodes, one placed in the inflow tube of the flow chamber and the other placed in the musculature near the dissection site for the chorda tympani. An ammeter was placed in series with the two electrodes. The polarity of the current could be reversed by means of the toggle switch on the CCU, and the magnitude of the current could be preset on the meter of the CCU.

Current was passed through various subthreshold tastants (generally 0.001 M) flowed through a flow chamber enclosing the anterior tongue. An estimate of the area of a rat's tongue contained within the flow chamber was calculated by dividing the tongue into four surface areas—ventral, dorsal, and left and right sides—calculating the area of each surface, and adding them together. This value (~1 cm²) was then used to calculate current density. All electrical stimulations were achieved with a single pulse of DC current lasting 2–3 min. These pulses, one per response, varied in magnitude and polarity, although most stimuli were anodal current within the range of 10–100 μA. For high current densities (>100 μA/cm²), it was necessary to use higher concentrations (0.005 and 0.01 M), which brought about small increases in the baseline activity. These increases were subtracted out of the integrated whole-nerve activity. However, most of the higher current densities used in this study occurred after treatment of the tongue with iodoacetic acid (IAA) or N-ethyl maleimide (NEM), so that the increases in baseline activity did not occur. Chemical stimulations were achieved by flowing suprathreshold solution through the chamber. The concentrations of the chemical stimuli were chosen to represent the response function of the chorda tympani from just above threshold to saturated response levels. For the salts tested, this corresponds to 0.01–1.0-M solutions. For acid stimuli, the highest concentration did not exceed 0.05 M (pH 1.3), as irreversible damage to the receptors can occur at higher concentrations. The magnitudes of the current used to produce electrical responses were similarly chosen as those that just began to produce measurable responses (approximately ±10 μA) to those that elicited saturated response magnitudes at the maximal level (close to 100 μA). Stimulations were generally given at 2-min intervals and responses were normalized to 0.1 M NaCl responses. This standard was given every fourth stimulation to check the viability and reproducibility of the preparation. The response to the standard did not vary by >10% during the course of the experiment.

**Experimental Treatments**

To assess the role of taste cells in electrical stimulation, the tongue was subjected either to removal of the dorsal epithelium with collagenase or to inactivation of taste cells by treatment with either IAA or NEM. The response profile of the chorda tympani was obtained to electrical stimulation through 0.001 M NaCl both before and after treatment.

Collagenase was applied to the tongue by injection of 0.5 ml of a 1% solution under the dorsal surface of the tongue epithelium. Care was taken to avoid injecting too deeply into the underlying cell layers, as this resulted in heavily damaged tissue. When the injection was confined to the subepithelial layer, little visible damage occurred and the tongue epithelium could be easily stripped off the tongue after ~30 min. To minimize the unavoidable swelling that occurs after removal of the lingual epithelium, only the side of the tongue ipsilateral to the chorda tympani from which responses were obtained was treated. If swelling was not minimized, the flow of solutions through the flow chamber could be impeded.

IAA and NEM were delivered to the tongue through the flow chamber. For an individual experiment, treatment consisted of a 3-min stimulation with either 0.26 M (5%) IAA or 0.06 M NEM. In the post-treatment responses, high-intensity stimulations were given last to avoid irreversible damage to free nerve endings. Responses were obtained only to anodal current up to a maximum of 1,000 μA/cm².
Neural Evidence on Electric Taste

**Lingual Nerve**

Recording from the lingual nerve in the rat was accomplished using the ventral approach, as in the chorda tympani dissection. The lingual nerve was dissected free from the point at which the chorda tympani branches off to the foramen ovale, where it enters the tympanic bulla. The activity of the lingual nerve was amplified and quantified using procedures identical to that used for the chorda tympani. Thermal stimulations (generally 30°C distilled water) were interspersed among electrical stimulations of the tongue in order to assess the viability of the preparation. Cathodal and anodal currents were alternated with one another and high current intensities were tested last to avoid damage to nerve fibers. Data were not obtained from animals that began to show signs of neural damage evidenced by a decrease in the neural response magnitude and an increase in the latency of the response.

**Latency**

The response latency to electrical stimulations was measured through 0.001 M NaCl both before and after treatment with IAA (0.26 M) or NEM (0.06 M) applied for 1 min. The current densities used ranged from 20 to 1,000 μA/cm². The latencies were measured by recording and storing the neural data on magnetic tape. Typically, 1 s of activity was recorded before and after the onset of the stimulus and stimulations were always followed by at least a 1-min rest. Higher intensities were allowed longer rests. After the data were recorded, they were photographed by a kymograph camera. The latency was measured from the start of the artifact to the first mark of activity well above the noise level.

**Results**

An anodal current passed through the tongue increased the neural activity in the chorda tympani nerve of the rat. When this current was carried through the flow chamber by a dilute (0.001 M NaCl) solution, the neural response produced was similar in shape to that produced by a suprathreshold stimulation with NaCl (Fig. 1). Similarly, if the current was passed through a dilute HCl solution, the response was similar to the acid response produced by a 0.01 N HCl solution. Note that both the chemically and electrically applied acid stimulations resulted in the OFF response characteristic of acid stimulation in the rat.

Cathodal currents, passed through dilute salt or acid solutions, produce inhibitions in the resting activity of the chorda tympani at the onset of the current flow and produce a transient OFF response at the break of the current. The focus of the experiments reported here was on anodal currents with dilute salts and acids; hence, cathodal responses (with the exception of those obtained from the lingual nerve) are not reported here.

The electrical responses increased in magnitude with increasing stimulus intensity within the tested range of 0–100 μA/cm². When the electrical responses for NaCl stimulation are plotted against the current density, a hyperbolic curve is obtained (Fig. 2). The data obtained from electrical stimulation can be described by the taste equation (Beidler, 1954):

\[ \frac{C}{R} = \frac{C}{R_m} + \frac{1}{KR_m}, \]

which relates, in this instance, the increasing magnitude of the neural response, \( R \), to the increasing current intensity, \( C \). \( R_m \) is the maximum (i.e., saturated) response and \( K \) is the equilibrium constant. Fig. 2B shows the neural response...
from the same preparation to chemical stimulation with NaCl; it is presented to show the similarity between the response profiles for chemical and electrical stimulation with NaCl. The reciprocal plot of the taste equation (Fig. 2C) for these data shows two relatively parallel lines, which indicates that these curves saturate at approximately the same maximum response level.

The response profiles for other electrolytes to electrical and chemical stimulation are presented in Fig. 3. The tested compounds were LiCl (A), KCl (B), HCl (C), and CaCl₂ (D); each response profile was obtained from a different preparation. The responses to chemical and electrical stimulation for any individual salt could be described by one curve saturating at the same response level for the two forms of stimulation. Table I gives the values of the calculated maximum responses, \( R_m \), and the equilibrium constants, \( K \), for all five compounds to chemically and electrically applied stimulations. These values were calculated from the taste equation by performing a linear regression analysis on the data plotted in the reciprocal (i.e., linear) form. The chemical and electrical curves were calculated independently. To align the two abscissae for one plot, the two equations were then solved simultaneously by setting \( R_{\text{chem}} \) equal to \( R_{\text{elect}} \). In this manner, a particular current density–concentration equality was obtained that became the basis for the alignment of the two abscissae. The reciprocal plots (not shown) for these salts yielded a set of parallel lines, which indicates that the curves saturated at the same response level in all cases. The one noticeable exception is HCl (Fig. 3C). The chemical and electrical curves for the acid HCl do not match, as do those for the salts; they are dissimilar and must be described by two curves that approach different asymptotes. The chemical responses obtained much larger magnitudes than did the electrical responses.
FIGURE 2. (A) The summated whole-nerve response of the rat chorda tympani plotted as a function of the current density when anodal current was passed through 0.001 M NaCl. The response was normalized to 0.1 M NaCl. The current density is expressed as microamperes per square centimeter. (B) The summated whole-nerve response from the same animal as in A plotted as a function of the molar concentration of NaCl applied to the tongue. The response was normalized to 0.1 M NaCl. (C) Graph of the data in A and B plotted in the reciprocal form of the taste equation. Note that C/R has different scales for the electrical and chemical lines since in one case C is given in units of microamperes per square centimeter and in the other case the units of C are moles per liter. The lines were calculated by linear regression analysis. O, NaCl electrical; ●, NaCl chemical.
FIGURE 3. The response profile for chemical (solid symbols) and anodal electrical (open symbols) responses from the rat chorda tympani to various electrolytes. Each graph represents data obtained from a different animal. The responses were normalized to 0.1 M NaCl and the current density is expressed as microamperes per square centimeter. The curves were calculated from the taste equation. The electrolytes used were (A) LiCl, (B) KCl, (C) HCl, and (D) CaCl₂. (The abscissa on the CaCl₂ graph has been expanded.) Note that the chemical and electrical responses match for all cases except HCl.
The Anion's Effect in Anodal Electric Taste

Differing response profiles from the chorda tympani were obtained when current was passed through subthreshold solutions of various sodium salts. Such curves are shown in Fig. 4A for the salts sodium chloride, sodium acetate, and sodium butyrate. Below the saturated level of response, current passed through sodium butyrate produced larger responses than sodium acetate and sodium chloride at a particular current density. The three curves, however, all saturated at the same maximum response level. The data presented here produced the following series for sodium salts in descending order of stimulating effectiveness with anodal current:

butyrate > acetate > chloride.

Hence, the larger, bulkier anions produced larger neural responses. These data can be interpreted on the basis of the transference of the stimulating solutions.

| Compound | R_m,chem | R_m,elec | K_chem | K_relect | ΔG_chem |
|----------|---------|---------|--------|---------|---------|
| NaCl     | 1.9     | 2.0     | 12.9   | 0.061   | -1.5    |
| LiCl     | 2.2     | 2.2     | 9.6    | 0.072   | -1.3    |
| CaCl_2   | 0.8     | 0.9     | 10.5   | 0.039   | -1.4    |
| KCl      | 1.6     | 1.6     | 2.5    | 0.016   | -0.5    |
| HCl      | 1.4     | 0.8     | 148.9  | 0.039   | -3.0    |

Since the larger anions of this series have lower electric mobilities, they travel more slowly. Hence, there is a greater percentage of the current carried by the sodium cation for solutions of heavier anions. When these data are replotted as a function of the sodium current, the points fall onto one curve (Fig. 4B). The neural response thus appears to be proportional to the amount of sodium current contained within the total current. (The total current in an ionic conductor can be divided into two parts, that carried by the positive charges and that carried by the negative charges. In a solution of 0.001 M salt at neutral pH, the hydrogen transference is negligible compared with the sodium transference; thus, for these solutions, one can use the terms sodium current and positive current synonymously.) Therefore, equal current densities distribute themselves differently between the positive and negative charge carriers in the sodium solutions with different anions; the receptors appear to respond directly to the amount of the positive (i.e., sodium) current. The anion of these sodium solutions appears to influence the neural response by affecting the forward transference of sodium via its transport number.

If the same experiment is repeated for acid stimulation rather than salt, these differences fail to appear. Fig. 4C shows that current passed through 0.0005 N
Figures A, B, and C from the paper. Figure 4.
HCl, acetic acid, or butyric acid failed to show any differences in the response magnitude at the same current density. The electrical acid responses did, however, demonstrate the OFF response characteristic of acid stimulation. One would expect the transference effects with acids, if present, to be much smaller than those observed with salts because of the high mobility of hydrogen. This is discussed further in the Discussion.

**NaCl pH Series**

Anodal currents passed through either a dilute salt or dilute acid solution yielded response profiles that were different in character. To study these differences, anodal currents were passed through mixtures of salt and acid solutions. Three solutions of 1 mM NaCl were used at pH 10.3, 7.0, and 3.75. These pH values were obtained by titrating with either NaOH or HCl. Table II gives the molar concentrations of all ions involved for these solutions. The responses obtained from a solution of pH 7.0 were the largest at a particular current density, although they were only slightly larger than the solution at pH 10.3 (Fig. 5). The acidic solution, however, gave responses that were much lower than either the neutral or basic solution. When these data are regraphed on the reciprocal plot, the curves for pH 7.0 and 10.3 graph as parallel lines. They both have a slope of 0.56, which indicates that these two response profiles saturate at the same level, namely 1.8. The curve obtained from a pH 3.75 solution also graphs linearly on the reciprocal plot with a slope of 0.59. This response profile then

\[
\begin{array}{cccc}
\text{pH} & [\text{Na}^+] & [\text{Cl}^-] & [\text{H}^+] & [\text{OH}^-] \\
5.75 & 1 \times 10^{-3} & 1.18 \times 10^{-3} & 1.78 \times 10^{-4} & 5.62 \times 10^{-11} \\
7.0 & 1 \times 10^{-3} & 1 \times 10^{-4} & 1 \times 10^{-7} & 1 \times 10^{-7} \\
10.3 & 1.2 \times 10^{-3} & 1 \times 10^{-3} & 5.01 \times 10^{-11} & 2.0 \times 10^{-4} \\
\end{array}
\]


**Figure 4.** (opposite) (A) Anodal electrical responses obtained with subthreshold solutions (0.001 M) of Na butyrate (■), Na acetate (●), and NaCl (○). The current density is in microamperes per square centimeter and the responses were normalized to 0.1 M NaCl. The curves were calculated from the taste equation. These data were obtained from one animal. (B) Neural responses obtained from electrical stimulation through 0.001 M solutions of Na butyrate, Na acetate, and NaCl. Data are the same as those presented in A; however, they are replotted with consideration given to the transport numbers of the stimulating solution. The abscissa is the calculated sodium current (microamperes per square centimeter) or equivalently (for these solutions) the cationic current of the total current density used for stimulation. (C) Whole-nerve responses plotted as a function of the current density (microamperes per square centimeter) for HCl (■, chemical; ▲, electrical), acetic acid (△), and butyric acid (●). The current was delivered through 0.0005-N solutions. The chemical responses were obtained from HCl solutions of different concentrations. Responses were normalized to 0.1 M NaCl. Data were obtained from one animal.
saturates at a level of 1.7, which is slightly lower than the two solutions at higher pH.

**Lingual Nerve Responses**

The lingual nerve, after the chorda tympani had branched off, responded to anodal current only at much higher current densities than did the chorda tympani (Fig. 6). This observation corroborates those of other investigators (Nejad, 1960; Warren, 1965) for anodal current. It can be seen that although the chorda tympani had almost saturated by 100 μA/cm², the lingual nerve had barely reached one-fifth of its full response to anodal current. The response increased further with increasing current intensity up to 1,000 μA/cm², the highest tested value. Cathodal current, on the other hand, produced inhibitions in the resting activity of the lingual nerve within the range of 0–500 μA/cm², with the largest inhibition produced at 200 μA/cm². Beyond 500 μA/cm², the lingual nerve responded with increases in neural activity to increasing stimulus intensity. The inhibitions produced in the lingual nerve resembled the inhibitions produced by thermal stimuli above ambient temperature; there was an initial depression followed by a transient increase at the break of the stimulus, i.e., either ambient distilled water or the current break.

**Collagenase**

Collagenase treatment dramatically reduced the effectiveness of anodal current in producing neural responses (Fig. 7). The treatment has been shown to remove the epithelial layer of the rat’s tongue, including the taste bud, and to leave
intact the nerve endings in the base of the papillae (Beidler, 1965). Before removal of the epithelium, the chorda tympani responded with a near-maximal response by 100 µA/cm². After removal of the epithelium, however, the response curve much more closely resembled the curve in Fig. 6 for the lingual curve. For the collagenase treatment, a 100-µA/cm² stimulus produced a response that was only 10.7% of the maximum response obtained at 1,000 µA/cm². This value is within the range of 16.6% obtained for the lingual nerve. The chorda tympani response, after collagenase, increased with a regular slope as the current density increased over the tested range. These responses are in agreement with those reported by Warren (1965).

**I A A and N E M: *Neural Responses***

IAA applied to the tongue (1 min of a 5% solution) drastically reduced responses obtained to anodal current through a NaCl solution (Fig. 8), as well as to suprathreshold NaCl solutions. This reduction was irreversible, as no recovery was noted even hours after the application of IAA. Although in the untreated condition 100 µA/cm² was almost a maximum response, after treatment this same current density elicited a response that was only 13.3% of the maximum response obtained. The response after treatment continued to increase with an increasing stimulus intensity up to the maximum response tested at 1,000 µA/
Figure 7. The response profile of the chorda tympani to anodal stimulation through a NaCl solution before and after removal of the epithelium with an injection of 1% collagenase solution. The response is expressed in arbitrary units and the unit area on the abscissa is 1 cm². These response profiles are considered prototypical data from one animal; several animals were tested. The large difference between pre- and post-treatment is thought to indicate that the current stimulates the cells of the taste buds rather than the nerve fibers.

Figure 8. The response profile of the chorda tympani to anodal electrical stimulation of the tongue through a NaCl solution before and after treatment of the tongue with 5% IAA. The response is expressed in arbitrary units and the unit area of the abscissa is 1 cm². These response profiles are considered prototypical data from one animal; several animals were tested. When taste cells are inactivated, the response function drops dramatically. Direct stimulation of the nerve fibers by the current seems unlikely.
The increase in response magnitude was approximately linear over this range. Furthermore, the maximum response obtained in the post-treatment condition was larger than the pretreatment maximum response. Presumably, the post-treatment stimulation activated fibers nonspecifically, whereas the pretreatment stimulation activated only fibers with salt sensitivity. The results with NEM were similar (not shown). In the post-treatment condition, a 100-μA/cm² stimulus elicited a response 7.7% that of the maximum responses obtained at 800 μA/cm².

![Figure 9](image-url)

**Figure 9.** Prototypical latency data obtained from one animal, describing the whole-nerve response to anodal electrical stimulations of the tongue with 0.001 M NaCl before and after treatment with 0.06 M NEM. The unit area on the abscissa is 1 cm². At the current densities used for electric taste, there is a large difference between pre- and post-treatment latencies, whereas at higher current levels there is little difference. Direct neural stimulation appears to be likely only at the higher current densities.

**IAA and NEM: Latency**

Latencies were determined both before and after treatment with either IAA or NEM. The shapes of the curves before treatment resembled those of Nejad (1960) and Warren (1965). There was an initial plateau at 21 ms occurring between 20 and 200–300 μA/cm². Latencies to currents of <20 μA/cm² were long by comparison (up to 40 ms) and dropped rapidly as the current magnitude approached 20 μA/cm². As currents increased beyond the 200–300-μA/cm² range, the latency dropped further, although this decline was a much slower and more gradual one. A minimum latency of 10 ms was finally reached at the highest test value, 1,000 μA/cm².

After treatment with either IAA or NEM, the latencies to lower current densities were considerably lengthened, whereas the latencies to higher current densities were changed little if at all (Fig. 9). IAA increased the latency at 10
μA/cm² from 31.5 to 64 ms. This latency dropped until at ≥150 μA/cm² the post-treatment latency curve merged with the pretreatment curve to form one curve. The effect of NEM on the latency was slightly different in the lower current density range. 30 μA/cm² produced a latency of 49 ms and this latency dropped until at 300 μA/cm² it was the same as the pretreatment latency. From 300 to 1,000 μA/cm², the post-treatment latency was similar but consistently higher by a small amount than pretreatment values. Over this range, the latency dropped from 20 to 11 ms.

**DISCUSSION**

*Similarity Between Electrical and Chemical Responses*

Electrophysiological responses from the chorda tympani nerve of the rat show increases in activity to anodal stimulation that resemble the increase caused by suprathreshold concentrations of salts and acids. The shape of the integrated neural response implies a similarity between the two forms of stimuli. Anodal current through a dilute NaCl elicits a response with an abrupt onset and offset and with phasic and tonic components. This response mimics the response to 0.1 M NaCl (Fig. 1). Similarly, anodal current through a dilute acid solution mimics stimulation with rapid acid stimuli. The rat demonstrates a “water response” after stimulation with HCl, which also occurs after electrical stimulation through a dilute HCl solution upon cessation of the current flow. This anodal OFF response occurs only when the current is passed through a dilute acid solution and is never observed when dilute salt solutions carry the current. The hamster also shows an OFF response to acid stimulation and to anodal current passed through a dilute acid solution (Smith and Bealer, 1975). Thus, at a gross level, anodal responses show a marked similarity to their corresponding chemical counterparts.

At a more intricate level, subtle differences in the whole-nerve responses to electrical and chemical stimuli appear that have been noted previously (Bujas et al., 1979). These include a more abrupt rise in the response to its maximum value, a greater relative decrement in the tonic portion of the response, and a somewhat shorter latency for electrical responses. All were observed in the present set of data. The initial phasic portion of the response is thought to reflect the rate at which the stimulus is increased to its maximum value (Smith and Bealer, 1975; Marowitz and Halpern, 1977). The same stimulus presented at different flow rates yields smaller phasic responses to slower stimuli presentation but no differences in the tonic phase of the response.

Electrical responses to anodal current are thought to yield faster rise times since the electrical stimulus has a virtually instantaneous rise to its maximum value. Recruitment may also be a factor. A chemical stimulus flowing over the tongue does not stimulate all receptors simultaneously; it reaches some receptors before others as it flows across the tongue. An electrical stimulus passed through a flow chamber surrounding the tongue begins to pass the epithelium everywhere at the same time. Thus, the electrical stimulus has a more abrupt nature, which could easily explain the faster rise time of the electrical responses. Additionally, it may explain the somewhat shorter latencies to anodal stimulation.
Anodal latency to response is on the order of 20–25 ms (this communication; Bujas et al., 1979; Yamamoto et al., 1980), although one report of a shorter latency has appeared (14–15 ms, Beidler, 1975). Although latency to anodal stimulation is shorter than most chemical latencies, of the order of 35 ms, latencies with a very abrupt stimulus onset of 25 ms to chemical stimuli have been reported (Faull and Halpern, 1972; Marowitz and Halpern, 1977). Thus, the latency, as well as the rise time, is dependent upon how fast the stimulus is presented, and the abrupt nature of the electrical stimulus would warrant shorter latencies. In addition, chemical latencies are difficult to measure, as a reliable stimulus onset marker for a chemical stimulus is difficult to obtain. This problem may lead to a somewhat longer latency for a chemical stimulus. The more accurate onset marker of an electrical stimulus may also be a factor in the shorter latencies measured.

The data for electrical and chemical responses to the salts NaCl (Fig. 2), LiCl, KCl, and CaCl₂ (Fig. 3, A, B, and D) demonstrate quite clearly that the sensitivity of the rat’s receptors for these salts is the same for both forms of stimulation. The curves show the same increase in response to increasing current as in response to increasing concentration. Moreover, the curves show the same maximum response levels to an individual salt, whether that stimulus was presented electrically or chemically. Thus, anodal electric taste responses in the rat for salts appear to mimic those produced by chemical stimuli. This observation strongly supports the idea of an ion accumulation mechanism, that is, that the receptors are responding as if a suprathreshold sapid stimulus were presented to them.

When an acid electrical stimulus is used (Fig. 3C), such quantitative agreement does not appear; however, at a qualitative level the responses do show similarity, especially as evidenced by the OFF response. There may be several reasons for the lack of quantitative agreement. Hydrogen has different physical properties than other alkali metals and alkaline earth metals. It has a much smaller size than the other cations and is transported through solution by a chain mechanism that jumps from water molecule to water molecule, which is not possible with other cations. Because of these unique physical features, a significant portion of the hydrogen current may be shunted along nongustatory pathways through the tongue that are not available to those other cations. Hydrogen currents would thus distribute themselves much differently and would explain the observations that higher current densities are needed to produce acid electric taste responses and that acid electric taste response curves do not match those response curves produced by chemical stimulation. Thus, the notion of an ion accumulation mechanism is not negated by the results with acid electric taste. Although quantitative agreement is lacking, the electrical responses appear to be acid responses, and they support the idea that receptors are responding to the hydrogen iontophoresed by the anodal current to the taste cell membrane.

The Equilibrium Constant, \( K \)

A comparison of the equilibrium constants (also called the association constant) for chemical and electrical data presented in this paper does not reveal any
significant differences among the various ions used as stimuli. However, a discussion of the problems and limitations of extracting meaningful information from the $K$ values presented may prove useful. The equilibrium constant $K$ is a meaningful physical constant since, if the system is in thermodynamic equilibrium, one can calculate the change in the standard free energy, $\Delta G$, of the system from the equation

$$\Delta G = -RT \ln(K).$$

The change in free energy calculated from the taste equation provides an insight into the physical interactions between stimulus and receptor (e.g., see Beidler, 1954, 1970, 1971).

The variations in $K$ among the chemical data (Table 1) do not reveal any significant differences. They range from 2.5 to 148.9, and, if one calculates $\Delta G$, values of approximately $-0.5$ to $-3.0$ kcal/mol are obtained. These values are all consistent with weak physical interactions between stimulus and receptor, for example, van der Waals forces ($1-2$ kcal/mol) and hydrogen bonding ($\sim5-7$ kcal/mol). The present study obtained a value of 12.9 for $K$ describing NaCl stimulation. Beidler (1961) reported that $K$ values for NaCl in the rat are of the order of magnitude of 7–15, depending upon the particular experiment involved, and had previously published a value of 9.8 (Beidler, 1954). The $K$ value of this study is thus in good agreement with previously published values. The equilibrium constant for hydrogen is of greater magnitude, 148.9. One can calculate a value of 474.1 for HCl in the rat from previously published data (Beidler, 1971). These $K$'s have $\Delta G$'s of $-3.0$ and $-3.6$ kcal/mol, respectively. Thus, the equilibrium constants and the calculated changes in free energy for the chemical data are in good agreement with previously published data.

A discussion of $K$ values for the electrical data becomes more problematic since, in their present form, they possess unusual units. The taste equation, which is derived from a second-order reaction, yields $K$'s of the second order with units of (molar)$^{-1}$ and (microamperes per square centimeter)$^{-1}$ for $K_{\text{chem}}$ and $K_{\text{elec}}$, respectively. Because of its units, $K_{\text{elec}}$ cannot be used for calculating $\Delta G$ values. The reason lies in the distinction between the practical equilibrium constant, $K$, and the standard equilibrium constant in terms of concentration, $K^\circ$. The standard equilibrium constant, $K^\circ$, is the practical equilibrium constant, $K$, standardized to unitary molar concentration. If $K$ is expressed in terms of molarity, then $K^\circ$ is simply the numeric value of $K$ and hence is a unitless number (e.g., see Laidler and Meiser, 1982). (The same argument applies for gases with equilibrium constants standardized to unitary atmospheric pressure.) Strictly, it is the standard equilibrium constant, $K^\circ$, that is used for calculating $\Delta G$. Since one cannot directly standardize an equilibrium constant with units of inverse current density in terms of unitary molar concentration, $K_{\text{elec}}$ cannot be used to calculate directly the change in free energy, $\Delta G$. Neither are $K_{\text{chem}}$ and $K_{\text{elec}}$ directly comparable, since they have different units. A comparison of $K_{\text{elec}}$ values reveals no significant differences; these values are all within an order of magnitude ranging from 0.016 to 0.072. Mayer (1977) has previously related electrophysiological responses from the rat chorda tympani to the intensity of anodal
stimulation of the tongue by the taste equation and obtained a larger value of 0.248 for $K_{\text{elec}}$. This equilibrium constant, however, was calculated as a function of current rather than current density; also, the adapting fluid bathing the tongue was not stated. Although this value is 3–15 times larger than the $K_{\text{elec}}$ values of this communication, differences of this order of magnitude did not prove significant for $K_{\text{chem}}$ values. Reasonable agreement might therefore be assumed.

If any differences emerge among the $K$ values, even qualitatively, it might be between the $K$ values for HCl and the salts for chemical and electrical stimulation. For the chemical data, the $K$ value is larger than that of the salts (although this difference is not significant if $\Delta G$ is considered). For the electrical data, however, $K$ values for HCl and the salts are more homogeneous. One might speculate then that the reason electric acid responses are much smaller than chemical acid responses is that the stimulus-receptor interaction for hydrogen is weaker for an electrical presentation than for a chemical one, perhaps because the electrical polarization alters the "hydrogen receptor" in some manner. At present, however, this argument is tenuous at best. First, it is not known whether $K_{\text{elec}}$ values, in their present form, can be thought of as a direct representation of the stimulus-receptor interaction. However, a quantitative link involving Faraday's constant, which relates a current in amperes to a molar quantity of ions, may enable such information to be extracted from the $K_{\text{elec}}$ values. Second, the role of a paracelluar shunt for hydrogen, which is not as significant for the larger alkaline and alkaline-earth cations, may also be involved in the differences in response magnitudes during electrical stimulation. At present, further experimentation will be needed to assess the possible roles of each of these factors in reducing the magnitude of electrical acid responses relative to the chemically applied acid responses.

Direct Stimulatory Effect

Volta (1792, 1793) was the first to suggest that electric taste may operate by directly stimulating the nerve fibers within the tongue. Several treatments of the tongue were performed to address the question of direct neural stimulation of nerve fibers. The general consensus of these results is that the electric current has very little direct effect on the nerve fibers of the tongue. For example, the lingual nerve (after the chorda tympani has branched off) is poorly sensitive to chemical stimulation but innervates the same regions of the tongue as the chorda tympani. However, it responds only to much higher current densities than are necessary to produce responses in the chorda tympani. Although there is some small amount of stimulation at low (<100 $\mu$A/cm$^2$) current levels, the response of the lingual nerve does not match that of the chorda tympani until much higher (>300 $\mu$A/cm$^2$) current densities.

Since it is not known whether the thresholds of the fibers of the chorda tympani are directly comparable to those of the lingual nerve, attempts were made to record directly from the chorda tympani after inactivation or removal of the taste buds. Collagenase treatment allows the lingual epithelium to be removed with the taste buds, leaving the nerve fibers as free endings within the
papillae. After this treatment, the response profile was dramatically altered and more closely resembled that of the lingual nerve. However, since removal of the tongue epithelium drastically alters the permeability and electrical resistance of the tongue, it is possible that the stimulating current was shunted along a low-resistance pathway via the extracellular pathways. Stimulation of the nerve fibers would then occur only at high current densities. Inactivation of the taste receptors with topically applied IAA or NEM resulted in a stimulating situation with intact lingual epithelium but with taste receptors inactivated. The site of action of NEM has been proposed by several investigators (Mooser, 1976; Beidler, 1978) to be situated at some point beyond the exterior membrane surface, either within the membrane or at an intracellular site. Its specificity of action is much greater in gustation than in olfaction (Getchell and Gesteland, 1972), since at low concentrations (0.0001 M NEM) it inhibits sweet stimuli only, whereas it inhibits all qualities at higher concentrations. IAA has been used less frequently as an inhibitor of gustatory function; however, Nejad (1960) has concluded that IAA affects the exterior membrane proteins of the microvillus membrane. The site of action of both NEM and IAA has been implicated at the level of the taste receptor cells rather than at the taste nerve fibers. Thus, it can be assumed that the neural responses that resulted from electrical stimulation after treatment with IAA and NEM were the result of the action of the current on nerve fibers.

The response profiles of the chorda tympani nerve to anodal stimulation after IAA or NEM treatment show an increase in response magnitude with increasing stimulus intensity. These results corroborate those from the collagenase treatment and those from the lingual nerve recordings. In all cases, there are small responses obtained within the range of current densities used for electric taste (<100 μA/cm²), but the response magnitudes do not approach those of the untreated chorda tympani responses until much higher current densities. It is therefore highly unlikely that electric taste operates by directly stimulating the taste nerves in the tongue.

The latency data obtained before and after treatment with IAA or NEM (Fig. 9) agree well with the findings from the response magnitude curves, i.e., that direct stimulation occurs only at high current densities. Before treatment, the latency plateaued at ~21 ms, which is consistent with the latency of 23 ms reported by Yamamoto et al. (1980) and slightly longer than the 16-ms latency reported first by Nejad (1960) and later by Beidler (1975). As the current density increased, the latency dropped gradually, reaching a minimum value of 9 ms. After treatment, the latencies to low current densities were much longer, but the latencies to high current densities were either very similar or identical. Thus, when gustatory function is impaired, the low current density latencies reflect this impairment. The high current density stimulations are basically unaltered, which indicates direct neural stimulation. Thus, at low current densities, it can be assumed that direct stimulation contributes little if at all to the neural response.

Transference in Electric Taste

The transport experiments give some insight into the physical mechanism of electric taste by exploring how ion migration in the adapting fluid affects the
neural response magnitude. When an anodal current flows through the adapting fluid bathing the tongue, there is a migration of cations toward and anions away from the taste receptors. It is believed that the differing neural magnitudes obtained with various sodium salts during electrical stimulation (Fig. 4) can be explained on the basis on the salts' transport numbers. Other salts (e.g., potassium, calcium) were not tested. The transport numbers for several salts are listed in Table III. As the cationic transport number increases (the percentage of the total current carried by the cations of the solution), so does the neural response, until a saturated response level is reached (Fig. 4A). Hence, sodium butyrate (which has a cationic transport number of 0.6) produces larger responses than does sodium chloride (with a cationic transport number of 0.4). The cationic transport number would affect the neural response as follows: the total current flow through the solution bathing the tongue can be divided into two currents,

\[ I_{\text{total}} = I_{\text{cation}} + I_{\text{anion}}. \]

Since the physical basis of the disparity between anionic and cationic currents lies in the electric mobilities of the charged particles involved, larger, slower anions will have a smaller anionic current and hence a larger cationic current. This effectively means that there will be more sodium traveling toward the receptors for these salts. Hence, at equal current densities, sodium butyrate should (and does) yield larger neural responses than sodium chloride. Other data, not presented in this paper (Herness, 1981a), have yielded the following anion series in descending order of neural response at equal current densities:

\[ \text{butyrate} > \text{propionate} > \text{acetate} > \text{formate} > \text{chloride}. \]

These differences can be explained at a quantitative level to a good agreement solely on the basis of the sodium current. (Since these 0.001 M salt solutions are all at neutral pH, the amount of cationic current carried by hydrogen is negligible, largely because the sodium concentration is 10,000 times greater than...
hydrogen. Therefore, for these solutions, one can use the terms cationic current and sodium current synonymously.) If the data from Fig. 4A are replotted on the basis of the cationic current ($I_{\text{cation}}$ from Table III) rather than the total current, then the three curves merge to form one curve (Fig. 4B). This curve indicates that the response is proportional to the amount of sodium delivered by the current. Once a saturated level of response has been reached, the transference should have no further effect, since the difference in the sodium concentration at the receptors is now in excess of that needed to produce a maximum response.

As these electrical responses from different sodium salts all saturate at the same response level, it appears that the anion present in the adapting fluid exerts only an indirect influence on the response magnitude. This is in opposition to the anion's effect in determining the magnitude of chemically applied salt solutions, where it is postulated to exert an inhibitory influence by binding to the microvillus membrane. In electric taste, it appears that the anion of the adapting fluid does not exert this influence at the microvillus membrane; rather, it functions by influencing the cationic current and hence the response magnitude. During anodal stimulation, the anion of the adapting solution is traveling toward the anode and hence in the opposite direction of the taste receptors. The anion at the level of the membrane, necessary to maintain electroneutrality, must have a different source than the adapting solution. Possible sources would be the cell's interior or its extracellular fluid. Thus, the anion at the membrane during anodal stimulation is the same regardless of the anion present in the stimulating solution.

When the anion is varied during acid electric taste, the differences in response magnitude caused by transference effects are not apparent, as they are with the salts. Responses to equal current densities through dilute hydrochloric, acetic, or butyric acid are all equal. There may be two reasons for these empirical results. First, since the mobility of hydrogen is so great, varying the anion has less of an effect on the cationic current than for the salts. For example, the sodium currents for NaCl and Na butyrate are 40 and 60% of the total current, respectively. However, for HCl or butyric acid, the hydrogen percentages are 82.2 and 90%, respectively. Thus, moving from chloride to butyrate increases the sodium current by 20%, but increases the hydrogen current by only 8.8%. Second, the permeabilities of the lingual epithelium to sodium and hydrogen are probably not identical. Hydrogen, owing to its smaller size, may be more permeant than sodium. If this is the case, an anodally carried hydrogen current would distribute itself differently than a sodium current; more of the hydrogen would be shunted through the epithelium and consequently there would be less of a buildup at the receptors. The homogeneity of the responses with the acid anion series again reflects the differences between acid electrical and salt electrical responses.

The differences between salt and acid electric taste can be further investigated by ascertaining the effect on the neural response when mixtures of the two, i.e., NaCl at different pH, are used to stimulate. With mixtures, as the concentration of the hydrogen ion increases, it will carry more of the current than sodium, since its mobility is about seven times greater than sodium's (Table III). Thus,
one would expect that as the pH decreases, so should the neural response, since current carried by hydrogen produces smaller responses than current carried by sodium. Indeed, the lower pH (3.75) is reminiscent of the acid electric taste profile obtained through HCl, as evidenced by the size and shape of the response. There was a slight depression of the sodium response to anodal current with the higher pH solution (10.8). This depression can be explained in terms of the transference effects occurring between cationic and anionic currents. The total ion concentrations of the stimulating solutions are listed in Table II, and the transference numbers are given in Table III. Note that with a higher pH, the sodium current (34.2%) is less than at a neutral pH (40%). The small depression in the salt response of anodal current occurring with increasing pH can be appreciated as being similar to that of increasing the mobility of the anion, as observed in the anion series of various sodium salts. This reduced sodium current can account for the small depression observed in the alkaline response.

**Ion Accumulation Mechanism**

It is believed that the anodal responses in the rat chorda tympani are best explained by an ion accumulation theory of electric taste. A detailed account of this theory will be presented elsewhere (Herness, M. S., manuscript in preparation), although its essential features are summarized here. It is proposed that during electrical stimulation of the tongue, the current delivers and concentrates ions from a subthreshold adapting solution to the immediate vicinity of the taste cell microvillus membrane, and that these suprathreshold concentrations are transduced to neural responses in a manner similar to chemical stimulations. This accumulation process is hypothesized to occur in two phases. During the initial phase, cations from the adapting fluid are transported toward the exterior surface of the microvillus membrane of the taste cell, and anions from the adapting fluid migrate away from the exterior membrane surface. On the intracellular surface, the opposite phenomenon occurs: cations move away from and anions move toward the intracellular membrane surface. The cationic composition at the exterior membrane surface thus changes and the interfacial cation concentration from the adapting solution increases. Electroneutrality at the exterior membrane is maintained by anions traveling in the opposite direction; the presence of fixed negative charges at the membrane surface may also contribute to the accumulation of cations from the adapting solution at the membrane surface. During the initial phase, the accumulation begins to form and consequently a concentration gradient between the exterior membrane solution and the bulk solution also forms. As this concentration gradient forms, backward diffusion from the membrane surface into the bulk solution will also begin. During the steady state phase, it is hypothesized that conduction by the current and this backward diffusion are equal and opposite to one another, so that the accumulation at the membrane surface does not increase, regardless of the current duration.

In addition, the charging of membrane capacitance by an anodal current would result in an increase in the interfacial cationic concentration at the external membrane surface with cations from the adapting solution and an increase in
the interfacial concentration at the internal membrane surface with anions from the intracellular environment. Equal amounts of cations and anions accumulate on opposite sides of the membrane so that the membrane considered as a whole (i.e., the membrane and both membrane-solution interfaces) remains electroneutral. Thus, the membrane capacitance for an anodal current also results in delivering the adapting cation to the taste cell apical membrane. Membrane capacitance could thus be an augmenting factor in the ion accumulation mechanism. However, since each ionic species tested in anodal electric taste displayed a unique equi-response chemical concentration–electrical current density relationship (see the abscissae of Fig. 3, A, B, and D), it is not thought that membrane capacitance alone can explain the anodal responses. This point will be discussed further in a future communication (Herness, M. S., manuscript in preparation).

Other Proposed Electric Taste Mechanisms

The data from this experiment can be reviewed in terms of the four current theories of electric taste mechanisms. Of these four theories, the direct stimulation mechanism appears to be the least valid. Electrophysiological recordings clearly demonstrate that the current does not stimulate the taste nerves directly; therefore, the current must first interact with the taste cells of the taste bud. Latency measurements and whole-nerve responses after taste cell inactivation or removal provide strong corroboration that the current (at the current densities used for electric taste) is acting first on the taste cell, which in turn stimulates the nerve fibers. More recently, the direct stimulation mechanism has been extended to include possible direct actions of the current on the taste cell, either a direct depolarization or hyperpolarization of the transmembrane potential. If such actions are occurring, they cannot be directly assessed from the present data. However, for anodal current, this would mean that the apical region of the cell, where current enters the cell, would be hyperpolarized (analogous to the region of a nerve under the anode), and the basal region of the cell, where current exits, would be depolarized (analogous to the cathode). Hence, direct effects of an anodal current on the taste cell could result in neural activation, as the synaptic area would be depolarized. This hypothetical situation is very similar to that proposed by Kashiwayanagi et al. (1981), i.e., that anodal current depolarizes the synaptic area of the taste cell, although they do not propose that the apical region becomes hyperpolarized. It would be of interest to obtain intracellular recordings from taste cells under electrical stimulation. Such data would provide better insight into possible direct or indirect effects of the current on the cells of the taste bud. However, it can safely be concluded that direct effects of the current on the taste nerves are minimal.

Recently, an entirely new idea concerning salt transduction has been introduced by DeSimone et al. (1981, 1984), which has strong implications for the mechanism of electric taste. An active transport across the lingual epithelium, composed of Na and Cl, is activated by hyperosmotic NaCl concentrations and may also be activated by an electric potential placed across the epithelium. It has been suggested that this sodium transport is specific for sodium transduction, distinct from potassium, since it is selectively inhibited by the diuretic amiloride
The most compelling evidence for the distinction between sodium and potassium pathways comes from electrophysiological experiments using amiloride (Teeter et al., 1983; Brand et al., 1984; Heck et al., 1984). Amiloride blocks a large portion of the sodium neural response and in vitro ion transport stimulated by hyperosmolar sodium solutions, whereas it only affects to a small degree the potassium neural response and the ion transport stimulated by hyperosmolar potassium solutions. It is interesting that amiloride does not completely block the sodium response. The significance of this residual transduction pathway for sodium responses after amiloride treatment has not been assessed, but it could represent a common pathway for salt transduction. The data from the present set of experiments do not negate the idea that a sodium-selective pathway, distinct from potassium, may be involved in the transduction of sodium electric taste responses. The data imply that sodium electric taste uses a transduction scheme similar to that of chemically applied sodium responses and that potassium electric taste uses the transduction mechanism that suprathreshold potassium solutions use. It neither implicates nor refutes a common pathway (or distinct pathways) for salt transduction. It would be of interest to see whether amiloride blocks sodium electric taste responses in the same manner as it does chemical sodium responses and to see whether it leaves potassium electric taste responses unaffected. It is conceivable that sodium and lithium responses use one transduction pathway and that other salts (e.g., potassium and calcium) use another (or others). However, the electric taste data imply only that the electrical responses resemble their chemical counterpart. The possible role of an active transport process in the transduction of electric taste responses awaits further experimentation.

Kashiwayanagi et al. (1981) have proposed that ion accumulation of salts on the tongue surface is not the mechanism of responses to anodal current in the frog. They have proposed instead that the synaptic area of the taste cell is depolarized by an anodal current that opens voltage-dependent calcium channels and hence induces a release of a chemical transmitter from the taste cell. The electrical responses to anodal current in the frog demonstrate ion specificity, which has been observed in other animals. For example, the frog tongue is much more sensitive to MgCl₂ than to NaCl, whether these stimuli are presented chemically or electrically. Similarly, treatment of the tongue by the chemical modifiers 8-anilino-1-naphthalenesulfonate (ANS) or NiCl₂, which both enhance the chemical response to salts, enhance the anodally induced salt responses. Treatment with uranyl acetate enhanced chemical and electrical responses to NaCl and decreased monotonically chemical and electrical responses to MgCl₂. These results are very similar to the results presented here in that electrical responses demonstrated a great deal of ion specificity and that the effect of chemical modifiers that altered sapid salt responses also altered anodally induced salt responses. In the frog, the only anodal response that differed from a possible ion accumulation interpretation was that of a strong CaCl₂ stimulus after preadaptation to a 50 mM CaCl₂ solution. When a 1.0 M CaCl₂ solution replaces a 50 mM CaCl₂ solution, a small response is obtained that quickly adapts away. When a 700-µA anodal current is superimposed on a constant 50 mM CaCl₂...
stimulus, a large response is obtained (see Fig. 6 of Kashiwayanagi et al., 1981). These authors interpreted the responses to mean that an ion accumulation mechanism is not possible for anodal electric taste. Other factors may be occurring.

In the transport of ions by an electric current through a solution, there exists a critical current density that, when surpassed, results in a sharp increase in the hydrogen ion flux. This hydrogen ion flux then becomes a substantial fraction of the total current (e.g., Miller, 1978; Peers, 1956). Gregor and Peterson (1964) observed a sharp increase in the hydrogen transport with a 0.0005 M KCl solution at 4 mA/cm². The critical current density depends on many factors (e.g., the degree of stirring, concentration, and membrane ion exchange properties), but at the current level of 0.7 mA used in the frog experiments of Kashiwayanagi et al. (1981), it is reasonable that a larger hydrogen flux than expected might be occurring. This hydrogen flux, taken in conjunction with the recent report by Nagahama and Kurihara (1984) that frog gustatory responses to acids were greatly enhanced by calcium ions (a greater than sixfold increase), but were not enhanced by other stimuli (including Na⁺ or Mg²⁺) provides a basis for an alternative explanation of the set of calcium responses under discussion. Any small acid component of the anodal response would be greatly enhanced by the presence of calcium. Hence, the electrical response could well be an augmented acid response. Since acid responses in the frog IXth nerve are almost entirely phasic, the tonic portion of this anodally induced response might be of different origin. Kashiwayanagi et al. state that ions in the mucus covering the tongue must be taken into consideration. The observation that Na⁺ and Mg²⁺ do not enhance the acid response would explain why this hydrogen component of the anodal stimulus does not play any significant part in the anodal response using these cations. Presently, the parameters of electric taste are not well enough understood to invalidate any possible mechanism. More empirical evidence will be needed before one can assess whether different or similar mechanisms are operating in the frog and rat to anodal stimulations. Nevertheless, it is encouraging that the phenomenon of ion specificity is seen across species.

The above discussion of ion accumulation to anodal stimuli may not apply to cathodal stimuli in the frog. Kashiwayanagi et al. (1981) also reported cancellation of nonionic chemical responses (distilled water and ethanol) by cathodal currents. These responses should probably be considered partially ionic since ions contained within the mucus of the frog tongue must be taken into consideration as current carriers. It is interesting to note that similar cancellations of nonionic stimuli (sucrose and quinine) by cathodal currents have also been observed in the rat (Herness, M. S., unpublished observations). In addition, the cathodal OFF response in the rat appears to be ion-insensitive (Herness, M. S., manuscript submitted for publication). Hence, it might not be appropriate to think of anodal and cathodal stimuli merely as inverse forms of the same stimulus. One cannot rule out the possibility that different mechanisms may be operating for these different polarities.

An ion accumulation mechanism remains the simplest explanation for the experimental results obtained with anodal electric taste in the rat. The fact that the phenomenon of ion specificity is so striking can be taken as evidence that
ions from the adapting solution are presented to the receptors by the current. The fact that stimulus intensity–response magnitude relationships for a particular ionic species are so similar for chemical and electrical stimulations implies that a similar transduction process may be operating for both forms of stimulation. Transference experiments also provide biophysical evidence for an ion accumulation mechanism for anodal responses in the rat. While the generality of this proposed mechanism for anodal responses to other species cannot be assessed without further experimentation, it is concluded that the ion accumulation mechanism provides the most adequate description of the data in the rat.

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REFERENCES
Beidler, L. M. 1954. A theory of taste stimulation. J. Gen. Physiol. 38:133–139.
Beidler, L. M. 1961. Taste receptor stimulation. Prog. Biophys. Biophys. Chem. 12:107–151.
Beidler, L. M. 1965. Comparison of gustatory receptors, olfactory receptors, and free nerve endings. Cold Spring Harbor Symp. Quant. Biol. 30:191–200.
Beidler, L. M. 1970. Taste bud cells act as receptors and not merely as chemical filters. In Second Symposium on Oral Sensation and Perception. J. F. Bosma, editor. Charles Thomas, Springfield, IL. 100–108.
Beidler, L. M. 1971. Taste receptor stimulation with salts and acids. In Handbook of Sensory Physiology. Vol. IV: Chemical Senses; Pt. 2: Taste. L. M. Beidler, editor. Springer-Verlag, Berlin. 200–220.
Beidler, L. M. 1975. Taste receptors. In Sensory Physiology and Behavior. R. Galun, P. Hillman, I. Farnas, and R. Werman, editors. Plenum Publishing Corp., New York. 201–210.
Beidler, L. M. 1978. Biophysics and chemistry of taste. In Handbook of Perception. Vol. VIA: Taste and Smell. E. D. Carterette and M. P. Friedman, editors. Academic Press, Inc., New York. 21–49.
Brand, J. G., J. H. Teeter, and W. L. Silver. 1984. Effect of amiloride concentration on reduction of chorda tympani responses to alkali chlorides. The Sixth Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL. (Abstr.)
Bujas, Z. 1971. Electric taste. In Handbook of Sensory Physiology. Vol. IV: Chemical Senses; Pt. 2: Taste. L. M. Beidler, editor. Springer-Verlag, Berlin. 180–199.
Bujas, Z., M. Frank, and C. Pfaffmann. 1979. Neural effects of electrical taste stimuli. Sensory Processes. 3:355–365.
DeSimone, J. A., G. L. Heck, and S. DeSimone. 1981. Active ion transport in dog tongue: a possible role in taste. Science (Wash. DC). 214:1039–1041.
DeSimone, J. A., G. L. Heck, S. Mierson, S. K. DeSimone. 1984. The active ion transport
properties of canine lingual epithelia in vitro. Implications for gustatory transduction. J. Gen. Physiol. 83:653–656.

Faull, J. R., and B. P. Halpern. 1972. Taste stimuli: time course of peripheral nerve response and theoretical models. Science (Wash. DC). 178:73–75.

Galvani, L. 1791. Commentary on the effects of electricity on muscular motion. In Effects of Electricity on Muscular Motion. 1954. I. B. Cohen, editor. Burndy Library, Norwalk, CT.

Getchell, M. L., and R. C. Gesteland. 1972. The chemistry of olfactory reception: stimulus-specific protection from sulfhydryl reagent inhibition. Proc. Natl. Acad. Sci. USA 69:1494–1498.

Gregor, H. P., and M. A. Peterson. 1964. Electrolytic polarization of ion-exchange membrane systems. J. Phys. Chem. 68:2201–2205.

Heck, G. L., S. Mierson, and J. A. DeSimone. 1984. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science (Wash. DC). 223:403–405.

Herness, M. S. 1981a. The anion's affect in salty electric taste. The Third Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL. (Abstr.)

Herness, M. S. 1981b. Ionic events of electric taste. Soc. Neurosci. 7:664. (Abstr.)

Herness, M. S. 1982. Electric taste stimulation: neural evidence. The Fourth Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL. (Abstr.)

Kashiwayanagi, M., K. Yoshii, Y. Kobatake, and K. Kurihara. 1981. Taste transduction mechanism. Similar effects of various modifications of gustatory receptors on neural responses to chemical and electrical stimulation in the frog. J. Gen. Physiol. 78:259–275.

Kobatake, Y., and N. Kamo. 1973. Effects of stagnant layer on membrane phenomena. Rate of electroosmotic flow. In Fourth International Symposium on Fresh Water from the Sea. 3:91–98.

Laidler, K. J., and J. H. Meiser. 1982. Physical Chemistry. Benjamin/Cummings Publishing Co., Menlo Park, CA. 142–147.

Marowitz, L. A., and B. P. Halpern. 1977. Gustatory neural response of the chorda tympani to lick-duration stimuli. Chem. Sens. Flavour. 2:457–485.

Mayer, D. 1977. Beidler's taste equation applied to electric taste. Acta Inst. Psychol. Univ. Zagrebiensis. 83:51–54.

Miller, I. 1978. Electrolysis of aqueous solutions. In Techniques of Electrochemistry. E. Yeager and A. J. Salkind, editors. John Wiley & Sons, New York. 3:437–487.

Mooser, G. 1976. N-substituted maleimide inactivation of the response to taste cell stimulation. J. Neurobiol. 7:457–468.

Nagahama, S., and K. Kurihara. 1984. Enhancement of taste responses to acids by calcium ions. Comp. Biochem. Physiol. 77A:63–66.

Nejad, M. 1960. Factors involved in the mechanism of stimulation of gustatory receptors and bare nerve endings of the tongue of the rat. Ph.D. Dissertation. The Florida State University, Tallahassee, FL.

Ninomiya, Y., and M. Funakoshi. 1981a. Responses of rat chorda tympani fibers to electrical stimulation of the tongue. Jpn. J. Physiol. 31:559–570.

Ninomiya, Y., and M. Funakoshi. 1981b. Role of ions in generation of taste nerve responses to electrical stimulation in rats. Jpn. J. Physiol. 31:891–902.

Peers, A. M. 1956. Discuss. Faraday Soc. 21:124–125.

Pfaffmann, C., and T. Pritchard. 1980. Ion specificity of "electric taste". In Olfaction and Taste. H. van der Starre, editor. IRL Press, London. 7:175–178.

Pritchard, T., and C. Pfaffmann. 1981. Electro-chemical stimulation of taste. Soc. Neurosci. 7:730. (Abstr.)
Smith, D. V., and S. L. Bealer. 1975. Sensitivity of the rat gustatory system to the rate of stimulus onset. *Physiol. & Behav.* 15:303–314.

Sulzer, M. 1754. Recherches sur l’origine des sentiments agréables et désagréables. Troisième partie: des plaisirs des sens. Histoire de l’académie des sciences et belles lettres de Berlin (année 1752): quoted from E. Skramlik. 1926. Handbuch der Physiologie der niedereren Sinne. 1. Bd., S. 378. G. Thieme, Leipzig.

Teeter, J. H., W. L. Silver, and J. G. Brand. 1983. Effect of amiloride on chorda tympani responses to salts. *Soc. Neurosci. Abstr.* 9:1022. (Abstr.)

Volta, A. 1792. Briefe über thierische Electricität. A. J. Oettingen, editor. 1900. Ostwald’s Klassiker der exakten Wissenschaften. Engelmann, Leipzig.

Volta, A. 1793. Account of some discoveries made by Mr. Galvani of Bologna; with experiments and observations on them. *Philos. Trans. R. Soc. Lond.* 83:10–44.

Warren, J. F. 1965. A study of the responses of taste receptors of rat tongue to electrical stimulation. M.S. Thesis. The Florida State University, Tallahassee, FL. 78 pp.

Yamamoto, T., N. Yuyama, and Y. Kawamura. 1980. Response of cortical taste cells and chorda tympani fibers of anodal D.C. stimulation of the tongue in rats. *Exp. Brain Res.* 40:63–70.