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Electrophysiological properties of the tongue epithelium of the toad *Bufo marinus*

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Summary

The dorsal lingual epithelium from the tongue of the toad *Bufo marinus* was mounted in an Ussing-type chamber, and the short-circuit current (I_{sc}) was measured using a low-noise voltage clamp. With NaCl Ringer bathing the mucosal and serosal surfaces of the isolated tissue, an outwardly directed (mucosa-positive) I_{sc} was measured that averaged $-10.71\pm0.82\,\mu A\,cm^{-2}$ (mean ± S.E.M., N=24) with a resistance of $615\pm152\,\Omega\,cm^2$ (mean ± S.E.M., N=10). Substitution of chloride with sulfate as the anion produced no significant change in I_{sc}. Fluctuation analysis with either NaCl or Na_2SO_4 Ringer bathing both sides of the tissue revealed a spontaneous Lorentzian component, suggesting that the I_{sc} was the result of K^+ secretion through spontaneously fluctuating channels in the apical membrane of the epithelium. This hypothesis was supported by the reversible inhibition of I_{sc} by Ba^{2+} added to the mucosal Ringer. Analysis of the kinetics of Ba^{2+} inhibition of I_{sc} indicates that there might be more than one type of K^+ channel carrying the I_{sc}. This hypothesis was supported by power spectra obtained with a serosa-to-mucosa K^+ gradient, which could be fitted to two Lorentzian components. At present, the K^+ secretory current cannot be localized to taste cells or other cells that might be associated with the secretion of saliva or mucus. Nonetheless, the resulting increase in [K^+] in fluid bathing the mucosal surface of the tongue could presumably affect the sensitivity of the taste cells. These results contrast with those from the mammalian tongue, in which a mucosa-negative I_{sc} results from amiloride-sensitive Na^+ transport.

Key words: toad, *Bufo marinus*, tongue, lingual epithelium, K^+ channel, taste.

Introduction

The landmark studies of DeSimone et al. (1981, 1984) demonstrated that isolated canine lingual epithelium, mounted an Ussing-type chamber, develops a short-circuit current (I_{sc}) like that of the frog skin, which is the result of the active transport of Na^+ from the outer (mucosal) to the inner (serosal) surface of the tissue (Ussing and Zerahn, 1951). The I_{sc} could be inhibited by the pyrazine diuretic amiloride, which blocks epithelial Na^+ channels in the apical membrane of the epithelial cells, including the taste cells. The neural response of the chorda tympani nerve to NaCl solutions applied to the tongue was similarly reduced by the application of amiloride to the mucosal surface of the tongue *in situ*, and it was suggested that the transport of Na^+ across the lingual epithelium is a primary mechanism for Na^+ taste transduction in mammals. More recent studies on mammalian (rat and hamster) tongue indicate that, in addition to transcellular currents across specific taste cells, transcellular and paracellular transport of salts across the lingual epithelium also serves a role in salt taste transduction (Ye et al., 1991; Gilbertson and Zhang, 1998). Gilbertson and Zhang (1998) suggest that '…the whole lingual tissue may be viewed as a taste organ involving both taste receptor cells and non-taste epithelium in parallel'.

Among other vertebrate classes, the taste response of the amphibian tongue was studied by Pumphry (1935), who demonstrated that the application of salty or acidic solutions to the lingual surface stimulated the glossopharyngeal nerve of frogs. Sato (1976) has summarized the historical development of studies on the chemosensory responses of the amphibian tongue, and the morphology of the tongue has been described by Jaeger and Hillman (1976). Experiments to date with the isolated lingual epithelium of amphibians have not demonstrated a transepithelial voltage difference when the tissue was bathed on both sides with identical Ringer's solutions (Soeda and Sakudo, 1985). Detailed studies of acutely dissociated taste cells from frog tongue have been conducted by Avenet and Lindemann (1988), who utilized whole-cell patch-clamp methodology to demonstrate a cationic current across these cells. This current was inhibited by amiloride, suggesting that, like taste cells from mammalian tongue (DeSimone et al., 1981, 1984), epithelial Na^+ channels play a role in salt taste transduction. In the present study, we tested the hypothesis that the transepithelial current across the lingual epithelium of the toad *Bufo marinus* is similar to that of mammals. We observed that, in contrast to the mammalian

tongue, the short-circuit current across the lingual epithelium of *Bufo marinus* is outwardly directed and is carried by active transport of K^+ from the serosal to the mucosal surface of the tissue. Fluctuation analysis (for a review, see Van Driessche, 1994) of the short-circuit current demonstrates the presence of spontaneously fluctuating channels in the apical membrane of the epithelium that may be involved in K^+ secretion.

Materials and methods

Animal care and tissue preparation

Bufo marinus L. were obtained commercially and kept at 21–23 °C on a 12 h:12 h L:D photoperiod in a terrarium that contained moist soil, covered burrows and standing water. The toads were fed crickets twice per week. After doubly pithing the animals, the tongue was removed and pinned in a dish containing frog Ringer's solution (115 mmol l^{-1} NaCl, 2.5 mmol l^{-1} $KHCO_3$, 1 mmol l^{-1} $CaCl_2$; NaCl Ringer). All solutions had a pH of 8.0 at the ambient temperature (21–23 °C). The ventral epithelium and large muscles underlying the dorsal surface were trimmed, and the dorsal surface was mounted in an Ussing-type chamber that minimized edge damage and allowed continuous perfusion of both the mucosal and serosal epithelia (De Wolf and Van Driessche, 1986). The chamber had a cross-sectional area of 0.5 cm^2 and a volume of 1.5 ml. The perfusion rate of approximately 5 ml min^{-1} in the present experiments was regulated by the height of the perfusion reservoirs. Short-circuit current was applied with a low-noise voltage clamp (Van Driessche and Lindemann, 1978). This voltage-clamp and electrode arrangement has been successfully used to record I_{sc} and current fluctuations arising from K^+ transport across the isolated skin of the frog *Rana temporaria* (De Wolf and Van Driessche, 1986) in addition to the standard Ussing conditions with identical NaCl Ringer on both sides of the tissue (Baker and Hillyard, 1992).

Current measurements

The I_{sc} is classically defined as the direct current (μA cm^{-2}) that maintains the voltage across the tissue at 0 mV with identical Ringer bathing both sides of the tissue (Ussing and Zerahn, 1951). With asymmetric bathing solutions, the I_{sc} may also reflect passive diffusion of ions. The alternating component of the current was filtered and amplified for fluctuation analysis. This technique is based on the assumption that the macroscopic I_{sc} is the sum of currents through a population of individual ion channels that fluctuate between an open and a closed state, thereby inducing current fluctuations of a much smaller magnitude. Analysis of these fluctuations can be used to characterize an ionic current as passing through channels and can provide information about the kinetic properties of the channels. The methodology for this procedure has recently been reviewed (Van Driessche, 1994) and is briefly summarized as follows. The alternating current signal was filtered with a fundamental frequency of 0.5 Hz and a low-pass filter of 1 kHz. The signal was also passed through an anti-aliasing filter, amplified and converted to a digital signal that was subject to a fast Fourier transform. The resulting power spectrum is displayed as a double-logarithmic plot of spectral density, S_f (A^2 s cm^{-2}) *versus* frequency, f (Hz). The spectrum was fitted as the sum of a linear and a Lorentzian function:

$$S_f = (K_b/f^{\alpha}) + S_o/[1 + (f/f_c)^2] . \quad (1)$$

In equation 1, the linear function includes K_b, the power density at 1 Hz, and α is the slope of the line in the double-logarithmic plot. The linear function is also called '1/*f*' noise and is believed to result from the diffusion of ions through open channels (Conti et al., 1975). These authors studied the current fluctuations across isolated squid axon membranes and found the slope of the line to be 1, while the value of K_b increased as the voltage gradient increased current through K^+ channels. In epithelia, Van Driessche (1974) points out that there are numerous sources of 1/*f* noise and that they provide little information about specific ion channels.

The Lorentzian function includes S_o, which is the power density of the Lorentzian plateau at low frequencies, and the corner frequency (f_c), which is the frequency at which the power density has relaxed to half that of S_o. The presence of a Lorentzian function in a power spectrum is the result of changes in the conductance state of individual ion channels and is taken as evidence for a spontaneously fluctuating population of ion channels contributing to the I_{sc} (Conti et al., 1975; Lindemann and Van Driessche, 1977; Van Driessche, 1994). Equations 2–5 (see Discussion) were developed in detail by Van Driessche (1994). They are presented in this paper to facilitate the interpretation of the fluctuation analysis measurements in terms of models that describe the kinetic properties of ion channels.

Experimental protocols

I_{sc} measurements were begun with NaCl Ringer's solution bathing both mucosal and serosal surfaces of the tissue and with the command voltage set to 0 mV. Under these conditions, there are no voltage or concentration gradients across the tissue, and any currents that are passed by the voltage clamp represent the active transport of ions (Ussing and Zerahn, 1951). Four sets of experiments were performed: cation exchange, anion exchange, mucosal Ba^{2+} treatment and mucosal quinine treatment.

The cation exchange experiments involved replacing NaCl Ringer with KCl Ringer (112.5 mmol l^{-1} KCl, 2.5 mmol l^{-1} $KHCO_3$, 1 mmol l^{-1} $CaCl_2$) on the mucosal surface, re-equilibration with NaCl in the mucosal and serosal solutions and then replacement with KCl in the serosal solution. Under these conditions, passive concentration gradients for K^+ are established across the tissue, and the changes in current and fluctuation analysis parameters can be compared with those obtained in the absence of a K^+ gradient.

For the anion exchange experiments, the tissue was initially bathed in NaCl Ringer. The first step was to change both mucosal and serosal solutions to Na_2SO_4 Ringer (57.5 mmol l^{-1}

Na_2SO_4, 2.5 mmol l^{-1} $KHCO_3$, 1 mmol l^{-1} $CaCl_2$). When a stable I_{sc} had been obtained, a power spectrum was recorded. The mucosal and then the serosal solutions were exchanged with K_2SO_4 Ringer (57.5 mmol l^{-1} K_2SO_4, 2.5 mmol l^{-1} $KHCO_3$, 1 mmol l^{-1} $CaCl_2$) to duplicate the sequence of cation substitutions performed above with KCl. Chloride ions are known to be transported across many epithelia (Larsen, 1991). If the I_{sc} across the toad tongue is the result of Cl^- transport, substitution of Cl^- should eliminate that current.

Ba^{2+} and quinine are known blockers of K^+ channels, so the inhibition of I_{sc} by these substances would support the hypothesis that the I_{sc} is the result of K^+ transport. The effects of Ba^{2+} on the I_{sc} were first recorded with NaCl Ringer bathing the mucosal and serosal surfaces of the tissue. The mucosal Ringer's solution contained, sequentially, 0.5, 1, 2, 5 and 10 mmol l^{-1} $BaCl_2$. After maximal inhibition of I_{sc} had been observed, the mucosal solution was replaced with Ringer containing no Ba^{2+}. In a second set of experiments, the inhibition of I_{sc} by mucosal Ba^{2+} was recorded from tissues bathed at the serosal surface with KCl Ringer and at the mucosal surface with NaCl Ringer.

Quinine experiments were performed with mucosal applications of 10 μmol l^{-1} followed by 100 μmol l^{-1} quinine with NaCl Ringer as the mucosal and serosal solutions. When the quinine solution had been washed out, 10 mmol l^{-1} $BaCl_2$ was added to the mucosal side and then washed out. I_{sc} was continuously recorded on a chart recorder, and noise analysis was conducted upon equilibration of I_{sc} after each solution change described above.

All data are reported as means ±1 S.E.M.

Results

Cation substitution

The initial I_{sc} with identical NaCl Ringer bathing both sides of the tissue was –10.71±0.82 μA cm^{-2} (N=24). The chart tracing from a typical experiment is shown if Fig. 1. Negative values for I_{sc} represent outward movement (serosa to mucosa) of positive charge, which could arise either from the outward transport of a cation (K^+) or from the inward transport of an anion (Cl^-). In 10 preparations, the tissue resistance was calculated by recording the current change associated with a 10 mV current pulse and was found to be 615±152 Ω cm^2. The resistance values were highly variable among preparations and following ion substitutions during a given experiment. We report here the initial resistance values obtained with NaCl Ringer in the mucosal and serosal solutions for comparisons with other species. In most experiments, and as seen in Fig. 1, the current started near –20 μA cm^{-2} before decreasing to a stable value. A representative power spectrum is shown in Fig. 2A. In all three panels of Fig. 2, the arrows represent the

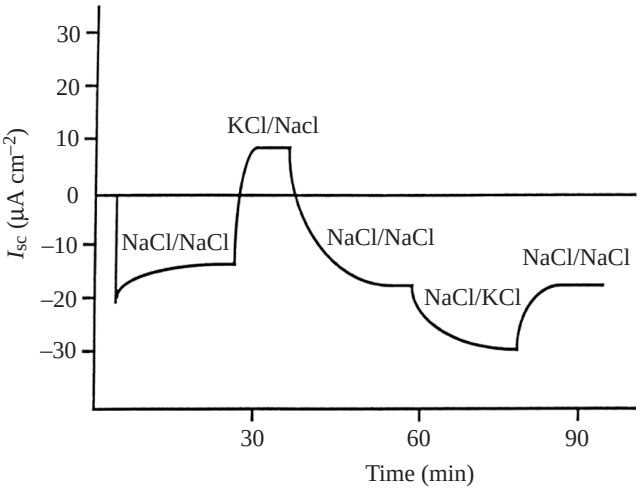

Fig. 1. A recording from a typical experiment in which NaCl and KCl Ringer were exchanged sequentially as the mucosal and serosal bathing solutions. The solution changes (mucosal/serosal) are indicated on the figure. I_{sc}, short-circuit current.

Table 1. *Effects of cation (K^+ for Na^+) substitution in the mucosal and serosal bathing solutions on* I_{sc}, S_o *and* f_c

Mucosa/serosa Ringer's solution	I_{sc} (μA cm^{-2})	S_o (A^2 s cm^{-2})	f_c (Hz)
NaCl/NaCl	–11.54±2.23	$12.71\times10^{-20}\pm3.43\times10^{-20}$	9.73±0.94
KCl/NaCl	9.45±1.97	$2.68\times10^{-20}\pm0.92\times10^{-20}$	19.03±6.81
	P<0.0001	P=0.02	P=0.02
	N=6	N=6	N=6
NaCl/NaCl	–11.76±1.94	$11.67\times10^{-20}\pm3.90\times10^{-20}$	9.41±0.36
NaCl/KCl	–33.52±6.75	$18.68\times10^{-20}\pm3.61\times10^{-20\dagger}$	6.39±0.58†
		$4.78\times10^{-20}\pm1.66\times10^{-20\dagger}$	47.40±7.34†
	P=0.02	P=0.22*	P=0.18*
	N=7	N=6	N=6

I_{sc}, short-circuit current; f_c, corner frequency; S_o, power density of the Lorentzian plateau at low frequency.

All values are means±1 S.E.M.

The effects of KCl substitution in the mucosal solution were completely reversible. A dagger indicates S_o and f_c values for six experiments in which two Lorentzian functions could be fitted to the power spectra. Asterisks indicate statistical comparison between the S_o and f_c values obtained with NaCl/NaCl and the lower-frequency Lorentzian function in spectra obtained with NaCl/KCl conditions.

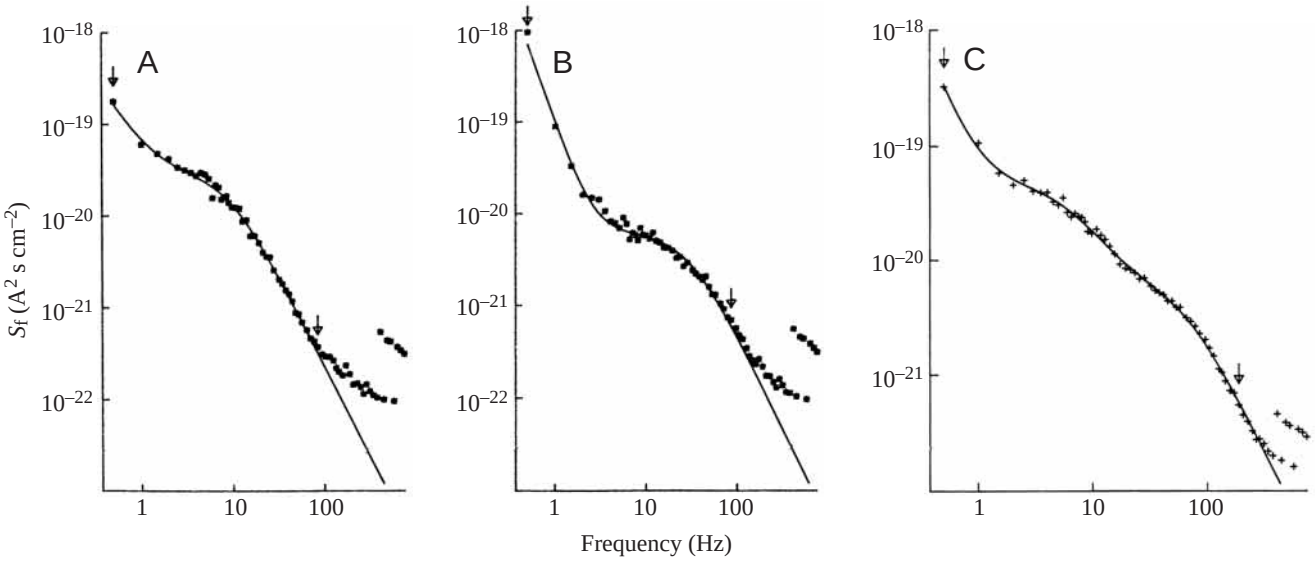

Fig. 2. Power spectra obtained from experiments with (A) NaCl Ringer as the mucosal and serosal bathing solutions, (B) NaCl Ringer as the serosal solution and KCl Ringer as the mucosal solution and (C) NaCl Ringer as the mucosal solution and KCl Ringer as the serosal solution. Note the presence of two Lorentzian components in C. The spectra from A and B are from sequential treatments of the same preparation as depicted in Fig. 1. S_f, spectral density. The arrows on the spectra indicate the range of data points selected for the computer fit of the spectra using equation 1.

range selected for fitting according to equation 1. Initial values for S_o and f_c were $18.16\times10^{-20}\pm2.41\times10^{-20}$ A^2 s cm^{-2} and 10.19±1.09 Hz, respectively (N=24). Values for I_{sc}, S_o and f_c for the cation substitution experiments are presented in Table 1.

Replacement of mucosal NaCl Ringer's solution with KCl Ringer resulted in significant changes in I_{sc}, S_o and f_c: I_{sc} became positive, S_o declined and f_c shifted to a higher frequency. Statistical comparisons, unless otherwise specified, were with Fisher's Protected Least Significant Difference (PLSD) test using StatView Version 4.5 for Windows. A representative power spectrum obtained with KCl Ringer as the mucosal solution is shown in Fig. 2B. The spectrum in Fig. 2B is from the same preparation as that of Fig. 2A and illustrates the decline in S_o and the increase in f_c presented in Table 1. All these effects of mucosal KCl were reversed by changing the solution back to NaCl Ringer.

When the serosal solution was changed from NaCl to KCl, I_{sc} became significantly more negative (Fig. 1; Table 1). This change in current was reversed by switching the serosal solution back to NaCl. With Cl^- as the anion, power spectra obtained with a serosa-to-mucosa K^+ gradient displayed two Lorentzian components in six of seven experiments (Table 1). One Lorentzian component had an f_c value that was somewhat smaller than that obtained with NaCl Ringer on both sides of the tissue, and S_o was increased but was highly variable among preparations and not significantly greater than with NaCl solutions on both sides of the tissue (Table 1). The second Lorentzian component had a higher f_c and lower S_o than the first. A representative power spectrum fitted with two Lorentzian components is shown in Fig. 2C.

Anion substitution

Substitution of NaCl Ringer's solutions with Na_2SO_4 Ringer in both the mucosal and serosal baths produced no significant change in any of the three variables (Table 2). Substitution of Na_2SO_4 Ringer on both sides of the tissue with mucosal K_2SO_4 Ringer's solution produced results similar to those seen with mucosal KCl: I_{sc} increased to positive values, S_o declined and f_c increased, all significantly (Table 2). As with the addition of mucosal KCl, these results were reversed by changing the mucosal solution back to Na_2SO_4. When the serosal solution was changed from Na_2SO_4 to K_2SO_4, with Na_2SO_4 Ringer bathing the mucosal surface, I_{sc} became significantly more negative. Thus, the general pattern of I_{sc} changes depicted in Fig. 1 also apply to experiments with SO_4^{2-} as the anion. Three of the seven power spectra obtained under these condition could be fitted with either one or with two Lorentzian components like that shown in Fig. 2C. The values for the single Lorentzian fits are presented in Table 2, with the understanding that the higher-frequency Lorentzian component, when present, is less prominent than that observed with Cl^- as the anion.

Ba^{2+} inhibition of I_{sc}

With NaCl Ringer in the mucosal and serosal bath solutions, the addition of increasing concentrations of $BaCl_2$ to the mucosal surface resulted in a dose-dependent inhibition of the negative I_{sc} from an initial value of -13.00 ± 2.22 μA cm^{-2} (N=6) (Fig. 3). Typically, the inhibition of I_{sc} was rapid, and a stable value for each concentration was achieved within 2–3 min. Upon removal of the $BaCl_2$ solution from the mucosal surface, the I_{sc} recovered to a level not significantly different from its pre-treatment value, -11.87 ± 0.82 μA cm^{-2} (N=6).

Table 2. *Effects of anion substitution in the mucosal and serosal solutions on* I_{sc}, S_o *and* f_c

Mucosa/serosa Ringer's solution	I_{sc} ($\mu A\,cm^{-2}$)	S_o ($A^2\,s\,cm^{-2}$)	f_c (Hz)
NaCl/NaCl	–9.34±1.47	$19.55\times10^{-20}\pm4.90\times10^{-20}$	9.21±1.17
Na_2SO_4/Na_2SO_4	–6.80±0.21	$12.34\times10^{-20}\pm3.33\times10^{-20}$	9.62±0.79
	P=0.21	*P*=0.24	*P*=0.79
	N=8	*N*=8	*N*=8
Na_2SO_4/Na_2SO_4	–5.70±1.03	$8.31\times10^{-20}\pm2.21\times10^{-20}$	9.76±1.24
K_2SO_4/Na_2SO_4	10.25±1.09	$0.51\times10^{-20}\pm0.11\times10^{-20}$	22.97±5.53
	P=0.0001	*P*=0.004	*P*=0.04
	N=7	*N*=7	*N*=7
Na_2SO_4/Na_2SO_4	–8.85±2.43	$13.80\times10^{-20}\pm2.91\times10^{-20}$	8.47±1.41
Na_2SO_4/K_2SO_4	–31.66±7.20	$20.64\times10^{-20}\pm6.04\times10^{-20}$	9.12±1.41
	P=0.01	*P*=0.33	*P*=0.69
	N=7	*N*=7	*N*=7

Values are means ± 1 S.E.M.

I_{sc}, short-circuit current; f_c, corner frequency; S_o, power density of the Lorentzian plateau at low frequency.

The effect of substituting K^+ for Na^+ as the mucosal cation is reversible.

As indicated in the text, three of the seven spectra obtained under Na_2SO_4/K_2SO_4 conditions could be fitted by a double or a single Lorentzian function. The values presented for S_o and f_c are from the single-fit values for all seven experiments.

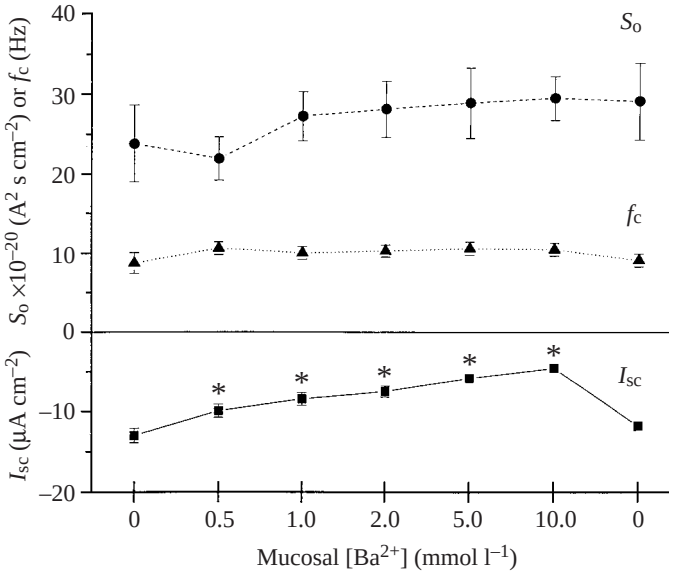

Fig. 3. The inhibition of short-circuit current (I_{sc}) by the addition of Ba^{2+} to the mucosal Ringer's solution. The *x* axis scale reflects the sequence of treatments, which were applied at approximately regular intervals. In these experiments, the mucosal and serosal baths were NaCl Ringer. I_{sc} values marked with an asterisk are significantly inhibited relative to the control value. Removal of Ba^{2+} restores I_{sc} to its control value. The changes in the power density of the Lorentzian plateau at low frequencies (S_o) and the corner frequency (f_c) are not significant. Values are means ± S.E.M., *N*=6.

Statistical analysis using a non-parametric paired sign test shows the inhibition of I_{sc} to be significant at each concentration change (*P*=0.0313). The pattern of Ba^{2+} inhibition was further analyzed with a direct linear plot (Eisenthal and Cornish-Bowden, 1979). This is a modification of the Michaelis–Menten equation for a pseudo-first-order inhibitor. Graphically, a family of lines is drawn between the points representing the inhibitor concentrations, as negative values, on the abcissa and the decrement in I_{sc} for each blocker concentration on the ordinate (Fig. 4A). If the inhibitor is binding to a single inhibitory site, the lines, extended to positive *x* axis values, should intersect at a common point that describes the inhibitory constant (K_i) as the *x* coordinate and the amount of inhibitor-sensitive current as the *y* coordinate. It can be seen in Fig. 4A that the lines for inhibition by 0.5, 1 and 2 mmol l^{-1} Ba^{2+} intersect near a common point but that the lines for 5 and 10 mmol l^{-1} Ba^{2+} intersect at points that indicate a higher binding constant and thus a lower binding affinity.

The inhibition of I_{sc} by Ba^{2+} was also examined with KCl as the serosal solution. In these experiments (*N*=4), the I_{sc} was $-30.37\pm5.14\,\mu A\,cm^{-2}$, and a similar pattern of inhibition was seen when Ba^{2+} was added to the mucosal solution. Specifically, the lines of the direct linear plot intersect at a common point at lower Ba^{2+} concentrations of 0.5–5 mmol l^{-1}, but the point of intersection at 10 mmol l^{-1} Ba^{2+} indicates a lower-affinity blockage (Fig. 4B). If the amount of Ba^{2+}-sensitive I_{sc} is compared between the mean values for 10 mmol l^{-1} Ba^{2+} data points in Fig. 4A and B, it can be seen that –8.46 of $-13.00\,\mu A\,cm^{-2}$ (65 %) is inhibited with no K^+ gradient across the tissue while 11.72 of $30.37\,\mu A\,cm^{-2}$ (38.6 %) is inhibited in the presence of a serosa-to-mucosa K^+ gradient, i.e. the increased negative I_{sc} is largely blocker-insensitive.

The fluctuation analysis results obtained for Ba^{2+} inhibition of I_{sc} with NaCl Ringer on both sides of the tissue showed no significant changes in either f_c or S_o as I_{sc} was inhibited (Fig. 3).

Quinine inhibition of I_{sc}

The addition of 10 μmol l^{-1} quinine, a bitter tastant as well

as a K^+ channel blocker, to the mucosal surface consistently produced a decrease in the mean I_{sc} value from –14.53 to –11.62 μA cm^{-2} (Fig. 5). Analysis of these data with a non-parametric paired sign test showed this to be significant (P=0.0313). Further inhibition of the current is seen with 100 μmol l^{-1} quinine (P=0.0022). Wash-out of the quinine does not produce a recovery of the initial current. Addition of 10 mmol l^{-1} Ba^{2+} to the mucosa, after the quinine wash-out, causes a further inhibition of the I_{sc} (P<0.0001), which is reversible upon its removal.

The fluctuation analysis experiments with quinine and Ba^{2+} showed that S_o values declined as I_{sc} was inhibited and increased when Ba^{2+} was washed out. The changes in f_c were very small, however, and did not correlate with the changes in I_{sc} or S_o (Fig. 5).

Discussion

Macroscopic I_{sc}

Similar values for the outwardly directed I_{sc} were observed with either Cl^- or SO_4^{2-} as the anion, and the I_{sc} could be reversibly inhibited by the addition of Ba^{2+} to the mucosal bath. Both these properties suggest that the I_{sc} is the result of active K^+ secretion that involves K^+ channels in the apical membrane of cells of the lingual epithelium. This pattern of transport differs qualitatively from that of the mammalian tongue, in which the I_{sc} recorded in Ussing chamber preparations is mucosa-negative as the result of Na^+ absorption that can be inhibited by amiloride (DeSimone et al., 1981; Gilbertson and Zhang, 1998). The qualitative difference between ionic currents across the toad and mammalian tongue epithelium thus provides an interesting model for comparative studies.

As noted above, Soeda and Sakudo (1985) conducted Ussing chamber experiments with the isolated lingual epithelium of bullfrogs but detected no spontaneous potential difference across the tissue bathed with identical Ringer on both sides. They measured a resistance of 720±180 Ω cm^2, which is very similar to the value of 615±152 Ω cm^2 for *B. marinus* tongue (present study) and 576±127 Ω cm^2 for dog tongue (DeSimone et al., 1981). It is generally assumed that the lingual epithelium of mammals is a low-resistance tissue with both transcellular and paracellular pathways for ion transport and sensory transduction (Ye et al., 1991; Gilbertson and Zhang, 1998). Given the similar low resistance values for frog and toad tongue, it appears that these epithelia also have paracellular as well as transcellular transport pathways. Soeda and Sakudo (1985) also observed that the addition of 200 mmol l^{-1} NaCl, 5 mmol l^{-1} acetic acid or 5 mmol l^{-1} quinine to the mucosal bath increased the potential across the bullfrog tongue, and they suggested that the change in transepithelial potential might be associated with a chemosensory function. We have conducted preliminary experiments with the lingual epithelium of the frog *Rana pipiens* and have noted a similar pattern of K^+ secretion as was noted above for the toad tongue, i.e. an outwardly directed I_{sc}, Ba^{2+}-sensitivity and a Lorentzian component in power spectra. These observations suggest a mucosa-positive I_{sc} that is mediated by K^+ secretion to be the pattern for the lingual epithelium of at least the bufonid and ranid families of the Anura. It is not clear why Soeda and Sakudo (1985) did not observe this pattern of transport.

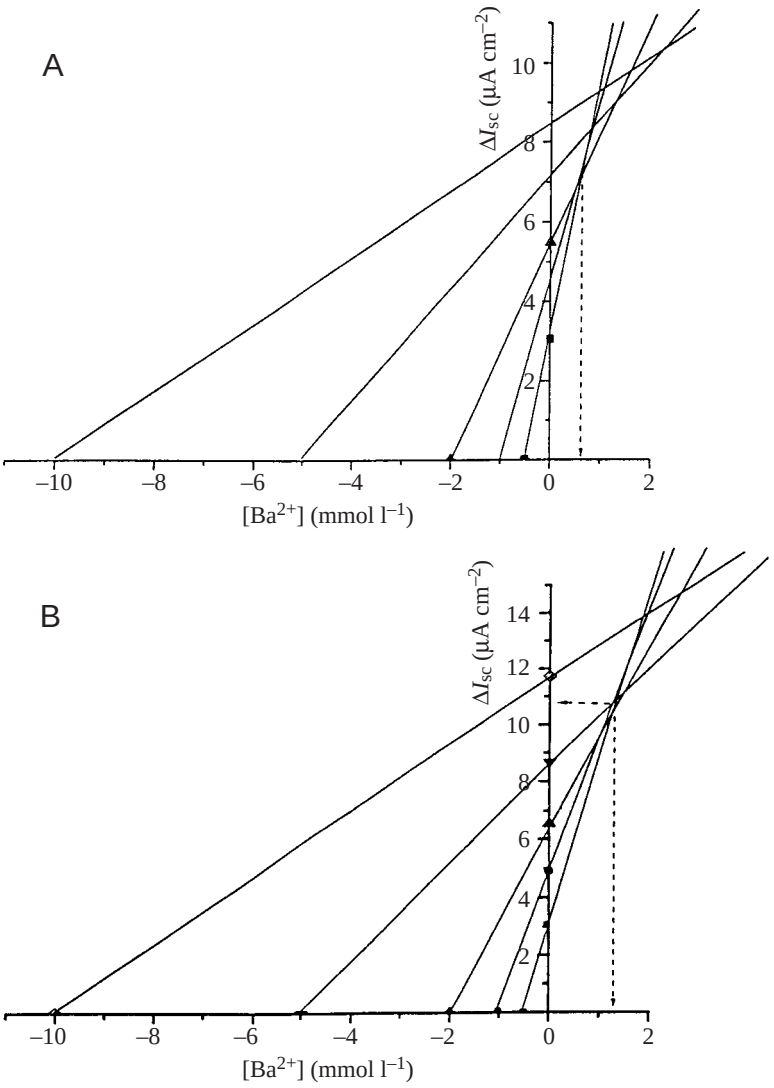

Fig. 4. Direct linear plots for Ba^{2+} inhibition of short-circuit current (I_{sc}). (A) Experiments with NaCl as the mucosal and serosal bathing solution; the mean control value of I_{sc} was –13.00 μA cm^{-2} (the y intercepts are the means for six experiments). (B) Experiments with NaCl and KCl as the mucosal and serosal solutions, respectively (N=4). The mean control I_{sc} for these experiments was –30.37 μA cm^{-2}. Note that the lines do not all intersect at a common point as would be predicted if the Ba^{2+} inhibition were due to binding to a single site with a single binding affinity. The broken lines indicate the x intercept in A and B, which is the K_i value for Ba^{2+} inhibition at lower concentrations. In B, the broken horizontal line indicates the blocker-sensitive I_{sc} (not drawn in A).

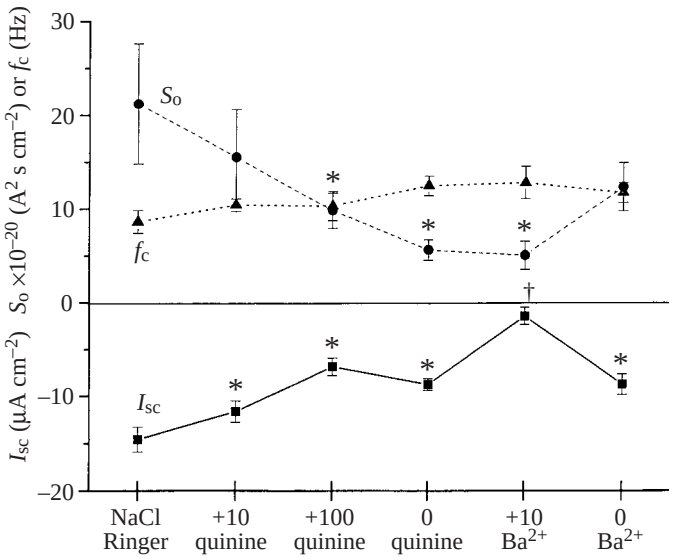

Fig. 5. The inhibition of short-circuit current (I_{sc}) by 10 and 100 μmol l^{-1} quinine added to the mucosal Ringer. Note that removal of quinine does not reverse the inhibition of I_{sc}, while the addition of 10 mmol l^{-1} Ba^{2+} does produce a further inhibition that is reversible. In all experiments, NaCl Ringer was the mucosal and serosal bathing solution. The values for the Lorentzian plateau at low frequencies (S_o) decrease with I_{sc} as the quinine concentration is increased and remain low after quinine wash-out and during Ba^{2+} treatment. S_o does increase when I_{sc} values return to more negative values following the removal of Ba^{2+}. Values marked with an asterisk indicate that I_{sc} and S_o are reduced significantly relative to the control values. The value marked with a dagger indicates that the value of I_{sc} following treatment with 10 mmol l^{-1} Ba^{2+} is significantly different from the quinine wash-out value. The corner frequencies (f_c) do not change significantly over the course of the mucosal solution changes. Values are means ± S.E.M., N=6.

We observed no effect of 10 μmol l^{-1} amiloride on I_{sc} across the toad tongue bathed with NaCl Ringer on both sides of the tissue. The sensory processes of taste cells make up a small percentage of the membrane area of the taste discs and an even smaller percentage of the membrane area of the lingual epithelium (Jaeger and Hillman, 1976). It is possible that a small amiloride-sensitive component to the measured I_{sc} that might be carried by inward Na^+ transport across taste cells would not be resolved at the microampere level. Avenet and Lindemann (1988) estimated the Na^+ current per taste cell to vary between 10 and 700 pA when clamped to –80 mV. The minimum number of cells required to produce 1 μA of current is thus greater than 1000, assuming that toad and frog taste cells have a similar Na^+ transport capability and that the *in situ* membrane potentials are of the order used in the patch-clamp study. Without this information and a detailed histological examination of the tissue used in specific experiments, it is not possible to speculate further. It is noteworthy that Kitada and Mitoh (1997) observed no effect of amiloride on the activity of the glossopharyngeal nerve of the frog during exposure of the tongue to NaCl concentrations between 0.1 and 0.5 mol l^{-1}. DeSimone et al. (1981) imposed inwardly directed Na^+ gradients across the canine lingual epithelium to obtain larger amiloride-sensitive I_{sc} values. This procedure might produce a sufficiently large Na^+ current in toad tongue to be detected at the microampere level. We have conducted a few measurements of the *in situ* potential across the lingual epithelium of pithed toads, and the values we obtained are similar to that observed during the initial open-circuit voltage in the Ussing chamber experiments, approximately 10–15 mV. Thus, the electrical properties of the tongue *in situ* appear to be similar to those of the isolated tissue.

Avenet et al. (1988) have also used patch-clamp methodology to demonstrate the presence of three types of K^+ channel in isolated frog taste cells. The direct linear plots and the presence of two Lorentzian functions in power spectra from the present study are consistent with the presence of multiple K^+ channels in the apical membrane of the lingual epithelium which could include both taste and non-taste cells working in parallel.

Spontaneous current fluctuations

The presence of a single Lorentzian component in power spectra obtained with NaCl Ringer bathing both sides of the lingual epithelium supports the hypothesis that spontaneously active K^+ channels are associated with the outward K^+ current. The reduction in S_o that resulted from the addition of KCl Ringer to the mucosal bath is predicted from a reduction in the chemical gradient for K^+ secretion across the apical membrane and a reduction in the single-channel current, as calculated from the equation:

$$S_o = 4Iik_{01}/(k_{10} + k_{01})^2 . \quad (2)$$

In equation 2, I is the macroscopic current through conducting channels, i is the single-channel current and k_{01} and k_{10} are, respectively, the rate constants for spontaneous channel closing and opening. It is not possible, however, to determine how much of the reversal of I_{sc} is due to a reduction in transcellular secretion *versus* paracellular diffusion of K^+ from mucosa to serosa. There was a significant increase in f_c with KCl Ringer in the mucosal bath. Under these conditions, there will be both an increase in K^+ concentration at the extracellular face of the K^+ channels and a depolarization of the apical membrane. Either or both of these changes might modify the gating kinetics of the channels. For spontaneous current fluctuations, f_c is related to k_{01} and k_{10} by the equation:

$$2\pi f_c = k_{01} + k_{10} . \quad (3)$$

An increase in f_c in conjunction with a decrease in current would indicate an increase in the rate of closing (k_{10}).

The addition of K^+ Ringer to the serosal bath augmented I_{sc} and resulted in the appearance of a second Lorentzian function in the power spectra in 10 of 14 experiments. This observation suggests the presence either of two types of K^+ channel or of multiple conductance states with different kinetics and affinity for Ba^{2+} inhibition. This procedure should depolarize the basolateral membrane, which would allow a voltage clamp of the apical membrane (Lindemann and Van Driessche, 1977). The Ba^{2+}-sensitive I_{sc} with a serosa-to-mucosa K^+ gradient

amounted to 38.6 % of the total compared with 65 % for K^+ secretion in the absence of a concentration gradient. Thus, the bulk of the enhanced current is Ba^{2+}-insensitive and may be paracellular. For this reason, there may not be substantially larger values for I or i *via* a transcellular pathway and, from equation 2, S_o would not be larger in these preparations relative to the control condition with NaCl Ringer on both sides of the tissue. In experiments with double Lorentzian components, the variability in f_c was large, and we have not analyzed them further.

Effects of blockers on current fluctuations

The inhibition of I_{sc} by Ba^{2+} occurred in the absence of change in f_c. A linear increase in f_c would have been expected if the inhibition of the apical K^+ channels obeyed pseudo-first-order kinetics and the kinetics of a single channel type was the source of the current fluctuations. In a two-state model of channel inhibition, channels fluctuate between an open and a blocked state. Increasing the blocker concentration will increase f_c in a linear fashion, as described by the equation:

$$2\pi f_c = k_{02}[B] + k_{20}\,, \tag{4}$$

where $[B]$ represents the blocker concentration and k_{02} and k_{20}, respectively, represent the association and dissociation rates of the blocker. Ba^{2+} inhibition of an apical K^+ channel in frog skin has been shown to occur, with a linear increase in f_c with increasing Ba^{2+} concentration (DeWolf and Van Driessche, 1986). These authors analyzed the inhibition of I_{sc} with a direct linear plot (Eisenthal and Cornish-Bowden, 1979) and obtained a set of lines that intersected at a single point, as would be expected for a single channel type that obeys pseudo-first-order kinetics for channel blockage. Application of the direct linear plot to the Ba^{2+} inhibition of I_{sc} in the present study gives a set of lines that do intersect near a common point at 0.5, 1 and 2 mmol l^{-1} Ba^{2+}, but deviate from that point at 5 and 10 mmol l^{-1}, suggesting the presence of more than one K^+ channel type so that the inhibition of the macroscopic current does not follow pseudo-first-order kinetics. Similarly, S_o did not change significantly as I_{sc} was inhibited by Ba^{2+}. For a two-state model of blocker-induced current fluctuations, S_o is calculated from the equation:

$$S_o = 4Iik_{02}[B](2\pi f_c)^{-2}\,. \tag{5}$$

The terms in equation 5 are the same as defined in equations 1–4. A feature of this equation is that, at blocker concentrations below the half-maximal (K_i) value, values for S_o will increase, even as I decreases (Van Driessche, 1994), while at higher concentrations I_{sc} and S_o will both decline. Thus, at lower blocker concentrations, an increase in S_o from inhibition of low-affinity channels could offset the decrease in S_o that occurred as the high-affinity channels were inhibited. Van Driessche (1994) also notes that Lorentzian components can appear in power spectra from epithelia in the presence of very small currents. It is possible that the source of the current fluctuations that produce the spontaneous Lorentzian components is not those channels that are inhibited by Ba^{2+} or that the blocker-induced fluctuations result in a small contribution to the current spectral density that cannot be resolved. This level of analysis is beyond the potential of the two-state model that was used and is beyond the scope of the present study.

Quinine treatment produced a decrease in I_{sc} that did not wash out. Van Driessche and Hillyard (1985) showed that quinidine, a stereoisomer of quinine, inhibited K^+ channels in the basolateral membrane of larval frog skin but only when applied to the mucosal side of the tissue. The inhibition was not readily reversible, and it was suggested that inhibition occurred after the blocker had diffused into the cell and become ionized at the lower pH of the cytosol and, thus, could not be entirely washed out, as had been suggested by Yeh and Narahashi (1976) for experiments with K^+ channels in squid axon membranes. From these experiments, it would appear that inhibition occurred as the result of blockage at the cytosolic face of the membrane, in contrast with Ba^{2+} which binds at the extracytoplasmic face of the channels and is readily washed out. We cannot rule out the possibility that quinine might be blocking channels in the basolateral as well as the apical membranes of the lingual epithelium.

The reduction in S_o during quinine treatment roughly paralleled the inhibition of I_{sc} and persisted after quinine had been washed out, as would be expected from equation 5. The addition of Ba^{2+} further reduced I_{sc} but not S_o, which is consistent with the results in Fig. 3 that also show Ba^{2+} to inhibit I_{sc} without lowering S_o. S_o did increase in concert with an increase in I_{sc} when Ba^{2+} was washed out. The changes in f_c were small and not consistent with the two-state model described by equation 4. This also suggests that the Lorenzian function that is fitted in the power spectra represents a spontaneously fluctuating K^+ channel that is blocker-insensitive. As noted above, the analysis of blocker-induced current fluctuations that are not compatible with a two-state model is beyond the scope of the present study.

Given the complex cellular structure of the toad tongue, it is premature to describe a cellular model for K^+ secretion. The simplest model would be one like that of the distal nephron or colon in which K^+ is transported into the epithelial cells by the Na^+/K^+ pump in the basolateral membrane and expelled from the cells through apical K^+ channels (Berne and Levy, 1988). The role of the Na^+/K^+ pump in K^+ secretion has been difficult to study because this enzyme in bufonid anurans is resistant to ouabain inhibition (Jaisser et al., 1992), presumably to protect the enzyme from alkaloid substances in the skin secretions and body fluids that are used for protection from predators (Butler et al., 1996).

Functional significance of the K^+ current

Kinnamon (1992) has suggested that K^+ channels serve a chemosensory function in the taste cells of both mammalian and amphibian tongue epithelia. Bitter tastants block K^+ secretion and depolarize the taste cells. As noted above, the proportion of the I_{sc} carried by K^+ transport across taste cells

versus other epithelial cells cannot be resolved from our measurements. Another source for K^+ secretion across the lingual epithelium is salivary and mucus secretion (Jaeger and Hillman, 1976). The secretion of both mucus and saliva in mammalian digestive systems is accompanied by an elevated K^+ concentration in the secretion (Berne and Levy, 1988). A coating of mucus on the tongue is required for the capture of insects, which raises the question of the function of taste modalities in anurans that feed in this manner. Specifically, insects are captured on a thick mucous layer at the dorsal surface of the tongue, held briefly in the mouth and then swallowed whole. Taste buds are thought to provide sensory input about the quality of food being ingested and, as pointed out by Lindemann (1996), chemosensory cells respond to relatively high concentrations of chemical tastants.

Many anuran species, including ranids and bufonids, swallow prey, primarily insects, whole, and most nutritive components are contained within an exoskeleton that is only partially degraded after ingestion (Larsen, 1992). Thus, the taste buds on the tongue would be unable to assess nutritional value. It is possible that tastants on the cuticular surface might provide some measure of prey selection. Mikulka et al. (1981) were able to show that coating prey insects with lithium chloride elicited an aversion to feeding. However, Larsen (1992) observed anecdotally that toads will swallow glass beads placed in the mouth. The thick layer of mucus required to capture the prey and the short time that prey are retained in the mouth would allow for minimal diffusion of tastant molecules to taste cells and appears to be an unreliable mechanism for evaluating food quality. Takeuchi et al. (1994) observed a rejection of gelatine capsules containing $1\,mmol\,l^{-1}$ quinine by the urodele amphibian *Ambystoma mexicanum* and that the presence of $100\,mmol\,l^{-1}$ NaCl in the capsules reduced the proportion rejected. It was suggested that the salamanders were able to discriminate between the taste qualities of these stimuli. The authors also noted that this salamander has a non-distensible tongue and feeds by snapping its jaws to capture food items dropped into the water. Thus, the feeding behavior does not require a layer of mucus covering the taste cells, and a limited amount of mastication occurs.

Recent studies by Sato et al. (2000, 2001) propose an interesting function for salivary secretion in chemosensory transduction. They observed that stimulation of the frog glossopharyngeal nerve elicited slow potentials at the tongue surface and in taste cells in the fungiform papillae. This was blocked by atropine, suggesting that the effect was mediated by parasympathetic nerve fibers. Depolarizing slow potentials enhanced the receptor potential response to exposure to $0.5\,mol\,l^{-1}$ NaCl, and it was suggested that the parasympathetic stimulation had produced salivary secretions whose ionic composition modified the liquid junction potential across the lingual epithelium. Assuming that salivary secretion in toads involves K^+ secretion, as in mammals (Berne and Levy, 1988), K^+ secretion could contribute to the measured I_{sc} and also to the chemosensory function of the tongue.

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References

Avenet, P., Hoffmann, F. and Lindemann, B. (1988). Transduction in taste receptor cells requires CAMP-dependent protein kinase. *Nature* **331**, 351–354.

Avenet, P. and Lindemann, B. (1988). Amiloride-blockable sodium currents in isolated taste receptor cells. *J. Membr. Biol.* **105**, 245–255.

Baker, C. A. and Hillyard, S. D. (1992). Capacitance, short-circuit current and osmotic water flow across different regions of the isolated toad skin. *J. Comp. Physiol. B* **162**, 707–713.

Berne, R. M. and Levy, M. N. (1988). *Physiology*. Second edition. St Louis, Washington, Toronto: C. V. Moseby.

Butler, V. P., Morris, J. F., Akizawa, T., Matsukawa, M., Keating, P., Hardart, A. and Furman, I. (1996). Heterogeneity and lability of endogenous digitalis-like substances in the plasma of the toad, *Bufo marinus*. *Am. J. Physiol.* **40**, R325–R332.

Conti, F., De Felice, L. J. and Wanke, E. (1975). Potassium and sodium ion current noise in the membrane of the squid giant axon. *J. Physiol., Lond.* **248**, 45–82.

DeSimone, J. A., Heck, G. L. and DeSimone, S. K. (1981). Active ion transport in dog tongue: a possible role in taste. *Science* **214**, 1039–1041.

DeSimone, J. A., Heck, G. L., Mierson, S. and DeSimone, S. K. (1984). The active ion transport properties of canine lingual epithelia *in vitro*. Implications for gustatory transduction. *J. Gen. Physiol.* **83**, 633–656.

De Wolf, I. and Van Driessche, W. (1986). Voltage-dependent Ba^{2+} block of K^+ channels in apical membrane of frog skin. *Am. J. Physiol.* **20**, C696–C706.

Eisenthal, R. and Cornish-Bowden, A. (1979). The direct linear plot. A new graphical procedure for estimating enzyme kinetic parameters. *Biochem. J.* **139**, 715–720.

Gilbertson, T. A. and Zhang, H. (1998). Characterization of sodium transport in gustatory epithelia from the hamster and rat. *Chem. Senses* **23**, 283–293.

Jaeger, C. B. and Hillman, D. E. (1976). Morphology of gustatory organs. In *Frog Neurobiology*, chapter 19 (ed. R. Llinas and W. Precht), pp. 588–606. Heidelberg, New York: Springer-Verlag.

Jaisser, F., Canessa, C. M., Horisberger, J.-D. and Rossier, B. C. (1992). Primary sequence and functional expression of a novel ouabain-resistant Na^+–K^+ ATPase: the β subunit modulates potassium acivation of the Na,K pump. *J. Biol. Chem.* **267**, 16895–16903.

Kinnamon, S. C. (1992). Role of K^+ channels in taste transduction. In *Sensory Transduction*, chapter 16 (ed. D. P. Corey and S. D. Roper), pp. 261–270. New York: The Rockefeller University Press.

Kitada, Y. and Mitoh, Y. (1997). Amiloride does not affect the taste responses of the frog glossopharyngeal nerve to NaCl. *Chem. Senses* **22**, 720.

Larsen, E. H. (1991). Chloride transport by high-resistance hetero-cellular epithelia. *Physiol. Rev.* **71**, 235–283.

Larsen, L. O. (1992). Feeding and digestion. In *Environmental Physiology of the Amphibians*, chapter 13 (ed. M. E. Feder and W. W. Burggren), pp. 378–394. Chicago, London: University of Chicago Press.

Lindemann, B. (1996). Sweet and salty: transduction in taste. *News Physiol. Sci.* **10**, 166–170.

Lindemann, B. and Van Driessche, W. (1977). Sodium-specific membrane channels of frog skin are pores: current fluctuations reveal high turnover. *Science* **195**, 292–294.

Mikulka, P., Vaughan, P. and Hughes, J. (1981). Lithium chloride-prey aversion in the toad (*Bufo americanus*). *Behav. Neural Biol.* **33**, 220–229.

Pumphry, J. (1935). Nerve impulses from receptors in the mouth of the frog. *J. Cell. Comp. Physiol.* **6**, 457–467.

Sato, M. (1976). Gustatory system. In *Frog Neurobiology*, chapter 18, *Physiology of the Gustatory System* (ed. R. Llinas and W. Prechtpp), pp. 576–587. Heidelberg, New York: Springer-Verlag.

Sato, T., Toda, K., Miyamoto, T., Okada, Y. and Fujiyama, R. (2000). The origin of slow potentials on the tongue surface induced by frog glossopharyngeal efferent fiber stimulation. *Chem. Senses* **25**, 583–589.

Sato, T., Toda, K., Miyamoto, T., Okada, Y. and Fujiyama, R. (2001). Non-

synaptic transformation of gustatory receptor potential by stimulation of the parasympathetic fiber of the frog glossopharyngeal nerve. *Chem. Senses* **26**, 79–84.

Soeda, H. and Sakudo, F. (1985). Electrical responses to taste chemicals across the dorsal epithelium of bullfrog tongue. *Experientia* **41**, 50–51.

Takeuchi, H. T., Masuda, T. and Nagai, T. (1994). Electrophysiological and behavioral studies of taste discrimination in the axolotl (*Ambystoma mexicanum*). *Physiol. Behav.* **56**, 121–127.

Ussing, H. H. and Zerahn, K. (1951). Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**, 110–127.

Van Driessche, W. (1994). Noise and impedance analysis. In *Methods in Membrane and Transporter Research*, chapter 2 (ed. J. A. Schafer, G. Giebisch, P. Kristensen and H. H. Ussing), pp. 27–80. Georgetown, TX, USA: R. G. Landes.

Van Driessche, W. and Hillyard, S. D. (1985). Quinidine blockage of K^+ channels in the basolateral membrane of larval bullfrog skin. *Pflügers Arch.* **405 (Suppl. 1)**, S77–S82.

Van Driessche, W. and Lindemann, B. (1978). Low-noise amplification of voltage and current fluctuations arising in epithelia. *Rev. Sci. Instrum.* **49**, 52–57.

Ye, Q., Heck, G. L. and DeSimone, J. A. (1991). The anion paradox in sodium taste reception: resolution by voltage clamp studies. *Science* **254**, 724–726.

Yeh, J. Z. and Narahashi, T. (1976). Mechanisms of action of quinidine on squid axon membranes. *J. Pharmac. Exp. Ther.* **196**, 62–70.