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Amiloride-Blockable Sodium Currents in Isolated Taste Receptor Cells

Patrick Avenet and Bernd Lindemann
Second Department of Physiology, University of the Saarland, D-6650 Homburg, West Germany

Summary. Isolated taste receptor cells from the frog tongue were investigated under whole-cell patch-clamp conditions. With the cytosolic potential held at −80 mV, more than 50% of the cells had a stationary inward Na current of 10 to 700 pA in Ringer's solution. This current was in some cells partially, in others completely, blockable by low concentrations of amiloride. With 110 mM Na in the external and 10 mM Na in the internal solution, the inhibition constant of amiloride was (at −80 mV) near 0.3 μM. In some cells the amiloride-sensitive conductance was Na specific; in others it passed both Na and K. The Na/K selectivity (estimated from reversal potentials) varied between 1 and 100. The blockability by *small* concentrations of amiloride resembled that of channels found in some Na-absorbing epithelia, but the channels of taste cells showed a surprisingly large range of ionic specificities. Receptor cells, which *in situ* express these channels in their apical membrane, may be competent to detect the taste quality "salty." The same cells also express TTX-blockable voltage-gated Na channels.

Key Words sensory cells · taste- and chemoreception · gustatory senses · salt-taste · patch-clamp whole-cell recording · Na channels · amiloride

Introduction

The tongue mucosa of rat and dog has a Na- or Li-dependent component of the *transepithelial short-circuit current*. This component is, in part, abolished by the common blocker of epithelial-type Na channels, amiloride, at concentrations of 100 μM (e.g. [10, 11, 22, 37]). Recordings from the *sensory nerve* of the tongue [8, 22] and sensory experiments with humans [53, 54] supported the suggestion that amiloride-blockable Na channels may provide the transducer mechanism for the taste quality "salty" [22].

An obvious prerequisite for a transducer function of the epithelial Na channel is the demonstration of this channel in gustatory receptor cells. While previous patch-clamp investigations of taste receptor cells isolated from the tongue mucosa did not establish amiloride-blockable currents [5, 30], we can now show that in the frog a large part of the high conductance of gustatory cells, which were previously rejected as "leaky," is due to amiloride-blockable channels. Of the cells investigated, 56% had stationary inward Na currents blocked by amiloride at a half-maximal concentration near 0.3 μM. A depolarization caused by these currents may be the transducer effect of salt perception.

Materials and Methods

The procedures used for isolation of taste receptor cells from the tongue of *Rana esculenta/ridibunda* and for patch-clamp recording [20] from these cells were described before [5]. Briefly, animals were decapitated and pithed, the tongues removed and 100–200 taste buds per tongue clipped off with fine scissors under the dissection microscope. A low Ca/collagenase/mechanical agitation treatment dissociated the taste buds into single cells. Collagenase was used at 0.4–1 mg/ml in the presence of trypsin inhibitor (0.1 mg/ml). In some cases 30 μM amiloride was present in the dissociation medium to protect the amiloride receptor from enzymatic attack [18].

Sensory cells, recognized by their typical bipolar shape, attached to the glass bottom of the chamber and were patch clamped [20] on dendrite or soma under microscopic control. The bath volume (100 μl) in the chamber was exchanged by slow perfusion (gravity feed and suction, 12 bath volumes/min), in order to change extracellular concentrations. Patch pipettes, optics and electronics were as described before [5].

The extracellular Ringer's solution contained in mM: NaCl 110, $CaCl_2$ 1, $MgCl_2$ 0.5, KCl 1, Tris-HEPES buffer 10 (pH 7.2). Sodium ions were replaced by N-methyl-D-glucamine (NMDG, Sigma) where indicated (NMDG-Ringer's). The standard pipette filling solution (220 mOsm) contained KCl 110, $MgCl_2$ 2, EGTA 0.1 (Sigma), Na_2ATP 5 (Sigma), or $Tris_2ATP$ 5 (Sigma) for lowering the Na concentration, KOH-HEPES buffer 10 (pH 7.2). Potassium was replaced by Tris, Tris + Na, NMDG or Cs. In the 110 mM Cs_2SO_4 solution, the activity of Cs was near 100 mM [46]. Chlorine was replaced by glutamate, EGTA or SO_4. KCl was usually substituted on a molar basis, except for replacement by NMDG-EGTA, which was used at 80 mM (240 mOsm). All solutions were used at room temperature (19–22°C).

The following chemicals (abbreviation, type, source in parentheses) were used in addition: collagenase (type I, Sigma; or Boehringer), trypsin inhibitor (type IS, Sigma), tetrodotoxin

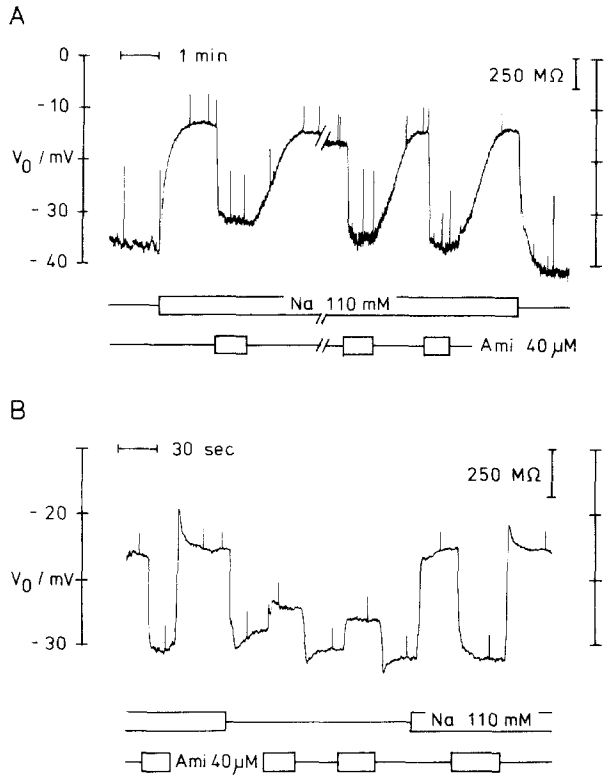

Fig. 1. Voltage response of two isolated taste receptor cells to amiloride and to changes in the bath Na concentration. Whole-cell clamped to zero current, except for brief pulses for measurement of membrane resistance (upward deflections). (*A*) The superfundate was changed from 0 Na (NMDG-Ringer's) to 110 mM Na (Na-Ringer's). Note that 40 μM amiloride, which is a maximal concentration (*see* Fig. 5), did not fully reverse the depolarization caused by Na. Pipette: standard filling solution, but with Cl replaced by glutamate. Outward current pulses of 26 pA were used to monitor membrane resistance. (*B*) This cell responded to removal of Na and to removal of amiloride with *overshooting* voltage transients (*compare* Fig. 3*A*). Chamber perfusion was faster than in *A*. Note that 40 μM amiloride caused hyperpolarization in the presence of Na but depolarization in the absence of Na (replaced by NMDG). Pipette: standard KCl filling solution. Outward current pulses of 15 pA were used to monitor membrane resistance

(TTX, Sigma), cyclic adenosine monophosphate (cAMP, Sigma). Amiloride (3,5-diamino-6-chloro-N-(diaminomethylene)-pyrazinecarboxamide) was a gift from Sharp & Dohme GmbH, FRG.

Results

Response of Membrane Voltage to Na

Many of the spindle-shaped receptor cells held under whole-cell current-clamp conditions did not respond to replacement of extracellular Na by NMDG, or responded with a hyperpolarization of only a few mV. However, in about 70% of the cells tested, a hyperpolarization of more than 5 mV was noted. Such cells had a "depolarized" resting voltage in NaCl Ringer's.

The voltage record of Fig. 1*A* was obtained from a taste receptor cell superfused with a Ringer's solution containing 110 mM NMDG-Cl rather than NaCl. Under these conditions the membrane voltage was −39 mV. On replacing the NMDG by Na, a depolarization to −20 mV was seen while the membrane resistance decreased to 1/3 of the previous value. Repolarization to −40 mV ensued when Na was resubstituted by NMDG (*see* end of record).

Response of Membrane Voltage to Amiloride

When, instead of Na replacement, 40 μM amiloride was added to the Na solution, the membrane repolarized and the resistance increased. These were maximal effects in that 12 and 80 μM amiloride caused the same responses. Nevertheless, in the experiment of Fig. 1*A* the repolarization was *incomplete,* instead of −40 mV it only reached −35 mV, and the recovery of resistance was also incomplete. Often, but not always, removal of Na caused a larger hyperpolarization than 40 μM amiloride (*see* Fig. 2*B,C*). Where this occurred, it may be indicative of several Na-conductive pathways, at least one of which is not sensitive to amiloride.

There was a tendency for the amiloride effect to be larger when the resting potential was low, as shown in Fig. 2*A*. This is to be expected if the Na-permeable pathways cause a stationary depolarization of the isolated cells.

When amiloride was added to an external solution containing NMDG instead of Na, it caused a small but significant *depolarization* (1.8 mV in Fig. 1*B*). This may indicate that the amiloride-blockable pathway is not highly Na selective but also permeable to K ions. Indeed, while amiloride *hyperpolarized* the membrane in the presence of external Na, it *depolarized* it in the absence of external Na, provided the pipette solution had a high K concentration.

It is interesting to note that in Fig. 1*B* the depolarization caused by amiloride in the absence of Na was accompanied by an unexpectedly small increase (only 20%) in membrane resistance. This may be explained by the voltage dependence of stationary K-outward currents, which tended to decrease the resistance when the cell depolarized.

For more than half of the taste-receptor cell population the above results suggest the presence of several electrogenic Na transport pathways, some of which are blockable by amiloride. Even though

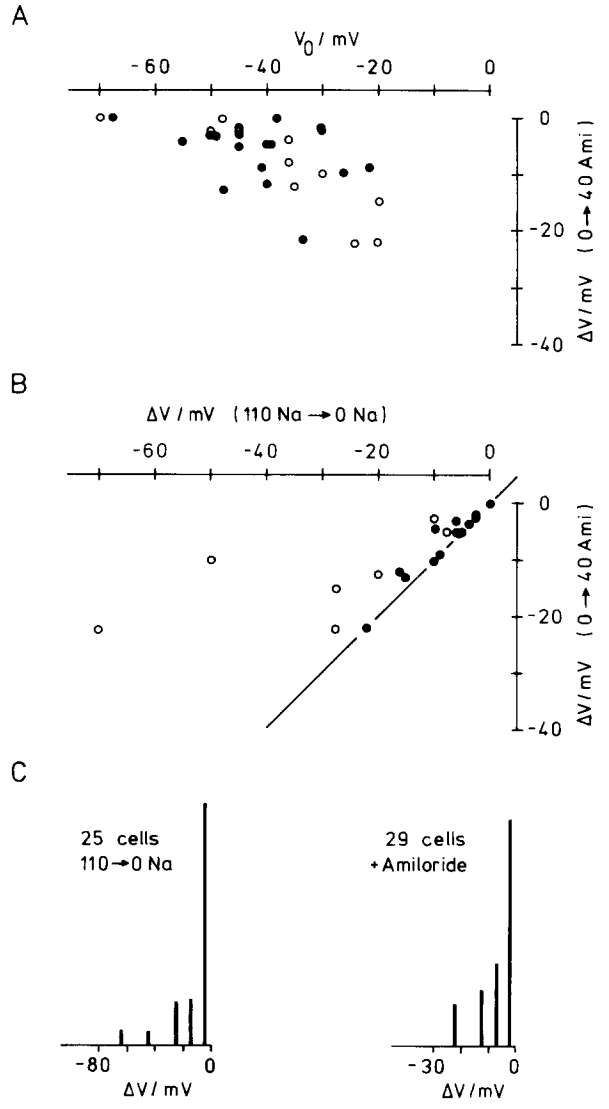

Fig. 2. (*A*) Amiloride-induced hyperpolarization (ΔV, ordinate) *vs.* resting membrane voltage (V_0) of 29 taste receptor cells. Whole-cell current clamp mode. The more depolarized cells tended to respond better to amiloride (40 μM in NaCl Ringer's). Pipette: standard KCl filling solution (●) or same solution with glutamate replacing Cl (○). (*B*) Comparison of the amiloride-induced hyperpolarization (40 μM in Na-Ringer's) with the hyperpolarization caused by removal of Na (NMDG-Ringer's) in the superfundate. The straight line is the line of identity. Data from 25 cells in the whole-cell current clamp mode. Pipette solution and symbols as in *A*. Note that the Na effect was usually larger than the amiloride effect. (*C*) Distribution histograms of hyperpolarizations caused by Na replacement (left) and by 40 μM amiloride in the presence of 110 mM Na (right). Cumulated data are from *A* and *B*

these pathways are often not highly Na specific, they may be referred to as "Na channels" because, as will be shown, under standard ionic gradients inward flow of Na through these channels dominates.

Magnitude of Na-Inward Current

Taste cells which have a voltage response to Na and amiloride typically draw a stationary inward current when held at −80 mV under voltage-clamp conditions. The current gives the impression of a "leak" current, but its Na dependence and the sensitivity to low concentrations of amiloride suggest that it is mediated by Na channels. The inward current was typically noisy, and in some but not all experiments the noise decreased considerably when the current was blocked with 30 μM amiloride (Fig. 3*A*).

In Fig. 3*C* the total cellular inward current observed at −80 mV (abscissa) is plotted against the fraction of current blocked by 30 μM amiloride, as measured in the first 10 min of whole-cell recording. Of 75 cells tested with amiloride, 42 (56%) responded to amiloride with a significant decrease in inward current. Like the total current, the amiloride-blockable current was rather variable among cells. The largest amiloride-blockable currents were observed with K glutamate (open symbols) rather than KCl in pipette and cell. With glutamate, most of the total inward current was accounted for by the amiloride-blockable current (Fig. 3*C*, ordinate values of open symbols above 0.5) in those cells where the amiloride-blockable current was preserved.

For pipette solutions of high Cl concentration, Fig. 3*C* shows (filled symbols) that, in the mean, less than half of the total inward current is due to the amiloride-blockable conductance. The remainder may be Cl current or Na current not blocked by amiloride. This problem is presently under investigation.

In a given cell, the amiloride-blockable fraction of current often changed with time. While some cells lost this current within an hour of whole-cell recording (run-down), others increased their amiloride-blockable current for unknown reasons.

Two Types of Na-Inward Current

Taste receptor cells generate action potentials [4, 29] and express Na channels which are retained in the membrane during cell isolation, and which support a *transient* voltage-gated Na-inward current blockable with TTX [5, 30]. The voltage ranges of the activation and inactivation assure that this current is zero at −80 mV [5]. It is interesting to note that amiloride-blockable Na channels are found in the same cells. The amiloride-blockable channels maintain a *stationary* inward current which is strong at −80 mV, only weakly voltage-dependent but not voltage-gated (*see below*, Fig. 6), and which tends to depolarize the cell in the current-clamp mode (Fig. 1).

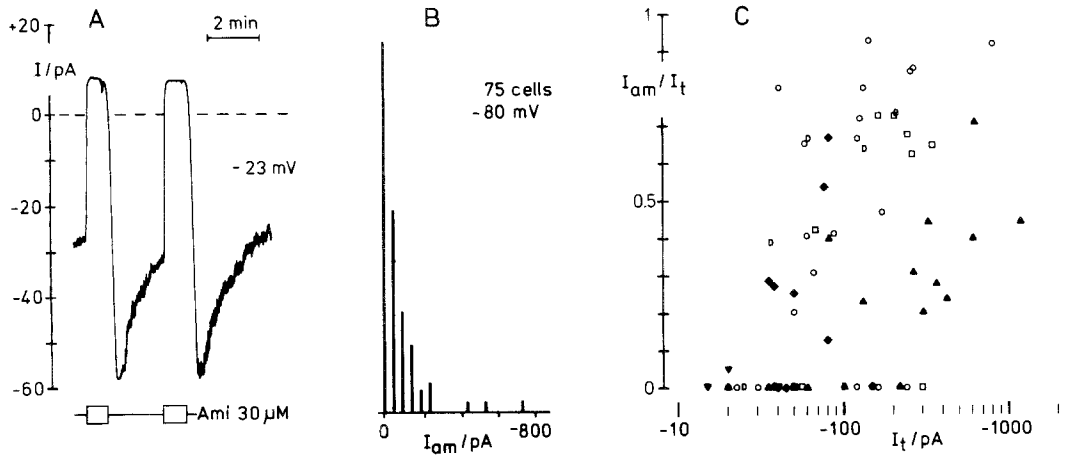

Fig. 3. Response of whole-cell current to amiloride. (*A*) Time course. Superfusion with amiloride (30 μM in Na-Ringers) caused a rapid decrease in inward current and in current noise. Note the overshooting transients on washout of amiloride. Pipette: standard filling solution, but with Cs replacing K and sulfate replacing Cl. Pipette voltage was clamped to −23 mV. (*B*) Amplitude distribution of amiloride-blockable inward currents measured within 20 min after breaking the patch. 75 taste receptor cells. Whole-cell clamp voltage was −80 mV. Bath and pipette solution were as in *C*. (*C*) Amiloride-blockable current, expressed as fraction of total current, plotted *vs.* total inward current. 75 cells in whole-cell mode, clamped to −80 mV. Bath: Na-Ringer's or Na-Ringer's + 20 mM $BaCl_2$; amiloride, 20 to 40 μM. Pipette: standard filling solution (▲), or same with KCl replaced by NMDG-Cl (▼), NMDG-EGTA (◆), K glutamate (○), Tris glutamate (□) or 70 mM Tris glutamate + 40 mM Na glutamate (□)

The coexistence of both types of Na-inward current is demonstrated in Fig. 4. Under voltage-clamp conditions, depolarizing voltage steps elicited a decrease of the stationary current, with a transient enhancement of inward current superimposed. The transient component was blocked by TTX (Fig. 4*A*) and the stationary component by amiloride.

The largest amiloride-blockable Na current, observed in the experiment of Fig. 4*B*, approached 0.7 nA at −80 mV. It was three times larger than the TTX-blockable current of the same cell at 0 mV. If the two types of Na pathways were independent, their currents would be additive. This, however, was often not the case. In Fig. 4*B* the transient Na current was larger in magnitude when the stationary Na current was blocked by amiloride. The phenomenon is shown more clearly in Fig. 4*C*. Possibly the two types of Na channels compete for Na ions in the unstirred layer at the outer surface of the membrane. This would imply that the two types of channels are located in close vicinity to one another.

Relaxation of Tail Currents

On washing out the amiloride while pulsing the voltage to 0 mV every 3 sec for 17 msec, pronounced exponential "tail currents" were noted (Fig. 4*B*). Their time constant increased as the amiloride concentration decreased, such that the tail currents disappeared when amiloride was completely removed. By fitting exponentials to the "tail currents" we obtained time constants of exponentials increasing from 2 to 7 msec, while amiloride was washed out (Fig. 4*B*). As described previously for apical Na channels of toad urinary bladder [44, 62], this type of current relaxation is due to the voltage dependence of channel blockage by amiloride.

Amiloride Concentration-Response Relationship

The concentration dependence of blockage of stationary Na-inward currents by amiloride was investigated in the whole-cell voltage-clamp mode at −80 mV. The pipettes contained 110 mM K glutamate, the external solution 110 mM NaCl (plus 20 mM Ba to block K channels), and the amiloride concentration was increased stepwise by superfusion. Figure 5*B* pools results from six cells. Typically, the inhibition was complete at 12 μM amiloride, in that there was no further decrease in current when increasing the concentration to 40 μM. The low inhibition constant, near 0.3 μM, suggests that channels rather than electrogenic exchange systems transported the current [6, 49].

The amiloride-blockable Na pathway is also somewhat permeable to K ions, as indicated by the whole-cell voltage recordings in the absence of Na (Fig. 1*B*) and by estimates of reversal potentials

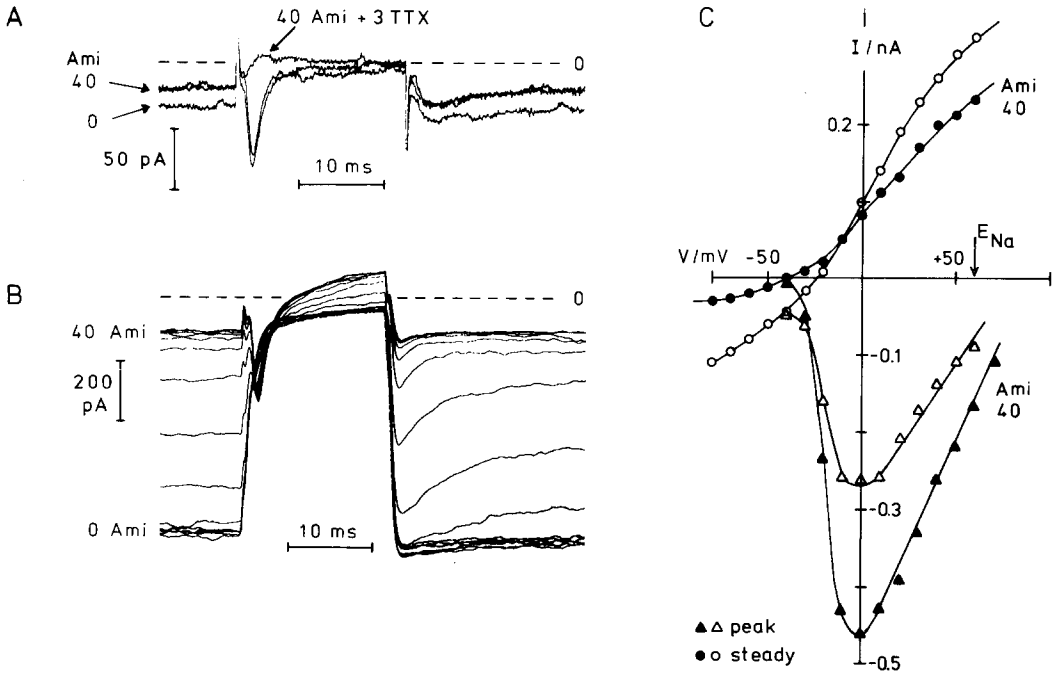

Fig. 4. Coexistence of two pathways for Na inward current under whole-cell voltage-clamp conditions. Data from three receptor cells. (*A*) Current time course during a voltage pulse $-80 \rightarrow 0 \rightarrow -80$ mV. The three sweeps were obtained without added blockers (control, Na-Ringer's), with 40 μM amiloride and with 40 μM amiloride + 3 μM TTX added to the superfundate. Pipette: standard filling solution with NMDG-glutamate replacing KCl. (*B*) In this cell the amiloride-blockable, stationary current (I_{am}) at the holding voltage of -80 mV was three times larger than the voltage-gated peak-inward current measured at 0 mV. With this exceptionally large I_{am} the *transient* Na inward current at 0 mV was largely suppressed. It was revealed only when I_{am} was blocked with amiloride. Note that the current traces recorded after changing to 0 mV cross each other. Bath: Na-Ringer's with 40 μM amiloride where indicated. Pipette: standard filling solution with K-glutamate replacing KCl. (*C*) Voltage dependence of steady-state currents (upper curves) and peak transient inward currents in the absence (open symbols) and presence of 40 μM amiloride in Na-Ringer's. Voltage pulse protocol as in *A* and *B*. *Note* the increase in voltage-gated currents in the presence of amiloride. Bath and pipette solution as in *B*

(discussed below). However, at negative membrane voltages, where the whole-cell preparations were stable, the magnitude of stationary K-outward currents sensitive to amiloride was small, precluding the estimation of the inhibition constant. Thus we cannot presently decide whether amiloride blocks stationary Na and K currents with similar efficacy.

Current-Voltage Relationship and Na-Selectivity

For investigation of the voltage dependence of amiloride-blockable currents, the current through K channels was preferentially diminished by maintaining 20 mM Ba in the outside solution, or by replacing cellular K by another cation as indicated below. Under *whole-cell* voltage-clamp conditions, cells were selected which, in the presence of external Na, showed an inward current blockable by 30 μM amiloride. As shown in Fig. 3*A*, blockage of this current component resulted in a pronounced decrease in current noise. Suitable cells were then depolarized in steps of 10 mV with pulses lasting 30–40 msec and separated by 3 sec, starting at a holding voltage of -80 mV. After obtaining the current response to one set of pulses the procedure was repeated in the presence of 30 or 40 μM amiloride (Fig. 6*A*,*B*). The stationary values of the second set of currents was then subtracted from the first to obtain the current-voltage relationship of the amiloride-blockable current.

Examples of the resulting curves are shown in Fig. 6*C–F*. The amiloride-blockable current was always most pronounced at negative membrane voltages, becoming less negative when the cells were depolarized. The $I(V)$-relationship tended to be slightly nonlinear, showing qualitatively the curvature of a Goldman-Hodgkin-Katz (GHK) function [19, 27]. The reversal potential was in most cases less positive than the Na equilibrium potential (designated as E_{Na} in the figures), indicating that Na was not the only ion that passed through the amiloride-blockable conductance. The data points were fitted with the sum of two GHK functions representing independent cationic currents (solid curves). These

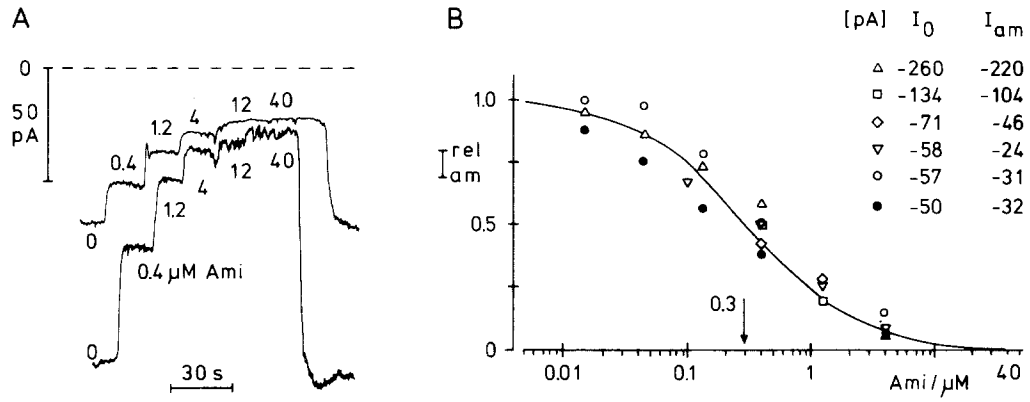

Fig. 5. (*A*) Response of Na-dependent stationary inward current to increasing concentrations of amiloride. Two taste receptor cells in whole-cell, voltage clamped to −80 mV. Bath: Na-Ringer's + 20 mM $BaCl_2$ and amiloride as indicated. Pipette: standard filling solution, with glutamate replacing Cl. (*B*) Concentration-response curve of amiloride. The *relative* amiloride-blockable inward current remaining at various submaximal concentrations of amiloride was obtained as $(I_c - I_{40})/(I_0 - I_{40})$, where the subscripts are amiloride concentrations in μM. Data from six taste receptor cells. Experimental conditions were as in *A*. The currents obtained with 12 and 40 μM amiloride were identical except for one case shown in *A*. The drawn-out line is the theoretical dose-response curve for an inhibition constant of 0.3 μM. The table (inset) specifies total currents (I_0, in the absence of amiloride) and the amiloride-blockable current ($I_{am} = I_0 - I_{40}$) for each cell used

fits were not satisfactory in most cases, in that the measured relationships had more curvature near 0 mV than could be accounted for with the GHK formalism.

This is particularly clear in Fig. 6*C* (pipette contained the standard KCl filling solution). For these curves, the reversal potential of +19 mV indicated a Na/K permeability ratio of 2.6. With the pipette containing K glutamate (Fig. 6*D*), a *range* of reversal potentials between 0 mV and values close to E_{Na} (+60.4 mV) was obtained from six cells. The Na/K permeability ratio varied from 0.9 to 104.

Attempts were made to suppress outward current by replacing cellular K glutamate with Tris glutamate and outside Cl by glutamate. When the cellular Na was only 1 mM (E_{Na} +118 mV), outward currents became small to negligible (Fig. 6*E*). This made the estimated reversal potentials less reliable. However, their values near +60 mV were clearly smaller than E_{Na}, indicating that Tris permeated the amiloride-sensitive pathway to some extent.

When the cellular Na was increased to 50 mM at the expense of Tris, outward currents appeared and lower reversal potentials (0 to +20 mV) were now found (Fig. 6*F*). In contrast to Fig. 6*D* (cells containing K), the outward currents *saturated* with increasing voltage, indicating that Tris permeated less easily than K and apparently tended to block outward Na current. Estimates of the Na/Tris permeability ratio ranged from ∞ to 1 under these conditions. In similar experiments Cs was also found to permeate the amiloride-blockable conductance of some cells.

Sensory Specificity of Taste Cells

Generation of cAMP in taste receptor cells occurs in response to sweet agents [31] and perhaps also in response to agents of other taste qualities. The cyclic nucleotide induces closure of K channels and thereby membrane depolarization [3, 5, 42, 57–59]. Do isolated receptor cells, which have a Na-dependent stationary inward current and, therefore, probably respond to the taste quality "salty," have in addition the means to detect taste qualities which involve cAMP-dependent membrane events?

In the whole-cell current clamp mode, 17 taste cells were screened for a hyperpolarizing effect of 40 μM amiloride, which would indicate the presence of amiloride-blockable Na inward currents. Following the washout of amiloride, each cell was also screened for a depolarizing effect of 5 mM cAMP added to the superfundate. The majority of cells responded to both agents, as summarized in Fig. 7.

This result is in agreement with previous microelectrode recordings from taste cells *in situ*, which indicated that the specificity of individual receptor cells with respect to different taste qualities is not large [1, 40, 50, 51], and in particular that individual taste cells may respond to both NaCl and sucrose, but do so with different ratios of response magnitudes when cells are compared [57, 59].

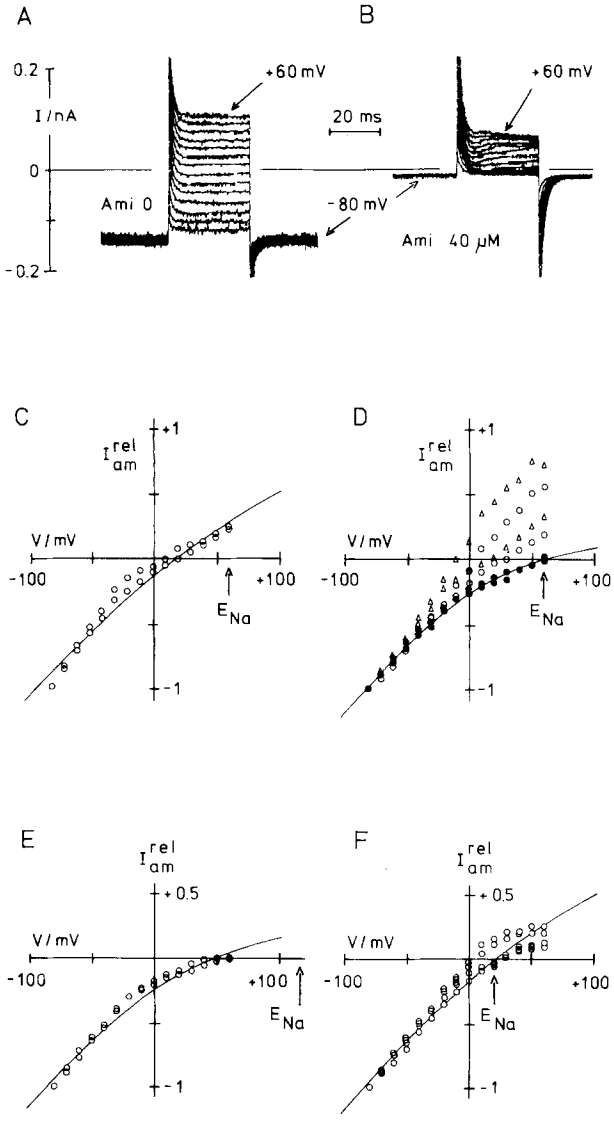

Fig. 6. Voltage dependence of amiloride-blockable currents recorded in the whole-cell voltage-clamp mode. (*A*,*B*) Time course of currents responding to depolarizing voltage pulses without (*A*) and with 40 μM amiloride (*B*). Bath: Na-Ringer's. Pipette: standard K filling solution with glutamate replacing Cl. Note that amiloride affects the inward currents more than the outward currents. For any voltage V, I^{rel}_{am} was evaluated from $(I^V_0 - I^V_{40})/(I^U_0 - I^U_{40})$ where U was the reference voltage (usually −80 mV), and plotted as shown below. The drawn-out curves in panels *C*–*F* are GHK relationships with permeabilities adjusted to fit the reversal potentials V_{rev} of the data (*see* text). Note that the fit is not satisfactory. (*C*) Bath: Na-Ringer's; pipette: standard KCl solution. Two cells. E_{Na}, 60.4; V_{rev}, 19 mV: P_{Na}/P_K, 2.6. (*D*) Bath: Na-Ringer's; pipette: standard K filling solution with glutamate replacing Cl (conditions of *A* and *B*). Six cells. E_{Na}, 60.4; V_{rev}, 0–60 mV: P_{Na}/P_K, 1–100. (*E*) Bath: Na-Ringer's with Cl replaced by glutamate; pipette; K was replaced by 109 mM Tris + 1 mM Na, Cl by glutamate. Two cells. E_{Na}, 118; V_{rev} 50 mV, P_{Na}/P_{Tris}, 7. (*F*) Bath: Na-Ringer's; pipette: K was replaced by 70 mM Tris + 40 mM Na, Cl by glutamate. Four cells. E_{Na}, 20; V_{rev}, 0–20 mV; P_{Na}/P_{Tris}, 1 − ∞

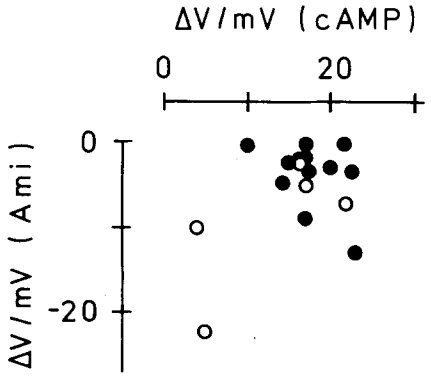

Fig. 7. Voltage response to amiloride (hyperpolarization) *vs.* voltage response of same cell to cAMP (depolarization). Seventeen taste receptor cells in the whole-cell current clamp mode. Bath: the superfundate was Na-Ringer's to which 40 μM amiloride or 5 mM cAMP was added. Pipette: standard KCl filling solution (●) or same with glutamate replacing Cl (○)

Discussion

Results from the Intact Mucosa

A number of experiments with the isolated mucosa of the tongue and with animals and human volunteers have suggested that amiloride-blockable Na transport may provide the transducer mechanism for the taste quality "salty":

1) The tongue mucosa of rat, dog and rabbit has a Na- or Li-dependent component of the *transepithelial short-circuit current* [e.g. 10, 11, 22, 55]. This component is, in part, abolished by the common blocker of epithelial-type Na channels, amiloride, at concentrations of 100 μM [22, 37, 55]. The inhibition constant was recently estimated to be 1 μM [54].

2) Recent microelectrode recordings from taste cells of the frog tongue showed that 100 μM amiloride caused hyperpolarization and an increase in cell resistance [24]. At 1000 μM, amiloride was found to hyperpolarize the cells from −22 to −32 mV [41].

3) Recordings from the *sensory nerve* (chorda tympani, adult rat) and higher taste-sensory centers also showed an amiloride-sensitive component [8, 22, 23, 26], which was enhanced by the Na-channel activator bretylium tosylate [53]. The inhibition constant for amiloride was estimated to be 4 μM [9], 1 μM [25] and less than 1 μM [8].

4) Humans fail to *sense "salty"* following preexposure of the tongue to an amiloride solution [52]. The Na-channel activator bretylium tosylate amplifies the subjective taste of Na and Li salts [53].

Thus, different methods have provided sugges-

tive but still indirect evidence for a primary role of amiloride-blockable transport, presumably apical Na channels, in salt perception. However, amiloride itself was reported to cause a nerve response in the absence of mucosal Na [64], and also to block the taste response to KCl [24, 64] and, in the presence of NaCl, to sucrose [52]. Furthermore, on the taste-nerve response of *Necturus* amiloride was found to be without effect, even though these animals are able to taste salt [36]. Therefore, it seemed prudent to search for stationary Na currents and amiloride-blockable currents in the taste receptor cells themselves.

Results with Isolated Taste Cells

In a recent study we did not observe effects of amiloride in isolated receptor cells from the frog tongue [5]. This was probably owing to the rejection of depolarized, low-resistance cells. Our present data show clearly that more than 50% of the isolated cells under whole-cell recording conditions have a stationary Na-dependent inward current. For frog taste cells *in situ* Tonosaki and Funakoshi recently reported a similar current response to mucosal NaCl [59]. In our experiments, the stationary inward current, which was quite variable in magnitude, was blocked partially or completely by amiloride. The inhibition constant had a low value, near 0.3 μM.

Similar or smaller inhibition constants of amiloride were found for some of the "epithelial-type apical Na channels" [e.g. 2, 6, 17, 45, 60], while at the classical apical Na channels of frog skin and toad urinary bladder, the values range from 0.45–2 μM when the mucosal solution contains more than 50 mM Na [2, 6, 33–35, 49]. Native vesicles derived from toad bladder indicate two different inhibition constants, 0.017 and 10 μM [2].

Concentrations of amiloride in excess of 2 μM are needed for the blockage of other transport systems. For instance, the apparent inhibition constants are 20 μM for Na/H-exchange [14], 1–2 mM for Na/Ca exchange [28], 30 μM for the T-type Ca channel [56] and 10–20 μM for olfactory reception [15]. Thus apical Na channels are the only transporters presently known that amiloride blocks with apparent inhibition constants below 2 μM. It is tempting, therefore, to suggest that epithelial Na channels are present in the apical membrane of taste cells. However, a final classification will require a comparison of channel structures based on molecular-biological data. The efficacy of a blocker is no more than suggestive.

Channel blockage by the cationic amiloride is usually voltage dependent [e.g. 21, 44, 61, 62]. In consequence, the Na current shows exponential relaxation following pulse perturbation of voltage in the presence of submaximal concentrations of amiloride. This phenomenon, which we also observed in taste receptor cells, allows a convenient estimate of the blocking rate constants [44, 62]. Thereby a more detailed comparison to amiloride blockage in Na absorbing epithelia will be possible.

The ionic selectivity of apical epithelial Na channels is usually high [43]. Therefore, it was surprising to find that the channels of taste cells, in addition to Na, also conduct K, Tris and Cs. However, some other epithelial Na channels are also of low specificity [21, 32, 60, 63]. Furthermore, the low specificity is in agreement with reports that amiloride, applied to the intact mucosa, affects the taste nerve response to K salts [24] and even to salts of organic cations [64]. Also surprising was the finding that the ionic specificity of these channels is rather variable when different cells are compared. We are at present unable to explain this observation.

Not all of the Na-dependent stationary inward current of single isolated receptor cells was blocked by amiloride. Similarly, amiloride blocks only part of the Na-dependent short-circuit current of the intact mucosa [11, 54] and only part of the nerve response to salt taste [8, 9, 13]. Future experimentation will show whether amiloride reduces the conductance of individual channels to values that remain larger than zero, or whether a fraction of the population of Na channels is not sensitive to amiloride. The latter possibility is interesting in that the salt taste of some animals appears unaffected by amiloride [26, 36].

Alterations in Channel Topology

Isolation of epithelial cells for patch clamping may alter them both morphologically and functionally. For taste cells of the frog, it was shown that the cytoskeleton tends to undergo changes when the cells are isolated. In consequence, some of the cells round off by the shortening of their apical process. They also start to disseminate the "rod-organelle," a dense bundle of *f*-actin which supports the chemosensory apical membrane [48].

On the surface of isolated epithelial cells, apical membrane proteins start to spread over the basolateral surface soon after opening of the tight junctions [7, 12, 47], and basolateral membrane material may be internalized [16]. Thus the loss of geometrical polarity is followed by a decline in functional polarity. It may be suspected that a migration of apical membrane proteins into basolateral domains will also occur in isolated taste cells. Furthermore,

in the isolated cells replacement of membrane proteins may not occur at the regular location.

For these reasons, experiments with isolated cells will not normally show where a given transport pathway is localized under conditions of maintained functional polarity [39]. Our observation of kinetic interference of voltage-gated and ungated Na-inward currents suggests that the TTX-blockable and the amiloride-blockable channels are located close to each other on the surface of isolated cells. This would enable them to compete for Na ions in the unstirred layer at the outer membrane surface.

However, it seems quite plausible that the amiloride-blockable channels *normally* have the apical location suggested by the results from intact mucosa quoted above. The presence of these channels on the basolateral membrane would depolarize the cells and render them unfunctional. Thus, a mixing of previously separated membrane proteins probably occurred in response to the opening of the tight junction.

Taste Cell Specificity and Conclusion

The depolarization caused by increasing the outside Na concentration from 0 to 110 mM was larger than 10 mV in eight out of 25 taste cells, and larger than 20 mV in five out of 25 cells. This result is compatible with previous reports that an increase in the NaCl concentration on the frog tongue from 10 to 100 mM causes a depolarization of impaled receptor cells by 10 mV or more [e.g. 1, 57, 59].

In taste receptor cells, sweet agents evoke the generation of cAMP [31], which, in turn, stimulates phosphorylation and thereby closure of K channels [3, 5, 58] and a membrane depolarization of up to 25 mV [3, 5, 42, 58][1]. We found a number of isolated receptor cells which respond to cAMP by depolarization but are in addition sensitive to amiloride. Thus, the same cell may detect two or more taste qualities, for instance salty and sweet (*compare* [59]), or salty and another quality requiring cAMP. In humans, this property could explain that low NaCl concentrations taste sweet [9] and that the sweet sensation is affected by amiloride [52].

In conclusion, a large fraction of taste receptor cells isolated from the frog tongue shows a stationary Na-dependent inward current which is sufficiently strong to depolarize the cells to threshold. The channels, which are thought to normally reside in the apical membrane, are in part blocked by low concentrations of amiloride. Current through these channels may be the primary event in reception of the taste "salty."

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[1] Nagahama and Kurihara [38] reported that cyclic nucleotides modify responses to many taste agents without themselves causing excitation. Therefore, cAMP and cGMP were classified as *modifiers* rather than messengers. However, more recent studies show cyclic nucleotides injected into taste receptor cells *in situ* to cause depolarization [42, 58]. In time-course and amplitude this response is comparable to that caused by sucrose [58]. Isolated taste cells respond similarly to cAMP [3, 5]. Based on these observations and the activation of the adenylate cyclase by sweet agents [31], it was suggested that cAMP is an intracellular messenger of sweet taste in the receptor cells [3, 31, 58]. Thereby its function as messenger of other tastes was not excluded. It may be expected, however, that messengers of one taste act as modifiers of another taste within the same cell.

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