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Noninvasive Recording of Receptor Cell Action Potentials and Sustained Currents from Single Taste Buds Maintained in the Tongue: The Response to Mucosal NaCl and Amiloride

Patrick Avenet and Bernd Lindemann
Department of Physiology, Universität des Saarlandes, D-6650 Homburg/Saar, Germany

Summary. Apical membrane currents were recorded from the taste pore of single taste buds maintained in the tongue of the rat, using a novel approach. Under a dissection microscope, the 150-μm opening of a saline-filled glass pipette was positioned onto single fungiform papillae, while the mucosal surface outside the pipette was kept dry. Electrical responses of receptor cells to chemical stimuli, delivered from the pipette, were recorded through the pipette while the cells remained undamaged in their natural environment. We observed monophasic transient currents of 10-msec duration and 10–100 pA amplitude, apparently driven by action potentials arising spontaneously in the receptor cells. When perfusing the pipette with a solution of increased Na but unchanged Cl concentration, a stationary inward current (from pipette to taste cell) of 50–900 pA developed and the collective spike rate of the receptor cells increased. At a mucosal Na concentration of 250 mM, the maximal collective spike rate of a bud was in the range of 6–10 sec^{-1}. In a phasic/tonic response, the high initial rate was followed by an adaptive decrease to 0.5–2 sec^{-1}. Buds of pure phasic response were also observed. Amiloride (30 μM) present in the pipette solution reversibly and completely blocked the increase in spike rate induced by mucosal Na. Amiloride also decreased reversibly the stationary current which depended on the presence of mucosal Na (inhibition constant near 1 μM). During washout of amiloride, spike amplitudes were first small, then increased, but always remained smaller than the amiloride-blockable stationary current of the bud. This is understandable since the stationary current of a bud arises from a multitude of taste cells, while each current spike is presumably generated by just one taste cell. We suggest that, in a Na-sensitive receptor cell, (i) the apical amiloride-blockable Na inward current serves as a generator current causing cell depolarization and firing of action potentials, and (ii) each current spike recorded from the taste pore arises mainly from a modulation of the apical Na inward current of this cell, because the action potential generated by the taste cell will transiently decrease or abolish the driving force for the apical Na inward current. The transients are indicators of receptor cell action potentials, which appear to be physiological responses of taste cells *in situ*.

Key Words: sensory cells · chemoreception · taste · gustatory senses · taste receptor cells · epithelial action potentials · amiloride

Introduction

Taste receptor cells (TRCs) are epithelial cells which, rather surprisingly, fire action potentials. This property was first discovered in the frog [20] and in *Necturus*, where the large cell volume facilitated impalement with microelectrodes [1, 26, 27]. In mammalian TRCs action potentials were observed by patch clamping cells isolated from the tissue [9]. However, the isolation of taste buds from the tongue and of TRCs from the taste buds caused problems of its own [25]. A more convenient and preferably noninvasive method of recording, suitable for taste cells maintained in the taste bud and in the tongue epithelium, is needed to investigate TRC electrical responses to gustatory stimuli. Using the example of salt taste, we demonstrate the feasibility of a noninvasive approach proposed some time ago (*see* appendix of ref. [4]).

We show that the presence of mucosal Na ions allows the flow of amiloride-blockable sustained currents which apparently depolarize the receptor cells and thus induce the firing of TRC action potentials. We suppose that in Na-sensitive receptor cells the action potentials will be ohmically conducted to the mucosal space by means of voltage modulation of the apical inward current flowing through the amiloride-blockable channels. Based on the noninvasive method used with *in situ* receptor cells, we confirm that the action potentials are indeed physiological responses of these cells. They probably serve to trigger release of transmitter at the synapses of TRCs with sensory axons. Some results of these experiments appeared in abstract form [6].

Materials and Methods

Wistar rats weighing 200–250 g were killed by decapitation or dislocation of the cervical vertebrae. Tongues were removed im-

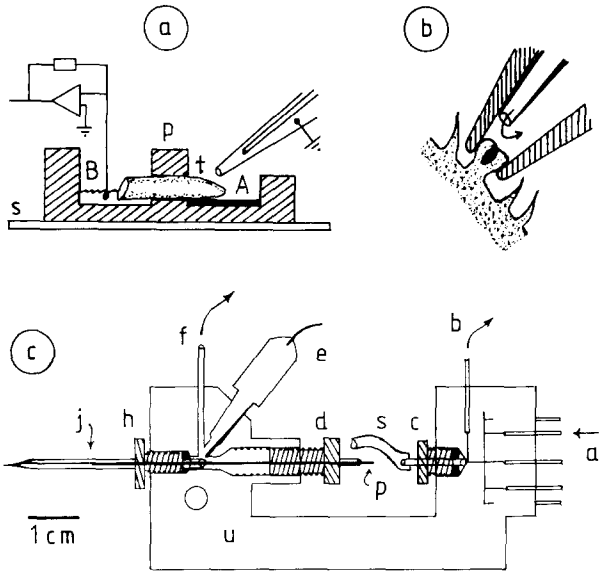

Fig. 1. (*a*) Scheme of bipartitioned chamber, made of Sylgard® cast onto a glass microscope slide (*s*). *p*: Partition containing a tunnel of 10-mm length and 4-mm diameter. Tunnel wall was sealed to middle section of rat tongue surface with injected silicon grease. *t*: Tip of tongue kept in air and resting on cushion of filter paper in compartment *A*. The pipette interior was grounded (reference electrode). Compartment *B* contained the cut edge of the tongue, immersed in a pool of Tyrode, and an agar bridge leading to the current-recording electrode. (*b*) A fungiform papilla, typically containing only one taste bud, was partially pulled into the pipette orifice (outer diameter 150–200 μm). The distance from papillary surface (or pipette orifice) to inner tube orifice (outer diameter 30–50 μm) was adjusted to about 50 μm with the distance micrometer (labeled *d* in panel *c*), using visual control through a dissection microscope. (*c*) Holder for internal perfusion of micropipette with up to eight solutions. This device is a refinement of the holder used by Behe et al. [8] for chemical stimulation of single taste buds. *u*: U-shaped Lucite frame. *a*: Set of eight inlets, pressurized, which joined with thin-bore channels at one point. *b*: Outlet for washing dead space. *c*: Gasket with O-ring, which sealed on removable steel capillary. *s*: Silicon tube, for connecting the two steel capillaries (shown disconnected). *p*: First part of inner perfusion pipe (polyethylene); outer diameter slightly larger than inner diameter of *s*, such that *p* seals to the inner surface of *s* when *s* is connected to *d* and *p* pushed to the right. *d*: Distance micrometer. The fine thread was sealed with heavy silicon paste. The steel capillary on the right of *d* was permanently fixed to *d*. *e*: Grounding electrode with conical fitting; Ag-AgCl pellet in 3 M KCl, connected to flow system with 3 M KCl agar. *f*: Outlet for waste collection and generation of negative pressure. *h*: Gasket with O-ring which seals onto outer glass pipette. *j*: Junction between 1st and 2nd part of inner perfusion tube. The thinner 2nd part (quartz tube) is shown on the left. Related schemes of perfused pipettes were previously developed by others [8, 22, 29].

mediately (length near 2 cm) and put into a beaker containing ice-cold Tyrode solution for storage (no more than 20 min).

For recording, tongues were placed into a bipartitioned chamber, made of Sylgard® cast onto a glass microscope slide (Fig. 1*a*). The partition had a width of 1 cm, containing a 4-mm diameter tunnel which connected compartments *A* and *B*. By means of a thread, the tongue was pulled (cut end first) into the tunnel. Three thin channels cast into the Sylgard® allowed silicon grease to be pressed into the gap between tongue and tunnel wall. The cut surface of the rear part of the tongue protruded into compartment *B*, where it made contact with electrode *B* (an agar bridge connected to a Ag/AgCl pellet) through a shallow pool of Tyrode. The tip of the tongue protruded into compartment *A*, where it was rinsed with distilled water and then laid on a cushion of dry filter paper for "continuous blotting." The tip was viewed from above with a dissection microscope at 12- to 25-fold magnification, using glass fiber illumination. One of the fungiform papillae, visible in circular areas free of filiform papillae, was pulled into the orifice of the pipette (Fig. 1*b*), which was positioned with a hydraulic 3-D manipulator. Experiments were done at room temperature.

Pipettes were pulled with a vertical two-stage puller (like patch pipettes) from thick-walled borosilicate glass of 2-mm outer diameter. To enlarge the pipette orifice to 150–200 μm (outer diameter), glass material was removed by touching the tip against the hot wire of a microforge. The pipette was fitted into the *multiple-perfusion Lucite holder* (*see* Fig. 1*c*) with gasket (*h*). With gasket (*c*) loosened, the distance of inner tube orifice to pipette orifice was adjusted to be about 50 μm, by manually twisting part (*d*) of the holder (a heavily greased fine-thread Lucite screw used as "distance micrometer"), while observing the tips with the dissection microscope. Gasket (*c*) was then tightened and pipette perfusion started. A thin suction tube, connected to outlet (*f*), led to a 10-ml buffer compartment. The dropping of outflowing perfusate into this compartment served to monitor the rate of pipette perfusion. Suction was applied to the buffer compartment first by mouth (to pull a papilla into the pipette opening), then with a small pump (for long-term recording).

The tip of the inner tube was a quartz capillary covered with plastic material (Polymicron Technologies, outer diameter 244 μm, inner diameter 100 μm), as introduced for perfusion of pipettes by Lapoint and Szabo [22]. In an alcohol flame, it was pulled to a length of 2 cm and an orifice diameter of 30–50 μm. Its rear end was sealed into a polyethylene tube (outer diameter 0.9 mm) with heated wax (*j*). With the silicon tube (*s*) connected to (*d*), the rear end (*p*) of the polyethylene tube fitted tightly into (s), which had an inner diameter <0.8 mm.

A pressure of 0.5–1 bar was needed to perfuse a pipette (containing an inner tube with an orifice of 40 μm diameter) at a rate of 0.3 ml/min. At this rate, the time required for the bolus of a new solution to move from (*a*) through (*c*), (*s*) and the inner tube up to the tip of the pipette was 3 sec. The concentration change in the tip of the pipette, which took place when the bolus began to leave the perfusion pipette, was probably faster. The concentration change occurring at the apical membranes of receptor cells was delayed further by diffusion processes (and, perhaps, binding of Na ions) in the mucus plug of the taste pore. Measured delays from switching the flow to first electrical response ranged from 5 to 10 sec). The mean delay in eight measurements was 7.6 sec. Flow was switched on and off with hemostat clamps.

Due to decompression there was a tendency for air bubble formation in the solution which had passed the orifice of the inner tube. Formation of bubbles was avoided by using two feeding reservoirs in series for each pipette solution. Only the first reservoir (50 ml) had thus an interface with the pressurizing gas phase. The second reservoir (100 ml), which fed the pipette, then retained low concentrations of gases. Feeding tubes from reservoir to pipette contained small bacterial filters (Millipore).

For recording of currents from single taste pores, a patch-

clamp amplifier was set to voltage-clamp mode (input stage feedback resistor 100 MΩ), a pipette potential of 0 mV and a gain of 20 mV/pA. The input stage was connected to electrode *B*. Electrode *A* of the multiple perfusion holder (labeled (*e*) in Fig. 1) was an Ag/AgCl pellet connected to the outflowing solution through a short bridge of a 3 M KCl-agar. This electrode was grounded, shielding the pipette orifice from 50 Hz pickup through perfusion reservoirs and feeding tubes.

For display, signals were low-pass filtered at 500 Hz. They were stored digitally on a DAT-recorder. During playback the data were low-pass filtered at 300 or 500 Hz and redigitized at 5 kHz for computer processing. Data were subjected to recursive digital filtering. Thereby, slowly changing or sustained currents (i.e., the dc component of the short-circuit current) and the fast transient currents of an experiment were obtained as separate records. Alternatively, the slowly changing currents were blocked from the record by using the "tracking" mode of the voltage clamp. Slowly changing currents were then obtained in separate experiments, using a low-pass filter of 10–100 Hz and no tracking.

Solutions contained (in mM): Tyrode, NaCl 140, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10, pH 7.4, adjusted with Tris base. Adaptation medium: NaCl 50, HEPES 10, pH 7.0, adjusted with NaOH. For Na stimulation, 200 mM NaCl were added to the saliva-like adaptation medium. In such experiments, the control solution contained 200 mM N-methyl-D-glucamine Cl (NMDG-Cl) to keep the Cl concentration constant while the Na concentration was changed. The solution was titrated with HCl to pH 7.0. Amiloride (3,5-diamino-6-chloro-N-(diaminomethylene)-pyrazinecarboxamide) was a gift of Sharp & Dohme GmbH (München, Germany).

Results

Basic Approach

To record from the single taste pore of a fungiform papilla with an acceptable signal-to-noise ratio, the surface of the keratinized epithelium around the papilla should be of low conductance. This was achieved by an initial rinse of the mounted tip of the tongue with distilled water. Thereafter, the tip was kept in air and subjected to "continuous blotting": by resting the ventral side on a cushion of filter paper, fluid was removed from the dorsal surface by capillarity. Recording was possible for 1–2 hr after mounting, then the tissue lost some of its elasticity, such that contact between pipette and papilla deteriorated.

When approaching a fungiform papilla, pipette perfusion was stopped and the meniscus at the tip of the pipette was slightly pulled in by suction, such that only a little fluid emerged from the tip when it first touched the papilla. With stronger suction (also by mouth), the papilla was partially pulled into the orifice (Fig. 1*b*). At this stage, the input resistance was on the order of 5 MΩ. Perfusion with control solution was begun by connecting the suction tube (*f*) to the pump and applying pressure to the reservoirs. Within 5–10 min, the input resistance increased to about 50 MΩ, presumably because of drying of the epithelial surface around the pipette. The current noise diminished accordingly. A "seal" resistance of 50 MΩ, in combination with a pipette resistance of 1 MΩ, allowed 98% of the membrane current to be recorded [16, 17]. However, since such a low seal resistance was strongly *shunting* the transcellular current pathways, the apical membrane potentials of taste cells could not be affected by changing the pipette potential. For the same reason, the shunt current was a significant part of the recorded short-circuit current. Therefore, changes in shunt current, as caused by alterations in liquid junction potentials, had to be minimized.

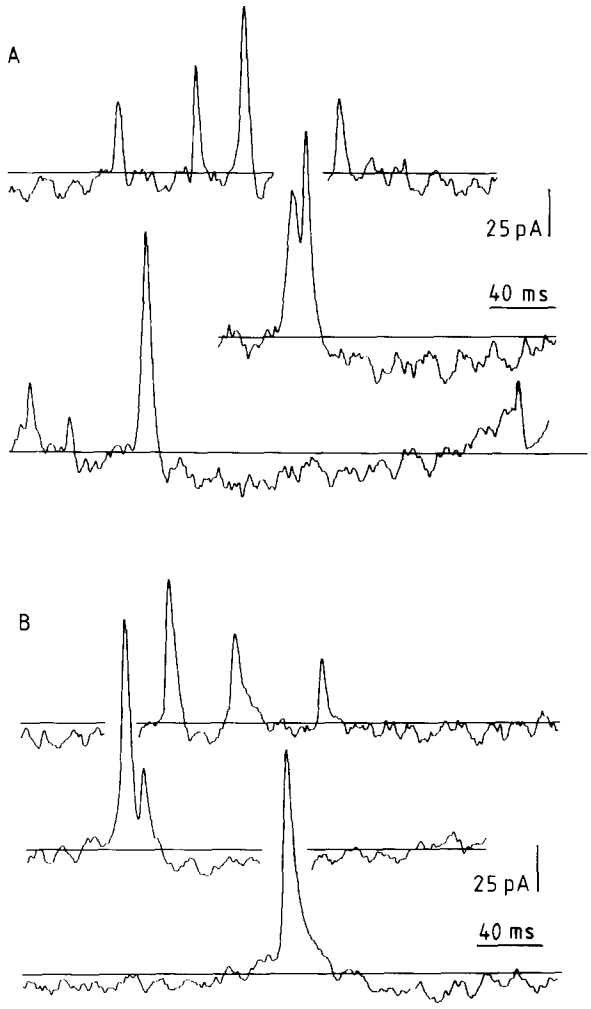

Fig. 2. Current transients recorded from single taste buds. Inward current through TRC apical membranes (i.e., from pipette to taste cell) is plotted downward. The larger "monophasic" transients were followed by periods of slowly changing inward current. Current transients with this secondary slow phase are, strictly speaking, biphasic. Records low-pass filtered at 500 Hz.

Monophasic transient currents (spikes) were often observed (Fig. 2) immediately after making con-

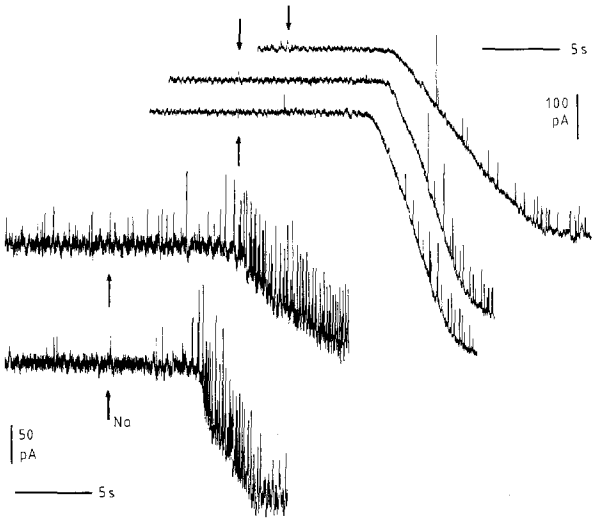

Fig. 3. Delay times and initial responses of single taste buds to an increase of mucosal NaCl from 50 to 250 mM (replacing NMDG-Cl). Inward current through TRC apical membranes (i.e., from pipette to taste cell) is plotted downward. For these recordings, fast and slow current components were not separated by filtering. High frequency cut off, 500 Hz. The vertical arrows indicate time of switch over to the perfusate of higher Na concentration. After a delay of 5 sec or more (*see* Materials and Methods), inward current increased sharply. On this time scale, the monophasic current transients are seen as vertical lines. They appeared at higher frequency while the slow inward current evolved. In the two lower traces the dc response was small and the frequency of transients large (up to 7 sec^{-1}), suggesting that most of the cells which drew apical inward current also generated action potentials. The upper three traces show large dc responses but only few transients. In this case some cells may have drawn inward current without firing action potentials. Note in traces *1*, *2* and *5* especially large transients near the onset of the response.

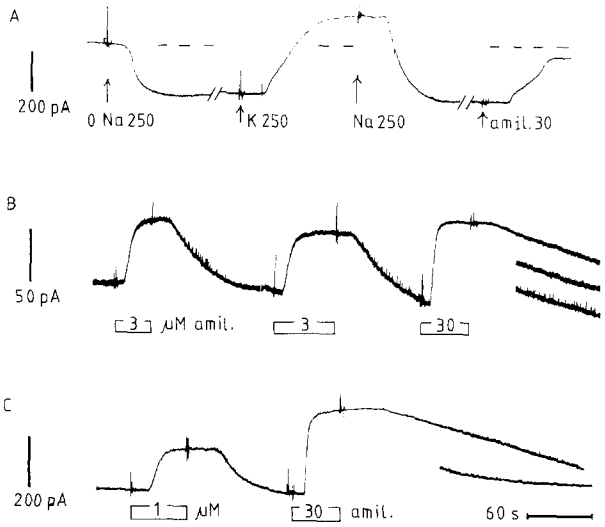

Fig. 4. Time evolution of sustained current (dc) of single taste buds in response to Na, K and amiloride applied at the taste pore. The chloride concentration was kept constant throughout, in order to avoid large changes of liquid junction potentials. Records low-pass filtered somewhat below 300 Hz, such that amplitudes of fast transients were rather small in these taste buds, about 15 pA in the absence of amiloride. The large deflections are switching artifacts. Current directed inward through the apical membrane (flowing from pipette to taste cell) is plotted downward. (*A*) Response to increasing Na from 0 to 250 mM (replacing NMDG), to replacement of Na by K and to 30 μM amiloride in the presence of 250 mM Na. The dashed line indicates the current level obtained in the absence of mucosal Na, K and amiloride. (*B*) and (*C*) Response to mucosal amiloride in the presence of 250 mM of mucosal NaCl.

tact with the papilla. They may have been responses to mechanical stimulation and to chemical stimulation exerted by the control solution. Within 1–2 min the initial spiking activity decreased, often ceasing completely. The transient currents were directed from electrode *B* to *A*, i.e., they were transient increases in outward currents or transient decreases of inward current flowing through the apical membranes of TRCs. Amplitudes were 10–50 pA, durations were 7–12 msec and the time course resembled that of action potentials recorded from isolated TRCs of the rat under whole-cell patch-clamp conditions [9]. The larger monophasic transients were followed by periods of slowly changing inward current (Fig. 2), suggesting that the action potentials were followed by periods of slow hyperpolarization.

Response of Sustained Currents

When the mucosal solution at the taste pore (i.e., the solution perfusing the pipette) was switched to one of increased Na concentration (Na replacing NMDG), the dc level of the recorded current (short-circuit current) changed as shown in Fig. 3: following a delay of 5–10 sec, a sustained current of 50–900 pA developed, which was directed from pipette to taste bud. The time evolution of this current was monotonic (no overshoot). Replacement of the NMDG in the pipette solution with K caused a current change opposite to that induced by replacement with Na (Fig. 4*A*). Ionic substitutions of this magnitude may be expected to induce significant changes in diffusion currents at the agar bridge within the pipette and at the current pathways which exist in parallel to the apical membranes of taste cells. Therefore, it was not certain to what extent the recorded changes in sustained currents reflected transport phenomena of taste cells.

When amiloride, a blocker of apical epithelial Na channels, was added to the NaCl-containing pipette solution at a concentration of 30 μM, the current decreased approximately by the amount induced with mucosal Na (Fig. 4*A*). Thus, a large part of the Na-dependent current was amiloride-blockable Na current flowing inwards through apical membranes of taste cells. The onset of blockage by

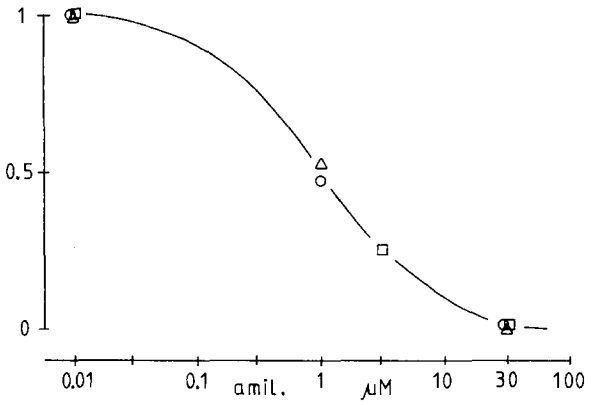

Fig. 5. Normalized dose-response curve for the blocking of sustained Na currents by amiloride. Data from three taste buds which had 128, 200 and 330 pA of amiloride-blockable steady-state inward currents at a mucosal Na concentration of 250 mM. The continuous curve was drawn for an inhibition constant of 1 μM.

amiloride was rapid, washout kinetics were less rapid, and reversibility was complete (Fig. 4*B* and *C*). The use of submaximal concentrations indicated an apparent inhibition constant near 1 μM in the presence of 250 mM NaCl (Fig. 5).

Response of Spike Rate

In response to a step increase in Na concentration at the taste pore, an increase in the repetition rate of monophasic transients was observed with many taste buds. The increase in collective spike rate[1] to values of 6–10 sec^{-1} occurred in the period of 10–15 sec in which the sustained current evolved (Fig. 3). A subsequent adaptive decrease in spike rate typically developed before or after the sustained current had reached its maximum amplitude, the rate of adaptation being variable. In the experiments of Fig. 6, the collective rate responses of trace *A* and *B* were both phasic/tonic, but clearly differed in the rate of adaptation. Phasic/tonic time courses are also known from the integrated responses of taste nerves to mucosal NaCl [10, 18, 19]. On washout of the high-Na solution, TRC spiking stopped completely for at least 30–50 sec, then resumed with the basal rate. It is noteworthy that unusually large monophasic transients (up to 100 pA) were often recorded at the onset of stimulation with mucosal Na. For the cases shown (Figs. 3 and 6*A* and *B*) we verified that the large current transients did not arise from superposition of smaller transients.

[1] The term "*collective* spike rate" draws attention to the fact that transients from a multitude of TRCs contribute to the rate response obtained from one taste bud.

Among the fast-adapting taste buds, some gave pure phasic responses to a step increase in mucosal Na. A step from 0 to 250 mM Na induced a faster adaptation than a step from 0 to 50 mM Na. Some buds appeared to be already fully inhibited at a mucosal Na concentration of 50 mM, which is near the threshold concentration of NaCl taste in the rat [7]. These buds did not respond to a Na step from 50 to 250 mM but gave a pure phasic response to a Na step from 0 to 50 mM.

When amiloride was added to a Na-containing perfusate at a concentration of 30 μM, the current noise decreased and generation of current transients ceased (Fig. 7). During washout of amiloride, the noise level gradually increased and the current transients reappeared. The lower traces of Fig. 7 are time expansions of the periods labeled *a–d* in the top trace. They show that the first transients observed during washout of amiloride barely exceeded the noise level, but increasingly larger ones were recorded as the washout proceeded[2].

Discussion

Stationary Inward Current

When the mucosal perfusate was changed from 50 mM NaCl plus 200 mM NMDG-Cl to 250 mM NaCl, a sustained current directed from perfusate to taste cell developed rapidly in many taste buds. In the rat, the NaCl concentration of saliva is below 50 mM, the NaCl taste threshold near 50 mM [7], and 250 mM NaCl a submaximal concentration [10]. Therefore, the Na step of 50 to 250 mM will address the salt taste reception system. A mammalian taste bud contains 50–70 taste cells [11, 12, 24]. If the bud responds to Na, an unknown though significant number of these cells may be expected to contribute to the observed Na-dependent current.

The sustained Na-dependent current was blocked by mucosal amiloride with a half-maximal concentration of 1 μM, suggesting conduction through apical Na channels [23]. While the change in current induced by substituting NMDG-Cl with NaCl was not a pure change in membrane current, but in part reflected altered liquid junction potentials, the current change induced by 30 μM ami-

[2] We noted that small current spikes continued to be recorded while the washout of amiloride progressed. However, during the washout small spikes may be generated by those TRCs which were subthreshold before. Thus, the mere occurrence of small spikes in this period cannot be taken to mean that spikes arising in one TRC during washout remain constant in amplitude. It is a disadvantage of our method that the spikes cannot be attributed to individual TRCs.

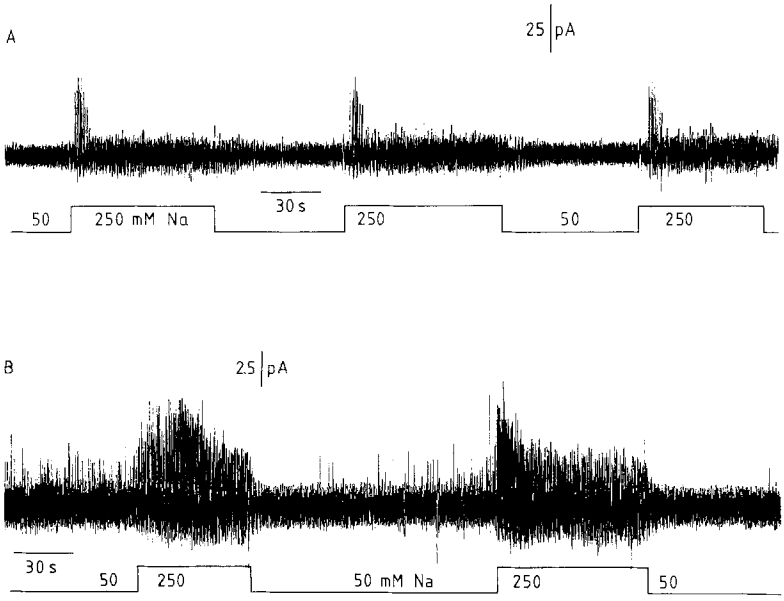

Fig. 6. Fast transient currents generated in response to changes of the Na concentration at the taste pore of a single taste bud. Inward current is plotted downwards. Slow currents were removed off-line by digital filtering (described in Materials and Methods). The high frequency cut off was at 300 Hz. In each case the taste bud was adapted to 50 mM of mucosal NaCl plus 200 mM NMDG-Cl. The latter was replaced with NaCl at the time indicated. Note, most clearly in (*A*), the large amplitude of transients which arose immediately after the step increase of mucosal Na. The large transients were not due to superposition, but were single monophasic transients. Peak and steady-state collective spike rates, measured during exposure to 250 mM Na, were near 10 and 0.5 sec^{-1} in *A* and near 10 and 2 sec^{-1} in panel *B*, where the basal rate was higher. In *A* adaptation of the collective spike rate was more rapid than in *B*.

loride was most likely free to be of such artifacts. The response of current to the removal of 30 μM amiloride was less rapid than the response to an increase in mucosal Na. The slow washout of amiloride and, in the case of increased mucosal Na, changes in shunt current, have so far prevented the estimation of the true time course of inward current of a taste bud in response to a stepwise increase in apical Na concentration or conductance. The time course is of interest because it may reflect the adaptation of TRCs to mucosal Na.

Our observations regarding the sustained current are in agreement with the findings of Heck, Mierson and DeSimone [18], who showed that the NaCl-dependent short-circuit current of the dorsal lingual epithelium of the rat is inhibited by amiloride. Since the integrated nerve response to 1 M NaCl was also suppressed by amiloride while that to 1 M KCl was not, Heck et al. [18, 19] suggested that amiloride-blockable Na channels, located in the apical membranes of receptor cells, are involved in salt taste. Stationary, amiloride-blockable currents were also found in taste cells of the frog [2, 3, 5]. Our results are not in accord with those of Yoshii, Kiyomoto and Kurihara (1986), who concluded that taste responses to salts are not induced by entry of cations via apical membranes of taste cells.

Nature of Transient Currents

Due to the increase in apical inward current caused by the availability of Na ions at the taste pore, the *in situ* TRCs will depolarize, as previously found in isolated taste cells of the frog [2]. The majority of isolated TRCs from rat fungiform papillae were found to contain voltage-gated Na and K channels which enabled them to fire action potentials when sufficiently depolarized [9]. The time course of the "fast" variety of these action potentials was very similar to the time course of the current transients observed in this study, suggesting that the transients are driven by TRC action potentials.

How an action potential will be picked up from the taste pore by a current-recording pipette depends on the distribution of channels between apical and basolateral membranes. This distribution is not known, except for taste cells of *Necturus* [21, 27]. Some possibilities are indicated in Fig. 8. In possibility *A* the apical membrane contains chemoreceptors but no active channels. Coupling to the recording electrode is capacitive, and the recorded current transients are biphasic. Such records were obtained from apical protrusions of olfactory receptor cells [16, 17]. In possibility *B* the apical membrane contains most of the voltage-gated K channels of the TRC, as described for *Necturus* taste cells [21, 27]. Generation of action potentials will induce transients of monophasic outward current. Should the apical membrane also contain some amiloride-blockable Na channels, then their blockage by amiloride will cause a decrease of depolarization and spike rate, while a decrease in the *amplitude* of the recorded transients will be small and limited to the decrease in amiloride-blockable current (*see below*).

Possibility *C* accounts for the observed extensive decrease in amplitude of recorded transients: in

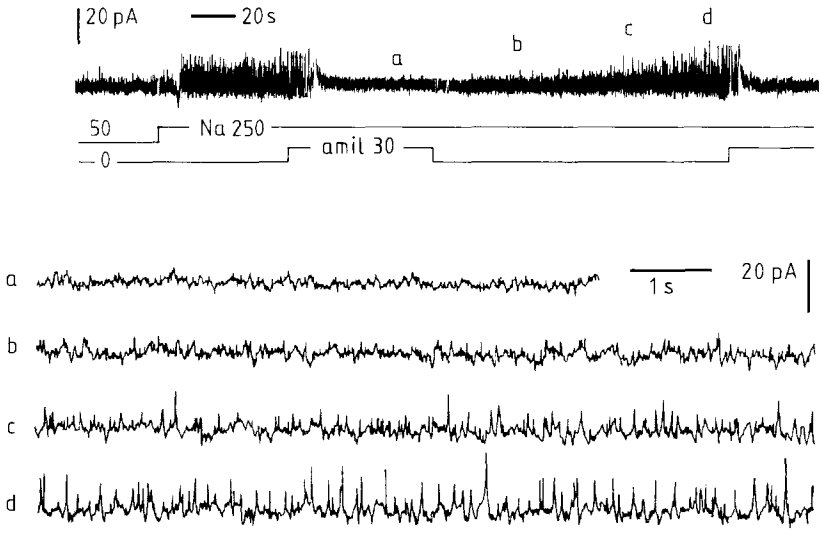

Fig. 7. Complete suppression of spiking activity by 30 μM amiloride (period *a*, part of which is seen below at higher time resolution). During the intentionally slow washout of amiloride (periods *b*, *c*, *d*), the noise level increased together with number and amplitude of the monophasic transients. Slow currents were suppressed using the tracking feature of the voltage clamp. The high frequency cut off was at 300 Hz.

this model the current spikes are modulations of the sustained inward current of individual TRCs which arise because a TRC action potential abolishes the driving voltage for apical Na uptake. Therefore, a cell with less sustained Na inward current will generate smaller current transients. This explains that large and small transients are recorded simultaneously (Fig. 2), and that transient amplitudes are strongly diminished by amiloride (Fig. 7). We suggest, therefore, that action potentials of Na-sensitive TRCs of the rat are transmitted to the mucosal space by ohmic conduction via amiloride-blockable Na channels. A small contribution by other conduction mechanisms cannot be excluded. The variable amplitude of recorded transients is not due to differences in the amplitude of TRC action potentials[3]. Rather, it is due to differences in apical conductance.

The current transients arising from receptor cells were by their shape and by their variable amplitude distinguished from the shorter and *biphasic* all-or-none transients arising from axons [15], in our case from sensory axons forming synapses with TRCs. Biphasic voltage transients arising from axons were previously recorded from taste buds of frog tongues [28] and from taste pores of buds on the tongue of the narcotized hamster [13, 14]. In those studies TRC action potentials were not noticed[4]. Under our recording conditions, the transients arising from sensory axons were either absent or remained within the noise level. Biphasic signals arising from axons were expected in the period following a monophasic transient, with a delay time due to synaptic transmission. We were unable to consistently observe such signals, possibly because the axons were not functional in the isolated, not vascularly perfused rat tongue. In future experiments the synaptic transmission ratio (i.e., the ratio of spike rates arising from a TRC and its sensory axon) will deserve attention.

Adaptation

Especially large current transients, of about 100 pA, were often recorded at the onset of stimulation with mucosal Na (Figs. 3 and 6). This may mean that directly after the step increase in Na concentration the Na inward current of the TRC generating the large transient was about 100 pA, but then decreased with time[5]. An increase of the cytosolic Na

[3] Since other taste qualities are mediated by other reception mechanisms [4] and, furthermore, require a different distribution of channels between apical and basolateral membrane, it may be expected that their action potential-driven current transients, when recorded from the taste pore, are of different shape. Indeed, we observed in preliminary experiments that some rat taste buds generated small *biphasic* current transients in response to sour stimuli. With frog taste buds we observed current transients which were inward, i.e., they were "upside down" compared to those shown here.

[4] When stimulating a taste bud chemically, the resulting postsynaptic spike sequence can be recorded from a *neighboring* taste bud, by virtue of collateral branching of the sensory axons. Such responses were obtained on stimulation with $CaCl_2$ [28] as well as with sweet and bitter compounds and with amino acids [13, 14]. NaCl stimulation was not yet used with this method. The published records are typical extracellular axonal recordings, the transients being pronounced biphasic, their amplitude all or none.

[5] In isolated taste receptor cells of the frog, amiloride-blockable currents of even several hundred pA were measured [2].

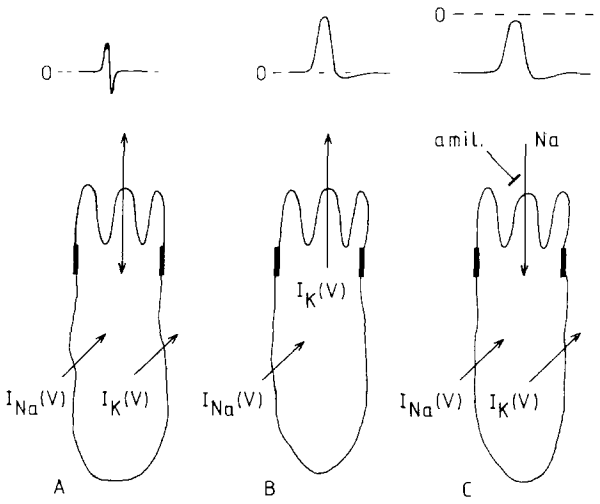

Fig. 8. Models of taste cells with different distributions of channels at apical (above) and basolateral (below) membrane. (*A*) Voltage-gated Na and K channels at basolateral membrane; no channels active at apical membrane. A pipette at the taste pore (Fig. 1*b*) will pick up biphasic current transients which are differentiated TRC action potentials (diagram above). (*B*) Voltage-gated Na channels at basolateral membrane and voltage-gated K channels at apical membrane. The current transients will be monophasic and outward. (*C*) Voltage-gated channels at basolateral membrane. The apical membrane contains amiloride-blockable Na channels (not voltage gated) which allow flow of Na inward current balanced by the action of a basolateral Na pump. When the basolateral membrane generates an action potential, the driving voltage for apical Na inflow is transiently turned off. The resulting interruption of inward current is shown above.

concentration might contribute to a decrease of this current with time. Furthermore, the apical Na permeability might be *downregulated* in this period, comparable, perhaps, to receptor inactivation in other systems. Indeed, in previous whole-cell experiments with taste cells of the frog we saw strongly "overshooting" inward currents in response to rapid removal of amiloride [2]. However, in the present experiments the evolution of recorded current, following a step increase in mucosal Na, did not show a secondary decrease in the period when the spike rate adapted.

As mentioned above, several technical problems prevented us from obtaining the true time course of this current. It may be significant, however, that the collective spike rate often adapted before the sustained inward current had reached its maximal value. In these cases, the rate with which the sustained current evolved was possibly not merely diffusion limited but already slowed down by an adaptive decrease in Na permeability. Then a secondary decrease in sustained current need not be expected. Furthermore, the sustained current is recorded collectively from many TRCs. These may adapt with different rates and to different degrees. Finally, not all TRCs which contribute to the Na inward current may generate action potentials and show an adaptive response. A clarification of these aspects requires the recording from single TRCs *in situ*. For the present, a downregulation of apical Na permeability contributing to the adaptation of Na-sensitive receptor cells remains speculative.

Signal Chain of Salt Reception

Our results are in accord with previous suggestions (reviewed in ref. [4]) that apical Na channels of the amiloride-blockable type serve as Na receptors in salt taste. The depolarization caused by apical Na inward current leads to the generation of receptor cell action potentials. Their function may be the activation of synaptic transmission, possibly involving voltage-gated basolateral Ca channels [9]. It is not known how many of the cells which draw amiloride-blockable inward current will generate action potentials. The signal chain also contains a negative feedback branch which serves for receptor cell adaptation. This process remains to be investigated.

Conclusion

We have shown that the action potentials fired by epithelial taste receptor cells can be recorded noninvasively from a single taste bud. The spikes were recorded as transient currents, while the bud remained in its natural position in the tissue. When taste stimuli (i.e., Na) were applied to the taste pore only, rate and amplitude of transient currents increased. Changes in sustained current were also observed. The method will be useful to investigate the relationship between taste stimulus and TRC-spike rate and to characterize the apical conductive pathways of TRCs *in situ*.

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