Current Recording from Sensory Cilia of Olfactory Receptor Cells In Situ

II. Role of Mucosal Na^+, K^+, and Ca^{2+} Ions

STEPHAN FRINGS, STEFAN BENZ, and BERND LINDEMANN

From the Department of Physiology, Universität des Saarlandes, D-6650 Homburg/Saar, Germany

ABSTRACT Action potential–driven current transients were recorded from sensory cilia and used to monitor the spike frequency generated by olfactory receptor neurons, which were maintained in their natural position in the sensory epithelium. Both basal and messenger-induced activities, as elicited with forskolin or cyclic nucleotides, were dependent on the presence of mucosal Na^+. The spike rate decreased to $\sim 20\%$ when mucosal Na^+ was lowered from 120 to 60 mM (replaced by N-methyl-D-glucamine$^+$), without clear changes in amplitude and duration of the recorded action potential–driven transients. Mucosal Ca^{2+} and Mg^{2+} blocked spike discharge completely when increased from 1 to 10 mM in Ringer solution. Lowering mucosal Ca^{2+} below 1 mM increased the spike rate. These results can be explained by the presence of a cyclic nucleotide–dependent, Ca^{2+}-sensitive cation conductance, which allows a depolarizing Na^+ inward current to flow through the apical membrane of in situ receptor cells. A conductance with these properties, thought to provide the receptor current, was first described for isolated olfactory cells by Nakamura and Gold (1987. *Nature (Lond.).* 325:442–444). The forskolin-stimulated spike rate decreased when *l-cis*-diltiazem, a known blocker of the cyclic nucleotide–dependent receptor current, was added to the mucosal solution. Spike rate also decreased when the mucosal K^+ concentration was lowered. Mucosal Ba^{2+} and 4-aminopyridine, presumably by means of cell depolarization, rapidly increased the spike rate. This suggests the presence of apical K^+ channels that render the receptor cells sensitive to the K^+ concentration of the olfactory mucus. With a slower time course, mucosal Ba^{2+} and 4-aminopyridine decreased the amplitude and caused rectification of the fast current transients (prolongation of action potentials). Abolishment of the apical Na^+ current (by removal of mucosal Na^+), as indicated by a strong decrease in spike rate, could be counteracted by adding 10 mM Ba^{2+} or 1 mM 4-aminopyridine to the mucosal solution, which re-established spiking. Similarly, blockage of the apical cation conductance with 10 mM Ca could be counteracted by adding 10 mM Ba^{2+} or by raising the mucosal K^+ concentration. Thus

mucosal concentrations of Na^+, K^+, and Ca^{2+} will jointly affect the sensitivity of odor detection.

INTRODUCTION

In a previous paper (Frings and Lindemann, 1991) we reported that the spike rate of olfactory receptor cells (ORCs) in situ increased when the ciliary membrane was superfused with permeant analogues of cAMP and cGMP or with forskolin or phosphodiesterase inhibitors. The response was attributed to the opening of an apical cation conductance, generating a depolarizing, inwardly directed receptor current. This interpretation was suggested by patch clamp experiments with isolated ORCs (Nakamura and Gold, 1987; Trotier and McLeod, 1987; Kurahashi and Shibuya, 1988; Suzuki, 1988, 1989; Firestein and Shepherd, 1989; Kolesnikov et al., 1990) which showed that odorants or cyclic nucleotides induce inward currents of low ionic specificity. However, after the opening of the tight junctions, isolated cells may redistribute their channels between the apical membrane and the cellular surface normally facing the interstitial space. Therefore, it is not clear where these cation channels are normally located: below the tight junction (in the somal and dendritic membrane of ORCs), or outside the tight junction on the apical membrane (either on the ciliary knobs or on the ciliary surface). The mucus-covered ciliary surface, of course, will be the site of primary contact with odorous stimuli.

For ORCs in situ, whose properties were often implied from electro-olfactograms (EOGs), there is still considerable uncertainty regarding the charge carrier of the receptor current. EOGs represent compound signals from in situ receptor cells and from glia-like sustentacular cells of the olfactory mucosa. The EOG response to mucosal cation substitution suggested that the charge carriers of odor-induced currents are either Na^+ (Takagi et al., 1969) or Ca^{2+} (Suzuki, 1978; Winegar et al., 1988). Alternatively, based on EOGs it was claimed that cations do not give rise to receptor currents (Yoshii and Kurihara, 1983).

Here we report results from ORCs maintained in the intact sensory epithelium, such that the sidedness of apical and somal membranes, which depends on the integrity of the tight junctions, was preserved (Frings and Lindemann, 1990b, 1991). The ciliated apical membrane represents > 80% of the cellular surface of ORCs in the frog (excluding the axon). We found that the increase in spike rate induced by cyclic nucleotides required the presence of mucosal Na^+. Mucosal Ca^{2+} and Mg^{2+} blocked the increase. At the normal concentrations of Na^+ and Ca^{2+} in the mucus, the block by divalent cations was already partially effective. The induced increase in spike rate was also blocked by l-cis-diltiazem.

These observations are in good agreement with the findings of Nakamura and Gold (1987), who first proposed that a nonspecific ciliary cation channel, activated by cyclic nucleotides and blocked by divalent cations, conducts the receptor current. At negative membrane potentials, the inward movement of Na^+ will be the main component of this current. Our finding that cyclic nucleotides increase the spike rate only in the presence of mucosal Na^+ suggests an apical location of the cation-conducting channel which is blocked by Ca^{2+}, Mg^{2+}, and l-cis-diltiazem.

Ciliary recording of spike rates and spike shapes also suggests that the ORC apical membrane contains potassium channels sensitive to barium and 4-aminopyridine

(4-AP). The K$^+$ conductance renders the ORCs sensitive to the potassium concentration of the mucus. This concentration may change with the secretory activity of the olfactory mucosa and with the electrical activity of the ORC apical membranes.

METHODS

The method of recording from olfactory cilia was used as described by Frings and Lindemann (1990b), with small modifications as specified in a previous paper (Frings and Lindemann, 1991). Briefly, frogs (*Rana esculenta/ridibunda*, from Yugoslavia) were kept at 4°C in tap water. Experiments were done from October to March. After killing a frog by decapitation, the dorsal olfactory mucosa was removed and stored at 4°C until placed into an experimental chamber where cilia were accessible to a recording pipette under microscope observation. The method of mounting the tissue allowed an independent perfusion of mucosal and interstitial sides, of the epithelium. Thus, when ion substitutions or blocker experiments on the ciliated side (mucosal solution) were performed, the medium bathing the interstitial side of the mucosa remained unaltered. Action potential–related fast biphasic current signals, representing mainly capacitive current (Frings and Lindemann, 1991), were recorded at an amplification of 200 mV/pA, stored on video tape, and analyzed with a computer program as described earlier.

Single-channel recordings were obtained in the patch clamp on-cell mode, with the pipette sealed to the apical knob of in situ receptor cells, as described before (Frings and Lindemann, 1990a). Sealing to ORCs was recognized by the recording of fast current transients, as opposed to sealing on sustentacular cells, which did not generate action potentials.

The basal medium used in most experiments on both sides of the mucosa was Na$^+$-Ringer solution. Its composition was (in mM): 120 NaCl, 4 NaOH, 3 KCl, 1 CaCl$_2$, 2 MgCl$_2$, 10 HEPES, 5 glucose, and 5 Na$^+$ pyruvate, pH 7.4. The measured osmolarity was 250 mosM. The pipette solution contained (in mM): 115 NaCl, 4 KOH, 1 CaCl$_2$, 2 MgCl$_2$, and 10 HEPES, pH 7.4. The measured osmolarity was 229 mosM. For the Na$^+$ substitution experiments the following solution was used (in mM): 4 KOH, 1 CaCl$_2$, 2 MgCl$_2$, and 10 HEPES, pH 7.4. To this solution NaCl and N-methyl-D-glucamine chloride (NMDGCl; Sigma Chemical GmbH, Munich, Germany) were added to give a combined concentration of 120 mM. The solution approaching the Na$^+$, K$^+$, and Ca^{2+} concentrations of amphibian olfactory mucus (Joshi et al., 1987) contained (in mM): 60 NaCl, 10 KCl, 3 CaCl$_2$ 1 MgCl$_2$, 60 NMDGCl, and 10 HEPES. Forskolin and 1,9-dideoxysforskolin (Sigma Chemical GmbH) were dissolved in ethanol (stock of 10 mM). Aliquots were added to Ringer solution to give the concentrations indicated. 0.1% ethanol had no effect on the spike rate. Other substances used were cpt-cAMP and 8BrcGMP (Sigma Chemical GmbH), D-600 (Knoll AG, Ludwigshafen, Germany), diltiazem (racemat; Sigma Chemical GmbH), l-cis-diltiazem and d-cis-diltiazem (Gödeke AG, Berlin, Germany), amiloride (Sharp Dohme GmbH, Munich, Germany), tetraethylamonium (TEA; Fluka, Neu-Ulm, Germany), 4-AP (Sigma Chemical GmbH), and dihydro-ouabain (Sigma Chemical GmbH).

RESULTS

Dependence of Spike Rate on Mucosal Sodium

When the mucosal Na$^+$ concentration was lowered from the value of the Ringer solution (120 mM) to 10 mM by replacement with NMDG$^+$, the generation of action potentials stopped within 15 s (Fig. 1 A). The ion substitution also elicited a diffusion current at the pipette orifice. The onset of this signal (as in Fig. 1 B, staircase function) coincided with the onset of the inhibition, showing that the 15 s were needed for the new solution to reach the recording site. The inhibition was observed for basal activity as well as for accelerated spike rates in the presence of 0.1 μM
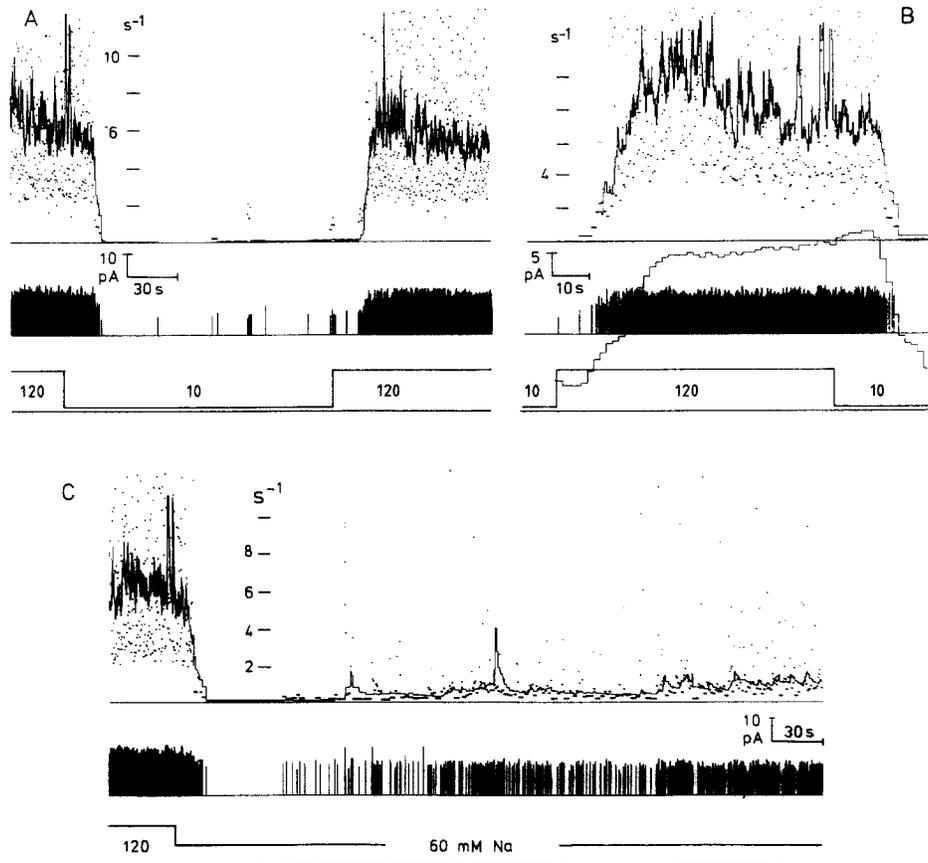
FIGURE 1.   (A) The effect of removing mucosal Na$^+$ (from Ringer solution) on the spike rate of olfactory receptor cells in situ. The upper part of the figure shows the spike rate as a function of time. Each point represents the inverse of the time interval between two spikes, given in s^{-1}. The baseline indicates zero activity and the solid line was created by calculating and connecting the means of 10 consecutive rate values. Below the rate plot, the spike record shows peak height and time of each detected current transient. The signals were recorded from three ORCs that were constantly stimulated with 0.1 μM mucosal forskolin. This concentration induced a maintained stimulation of the cells without excessive desensitization. Serosal Na$^+$ was held constant at 120 mM. When mucosal Na$^+$ was reduced from 120 to 10 mM by replacing it with NMDG$^+$, the spike rate immediately decreased. When the perfusion was switched back to 120 mM Na$^+$ after 2.5 min, spiking resumed in a phasic-tonic manner. (B) Phasic-tonic increase of spike rate on raising mucosal Na$^+$ from 10 to 120 mM. The staircase function (change in diffusion current at the pipette opening) indicates the time course of cation concentration changes at the site of recording (same calibration as for spike record). (C) Partial recovery of spike rate after decrease of mucosal Na$^+$. Ciliary recording from three ORCs in the presence of 0.1 μM mucosal forskolin. Spike rate dropped from ~6.5 s^{-1} to zero on reducing mucosal Na$^+$ from 120 to 60 mM, and subsequently rose to a steady level at 1.5 s^{-1}. Sodium was replaced with NMDG$^+$. A, B, and C are based on one experiment.

forskolin.[1] On returning to 120 mM mucosal Na^+, spiking resumed with a phasic-tonic time course (Fig. 1, *A* and *B*) as previously observed on exposure to cAMP (Frings and Lindemann, 1990*b*, 1991). The Na^+ dependence of the tonic component is shown in Fig. 2 *A*. The small slope at low Na^+ may be due to a voltage-dependent block of the cyclic nucleotide–induced conductance by divalent cations (see Discussion). Lowering the mucosal Na^+ concentration to 10 or 30 mM had no consistent effect on the amplitude and duration of the recorded transients.

After a step decrease of mucosal Na^+ from 120 mM to a lower value, spiking transiently ceased at 60 mM or below (Fig. 1 *C*). About 1 min later the spike rate increased back to the stationary value (for steady-state data see Fig. 2 *A*). A similar undershooting response was observed after washout of cyclic nucleotides (Frings and Lindemann, 1991). It may indicate a shift in the Na^+ equilibrium potential of the apical membrane (see Discussion).

The olfactory mucus has a lower Na^+ and a higher K^+ and Ca^{2+} concentration than Ringer solution (Joshi et al., 1987). When the mucosal side was superfused with 60 mM Na^+, 10 mM K^+, and 3 mM Ca^{2+} (see Methods), the basal rate dropped to 50% of the value in Ringer solution. Under these ionic conditions, the stimulated spike rate (0.1 μM forskolin) reached a mean value of 3.4 s^{-1} ($n = 4$), which is close to that found with Ringer solution (Frings and Lindemann, 1991).

To investigate whether the dependence of spike rate on mucosal Na^+ could be attributed to voltage-dependent, TTX-sensitive Na^+ channels present on the apical membrane surface, 1 μM TTX was added to the mucosal solution. 0.1 μM forskolin was also added to confirm contact of the TTX solution with the ciliary membranes of the ORCs from which records were made. In response to this solution, the spike rate increased and the amplitudes of recorded transients decreased (Fig. 2 *B*), as typically observed with forskolin (cf. Frings and Lindemann, 1991). Mucosal TTX without forskolin had no effect. Application of the TTX-forskolin solution to the interstitial side blocked the generation of action potentials dramatically (Fig. 2 *C*). Forskolin without TTX had no effect on this side. Therefore, (*a*) TTX-blockable, voltage-dependent Na^+ channels did not contribute to the conduction of action potentials on the ciliary membranes, and (*b*) the apical Na^+ conductance was not TTX sensitive. The nucleotide-gated channel described by Nakamura and Gold (1987) is the obvious candidate for this conductance.

Kolesnikov et al. (1990) observed that the cyclic nucleotide–gated conductance of isolated ORCs, like that of photoreceptor cells (Stern et al., 1986), is blocked by *l-cis*-diltiazem. In agreement with this report, we found that the spike rate of in situ ORCs, stimulated with 0.1 μM forskolin, was decreased by addition of 10 μM *l-cis*-diltiazem to the mucosal solution (five of five cells responded). When using 50 μM, the spike rate was suppressed by 80–100% (Fig. 2 *D*). The effect was reversible. In contrast to the blockage by mucosal Ca^{2+}, the inhibition by *l-cis*-diltiazem required ~1 min for completion. The washout was even slower, possibly because the agent penetrated through the lipid phase and acted at the cytosolic surface of the

---

[1] The analogue 1,9-dideoxyforskolin, which does not stimulate the adenylate cyclase (Laurenza et al., 1989), was ineffective at a mucosal concentration of 1 μM (12 ORCs tested). In contrast, 0.1 μM mucosal forskolin increased the spike rate of all ORCs tested.

A

100

% spike rate

60

20

0

0   30   60   90   120

mM mucosal Na

B   mucosal

TTX +Forskolin

20
pA   30 s

C   interstitial

TTX + Forskolin

D

8 — s^{-1}

6 —

4 —

30 s

10
pA

Forskolin   0,1 µM

l−cis−diltiazem 50µM
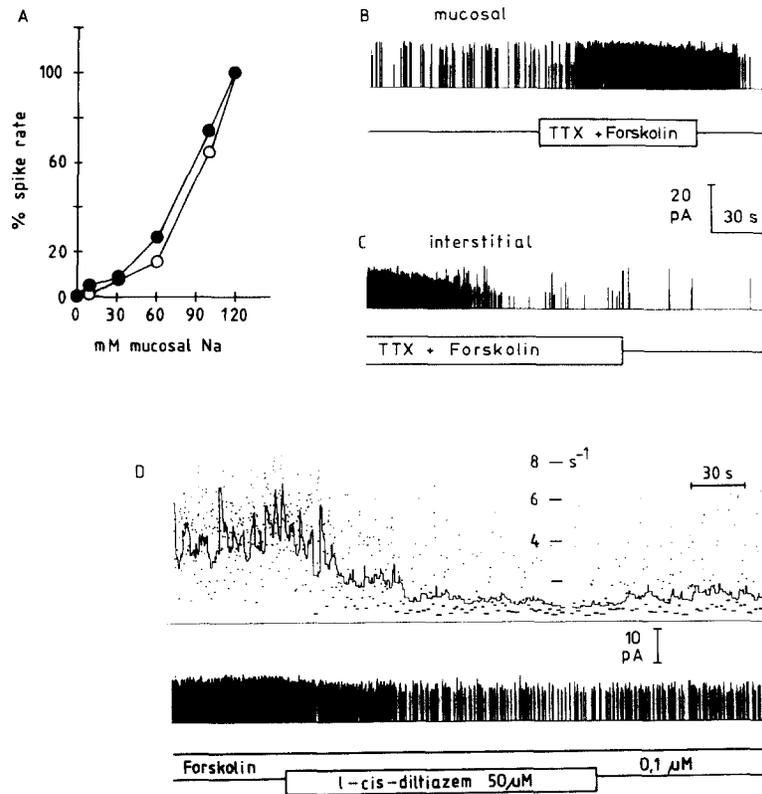
FIGURE 2. (*A*) Sodium dependence of stationary spike rate of ORCs. Rates represent the combined activity of three ORCs, recorded after pulling one or more cilia of each of the three cells into the recording pipette. All values were obtained during steady-state (plateau) periods of activity in the presence of 0.1 µM mucosal forskolin dissolved in Ringer solution. Two protocols of changing mucosal Na$^+$ were used: cumulative addition starting from Na$^+$-free solution (*filled circles*), and stepwise Na$^+$ depletion starting from 120 mM to each indicated concentration (*open circles*). In both cases, Na$^+$ was replaced with NMDG$^+$. (*B*) Effect of tetrodotoxin on the mucosal side of the olfactory epithelium. Ciliary recording from three ORCs. The interstitial side was continually perfused with Ringer solution, while the mucosal side was perfused with Ringer solution that contained, during the indicated period, 1 µM TTX and 0.1 µM forskolin. (Forskolin was added to mark the arrival of the test solution at the ciliary membrane.) The response shown is typical for forskolin stimulation and was not altered by the presence of TTX. (*C*) Time course of the effect of interstitial TTX in a ciliary recording from five to six ORCs. The interstitial compartment was perfused (through inlet i$_3$ of Fig. 1*A* of Frings and Lindemann, 1990*b*) with Ringer solution containing 1 µM TTX and 0.1 µM forskolin. The perfusion delay (due to dead space) was ∼2 min. When the test solution reached the tissue, most cells ceased spiking within 1 min. This delay may be interpreted as the time that the toxin needs to diffuse through the submucosa to reach the basal poles of the ORCs. During the presence of TTX, only very few spikes were seen. They were probably generated by cells whose axon hillocks were less easily accessible to TTX from the interstitial side. When TTX was washed out (not shown), spiking accelerated after 4 min (perfusion delay included) and reached the control value after 13 min. During the whole experiment, the ciliary side was washed with Ringer solution. Interstitial forskolin alone, at concentrations from 0.1 to 5 µM, had no effect on the spike rate. (*D*) Suppression of spike generation by mucosal *l-cis*-diltiazem. Record from two ORCs that were constantly stimulated with 0.1 µM mucosal forskolin. For the time indicated, 50 µM *l-cis*-diltiazem was added to the forskolin solution. Within 1 min, the mean spike rate decreased from 2.4 to 0.4 s^{-1}. It took 6 min of washout to restore the initial spike rate (not shown).

membrane, as suggested by Stern et al. (1986). The Ca^{2+}-antagonist *d-cis*-diltiazem (50 μM) had no effect on the spike rate.

Sensitivity to Mucosal Calcium and Magnesium

The Ringer solution used for this and the preceding study contained 1 mM Ca^{2+} and 2 mM Mg^{2+}. When the mucosal concentration of one of these ions was raised to 10 mM, the firing of action potentials was strongly inhibited (Fig. 3). This was true for the basal rate (i.e., in the absence of added stimuli), as well as for stimulation by forskolin, cyclic nucleotides, or phosphodiesterase inhibitors. Basal rate was lowered to zero by 10 mM Ca^{2+} (in 12 of 12 ORCs tested), while 10 mM Mg^{2+} decreased the basal rate by 87% (mean from four ORCs). Cells stimulated by mucosal forskolin (0.1–1 μM) responded to an increase of mucosal Ca^{2+} to 10 mM with a mean inhibition of spike generation by 95% (seven ORCs). Similarly, cells stimulated by mucosal cpt-cAMP (70 μM) were completely blocked by 10 mM mucosal Ca^{2+} (four cells). Spike amplitudes did not decrease. The inhibition by mucosal Ca^{2+} or Mg^{2+} may be caused by a direct block of the nucleotide-gated channels, resulting in a reduction of Na^+ inward current and hence a reduction of cell depolarization. The alternative, a hyperpolarization caused by Ca^{2+} inflow and an opening of Ca-activated K^+ channels, was excluded because Mg^{2+} had the same effect as Ca^{2+}.

It may be expected that a direct block of apical inward current by divalent cations will diminish the depolarization and can, therefore, be counteracted by depolarizing the apical membrane in other ways. This possibility was assessed as follows: Due to the presence of K^+ channels in the apical membrane (see below), and because this membrane represents at least 80% of the neuronal surface (excluding the axon), it should be possible to depolarize the neuron by increasing the mucosal K^+ concentration. Exposure of the ciliated surface to K^+ channel blockers should also have a depolarizing effect. The outcome of such experiments is shown in Fig. 3 *C*. The block of spiking activity caused by 10 mM mucosal Ca^{2+} was relieved reversibly with 10 mM Ba^{2+}, added to the mucosal solution together with the 10 mM Ca^{2+}. It was also relieved by raising mucosal K^+ to 20 mM (not shown).

When mucosal Ca^{2+} or Mg^{2+} was lowered, spike rate increased dramatically (Fig. 4 *A*). Reducing Ca^{2+} from 1 to 0.1 mM in the absence of added chemical stimuli usually accelerated the spike rate to the high values of 20 s^{-1} previously observed with cpt-cAMP or 8BrcGMP at mucosal concentrations of > 1 mM (Frings and Lindemann, 1991). Concomitantly, spike amplitudes decreased and finally vanished, indicating a strong depolarization of the neuron. All effects of divalent cations were readily reversible. Fig. 4 *B* shows the effect of mucosal Ca^{2+} on the basal spike rate of six ORCs. When tested with mucosal Ca^{2+} concentrations < 0.3 mM, 34 of 34 cells responded with high spike rates and 30 of 34 cells in addition responded with vanishing spike amplitudes. (In the latter case a quantitative rate evaluation rapidly became impossible.) The acceleration of spike rate by low Ca^{2+} was dependent on mucosal Na^+: in the absence of mucosal Na^+, a reduction of mucosal Ca^{2+} did not increase the spike rate (Fig. 4 *C*). Apparently, divalent cations block the apical Na^+ conductance, as expected from patch clamp studies of isolated ORCs (Nakamura and Gold, 1987). Reducing Ca^{2+} in the interstitial solution to 0.1 mM was without effect on spike rate.

Since it was reported that the EOG is affected by Ca^{2+} channel blockers (Winegar et
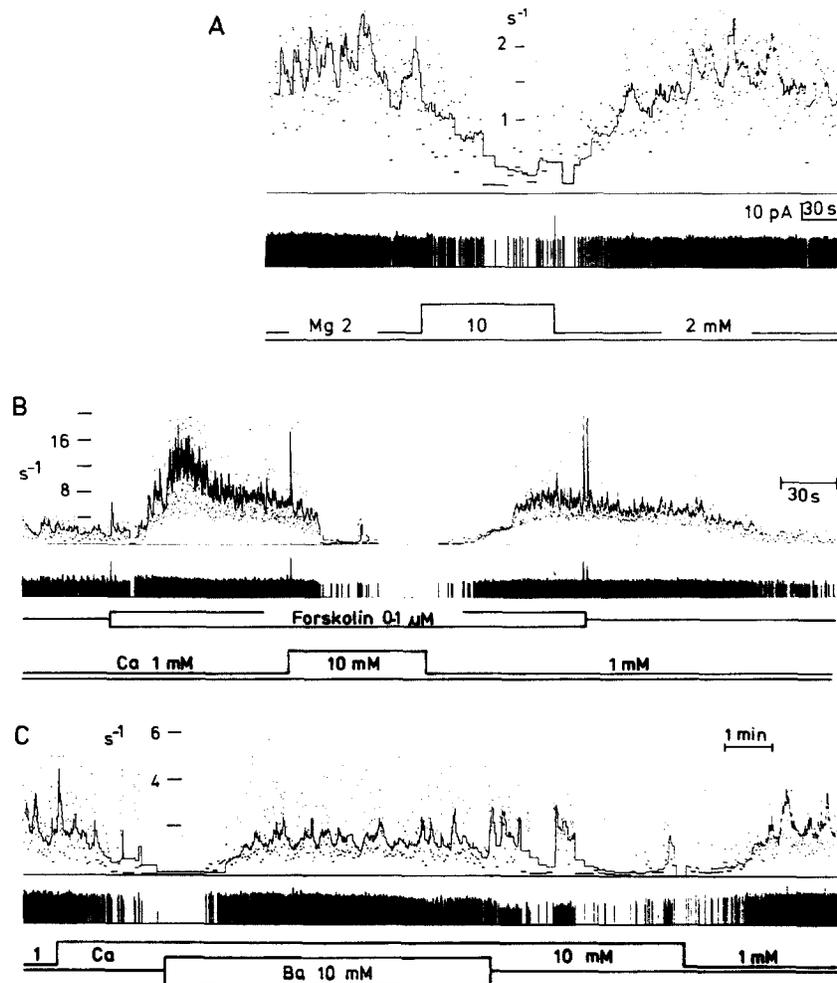
FIGURE 3. (A) Mg^{2+} block of forskolin-induced spiking. The combined activity of two ORCs was monitored in the presence of 0.1 μM forskolin, while mucosal Mg^{2+} was raised from 2 to 10 mM (in Ringer solution) during the period indicated. The response was slow due to slow mucosal superfusion. Suppression of spike generation was almost complete in the presence of 10 mM Mg. (B) Calcium block of forskolin-induced spike generation. Ciliary recording from two ORCs, showing the effect of 10 mM mucosal Ca^{2+} (in Ringer solution) on spike rate. When 0.1 μM forskolin was added to the mucosal perfusate, the combined spike rate rose from ~ 2 s^{-1} to ~ 13 s^{-1}, then declined to a plateau value of ~ 7 s^{-1}. Raising mucosal Ca^{2+} from 1 to 10 mM inhibited rapidly (both basal and forskolin-induced rate decreased). While the Ca^{2+} block developed, spike amplitudes did not decrease, indicating that spiking was not blocked by extreme depolarization of the cells. On returning to 1 mM mucosal Ca^{2+}, spike rate rose to the previous plateau level of 6 s^{-1}. The rate returned to 2 s^{-1} after washout of forskolin. (C) Release from Ca^{2+} block by mucosal Ba^{2+}. Ciliary recording from two ORCs in the presence of 0.1 μM mucosal forskolin. When Ca^{2+} was raised from 1 to 10 mM, spike rate dropped to zero. This response was slower, compared with B, because the rate of superfusion was slower. Addition of 10 mM mucosal Ba^{2+} restored activity, with spike amplitudes that were, during the first minute, slightly larger than before Ca^{2+} block. In the presence of mucosal Ba^{2+}, spike amplitudes gradually decreased by 30%, and spike shapes gradually rectified. This probably indicated that the ciliary membrane was hyperpolarized during Ca^{2+} block of the cyclic nucleotide–activated channels, and progressively depolarized as the Ba^{2+} block of ciliary K^+ channels took effect.
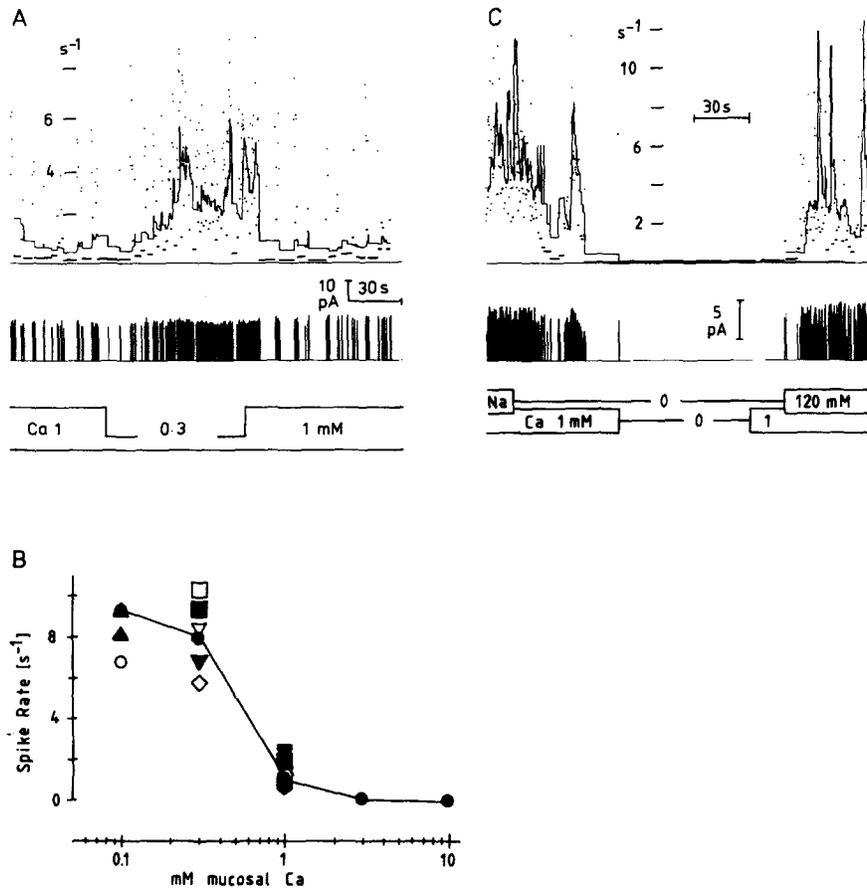
FIGURE 4. (*A*) Effect of decreased mucosal Ca^{2+} concentration on the basal spike rate of one ORC. Lowering mucosal Ca^{2+} (in Ringer solution) from 1 to 0.3 mM increased the spike rate reversibly. (*B*) Dose–response relationship of mucosal Ca^{2+} block in the steady state measured at five concentrations of mucosal Ca^{2+} (in Ringer solution). Different symbols indicate different cells. Mean rates were 8.45 ± 1.21 (4) at 0.1 mM, 8.09 ± 1.64 (6) at 0.3 mM, and 1.44 ± 0.71 (8) at 1 mM of mucosal Ca^{2+}. Mucosal Ca^{2+} concentrations less than 0.1 mM caused immediate depolarization, indicated by spike rates greater than 15 s^{-1} and vanishing spike amplitudes. (*C*) Lowering of mucosal Ca^{2+} concentration to 0 mM did not increase spike rate in the absence of mucosal Na^+. Combined response of two to three cells. The nominal value of 0 mM Ca^{2+} indicates that Ca was omitted from the Ringer solution. The Ca^{2+} value of the water used was near 30 μM.

al., 1988), we tested the effects of the following substances: D-600 (50 μM), *d-cis*-diltiazem (50 μM), amiloride (0.5 mM), $CoCl_2$ (5 mM), $NiSO_4$ (5 mM), $CdSO_4$ (1 mM), and $ZnSO_4$ (5 mM). Each agent was applied mucosally in Ringer solution alone, and together with 0.1 μM forskolin. Only with occasional ORCs were inhibitory effects on spike rate seen. In the majority of ORCs the response to forskolin was not affected by the Ca^{2+} channel blockers listed above. The compound that was effective, *l-cis*-diltiazem (see Fig. 2 *D*), is not known to block Ca^{2+} channels.

Apical Potassium Conductance

A putative apical or ciliary K^+ conductance was assessed by recording current spikes from cilia during changes of the mucosal K^+ concentration and during exposure of the mucosal side to known blockers of K^+ channels. Fast and delayed responses could be distinguished. When the mucosal K^+ concentration was decreased below the reference value of 3 mM, the basal rate responded very little. However, cells stimulated with forskolin decreased their spike rate promptly and reversibly on lowering the mucosal K^+ concentration to zero (Fig. 5 A). The spike rate first decreased to values near zero, then recovered to the basal rate which was observed before stimulation with forskolin. There was no change in the shape of the fast current transients during this experiment. The amplitudes of transients decreased during high spike rates in the presence of 3 mM K^+, but recovered when rates were low at low K^+.

When, in the absence of stimulators like forskolin, the mucosal K^+ concentration was raised from 3 to 20 mM, either at the expense of Na^+ or at constant Na^+, spike rate did not respond within the first 30 s. Only with a delay of 1 min or more did it decrease to some extent. At 20 mM K^+ the amplitudes of transients recorded from cilia became smaller, but the transients remained biphasic and rectification was not apparent (Fig. 7 A).

In the absence of stimulators like forskolin, application of the K^+ channel blocker Ba^{2+} (10 mM) increased the spike rate promptly. With a slower time course, the amplitude of the transients decreased (Fig. 5 B). The time evolution of these changes is shown in Fig. 5 C. Addition of 10 mM Ba^{2+} to the mucosal solution caused, after a flow-dependent delay of 15 s, a rapid increase in spike rate, from 1 to 7 s^{-1}. This was followed by a slow further increase to 12 s^{-1}. Initially the amplitudes of recorded transients were a = 15 pA (first phase) and b = −9 pA (second phase); the rectification ratio −b/a, therefore, was 0.6. Mucosal Ba^{2+} induced a slow decrease of both a and b, but the decrease of b was more rapid, such that rectification became more pronounced.

The increase in spike rate observed on adding 10 mM Ba^{2+} to the mucosal solution was also seen while all mucosal Na^+ was replaced with $NMDG^+$. Clearly, therefore, the presence of mucosal Na^+ is not essential for the generation of action potentials per se.

Mucosal application of 20 mM TEA was ineffective, while 4-AP (1–5 mM) usually caused a prompt increase in spike rate (Fig. 6, A and B), sometimes followed by a slow decrease in spike amplitude. In some ORCs strong rectification ensued (Fig. 7 C), while in other ORCs the rectification was weak or absent. Low mucosal concentrations of 4-AP (1–3 mM) typically induced a maintained increase in spike rate. The spikes tended to occur in bursts (Fig. 6 A). Acceleration of spike rate (and bursting) was also observed in response to 1 mM 4-AP (a) during replacement of all mucosal Na^+ by NMDG, or (b) in the presence of 10 mM mucosal Ca^{2+}.

Mucosal 4-AP at the higher concentration of 5 mM caused a strong increase in spike rate (Fig. 6 B), which faded within 100 s in the continued presence of the blocker, while the amplitudes of individual transients were not greatly decreased. (Similar, but faster "fading" [desensitization] was previously found in the response of ORCs to odorants [Frings and Lindemann, 1990b].) The same ORC responded to 1
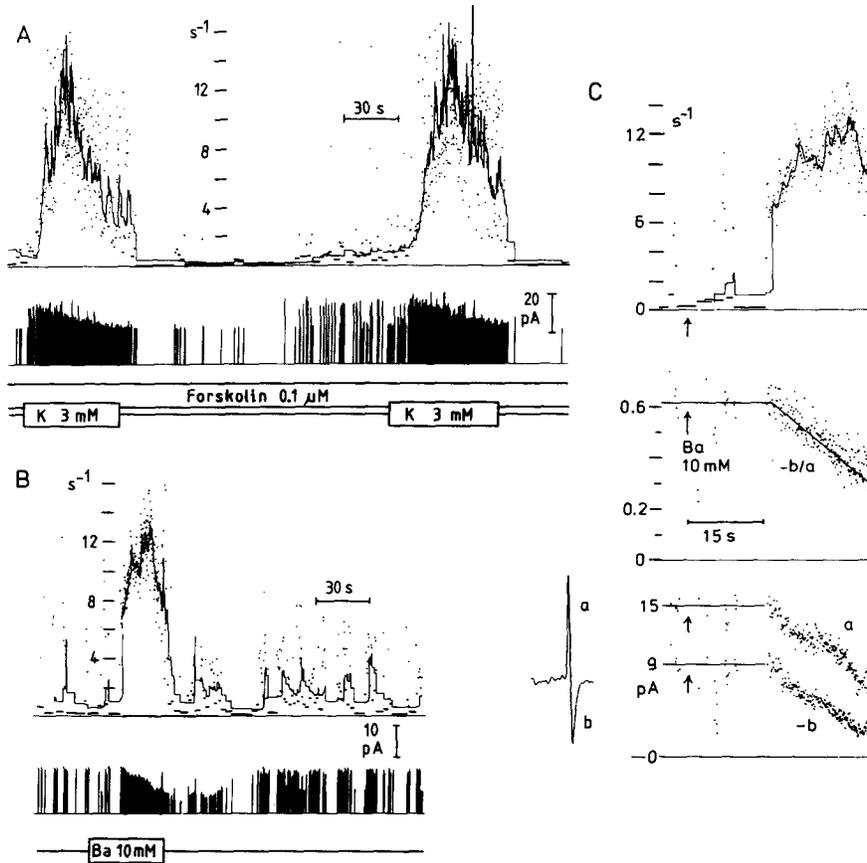
FIGURE 5. (*A*) Effect of decreased mucosal K⁺ concentration on the spike rate during continued strong stimulation with forskolin. Joint recording from three ORCs. Lowering the K⁺ concentration of the mucosal superfundate (Ringer solution) from 3 to 0 mM promptly decreased the spike rates. (*B*) Effect of mucosal Ba on basal spike rate. Joint recording from two ORCs. (*C*) Part of the experiment of *B*, shown at higher time resolution. *Upper curve*, evolution of spike rate in response to 10 mM of mucosal Ba^{2+} (in Ringer solution). Vertical arrows mark time of addition. After a delay of 15 s (due to dead space), a fast and a slow component of the increase in spike rate can be distinguished. Curves labeled *a* and *b* are the amplitudes of the first and second phases of the biphasic current transients (see inset). Curves labeled −*b/a* are the rectification ratios, obtained by dividing the negative of the amplitude of the second phase by the amplitude of the first phase.

and 3 mM 4-AP with maintained high spike rates. In a few cases 2 mM mucosal 4-AP abolished the spike rate abruptly without a transient increase in rate. The wide spectrum of responses suggested that the olfactory epithelia contained ORCs of different apical K⁺ conductance.

The blockers Ba^{2+} and, in some instances, 4-AP caused a change in the shape of the transients: both the early and later phases of the biphasic transients decreased their amplitude, but the later phase more so. In consequence, the transients soon

appeared rectified (Fig. 7, B and C). In contrast to the prompt increase in spike rate, these changes developed slowly. In Fig. 5 B, it took 30 s for the rectification caused by mucosal Ba^{2+} to become complete. Numerical integration of the capacitive current transients (Frings and Lindemann, 1991) showed deceleration of the repolarizing phase of the action potentials (Fig. 7, B and C), explainable by inhibition of voltage-dependent K^+ currents. None of the effects of mucosal Ba^{2+} were influenced by mucosal exposure to the Ca^{2+} channel blockers mentioned above.

Superfusion of the interstitial side of the mucosa with 10 mM Ba^{2+} caused a decrease in amplitude and progressive rectification of the recorded current tran-
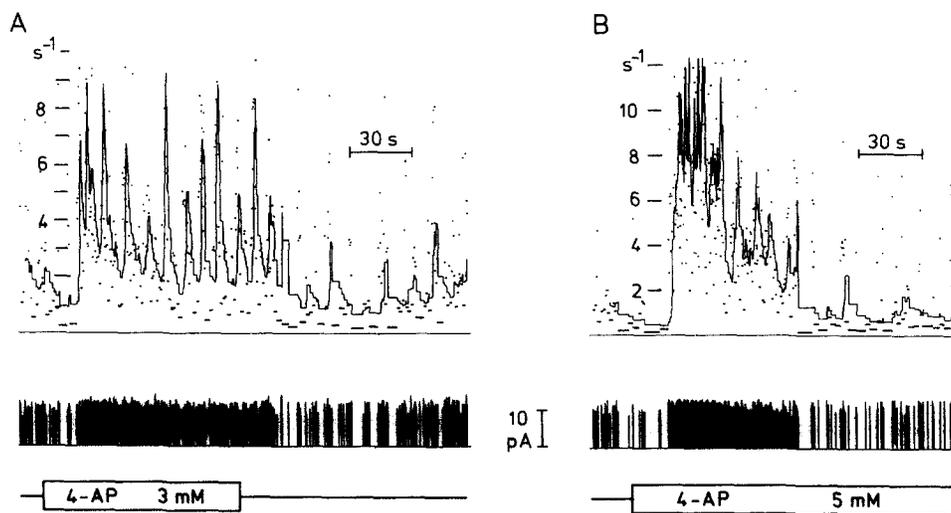
FIGURE 6.   Response of spike rate to mucosal superfusion with the K^+ channel blocker 4-AP dissolved in Na^+-Ringer. (A) In this cell 3 mM 4-AP induced a rapid increase in spike rate, followed by grouping of spikes into bursts. The burst frequency was 7–8/min, as previously observed with isolated ORCs in response to odorants (Frings and Lindemann, 1988). Similar maintained responses were observed with 1 mM 4-AP (nine cells tested). (B) Induced by 5 mM 4-AP, spike rate increased rapidly to ∼ 10 times the basal value, then reclined within 1 min to the basal rate while exposure with 4-AP continued. The amplitudes of recorded transients remained high. With 1 and 3 mM 4-AP, the response of this cell was maintained (not shown). Of seven cells tested, five responded to 5 mM 4-AP as shown. In one cell spike generation was completely blocked, while another ORC increased spike rate but decreased spike amplitude strongly.

sients. These changes developed slowly (with a rate comparable to the rate seen on changing mucosal Ba^{2+}). However, in contrast to the effect of mucosal Ba^{2+}, the spike rate did not increase much, and the change in spike rate was not rapid. The development of these changes was affected by the mucosal K^+ concentration. Lowering the mucosal K^+ concentration from 3 to 0 mM arrested the time evolution of decreasing transient amplitudes, and of rectification, which occurred slowly in the presence of Ba^{2+} applied to the interstitial side (Fig. 8). Apparently the depolarization caused by blockage of K^+ channels on the somal membrane with interstitial Ba^{2+} was rapidly conteracted by the hyperpolarization (shift in the K^+ equilibrium

potential) achieved by a decrease in the mucosal K^+ concentration. These responses indicate K^+ conductances on both somal and apical membranes of ORCs.

Are the Cyclic Nucleotide–gated Channels Also Located on Nonapical Membrane Areas?

Above, we described effects of mucosal Na^+, Ca^{2+}, and Mg^{2+} ions and of mucosal *l-cis*-diltiazem on the forskolin-induced spike rate, which are compatible with an
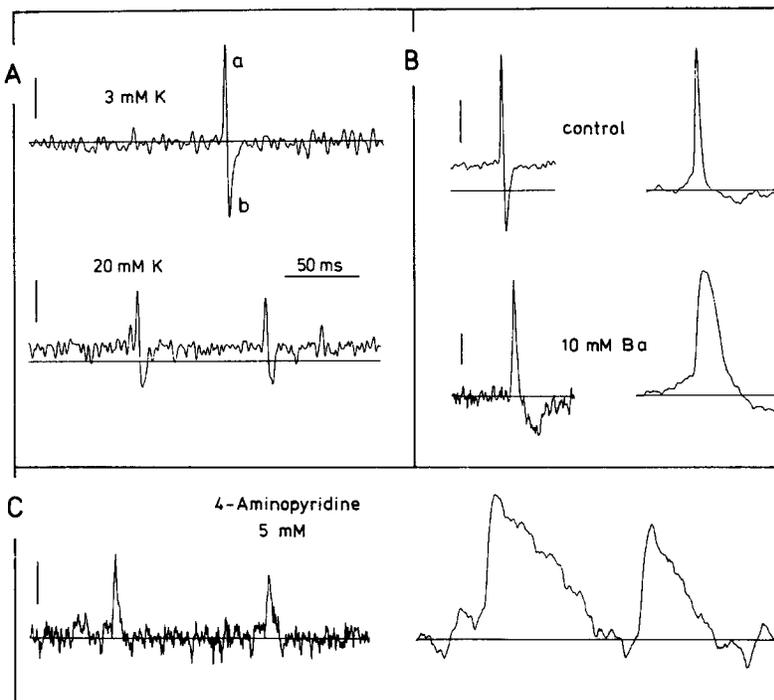
FIGURE 7. Shape of action potential–related current transients recorded from sensory cilia. (*A*) Spike amplitudes in the presence of 3 and 20 mM mucosal K^+. 17 mM Na^+ were replaced by K^+ in Ringer solution. The amplitudes (*a* and *b*) of both half-transients decreased by 45%. Vertical calibration was 10 pA. (*B* and *C*) Effect of K^+ channel blockers present in the mucosal solution. Isotonic aliquots of NaCl were replaced by 10 mM $BaCl_2$ or 5 mM 4-AP. The late phase (plotted downward) was prolonged and decreased in amplitude more than the early phase of the transients. Corresponding action potentials (results of numerical integration of the current transients) are on the right (cf. Frings and Lindemann, 1991). With 10 mM Ba^{2+}, the integrated signal had a prolonged repolarizing phase, as expected from blockage of K^+ outward current. Vertical current calibration was 10 pA. Vertical scaling of integrated signals was arbitrary. Time scale as in *A*.

apical location of the cyclic nucleotide–gated channels. However, the same channels might in addition be present on the basolateral (i.e., the somal and dendritic) membrane of the ORCs. If this were true, an increase in spike rate would occur even in the absence of mucosal Na^+, when the cellular concentration of cyclic nucleotides is increased, since interstitial Na^+ is available for basolateral channels. To assess this

possibility, mucosal Na^+ was first replaced with $NMDG^+$. This lowered the basal spike rate to zero. On adding 10 mM Ba^{2+} to the Na^+-free mucosal solution, the spike rate increased to steady values in the range of 1–4 s^{-1}. Under these conditions, small changes in spike rate should be observable. Yet addition of forskolin (0.1 μM) or of the permeant cyclic nucleotide 8BrcGMP (0.1 mM) to the mucosal solution had no discernible effect on the spike rate. (The same cells responded with rates approaching 20 s^{-1} when mucosal Na^+ was present.) Thus, these experiments produced no evidence for the presence of Na^+-conducting, cyclic nucleotide–gated channels on the basolateral membrane of ORCs in situ. This is in contrast to results obtained with isolated ORCs (Nakamura and Gold, 1987; Kolesnikov et al., 1990).
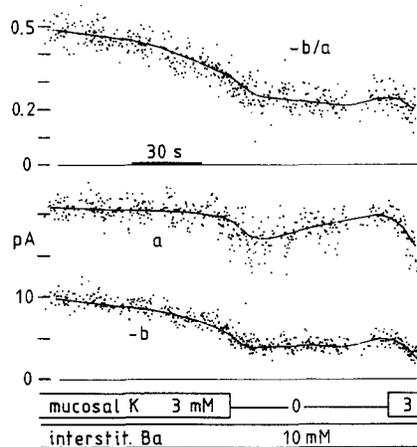
FIGURE 8. Response of one ORC to 10 mM Ba^{2+} applied from the interstitial side. The diffusion delay due to dead space was ∼1.5 min. a, Amplitude of first half-transient; b, amplitude of second half-transient; $-b/a$, rectification ratio. Interstitial Ba^{2+} caused a mild increase in spike rate, while the amplitudes of the second half-transients decreased (falling phase of action potentials longer). This change developed slowly, then more rapidly. The rapid change was accompanied by attenuation of the first half-transients (rising phase of action potentials longer). When the

rapid change was under way, the mucosal K^+ concentration was lowered to 0 mM. This caused arrest and slow recovery of the amplitude changes, presumably by means of cell hyperpolarization. When mucosal K^+ was restored to 3 mM, the initial changes continued rapidly.

DISCUSSION

Method and Interpretation

Using olfactory receptor neurons in situ, we investigated by means of extracellular ciliary recording the influence of mucosal cations on the spike rate of cells responding to forskolin or cAMP. We see the strength of this method in the facts that the receptor cells remain (a) in their natural position in the epithelium, surrounded by their glia-like "sustentacular cells," (b) undamaged by treatment with enzymes or low Ca^{2+}, or by trituration (used to isolate cells for patch clamping), and (c) undamaged by invasive recording procedures (like impalement with microelectrodes) and not subject to cytosolic washout (as in whole-cell patch clamping). Furthermore, (d) the sensory cilia remain in contact with mucus and, presumably, odorant-binding protein, and (e) the integrity of the tight junctions is maintained. Therefore, the distribution of transport molecules between apical and basolateral membrane areas is not disturbed, and the mucosal solution can be changed without directly affecting the

solution surrounding the soma and axon hillock of the receptor cells. Hence, one expects to investigate the true physiological performance of individual receptor cells. Odorant thresholds in the picomolar range were observed with this recording method (Frings and Lindemann, 1990*b*).

The disadvantage of our approach is that the variables causing the recorded changes in spike rate, i.e., membrane currents and changes in membrane voltage, are themselves not observed. Thus, we do not show directly that effects of mucosal Na^+ and Ca^{2+} on spike rate are due to modulation of the depolarizing receptor current, i.e., of an apical Na^+-inward current flowing through nonspecific cation channels gated by cyclic nucleotides. (The current, in turn, is thought to affect the membrane potential of the soma and axon hillock, and thereby the spike rate.) Other possibilities exist. For instance, the second messenger cAMP is known to cause depolarization through closure of K^+ channels in taste receptor cells (Avenet et al., 1988). A reduction in K^+ current also contributes to the late component of the excitatory postsynaptic potential of sympathetic neurons (Brown, 1988). Future work will clarify the contribution of K^+ channel closure to the receptor potential of ORCs.

The changes in spike rate, which we observed, are well explained by modulation of an apical receptor current, i.e., a cyclic nucleotide–dependent Na^+ inward current. The explanation is based on several investigations of isolated ORCs, which previously demonstrated these changes in receptor current in response to cyclic nucleotides (Nakamura and Gold, 1987; Trotier and McLeod, 1987; Suzuki, 1988, 1989; Firestein and Shepherd, 1989; Kolesnikov et al., 1990; Schild et al., 1990). The explanation is also strengthened by our observation that *l-cis*-diltiazem, a known blocker of the cyclic nucleotide–dependent receptor current (Kolesnikov et al., 1990), decreased the spike rate when added to the mucosal solution.

Our investigation deals with only one of the transduction mechanisms of ORCs, i.e., that involving adenylate cyclase and the nonspecific channels gated by cyclic nucleotides. The other known transduction mechanism, involving phospholipase C and inositol trisphosphate (Boekhoff et al., 1990; Breer et al., 1990; Restrepo et al., 1990), may have different ionic requirements. In this and the preceding study (Frings and Lindemann, 1991) all ORCs tested responded to forskolin and cAMP.

Mucosal Na^+ as Charge Carrier of the Cyclic Nucleotide Dependent Receptor Current

The presence of mucosal Na^+ was not an essential requirement for the generation of action potentials. While the basal spike rate dropped to zero when mucosal Na^+ was replaced by $NMDG^+$, spikes reappeared when 10 mM Ba^{2+} was added to the mucosal solution (see below). However, an increase in spike rate induced by forskolin required the presence of mucosal Na^+. When Na^+ was reduced below 30 mM by replacing it with the large monovalent cation $NMDG^+$, the spike rate was reduced to <10% of the control value at 120 mM Na^+, probably because membrane depolarization was significantly decreased. The dependence of spike rate on mucosal Na^+ may be explained by an inwardly directed receptor current, elicited by forskolin or cyclic nucleotides, which was carried by mucosal Na^+. This explanation is supported by the effect of mucosal *l-cis*-diltiazem, reported to block the receptor current induced by cyclic nucleotides (Kolesnikov et al., 1990), which abolished the increase in spike rate induced by forskolin in our experiments. The effects of mucosal Na^+ on the

forskolin-induced spike rate, and their sensitivity to mucosal l-cis-diltiazem, indicate an apical location of the cyclic nucleotide–gated channels.

Our finding is in agreement with the report of Takagi et al. (1969), that for the generation of the normal, negative EOG, mucosal and serosal Na^+ ions were necessary and could not be substituted by any of the mono-, di-, or trivalent cations that they used. Of course, the odorant-induced compound EOG, based on receptor currents of many ORCs (Ottoson, 1956, 1971), may contain non-Na^+ components in addition. As will be discussed below, Ca^{2+} is not the main charge carrier of cyclic nucleotide–dependent receptor currents and EOGs.

The rate of forskolin-induced spiking was linearly dependent on mucosal Na^+ in the range 60–120 mM. No saturation of spike rate was observed. Below 60 mM Na^+, activity was low. The dose–response curve (Fig. 2) shows near 60 mM an increase in slope with increasing mucosal Na^+ concentration. This may be due to a release of channels from voltage-dependent Ca^{2+} blockage, effected by depolarization of the apical or ciliary membrane. Nakamura and Gold (1987) have shown that the block by divalent ions is voltage dependent.

Several selectivity studies demonstrated that the cyclic nucleotide–gated channels of isolated ORCs conduct both Na^+ and K^+ (Nakamura and Gold, 1987; Suzuki, 1988, 1989; Kolesnikov et al., 1990). However, as we show here, $NMDG^+$ does not substitute for Na^+ in supporting a high spike rate, presumably because it does not permeate through the channel.

The secondary decrease in spike rate that followed the acceleration in response to a step increase in mucosal Na^+ may have been due to an accumulation of Na^+ within the small ciliary volume (< 10 fl for one cilium). (Here we assume, for simplicity of argument, that the nucleotide-gated channels are on the ciliary membrane rather than on the apical knobs. However, a ciliary location was not strictly demonstrated by us.) A decreased or inverted driving force of the receptor current also explains that the spike rate dropped transiently to zero when Na^+ was abruptly lowered. Later the spike rate assumed its stationary non zero value, presumably because the normal ciliary Na^+ gradient was re-established. These results suggest that accumulation of Na^+ in the cilia, or in other restricted volumes of the apical cell pole, may also contribute to phenomena like the desensitization observed in response to odorants and cyclic nucleotides.

The mechanism that removes Na^+ from cilia or apical cell pole when the nucleotide-gated channels close is not known. Anholt et al. (1986) identified the beta-subunit of the Na/K-ATPase in cilia preparations from $Rana$ $catesbeiana$ by immunostaining. However, in our experiments mucosal dihydro-ouabain (0.1 mM) had no effect on basal or cyclic nucleotide–stimulated spike rate (Frings, S. and B. Lindemann, unpublished results), indicating that this enzyme was not functional in the apical membrane of our preparation. Accumulated ciliary Na^+ may have diffused into the dendrite or soma of the ORC, where it was transported into the interstitial solution by a somal Na/K-ATPase. Thus an energy-dependent, vectorial transport of Na^+ from mucus to interstitium would be induced by odorants or cyclic nucleotides. Indeed, Persaud et al. (1987, 1988) demonstrated this odorant- and cAMP-induced active transport of Na^+ in the olfactory mucosa of $Rana$ $catesbeiana$. They also found mucosal ouabain to be without effect.

Sodium-dependent spiking, while sensitive to mucosal *l-cis*-diltiazem, was not sensitive to 1 μM mucosal TTX, a concentration that was sufficient to block the generation of action potentials when applied to the interstitial side of the tissue. This result is in good agreement with the observations that neither mucosal TTX (Tucker and Shibuya, 1965) nor mucosal cocaine (Ottoson, 1956) inhibit activity of ORCs or EOG generation at concentrations that block the generation of action potentials in the axons of ORCs. Thus we did not find a significant contribution of voltage-dependent Na^+ channels to apical or ciliary membrane events.

Mucosal Calcium Inhibits the cAMP-induced Receptor Current

Tucker (1963) reported the following observation from recordings from the tortoise olfactory nerve: "A solution free of Ca^{++} ions flowed through the olfactory cavity causes intense neural activity in the olfactory nerve, and if the flow is continued, the receptors are killed in a few minutes." We found a similar response in frog ORCs in situ. When the Ca^{2+} concentration was decreased below 1 mM, spike rate increased in a dose-dependent manner even in the absence of added exogenous stimuli. The response was reversible and occurred in all ORCs tested. Since the acceleration of spike rate in low Ca^{2+} solutions was dependent on mucosal Na^+ (Fig. 4 *C*), mucosal Ca^{2+} appeared to control current flow through the apical cation channels gated by cyclic nucleotides. This explains Tucker's finding: ORCs stimulated by Ca^{2+} removal will be permanently and strongly depolarized. In consequence, the voltage-dependent Na^+ channels of soma and axon hillock will be inactivated.

When mucosal Ca^{2+} was increased from 1 to 10 mM, spike generation stopped. In this case, a hyperpolarization following Ca^{2+} inflow, mediated by Ca^{2+} activated K^+ channels or a Ca^{2+}-dependent decrease of the cytosolic cAMP concentration, does not appear to be essential for the cessation of spike activity, because an increase of the mucosal Mg^{2+} concentration had qualitatively the same effect as an increase of the Ca^{2+} concentration. Therefore, an increased blockage of cation channels by divalent cations must have abolished the depolarization of the apical membrane, thus suppressing spike generation.

Our results match the findings of Nakamura and Gold (1987), who demonstrated that mucosal Ca^{2+} blocks the nucleotide-activated conductance, and those of Kurahashi (1989) and Kurahashi and Shibuya (1990), who found a 4.5-fold increase of odorant-induced inward current in newt ORCs when extracellular Ca^{2+} was removed. Our results are, however, at variance with several EOG studies which suggested that Ca^{2+} is the charge carrier of the receptor current (Suzuki, 1978; Winegar et al., 1988; Leveteau et al., 1989). In these studies it was usually observed that EOG responses to odorants were greatly diminished when mucosal Ca^{2+} was removed. This lack of odorant response may have been caused by excessive depolarization of ORCs in the absence of Ca blockage of the cyclic nucleotide–gated channels.

The receptor current induced in isolated ORCs by odorants has a transient time course when measured at a constant membrane potential of −40 to −80 mV in the continued presence of the stimulus (Trotier, 1986; Firestein and Werblin, 1989; Kurahashi and Shibuya, 1989, 1990). A transient evolution of cAMP concentration (Breer et al., 1990) may be responsible. However, the inward current recorded in response to a maintained high concentration of cAMP is also transient (Kurahashi

and Shibuya, 1988; Suzuki, 1989; Trotier et al., 1989). When extracellular Ca^{2+} and Mg^{2+} were removed, the transient response to cAMP changed into a tonic response (Kurahashi and Shibuya, 1988; Suzuki, 1989). The transient response may be explained by a delayed blockage with Ca^{2+} ions (and/or Mg^{2+} ions) from the outside: in the absence of cyclic nucleotides, the channels are closed and their Ca^{2+} occupancy is small. When the channels are suddenly opened at a maintained negative potential, inward current is initially large, but decreases when Ca^{2+} occupancy equilibrates to its steady-state value. Due to the voltage dependence, the degree of steady-state blockage would be smaller when the membrane potential is allowed to depolarize, as in our experiments.[2]

Joshi et al. (1987) measured the Ca^{2+} content in frog olfactory mucus spectrophotometrically. They found a mean concentration of 5.4 mM, which increased slowly during odorant or secretagogue stimulation to 8.1 mM. Similar values were obtained by Lorin et al. (1972) from human nasal secretion. In the mucus, Ca^{2+} activity may be significantly lower than Ca^{2+} concentration, due to binding of Ca^{2+} to mucopolysaccharides and proteins (Boat and Cheng, 1980). D. Trotier (personal communication) measured the Ca^{2+} activity in the mucus with Ca^{2+}-selective electrodes. The resting value was near 3 mM and stimulation with odorants decreased the mucosal Ca^{2+} activity. This may be due to Ca^{2+} uptake through ciliary channels. During long-term stimulation, the Ca^{2+} activity in the mucus compartment increased. It would be important to record, in response to stimulation with odorants, simultaneous changes in the activities of Ca^{2+}, Na^+, and K^+ in the olfactory mucus, which may all change and thereby influence the rate of firing.

In some 200 cells studied, the basal spike rate in the presence of 1 mM Ca^{2+} was remarkably constant, with values near 1.4 s^{-1}. Basal spike rates reported from extracellular single unit recordings, where recording electrodes were placed into the olfactory epithelium without superfusing the mucosal side, range from 0.05 to 1 s^{-1} (reviewed by Getchell, 1986). In a study with intracellular electrodes, Trotier and MacLeod (1983) found that the basal spike rate was dependent on the recorded resting potential with values between 0.3 and 2 s^{-1}. In all of these studies, the mucosal Ca^{2+} concentration was not known. Getchell (1974) investigated basal and odor-stimulated spike rates in frog olfactory epithelium while superfusing the mucosal surface with Ringer solution containing 2 mM Ca^{2+} and no Mg^{2+}. He measured basal rates between 0.07 and 1.8 s^{-1}, while 68% of the units discharged at < 0.42 s^{-1}. This value, obtained at a known mucosal Ca^{2+} concentration, is in good accordance with our observations (cf. Fig. 4 B) and further confirms the notion that mucosal Ca^{2+}, in concert with mucosal Na^+ and K^+, determines basal spike rate (see below).

---

[2] In addition, divalent cations might enter the cilia during stimulation and inhibit the cyclic nucleotide–gated channels from the inside (Kurahashi and Shibuya, 1990). In our experiments Mg^{2+} had qualitatively the same inhibitory effect as Ca^{2+}. It is obvious that the spike rate is exquisitely sensitive to mucosal divalent cations.

Apical Potassium Conductance

Reports on potassium channels from ciliary membrane preparations are scarce (reviewed in Labarca and Bacigalupo, 1988): Vodyanoy and Murphy (1983) found a 60-pS potassium channel, sensitive to 4-AP, in bilayer membranes that had been treated with rat olfactory mucosa homogenate. It was assumed that these channels were associated with chemoreception, for their open probability was increased by applying odorants. Labarca et al. (1988) fused ciliary vesicles from *Rana catesbeiana* with bilayer membranes and observed a 190-pS, Ca-activated potassium channel.

By patch-clamping, Maue and Dionne (1987) found three types of potassium channels on the ciliary knob of isolated mouse ORCs: (*a*) a 20-pS channel with long (>50 ms) open times, which was activated at negative membrane potentials; (*b*) an 80-pS, Ca-activated potassium channel that was not voltage dependent, but was blocked by Cs^+; and (*c*) a 130-pS, Ca-activated potassium channel that opened on depolarization, which was sensitive to Cs^+ but not to TEA and 4-aminopyridine. Since these channels were studied in isolated ORCs, i.e., after opening of the tight junctions, their location on cells in situ is not certain. Recently, Schild et al. (1990) reported hyperpolarizing responses which may be due to the opening of apical K^+ channels in *Xenopus* ORCs.

We found novel effects of mucosal K^+ and of K^+ channel blockers, which indicate a significant K^+ conductance in the apical membranes of ORCs in situ. Mucosal Ba^{2+} (Fig. 5 *B*) caused (*a*) a rapid increase in spike rate followed by (*b*) a slow further increase in rate, (*c*) a slow decrease in the amplitude of transients, and (*d*) a slow development of rectification of the transients, indicating a prolongation of action potentials.

The rapid increase in spike rate effected by mucosal Ba^{2+} is likely to be mediated by cellular depolarization, caused by Ba^{2+} blocking K^+ channels of finite open probability at the resting membrane potential.[3] The alternative, that a significant Ba^{2+} inward current flows through the nonselective apical channels, thus causing additional depolarization, is excluded since (*a*) the Ba^{2+} effect also develops while the nonselective channels are blocked by 10 mM Ca^{2+}, (*b*) the K^+ channel blocker 4-AP, like Ba^{2+}, increases the spike rate when added to the mucosal solution, and (*c*) the permeability of the nonselective channel for Ba^{2+} is lower than for Na^+ (Kolesnikov et al., 1990).

The subsequent slow effects of mucosal Ba^{2+} may be due to a change of K^+ currents at the somal membrane of ORCs. For instance, blockage of apical K^+ channels of sustentacular cells (Trotier and MacLeod, 1986) would diminish the ability of these glia-like cells to buffer the K^+ concentration of the interstitial space of the mucosa.

---

[3] In preliminary experiments, by sealing patch pipettes to apical knobs of ORCs in situ as described before (Frings and Lindemann, 1990*a*), we obtained single-channel recordings of several kinds of K^+ channels. Ca^{2+}- and voltage-independent channels of 6.5 and 45 pS were observed in the on-cell mode. A 35-pS channel was active only at negative potentials (inward rectifier). A 70-pS channel, not Ca^{2+}- or voltage-gated, opened only after excision of the patch. In patches from apical membranes of sustentacular cells (recognized by the absence of action potentials) at least five types of K^+ channels (14, 30, 65, 80, and 140 pS) were found (Frings, S., and B. Lindemann, unpublished observations).

Thereby, the ORCs may slowly develop an additional depolarization. Also, mucosal Ba^{2+} might diffuse into the interstitial space, thereby causing a slowly developing additional depolarization at the somal membrane of the ORCs.

It is interesting that changes in the mucosal K^+ concentration had, in several cases, little effect on the basal spike rate, but large effects on the forskolin-stimulated spike rate. In these cases, movement of K^+ through the cyclic nucleotide–gated channel may have enhanced the depolarization. Also, the apical membrane may have required some depolarization before its K^+-specific conductance became significant. Before an effect on spike rate, the elevation of mucosal K^+ decreased the amplitude of the recorded current transients, without, however, inducing rectification. The decrease in amplitude may be related to the shunting effect (decrease in space constant) of a K^+ conductance located on the ciliary knob and/or in the ciliary segment not shielded by the recording pipette. A general depolarization of the ORC may also have contributed, but the increase in spike rate expected from a general depolarization followed, rather than preceded, the attenuation of recorded transients.

Joint Effect of Mucosal Cations

Exposure of the ciliated surface to 10 mM Ba^{2+} abolished the block of spike rate caused by 10 mM of mucosal Ca^{2+}. Similarly, 10 mM Ba^{2+} or 1 mM 4-AP abolished the block of spike rate caused by replacement of mucosal Na^+ with $NMDG^+$. This may be explained by depolarizing effects of Ba^{2+} and 4-AP (due to blockage of apical K^+ channels) counteracting the hyperpolarization achieved by reduction of the Ca^{2+}-blockable Na^+ inward current. An increase of the mucosal K^+ concentration was also able to counteract the block by 10 mM Ca^{2+}.

The olfactory mucus in vivo has K^+ concentrations of 10–20 mM in the frog (Joshi et al., 1987). Elevation of mucosal K^+, like blockage of apical K^+ conductance, may be expected to depolarize the ORCs, thereby counteracting the Ca^{2+} block of the cyclic nucleotide–gated channels. Thus, through secretory adjustment of the mucosal K^+ and Ca^{2+} concentration, the sensitivity to odorant stimulation can be changed. In most of our experiments both the K^+ and Ca^{2+} concentrations were those of Ringer solution (i.e., lower than in mucus). Nevertheless, spike rates were as expected from microelectrode and single unit recordings with a preserved mucus composition (e.g., Trotier and MacLeod, 1983; Getchell, 1986). In our experiments, the K^+ equilibrium potential at the apical membrane was certainly more negative than in vivo, diminishing the depolarizing effects of inward currents and thereby decreasing spike rate. On the other hand, Ca^{2+} blockage of apical cation channels will have been less pronounced in mucosal Ringer solution (1 mM Ca^{2+}) as opposed to the mucus environment (3–6 mM Ca^{2+}). Therefore, inward current through the cation channels must have been increased, compensating in part for the effect of lowered mucosal K^+.

Apical K^+ channels, in conjunction with the elevated K^+ concentration of the mucus, apparently serve to bring the membrane potential close to threshold. By this predepolarization, the sensitivity of the neuron to odorants may be tuned. Interestingly, prolonged stimulation with odorants causes changes of mucosal ion concentrations: K^+ decreases and Ca^{2+} increases (Joshi et al., 1987). Both changes will tend to diminish odorant sensitivity in the continued presence of large stimuli.

REFERENCES

Anholt, R. R. H., U. Aebi, and S. H. Snyder. 1986. A partially purified preparation of isolated chemosensory cilia from the olfactory epithelium of the bullfrog, *Rana catesbeiana. Journal of Neuroscience.* 6:1962–1969.

Avenet, P., F. Hofmann, and B. Lindemann. 1988. Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature.* 331:351–354.

Boat, T. F., and P. W. Cheng. 1980. Biochemistry of airway mucus secretions. *Federation Proceedings.* 39:3067–3074.

Boekhoff, I., E. Tareilus, J. Strotmann, and H. Breer. 1990. Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO Journal.* 9:2453–2458.

Breer, H., I. Boekhoff, and E. Tareilus. 1990. Rapid kinetics of second messenger formation in olfactory transduction. *Nature.* 345:65–68.

Brown, D. A. 1988. M-currents: an update. *Trends in Neurosciences.* 11:294–299.

Firestein, S., and G. M. Shepherd. 1989. Olfactory transduction is mediated by the direct action of cAMP. *Society for Neuroscience Abstracts.* 15:749. (Abstr.)

Firestein, S., and F. S. Werblin. 1989. Odor-induced membrane currents in vertebrate-olfactory receptor neurons. *Science.* 244:79–82.

Frings, S., and B. Lindemann. 1988. Odorant response of isolated olfactory receptor cells is blocked by amiloride. *Journal of Membrane Biology.* 105:233–243.

Frings, S., and B. Lindemann. 1990a. Response of olfactory receptor cells, isolated and in situ, to low concentrations of odorants. *In* Chemosensory Information Processing. D. Schild, editor. Springer-Verlag, Berlin. 1–8.

Frings, S., and B. Lindemann. 1990b. Single unit recording from olfactory cilia. *Biophysical Journal.* 57:1091–1094.

Frings, S., and B. Lindemann. 1991. Current recording from sensory cilia of olfactory receptor cells in situ. I. The neuronal response to cyclic nucleotides. *Journal of General Physiology.* 97:1–16.

Getchell, T. V. 1974. Unitary responses in frog olfactory epithelium to sterically related molecules at low concentrations. *Journal of General Physiology.* 64:241–261.

Getchell, T. V. 1986. Functional properties of vertebrate olfactory receptor neurons. *Physiological Reviews.* 66:772–818.

Joshi, H., M. L. Getchell, B. Zielinski, and T. V. Getchell. 1987. Spectrophotometric determination of cation concentration in olfactory mucus. *Neuroscience Letters.* 82:321–326.

Kolesnikov, S. S., A. B. Zhainazarov, and A. V. Kosolapov. 1990. Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane. *FEBS Letters.* 266:96–98.

Kurahashi, T. 1989. Activation by odorants of cation-selective conductance in the olfactory receptor cell isolated from the newt. *Journal of Physiology.* 419:177–192.

Kurahashi, T., and T. Shibuya. 1988. Ca^{2+}-dependent adaptive properties in the solitary olfactory receptor cells of the newt. *Proceedings of the 22nd Japanese Symposium of Taste and Smell.* 22:245–248.

Kurahashi, T., and T. Shibuya. 1989. Membrane responses and permeability changes in the solitary olfactory receptor cells of newt. *Zoological Sciences.* 6:19–30.

Kurahashi, T., and T. Shibuya. 1990. Ca^{2+}-dependent adaptive properties in the solitary olfactory receptor cell of the newt. *Brain Research.* 515:261–268.

Labarca, P., and J. Bacigalupo. 1988. Ion channels from chemosensory olfactory neurons. *Journal of Bioenergetics and Biomembranes.* 20:551–569.

Labarca, P., S. A. Simon, and R. H. H. Anholt. 1988. Activation by odorants of a multistate cation channel from olfactory cilia. *Proceedings of the National Academy of Sciences, USA.* 85:944–947.

Laurenza, A., E. McHugh Sutkowski, and K. B. Seamon. 1989. Forskolin: a specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? *Trends in Physiological Sciences.* 10:442–447.

Leveteau, J., I. Andriason, D. Trotier, and P. MacLeod. 1989. Role of divalent cations in EOG generation. *Chemical Senses.* 14:611–620.

Lorin, M. I., P. F. Gaerlan, and I. D. Mandel. 1972. Quantitative composition of nasal secretions in normal subjects. *Journal of Laboratory and Clinical Medicine.* 80:275–281.

Maue, R. A., and V. E. Dionne. 1987. Patch-clamp studies of isolated mouse olfactory receptor neurons. *Journal of General Physiology.* 90:95–125.

Nakamura, T., and G. H. Gold. 1987. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature.* 325:442–444.

Ottoson, D. 1956. Analysis of the electrical activity of the olfactory epithelium. *Acta Physiologica Scandinavica.* 35(Suppl. 122):1–83.

Ottoson, D. 1971. The electro-olfactogram. *In* Handbook of Sensory Physiology IV. Olfaction. L. M. Beidler, editor. Springer-Verlag, Berlin. 95–131.

Persaud, K. C., J. A. DeSimone, M. L. Getchell, G. L. Heck, and T. V. Getchell. 1987. Ion transport across the frog olfactory mucosa: the basal and odorant-stimulated state. *Biochimica et Biophysica Acta.* 902:65–79.

Persaud, K. C., G. L. Heck, S. K. DeSimone, T. V. Getchell, and J. A. DeSimone. 1988. Ion transport across the frog olfactory mucosa: the action of cyclic nucleotides on the basal and odorant-stimulated states. *Biochimica et Biophysica Acta.* 944:49–62.

Restrepo, D., T. Miyamoto, B. P. Bryant, and J. H. Teeter. 1990. Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish (*Ictalurus punctatus*). *Science.* 249:1166–1168.

Schild, D., J. A. DeSimone, and S. Hellwig. 1990. Excitation and adaptation of frog olfactory receptor neurones upon stimulation with second messengers and natural odorants. *In* Chemosensory Information Processing. D. Schild, editor. Springer-Verlag, Berlin. 9–20.

Stern, J. H., U. B. Kaupp, and P. R. MacLeish. 1986. Control of the light-regulated current in rod photoreceptors by cyclic GMP, calcium, and 1-cis-diltiazem. *Proceedings of the National Academy of Sciences, USA.* 83:1163–1167.

Suzuki, N. 1978. Effects of different ionic environments on the responses of single olfactory receptors in the lamprey. *Comparative Biochemistry and Physiology.* 61A:461–467.

Suzuki, N. 1988. Cation selectivity of cyclic nucleotide-gated conductance in isolated olfactory receptor cells. *Zoological Sciences.* 5:1194.

Suzuki, N. 1989. Voltage- and cyclic nucleotide-gated currents in isolated olfactory receptor cells. *In* Receptor Events and Transduction in Taste and Olfaction. J. G. Brand, R. H. Cagan, M. R. Kare, and J. H. Teeter, editors. Marcel Dekker, Inc., New York. 469–493.

Takagi, S. F., H. Kitamura, K. Imai, and H. Takeuchi. 1969. Further studies on the role of sodium and potassium in the generation of the electro-olfactogram. *Journal of General Physiology.* 53:115–130.

Trotier, D. 1986. A patch-clamp analysis of membrane currents in salamander olfactory receptor cells. *Pflügers Archiv.* 407:589–595.

Trotier, D., and P. MacLeod. 1983. Intracellular recordings from salamander olfactory receptor cells. *Brain Research.* 268:225–237.

Trotier, D., and P. MacLeod. 1986. Intracellular recordings from salamander olfactory supporting cells. *Brain Research.* 374:205–211.

Trotier, D., and P. MacLeod. 1987. The amplification process in olfactory receptor cells. *Annals of the New York Academy of Sciences.* 510:677–679.

Trotier, D., J.-F. Rosin, and P. McLeod. 1989. Channel activities in *in vivo* and isolated olfactory receptor cells. *In* Receptor Events and Transduction in Taste and Olfaction. J. G. Brand, R. H. Cagan, M. R. Kare, and J. H. Teeter, editors. Marcel Dekker, Inc., New York. 427–448.

Tucker, D. 1963. Physical variables in the olfactory stimulation process. *Journal of General Physiology.* 46:453–489.

Tucker, D., and T. Shibuya. 1965. A physiologic and pharmacologic study of olfactory receptor cells. *Cold Spring Harbor Symposium on Quantitative Biology.* 30:207–215.

Vodyanoy, V., and R. B. Murphy. 1983. Single-channel fluctuations in bimolecular lipid membranes induced by rat olfactory epithelial homogenates. *Science.* 220:717–719.

Winegar, B. D., E. R. Rosick, and R. Schafer. 1988. Calcium and olfactory transduction. *Comparative Biochemistry and Physiology.* 91A:309–315.

Yoshii, K., and K. Kurihara. 1983. Role of cations in olfactory reception. *Brain Research.* 274:239–248.