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A Cyclic Nucleotide-dependent Chloride Conductance in Olfactory Receptor Neurons

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Abstract. Whole-cell membrane currents were recorded from olfactory receptor neurons from the neotenic salamander *Necturus maculosus*. Cyclic nucleotides, released intracellularly by flash photolysis of NPE-caged cAMP or NPE-caged cGMP, activated a transient chloride current. The chloride current could be elicited at constant voltage in the absence of extracellular Ca^{2+} as well as in the presence of 3 mM intracellular Ca^{2+}, suggesting that the current did not require either voltage or Ca^{2+} transients for activation. The current could be elicited in the presence of the protein kinase inhibitors H-7 and H-89, and in the absence of intracellular ATP, indicating that activation was independent of protein kinase A activity. These results suggest that *Necturus* olfactory receptor neurons contain a novel chloride ion channel that may be directly gated by cyclic nucleotides.

Key words: Chloride conductance — Cyclic nucleotide — Channel regulation — Cyclic AMP

Introduction

Odors are transduced by olfactory receptor neurons (ORNs) using several different intracellular cascades that alter channel activity and modulate neuronal excitability (Trotier, 1994; Dionne & Dubin, 1994). The best studied transduction cascade involves the direct activation of cyclic nucleotide-gated (CNG) cation channels by adenosine 3′,5′-cyclic monophosphate (cAMP) (Nakamura & Gold, 1987). In rats and postmetamorphic frogs, newts and salamanders, the CNG cation channel serves as an intermediary to allow Ca^{2+} influx and secondary activation of a Ca^{2+}-gated Cl^- conductance (Kleene, 1993; Kurahashi & Yau, 1993; Lowe & Gold, 1993); it is the Cl^- conductance that generates most of the receptor potential.

By contrast, in ORNs from the neotenic salamander *Necturus maculosus*, odors can modulate Cl^-, K^+, as well as nonselective cation conductances (Dubin & Dionne, 1993), but it is uncertain how these conductances are regulated. About half of the odor-elicited responses in *Necturus* ORNs involve changes in the Cl^- conductance, but while in some cells odors can increase the Cl^- conductance, in others they decrease it (Dubin & Dionne, 1993, 1994). We have examined the cyclic nucleotide dependence of the Cl^- conductance in *Necturus* ORNs and find evidence for a previously unreported type of Cl^- channel that may be directly gated by cyclic nucleotides. In *Necturus* ORNs this channel could participate in odor transduction, but if homologues of the channel also occur in other epithelial cells, its functions may be wide-reaching.

Materials and Methods

Whole-cell voltage-clamp data were digitally recorded from ORNs of the salamander *Necturus maculosus* using standard patch clamp methods (Hamill et al., 1981). Recordings were obtained both from dissociated ORNs and from ORNs in ~250-μm thick slices of olfactory epithelium using Axopatch 200A and 1D amplifiers. Dissociated cells were prepared without proteolytic enzymes (Dionne, 1992). Epithelial slices were prepared after the methods of Bigiani and Roper (1995). Voltage-dependent currents were elicited by step depolarization of the membrane potential; average leak currents, measured using 25 mV hyperpolarizing steps, were scaled and subtracted from the data. Cells in the slice preparation were filled with Lucifer Yellow CH dye (Sigma Chemical) during whole-cell recording to allow visual identification of cell type with fluorescent illumination.

Cyclic nucleotide-activated currents were elicited from dissociated ORNs by photoylsis of NPE-caged cAMP (adenosine 3′,5′-cyclic monophosphate, P-1-(2-nitrophenyl)ethyl ester) and NPE-caged cGMP (guanosine 3′,5′-cyclic monophosphate, P-1-(2-nitrophenyl)ethyl es-

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Table. Composition of extracellular and intracellular solutions

	APS	APS^{HO}	AIS	ZIS
Kgluconate			90	
KCl	2	2.5	25	
$CaCl_2$	8	3	5.0	0.23
$MgSO_4$		1	3	
$MgCl_2$				2
NaHEPES		10	10	
HEPES (free acid)	3			10
K_4BAPTA			10	
NMDG				100
Methanesulfonic acid				100
NaCl	112	130		10
EGTA				1.0
Glucose	5	5		
Na-pyruvate		5		
MgATP			3	2.5
GTP			0.1	0.5

Amounts are given in millimolar. Tris base (tris[hydroxymethyl]aminomethane) was used to titrate the ZIS, NaOH the APS. Abbreviations: NMDG N-methyl-D-glucamine ($C_7H_{17}NO_5$). HEPES free acid N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]. K_4BAPTA 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrapotassium salt. EGTA ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid.

ter) (Calbiochem-Novabiochem International). The caged compounds were first dissolved in dimethyl sulfoxide (DMSO), then suspended at concentrations of 400–800 μM in the saline filling the whole-cell recording pipettes. The photolytic reaction which hydrolyzed the caged compounds was produced with a 1-msec flash of ultraviolet light (Nerbonne et al., 1984) using a Hi-Tech Scientific Flash Lamp (Salisbury, UK) equipped with a fiber optic light guide. The peak concentration of free cyclic nucleotide following a single flash was estimated to be no more than 20 μM based upon a photoconversion efficiency of ⩽2% per flash, a value derived from previous reports (Nerbonne et al., 1984) and the physical constraints on the light source in our system. Most flash-activated membrane currents were recorded from dissociated ORNs held at constant membrane potentials. Control experiments using NPE-caged adenosine triphosphate (ATP) showed that neither the NPE caging moiety, nor the proton that is released during photolysis, nor the DMSO could elicit a conductance change (10 of 10 cells).

To isolate the Cl^- component from the total membrane current, outward currents carried through K^+ and cation channels were eliminated using an ''impermeant'' intracellular saline (here termed ZIS; *see below*) in which K^+ was replaced by the ion $NMDG^+$ from N-methyl-D-glucamine ($C_7H_{17}NO_5$) (Table). This ion is larger than the largest cations that permeate K^+ channels (Hille, 1992) or retinal CNG cation channels (Furman & Tanaka, 1990; Picco & Menini, 1993), and it does not permeate olfactory CNG cation channels (Frings, Lynch & Lindemann, 1992). With outward cation fluxes eliminated, the remaining outward current was carried by Cl^- influx.

Normal amphibian physiological saline (APS) (*see* Table for saline recipes) was formulated at 240 mOsm; in some experiments a 16% higher osmotic strength was used to counter cell swelling (APS^{HO}, data in Figs. 2 and 4*a* and *c*). Low Cl^- salines were prepared by equimolar substitution of NaCl with methanesulfonic acid titrated with NaOH; the Cl^- concentrations in these solutions are given in the text. Normal amphibian intracellular saline (AIS) was formulated with a Cl^- concentration that separated the reversal potentials of Cl^- and nonselective cation currents measured in APS; AIS contained 0.2 μM free Ca^{2+}. Experiments were also performed with an AIS formulation containing 1 mM BAPTA (0.2 μM free Ca^{2+}) as opposed to 10 mM, but no difference in the flash-induced response was seen. When recording from ORNs in the slice preparation, the AIS included 2 mg/ml Lucifer yellow CH. ZIS was formulated with EGTA instead of BAPTA to eliminate the K^+ counterions. To address concerns that EGTA is a slower Ca^{2+} buffer than BAPTA, we reduced free Ca^{2+} in ZIS to 40 nM, and in some experiments we used 10 mM EGTA and further reduced free Ca^{2+} to <3 nM. These changes in Ca^{2+} concentration had no effect on the cyclic nucleotide responses. Free Ca^{2+} concentrations were calculated using CHELATOR (Schoenmakers et al., 1992).

Results

The membrane currents in *Necturus* ORNs, as in other vertebrates (Kleene, 1993; Lowe & Gold, 1993; Kurahashi & Yau, 1993; Firestein & Shepherd, 1995; Corotto et al., 1996), are mediated by several conductances including those for cations and Cl^- (Dubin & Dionne, 1993, 1994). The Cl^- conductance has at least two components, one that is directly voltage-gated and a second that is Ca^{2+}-gated but which can masquerade as voltage-dependent when activated by Ca^{2+} influx through voltage-gated channels. These two Cl^- currents were readily seen in whole-cell recordings from ORNs in epithelial slices bathed in APS with ZIS intracellular saline (Fig. 1*a*). When the Cl^- concentration of the bath was lowered to 25 mM, the outward current was almost eliminated, demonstrating that Cl^- was the major current carrier. When Ca^{2+} influx was blocked with 200 μM Cd^{2+}, a concentration that fully inhibits Ca^{2+} currents in these cells (*unpublished observations*), the Cl^- current was only partially eliminated, suggesting that the Cl^- flux was carried through both voltage-gated and Ca^{2+}-gated Cl^- channels (Fig. 1*b*). The relative proportion of these two Cl^- components varied from cell to cell, but both components were present in all the cells tested ($n = 9$).

In whole-cell recordings with APS^{HO} in the bath and AIS plus NPE-caged cAMP in the pipette, a 1-msec flash of UV light activated a transient current (I_{FLASH}) having a well-resolved risetime, a duration of a few seconds, and a peak value up to several hundred pA (Fig. 2*a*, APS trace). The typical time-to-peak of I_{FLASH} was ~½ sec, while the typical half-decay time varied in the range of 1–10 sec. In many cells the half-decay time slowly increased with repeated flashes. I_{FLASH} was detected in 61% of cells tested with APS^{HO}/AIS solutions (28 of 46 cells).

With physiological salines bathing the cell membrane, I_{FLASH} appeared to have two ionic components, one Cl^- and the other cation. This was suggested by the reversal potential of the flash-activated response. The reversal potentials measured in different cells using the APS^{HO}/AIS solutions varied between the Cl^- equilibrium potential ($E_{Cl} = -35$ mV) and the reversal poten-

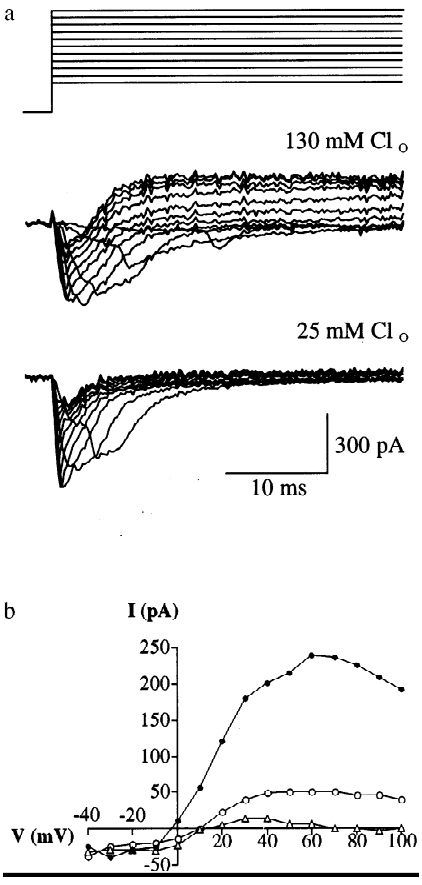

Fig. 1. Whole-cell membrane currents recorded in an olfactory slice preparation. (a) Two sets of whole-cell currents from the same ORN are shown beneath the voltage-step profile used to elicit them. When the slice was bathed in APS, transient inward (negative deflecting) currents carried primarily by Na^+ were observed followed by sustained outward (positive) currents carried by Cl^-. When bathed in low-Cl^- (25 mM) APS, the outward currents were nearly eliminated. V_{HOLD} = −87 mV; pipette contained ZIS. Test voltages ranged from −40 to +100 mV in 10 mV increments (only −40 to +60 mV shown). Because a long axonal process remains on neurons in the slice, part of the active membrane is not well voltage-clamped; this results in imperfect voltage control that affects Na^+ currents as seen in these data. (b) Current-voltage curves. Mean Cl^- currents were measured 30 msec after stepping the voltage, by which time the Na^+ currents had inactivated; the measurements were plotted against the voltage-step values. The three curves were measured in the same cell in different baths. The upper curve (●) shows data recorded in APS, the lower curve (△) in low Cl^- APS, and the middle curve (○) in APS containing 200 μM Cd^{2+} to block voltage-gated Ca^{2+} channels.

tial of the cation current (0 mV). The distribution of the reversal potentials of I_{FLASH} was shifted toward E_{Cl} *(Fig. 2b)* and had a mean value of −22.3 ± 7.5 mV (m ± SD, n = 28), suggesting that the Cl^- conductance was the more prominent of the two components. These features including the intercellular variability of the flash-activated response would be expected if a Cl^- and a CNG cation conductance, each with varying relative densities, were both activated.

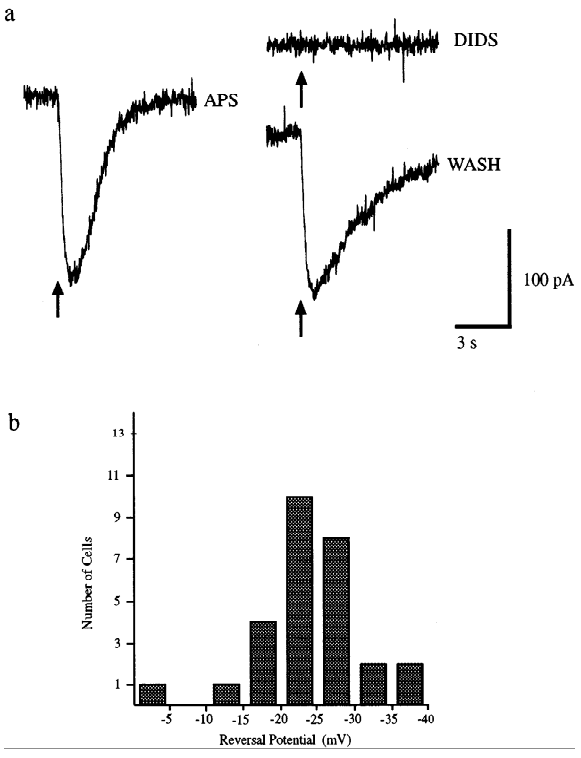

Fig. 2. Flash-activated currents from dissociated ORNs in physiological saline. The vertical arrows mark the 1 msec UV flash (here and in later figures). (a) I_{FLASH} recorded before, during, and after exposure to DIDS. Trace APS: cell held at −89 mV. The onset time-to-half-maximum was ~125 msec, and the time-to-peak was ~600 msec; the response returned to baseline within ~3.5 sec. Trace DIDS: recorded 1 min after addition of 100 μM DIDS. DIDS blocked the response, but had no effect on the baseline holding current. Trace WASH: recorded 1 min after washing out the DIDS-containing bath. The flash-activated response returned to 90% of its initial value. In this cell I_{FLASH} appeared to be almost completely carried by Cl^-. Bath: APS^{HO}; pipette: AIS with 800 μM NPE-caged cAMP. (b) A histogram showing the distribution of the reversal potentials of I_{FLASH} measured in 28 cells using the APS^{HO}/AIS salines.

The presence of a Cl^- component in the flash response was also suggested by experiments using DIDS (4,4′-diisothiocyanatostilbene 2,2′-disulfonic acid) which blocks Cl^- currents in these cells (Dubin & Dionne, 1994). We found that 100–300 μM DIDS blocked ⩾80% of I_{FLASH} (10 of 10 cells), and that the inhibition by DIDS was reversible (Fig. 2*a*). Surprisingly, in cells where the reversal potential of I_{FLASH} was close to E_{Cl}, the DIDS block appeared to be complete (Fig. 2*a*). This last observation suggests that in some cells the flash-activated current might be purely Cl^-, and we were led to look more carefully at how the Cl^- component of I_{FLASH} was activated.

The Cl^- component of I_{FLASH} was studied following elimination of outward cation currents using the ''impermeant'' intracellular saline ZIS. When ZIS and normal APS were used as the respective intracellular and extra-

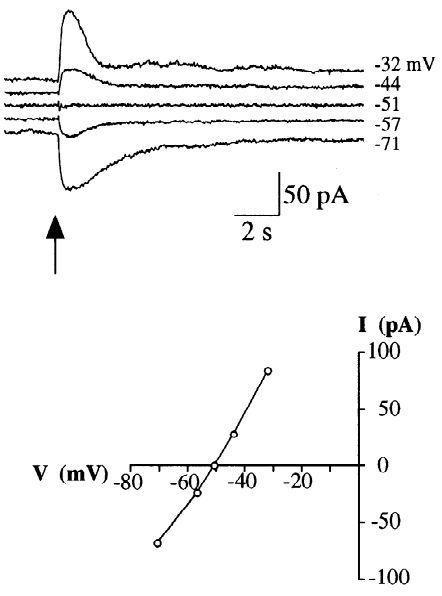

Fig. 3. Flash-activated currents carried by Cl^-. (a) Five flash-activated responses recorded from a cell held at membrane potentials between −32 and −71 mV. The baselines of the responses were offset from one another for the illustration. Over this voltage range the flash-activated current shifted from outward (positive deflecting) to inward (negative). Bath: APS; Pipette: ZIS with 800 μM NPE-caged cAMP. (b) The peak current/voltage curve shows a nearly linear relation with an apparent reversal potential of −51 mV. The difference between this value and the Cl^- equilibrium potential (−55 mV) is due to a liquid junction potential (Barry & Lynch, 1991).

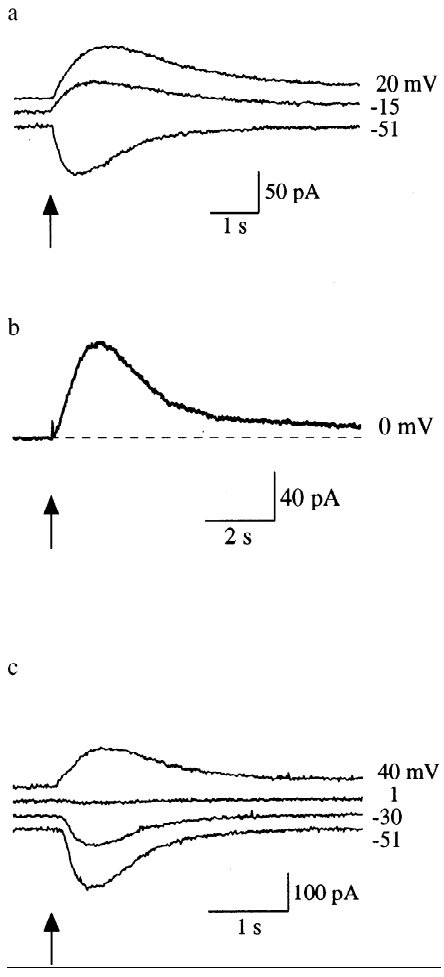

Fig. 4. The flash-activated Cl^- current in Ca^{2+}-free and high Ca^{2+} baths. (a) Flash-activated Cl^- currents recorded with Ba^{2+} substituted for Ca^{2+} in the APS bathing the cell (baselines offset). (b) Flash-activated Cl^- currents recorded in Ca^{2+}-free APS with no divalent ion substitute. Bath: Ca^{2+}-free APS with choline$^+$ substituted for Na^+; Pipette (panels *a* and *b*): ZIS plus 800 μM NPE-caged cAMP. (c) Flash-activated currents recorded with APS^{HO} containing 3 mM Ca^{2+} on both sides of the membrane (baselines offset).

cellular recording salines, the reversal potential for Cl^- was $E_{Cl} = -55$ mV; for other ions: $E_{Na} = +61$ mV, $E_{Ca} > +130$ mV, and $E_K > +60$ mV assuming trace contamination by K^+. With ZIS plus NPE-caged cAMP in the recording pipette, the photolytic release of cAMP elicited a transient current (Fig. 3*a*) with a linear dependence on voltage that reversed polarity very close to E_{Cl} (Fig. 3*b*). Since only Cl^- could carry outward current at the test voltages, these data reveal a Cl^- flux activated by cAMP; however, they give no indication of the specific mechanism by which the Cl^- conductance was gated. There are three possibilities to consider: (i) that the Cl^- conductance was actually Ca^{2+}-gated via an action of cAMP which elevated intracellular Ca^{2+}, (ii) that it was activated following phosphorylation by cAMP-dependent protein kinase (PKA), or (iii) that it was directly gated by cAMP.

To test whether the flash-activated Cl^- current was mediated by Ca^{2+}-gated Cl^- channels, measurements were made both in the absence of extracellular Ca^{2+} and in the presence of elevated intracellular Ca^{2+}. The flash-activated Cl^- current could still be elicited when extracellular Ca^{2+} was replaced by Ba^{2+} (Fig. 4*a*). With ZIS in the pipette, the currents observed in Ba^{2+}-substituted APS had similar magnitudes and activation kinetics to those seen in normal APS. These results suggest that the Cl^- current was not due to the activation of Ca^{2+}-gated Cl^- channels, assuming that Ba^{2+} is not an effective Ca^{2+} substitute in this regard (Barish, 1983). To test this assumption, measurements were made in a modified Ca^{2+}-free APS from which Na^+ was also eliminated (composition in mM: 112 cholineCl, 10 HEPES free acid, 1 EGTA, 5 glucose, pH 7.2 with Tris base). Although our recordings were stable for only a few minutes after normal APS was replaced with this Ca^{2+}-free saline, from 4 cells we recorded outward Cl^- currents in Ca^{2+}-free saline (Fig. 4*b*) that were similar to those seen in the same cells in normal APS. These data support the conclusion that the flash response was not activated as a consequence of Ca^{2+} entry.

The flash-activated current could also be elicited in cells when both the intracellular and the extracellular faces of the membrane were bathed in APS^{HO} containing 3 mM Ca^{2+} (Fig. 4*c*) ($n = 4$). The response reversed polarity at 0 mV as expected when identical salines bathed both sides of the membrane. Moreover, the reversal of the current showed that the responses could be elicited at voltages which drove Ca^{2+} influx as well as efflux, suggesting that neither accumulation nor loss of Ca^{2+} on the cytoplasmic face of the membrane was a factor in the response. Although small cations were not eliminated from APS^{HO}, the Ca^{2+} on both sides of the membrane would be expected to block the CNG cation conductance, leaving Cl^- as a major current carrier. The presence of a Cl^- contribution to the response was verified by measurements with APS^{HO} intracellular and low-Cl^- (10.5 mM) APS^{HO} in the bath which shifted the reversal potential to +29.0 ± 6.0 mV ($n = 6$; *data not shown*). The Ca^{2+} concentration in these experiments was about 5 orders of magnitude greater than the normal resting intracellular concentration, suggesting that changes of the intracellular Ca^{2+} concentration that mimicked physiological changes were not needed to elicit the flash-activated current. The ability of cAMP to activate a Cl^- conductance both in the absence of extracellular Ca^{2+} and in the presence of high intracellular Ca^{2+} strongly suggests that the response was not caused by Ca^{2+} acting on Ca^{2+}-gated Cl^- channels.

Four different experiments were conducted to assess whether the flash-activated Cl^- current was mediated by protein kinase activity. First, I_{FLASH} could be activated by NPE-caged cGMP (5 of 5 cells; *data not shown*). The cGMP-activated response, recorded in physiological salines as in Fig. 2, was indistinguishable from that activated by cAMP in terms of its reversal potential (range: −20 to −33 mV, $n = 5$) and block by DIDS (~100% block by 300 μM DIDS, reversible, $n = 2$). This suggests a lack of specificity in the cyclic nucleotide dependence of any putative kinase. Second, whole-cell recordings with ZIS plus NPE-caged cAMP in the pipette were obtained from ORNs incubated in the membrane-permeant PKA-selective inhibitor H-89. H-89 (17 μM) was added to the APS bath after measuring the flash-activated Cl^- current (Fig. 5). The flash-activated current was not blocked by H-89 (5 of 5 cells), even with incubation times exceeding 30 min and a bath concentration of inhibitor that was >350 times its K_i (48 nM) (Chijiwa, Mishima & Hagiwara, 1990). At this concentration, H-89 should also have been an effective inhibitor of cGMP-dependent protein kinase ($K_i = 0.48$ μM) (Chijiwa et al., 1990). As evidence that H-89 was otherwise effective, in its presence we noted a >50% reduction of the voltage-gated Na^+ current, which was partially reversible following washout of the inhibitor. Protein kinase inhibition experiments were also conducted with

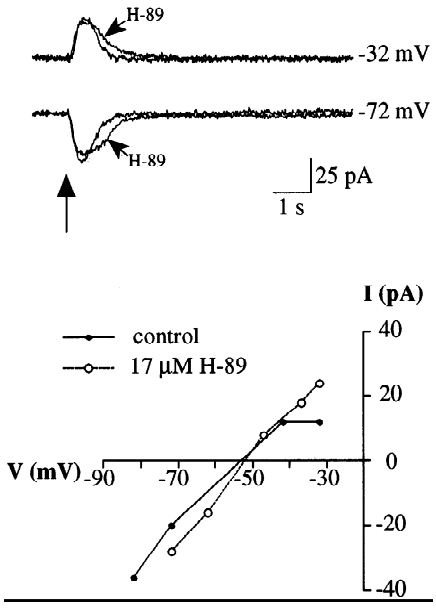

Fig. 5. Flash-activated Cl^- currents in the presence of a kinase inhibitor. (a) Flash-activated Cl^- currents were recorded from cells both in the absence and presence of H-89. Following control responses obtained in normal APS, this cell was exposed to 17 μM H-89 in APS for >10 min before the responses labeled H-89 were recorded. Pipette: ZIS plus 800 μM NPE-caged cAMP. (b) The peak Cl^- current/voltage curves were plotted for this cell using responses like those in panel (a). The reversal potential was unaffected by H-89.

H-7 which blocks protein kinases A, G, and C with K_is in the 3–6 μM range (Hidaka et al., 1984). H-7 (100 μM), was included in intracellular AIS together with NPE-caged cAMP (700 μM), allowing simultaneous access of both compounds to the interior of the cell, but H-7 failed to block the flash-activated current (5 of 5 cells, *data not shown*). Finally, ATP was eliminated from the ZIS intracellular solution to deprive any kinase of its necessary substrate (Fig. 6). Removal of ATP should have blocked a kinase-dependent response, but the flash-activated Cl^- current could be elicited repeatedly in these cells ($n = 14$), just as in cells with ATP. The ability of both cAMP and cGMP to activate the Cl^- current, the inability of two different kinase inhibitors to block the current, and the persistence of the response in the absence of intracellular ATP all indicate that activation of the Cl^- conductance is not dependent on phosphorylation by a cyclic nucleotide-dependent protein kinase.

We tested the flash-activated and voltage-dependent Cl^- currents for additivity in 7 cells, measuring the currents individually, then activating them together. The total Cl^- current activated by a depolarizing voltage-step in the presence of flash-released cAMP was greater than the sum of the two components activated individually (Fig. 7). The average of the simultaneously elicited currents was about 2-fold greater than the sum of the separate

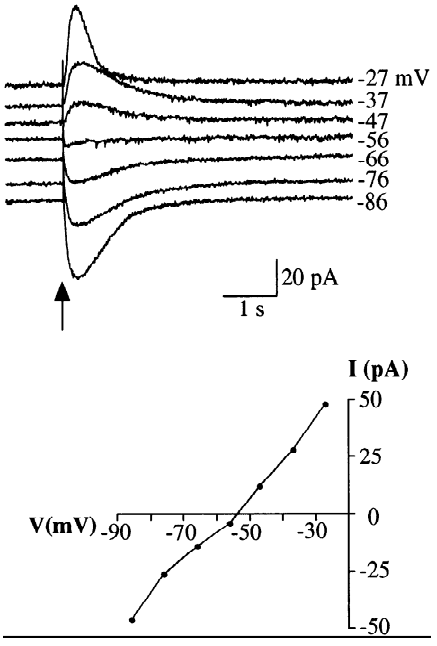

Fig. 6. Flash-activated Cl^- currents recorded without intracellular ATP. (a) Data recorded at holding potentials of −27 to −86 mV (baseline currents offset) with APS in the bath and ATP-free ZIS plus 800 μM NPE-caged cAMP in the pipette. (b) The peak current/voltage curve for the data above shows a near-linear relation with a reversal potential of −55 mV (E_{Cl} = −55 mV), indicating that a Cl^- current was activated in the absence of ATP.

components. This result was observed in normal APS and with APS containing Ba^{2+} substituted for Ca^{2+}, with and without H-89, and with and without ATP + GTP in the pipette saline, suggesting that Ca^{2+}-gated Cl^- channels and cyclic nucleotide-dependent kinases were not factors affecting additivity. Furthermore, in cells that did not have a flash-activated Cl^- current, the voltage-gated Cl^- current could still be detected and was insensitive to the release of cAMP (n = 7). This indicates that the supra-additive effect was not mediated by cAMP acting on voltage-gated Cl^- channels, and suggests that the voltage-gated and cyclic nucleotide-dependent Cl^- conductances must be separate entities. The supra-additive effect can be explained if the cyclic nucleotide-dependent channels, although not voltage-gated, are voltage-sensitive.

Discussion

Our results provide the first description of a novel Cl^- conductance found in *Necturus* ORNs. The Cl^- current mediated by this conductance could be activated by either cAMP or cGMP, but it was not activated by depolarization or by elevation of intracellular Ca^{2+}, nor did it depend upon protein kinase activity. These observations

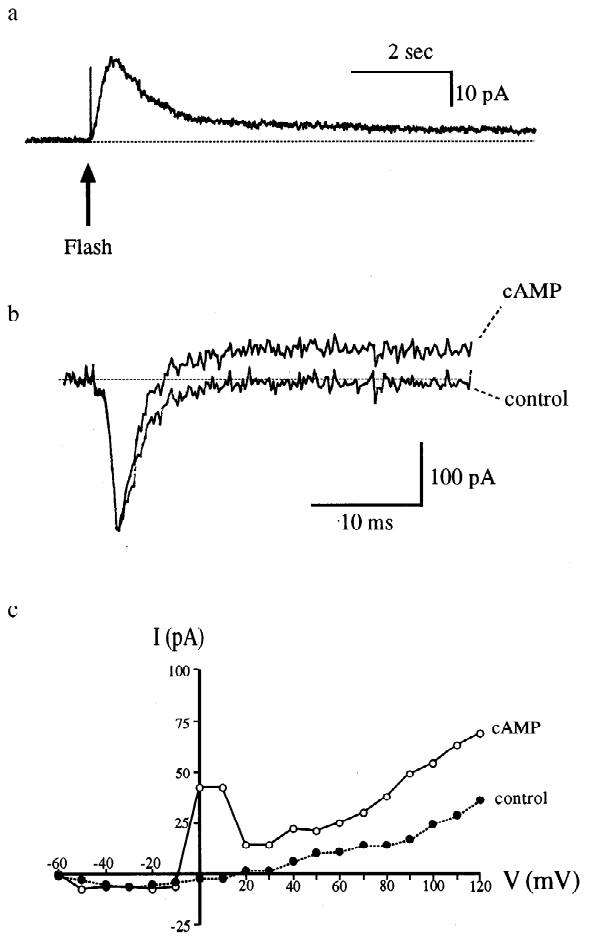

Fig. 7. Supra-additivity of the flash-activated and voltage-activated Cl^- currents. These data are from a single cell with ZIS plus 800 μM NPE-caged cAMP in the pipette. (a) A response recorded at 0 mV illustrates the magnitude (22 pA peak) and duration of the flash-activated current in this cell. 500 Hz filter. (b) Voltage-activated currents elicited by depolarizing voltage steps to 0 mV from a holding potential of −80 mV. The lower trace was recorded before the UV flash; the trace labeled cAMP was recorded <1 sec after the flash. Leak currents subtracted; 10 kHz filter. Note calibration differences here and in the panel above. (c) Current-voltage curves. Current-voltage curves were plotted from two series of voltage-activated currents, examples of which are shown in panel *b*. The voltage pulses were delivered at 1 sec intervals beginning with −60 mV and stepping to +120 mV in 10 mV increments. Mean currents measured 30 msec after each voltage step, by which time the Na^+ current had inactivated, were plotted against the step potential. During the series labeled cAMP, a UV flash was delivered between the −10 and 0 mV pulses. Following the flash, the outward Cl^- current was elevated as much as 40 pA and did not return to control levels before the pulse series ended.

strongly argue against two of the three most plausible cyclic nucleotide-dependent mechanisms known to activate ion channels, but leave extant the possibility that cyclic nucleotides may act directly on a dependent Cl^- channel to activate it. A comparable cation conductance was discovered in ORNs a decade ago (Nakamura & Gold, 1987).

Our data favor but do not establish unequivoably the existence of a cyclic nucleotide-gated Cl^- channel; proof will require single-channel recordings. We attempted to record single-channel activity associated with this conductance using inside-out patches of dendritic membrane; however, we were unsuccessful. Although K^+ channel activity was reliably seen, we found no cyclic nucleotide-dependent Cl^- channel activity in any of the membrane patches excised from the dendrites of 7 ORNs. It is plausible that these particular cells had no cyclic nucleotide-dependent Cl^- channels, or that the channels are not located on dendritic membrane in very high density. Alternatively, the single-channel conductance of these Cl^- channels may be so small that the unitary currents were not resolved.

Since *Necturus* ORNs have Cl^- conductances activated by depolarization and intracellular Ca^{2+} as well as by cyclic nucleotides, we questioned whether the cyclic nucleotide-dependent Cl^- conductance involved a novel type of channel, or whether cyclic nucleotides might activate voltage-gated or Ca^{2+}-gated Cl^- channels using an alternate, yet unrecognized mechanism. Our data strongly favor a novel channel type because, in cells where no cyclic nucleotide-dependent Cl^- current could be elicited, we still saw voltage-gated and Ca^{2+}-gated Cl^- currents. Although the cyclic nucleotide-dependent Cl^- conductance was not activated by depolarization alone, it was sensitive to voltage. When the conductance was activated by cAMP, depolarization caused further activation. This voltage-sensitivity might be mediated through effects on either the opening or the closing rates of the channels, both of which would affect the open probability.

Although direct gating of ion channels by cyclic nucleotides is not a new observation, this mechanism has been principally associated with cation (Fesenko, Kolesnikov & Lyubarsky, 1985; Nakamura & Gold, 1987) and K^+ channels (Bruggemann et al., 1993; Hoshi, 1995; Hatt & Ache, 1994). A cyclic nucleotide-gated Cl^- channel was recently described in cultured renal tubule cells (Darvish, Winaver & Dagan, 1995); however, unlike the one here, it was activated by cGMP with an EC_{50} near 1 mM, but it was insensitive to cAMP. It is plausible that, like the CNG cation channels, there is a family of CNG anion channels that help to regulate membrane permeability in different cell types.

The presence of the cyclic nucleotide-dependent Cl^- conductance in ORNs where odors can stimulate rapid, transient increases in cAMP (Breer, Boekhoff & Tareilus, 1990) suggests that the conductance may be involved in odor transduction. Yet the conductance was not present in all *Necturus* ORNs. In our recordings with ZIS in the pipette to isolate the Cl^- current, the cyclic nucleotide-dependent Cl^- conductance was detected in only 52% of ORNs where it showed a whole-cell conductance that ranged from <0.2 to >10 nS. During transduction of odors that stimulate adenylyl cyclase, this conductance should exert substantial effects on excitability.

Ca^{2+}-gated Cl^- conductances involved in odor transduction are thought to help insure the integrity of the transduction signal because Cl^- efflux can reliably produce a depolarizing receptor potential in a cation-deficient environment such as mucus (Lowe & Gold, 1993). But why should *Necturus* ORNs have this additional type of Cl^- channel? *Necturus* lives in fresh water lakes that are low in cations generally and in which the Ca^{2+} concentration is variable; this could render a Ca^{2+}-gated Cl^- conductance an unreliable mechanism. By contrast, a cyclic nucleotide-dependent Cl^- conductance might directly and efficiently generate a receptor potential in this environment. In addition, the combination of Ca^{2+}-gated and cyclic nucleotide-dependent Cl^- conductances may allow some form of differential control of excitability (Jaworsky et al., 1995) that satifies other demands such as odor discrimination or modulation of spontaneous activity. Odor transduction in these and other animals appears to be characterized by substantial heterogeneity of the odor-activated transduction mechanism and of the response that it elicits (Boekhoff et al., 1990; Dionne, 1992; Dubin & Dionne, 1993; Ache, 1994; Dubin & Dionne, 1994).

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References

Ache, B.W. 1994. Towards a common stratagy for transducing olfactory information. *Sem. Cell Biol.* **5:**55–63

Barish, M.E. 1983. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol.* **342:**309–325

Barry, P.H., Lynch, J.W. 1991. Liquid junction potentials and small cell effects in patch-clamp analysis. *J. Membrane Biol.* **121:**101–117

Bigiani, A., Roper, S.D. 1995. Estimation of the junctional resistance between electrically coupled receptor cells in *Necturus* taste buds. *J. Gen. Physiol.* **106:**705–725

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

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

Bruggemann, A., Pardo, L.A., Stuhmer, W., Pongs, O. 1993. Ether-a-go-go encodes a voltage-gated channel permeable to K^+ and Ca^{2+} and modulated by cAMP. *Nature* **365:**445–448

Chijiwa, T., Mishima, A., Hagiwara, M. 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *J. Biol. Chem.* **265:**5267–5272

Corotto, F.S., Piper, D.R., Chen, N., Michel, W.C. 1996. Voltage- and

Ca^{2+}-gated currents in zebrafish olfactory receptor neurons. *J. Exp. Biol.* **199:**1115–1126

Darvish, N., Winaver, J., Dagan, D. 1995. A novel cGMP-activated Cl^- channel in renal proximal tubules. *Am. J. Physiol.* **268:**F323–F329

Dionne, V.E. 1988. Membrane conductance mechanisms of dissociated cells from the olfactory epithelium of the mudpuppy. *Necturus maculosus. In:* Olfaction and Taste IX: From Reception to Perception, S. Roper and J. Aetma, editors. *New York Academy of Sciences* **510:**258–259

Dionne, V.E. 1992. Chemosensory responses in isolated olfactory receptor neurons from *Necturus maculosus. J. Gen. Physiol.* **99:**415–433

Dionne, V.E., Dubin, A.E. 1994. Transduction diversity in olfaction. *J. Exp. Biol.* **194:**1–21

Dubin, A.E., Dionne, V.E. 1993. Modulation of Cl^-, K^+ and nonselective cation conductances by taurine in olfactory receptor neurons of the mudpuppy *Necturus maculosus. J. Gen. Physiol.* **101:**469–485

Dubin, A.E., Dionne, V.E. 1994. Action potentials and chemosensitive conductances in the dendrites of olfactory neurons suggest new features for odor transduction. *J. Gen. Physiol.* **103:**181–201

Fesenko, E.E., Kolesnikov, S.S., Lyubarsky, A.L. 1985. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313:**310–313

Firestein, S., Shepherd, G.M. 1995. Interaction of anionic and cationic currents leads to a voltage dependence in the odor response of olfactory receptor neurons. *J. Neurophysiol.* **73:**562–567

Frings, S., Lynch, J.W., Lindemann, B. 1992. Properties of cyclic nucleotide-gated channels mediating olfactory transduction. Activation, selectivity, and blockage. *J. Gen. Physiol.* **100:**45–67

Furman, R.E., Tanaka, J.C. 1990. Monovalent selectivity of the cyclic guanosine monophosphate-activated ion channel. *J. Gen. Physiol.* **96:**57–82

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hatt, H, Ache, B.W. 1994. Cyclic nucleotide- and inositol phosphate-gated ion channels in lobster olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* **91:**6264–6268

Hidaka, H., Inagaki, M., Kawamoto, S., Sasaki, Y. 1984. Isoquinolinesulfonamines, novel and potent inhibitors of cyclic nucleotide dependent kinase and protein kinase C. *Biochemistry* **23:**5036–5041

Hille, B. 1992. Ionic Channels of Excitable Membranes. 2nd ed. Sinauer Associates, Sunderland, MA

Hoshi, T. 1995. Regulation of voltage dependence of the KAT1 channel by intracellular factors. *J. Gen. Physiol.* **105:**309–328

Jaworsky, D.E., Matsuzaki, O., Borisy, F.F., Ronnett, G.V. 1995. Calcium modulates the rapid kinetics of the odorant-induced cyclic AMP signal in rat olfactory cilia. *J. Neurosci.* **15:**310–318

Kleene, S.J. 1993. Origin of the chloride current in olfactory transduction. *Neuron* **11:**123–132

Kleene, S.J., Gesteland, R.C., Bryant, S.H. 1994. An electrophysiological survey of frog olfactory cilia. *J. Exp. Biol.* **195:**307–328

Kurahashi, T., Yau, K-W. 1993. Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature* **363:**71–74

Lowe, G., Gold, G.H. 1993. Contribution of the ciliary cyclic nucleotide-gated conductance to olfactory transduction in the salamander. *J. Physiol.* **462:**175–196

Lowe, G., Gold, G.H. 1993. Non-linear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* **366:**283–286

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

Nerbonne, J.M., Richard, S., Nargeot, J., Lester, H.A. 1984. New photoactivateable cyclic nucleotides produce intracellular jumps in cyclic AMP and cyclic GMP concentrations. *Nature* **310:**74–76

Picco, C., Menini, A. 1993. The permeability of the cGMP-activated channel to organic cations in retinal rods of the tiger salamander. *J. Physiol.* **460:**741–758

Schoenmakers, T.J.M., Visser, G.J., Flik, G., Theuvenet, P.R. 1992. CHELATOR: An improved method for computing metal ion concentrations in physiological solutions. *Biotechniques* **12:**870–879

Trotier, D. 1994. Intensity coding in olfactory receptor cells. *Seminars in Cell Biology* **5:**47–54