Ionic Currents and Ion Channels of Lobster Olfactory Receptor Neurons

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ABSTRACT The role of the soma of spiny lobster olfactory receptor cells in generating odor-evoked electrical signals was investigated by studying the ion channels and macroscopic currents of the soma. Four ionic currents; a tetrodotoxin-sensitive Na⁺ current, a Ca⁺⁺ current, a Ca⁺⁺-activated K⁺ current, and a delayed rectifier K⁺ current, were isolated by application of specific blocking agents. The Na⁺ and Ca⁺⁺ currents began to activate at -40 to -30 mV, while the K⁺ currents began to activate at -30 to -20 mV. The size of the Na⁺ current was related to the presence of a remnant of a neurite, presumably an axon, and not to the size of the soma. No voltage-dependent inward currents were observed at potentials below those activating the Na⁺ current, suggesting that receptor potentials spread passively through the soma to generate action potentials in the axon of this cell. Steady-state inactivation of the Na⁺ current was half-maximal at -40 mV. Recovery from inactivation was a single exponential function that was half-maximal at 1.7 ms at room temperature. The K⁺ currents were much larger than the inward currents and probably underlie the outward rectification observed in this cell. The delayed rectifier K⁺ current was reduced by GTP-γ-S and AlF₄⁻, agents which activate GTP-binding proteins. The channels described were a 215-pS Ca⁺⁺-activated K⁺ channel, a 9.7-pS delayed rectifier K⁺ channel, and a 35-pS voltage-independent Cl⁻ channel. The Cl⁻ channel provides a constant leak conductance that may be important in stabilizing the membrane potential of the cell.

INTRODUCTION

Olfactory receptor cells transduce chemosensory information into graded electrical signals and generate action potentials that propagate to the central nervous system. Because these are bipolar neurons, the soma is interposed between the transduction sites in the dendrite and the axon where action potentials are thought to originate (reviewed by Getchell, 1986). Somatic ion channels must therefore perform not only the usual functions of membrane repolarization and setting the membrane potential, but also facilitate the spread of odor-activated currents.

To date little information exists about the somatic ion channels and currents of olfactory receptor cells. Mouse olfactory receptor cells have two Ca⁺⁺-activated K⁺ channels, a type of inward rectifier, a delayed rectifier, a Cl⁻ channel, and a Ca⁺⁺
channel (Maue and Dionne, 1987). Collectively, amphibian olfactory receptor cells possess a Na⁺ current, a Ca++ current, a delayed rectifier current, an A current, a Ca++-activated K⁺ current, and perhaps an anion current (Trotier, 1986; Firestein and Werblin, 1987; Suzuki, 1987). These vertebrate channels and currents appear to have little activity in the critical range of membrane potentials between rest and spike threshold. This finding is consistent with the emerging idea (Hedlund et al., 1987) that olfactory receptor cells solve the problem of current spread by being electrotonically compact. Salamander olfactory receptor cells, for instance, have an average input resistance of several gigaohms and no equalizing time constants (Trotier, 1986; Firestein and Werblin, 1987).

By comparison, lobster olfactory receptor cells are electrotonically less compact. They have an average input resistance of 500 MΩ and an equalizing time constant (Schmiedel-Jakob et al., 1989), perhaps because the lobster has the longest dendritic arbor of the smallest diameter yet reported for olfactory receptor cells (Grünert and Ache, 1988). Lobster olfactory receptor cells might be forced to compensate by using active processes, such as a voltage-dependent channel carrying inward current, to effectively increase the space constant of the cell (Yoshii et al., 1988). To better understand the spread of odor-evoked currents within the cells, to properly interpret whole-cell recordings of these currents, and to further the comparative perspective of olfactory receptor cell function, we have investigated the ionic currents and channels of the soma of lobster olfactory receptor cells.

In this paper we characterize four voltage-dependent ionic currents and three ion channels found on the soma and correlate their properties with functions they might perform. The currents include a Na⁺ current, a Ca++ current, a delayed rectifier K⁺ current, and a Ca++-activated K⁺ current. The channels include a steady-state Cl⁻ channel, a Ca++-activated K⁺ channel, and a voltage-activated K⁺ channel. We found no evidence for inward currents or channels active at potentials below spike threshold, where they might contribute to the spread of odor-evoked inward currents. Our findings are therefore consistent with the idea that the somata of lobster olfactory receptor cells, like their vertebrate counterparts, contribute passively to the spread of odor-evoked currents.

METHODS

Preparation of Receptor Cells

Specimens of the spiny lobster, Panulirus argus, were collected in the Florida Keys and maintained in running seawater for up to 3 mo. Fresh cells were prepared daily by excising an olfactory organ (lateral filament of the antennule) and cutting it into 0.5-mm-long hemicylinders to expose the clusters of receptor cell soma that fill 50% of the lumen of the olfactory organ (Anderson and Ache, 1985). The hemicylinders were gently agitated with L-cysteine-activated papain (0.25 mg/ml; type IV; Sigma Chemical Co., St. Louis, MO) for 20 min and then with trypsin (2.5–5 mg/ml; type IX; Sigma) for an additional 20 min. Hemicylinders, or somata isolated from the hemicylinders by a single trituration step, were placed in a 35-mm culture dish for patch-clamp recording. Cells were viewed at 200× or 300× under brightfield or modulation contrast optics.
Patch-Clamp Recordings

For voltage-clamp recording in the whole-cell configuration, borosilicate glass pipettes (Boralex, Rochester Scientific, Rochester, NY) were pulled to a tip diameter of just over 1 μm (bubble numbers of 4.6 to 5.4, Mittman et al., 1987). Sylgard 184 (Dow Corning Corp., Midland, MI) was applied to the neck of the pipette to reduce electrode capacitance. After forming a seal and compensating the capacitance of the electrode, gentle suction was used to break into the whole-cell recording configuration and the series resistance error was compensated qualitatively. A commercial voltage-clamp and current-amplification circuit with a 1-GΩ head stage (model 8900; Dagan Corp., Minneapolis, MN) was used to record macroscopic currents from isolated somata. An IBM-XT computer with a D/A, A/D converter and accompanying software (pClamp; Axon Instruments, Inc., Burlingame, CA) was used both to apply voltage-step protocols and to digitize, store, and analyze macroscopic currents. Leak currents were recorded from each soma by applying half-amplitude voltage step protocols at hyperpolarized potentials where no active currents were elicited. Leak correction was then performed by multiplying these leak currents by ±2, as necessary, and subtracting them from the active current records. Cell capacitance was calculated by integrating the capacitative current transients evoked by voltage steps from -70 to -6 mV and dividing by 64 mV.

For single-channel recording, pipettes of the same glass were pulled and fire-polished to tip diameters of <1 μm (bubble numbers of 3–4). Sylgard was applied to the electrodes to reduce capacitance when attempting to record channels that were rapidly activated by step changes in holding potential. Single-channel currents were recorded from the plasma membranes of somata using a 10-GΩ head stage. Records were filtered at 10 kHz for storage on video tape (Bezanilla, 1985) and at 0.5–5 kHz for analysis using an eight-pole low-pass Bessel filter (Frequency Devices, Inc., Haverhill, MA). Single-channel records were digitized and analyzed using the same microcomputer system described above. Kinetic analysis was performed only on records from patches containing one active channel. Open and closed duration distributions were compiled as histograms and exponential probability density functions were fitted to them by a chi-square minimization method. Voltages reported for cell-attached patches are the voltages applied across the membrane, without reference to the contribution of the membrane potential of the cell (not measured), following the convention of inside as negative. Mean values are reported ±1 standard deviation. Inside-out patches were exposed to different solutions by placing them into a flow from a glass pipette (100 μm tip diameter) connected by a switching valve to six solution reservoirs.

Solutions

The compositions of patch solutions and salines are listed in Table I. HEPES and EGTA were obtained from Research Organics, Inc. (Cleveland, OH) and inorganic salts from Fisher Scientific (Fair Lawn, NJ). All other chemicals were obtained from Sigma Chemical Co. Calcium concentrations in EGTA solutions were calculated according to Hagiwara (1983). Salines were buffered to pH 7.4 and patch solutions to pH 7.0 with HEPES. Recordings were made at room temperature, which varied from 22 to 24°C.

RESULTS

Total Membrane Currents

In response to depolarizing voltage steps, isolated somata bathed in saline and perfused with normal patch solution showed an inward current followed by a much
TABLE I

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*NaOH or KOH was used to adjust the pH of solutions as appropriate to prepare Na+- and K+-free salines.

†TEA, tetraethylammonium; 4-AP, 4-aminopyridine.

larger outward current (Fig. 1). Hyperpolarizing voltage steps ranging from -40 to -120 mV, applied from holding potentials of -35 to -70 mV, failed to activate any currents in these somata. The inward current became apparent at slightly more negative potentials (-40 to -30 mV) than did the outward current (-30 to -20 mV). Specific blocking agents and impermeable ions were then used to isolate four component currents from the total membrane currents of these somata. In no instance did a soma fail to exhibit the appropriate current for the conditions used.

**FIGURE 1.** Total membrane currents of an isolated soma of a lobster olfactory receptor cell. (Top) Macroscopic currents evoked by depolarizing voltage steps (inset) showing a small inward current and a large outward current. Leak currents were not subtracted from these records. (Bottom) The I-V relationships of the inward current (circles) and the outward current (triangles) from this soma. Pipette: normal patch solution. Bath: saline.
Replacing the K⁺ in the patch solution with Cs⁺ and adding 4 mM Co²⁺ or Cd²⁺ to the bath allowed isolation of a fast, transient inward current \((N = 24\) somata) that peaked within 1 ms of the initiation of the voltage step (Fig. 2). This current was absent in the presence of submicromolar concentrations of tetrodotoxin (TTX; \(N = 111\) somata) and saxitoxin (\(N = 2\) somata), identifying this as a Na⁺ current. The current began to activate at \(-38\) to \(-30\) mV and reached maximum amplitude at \(-10\) to \(0\) mV. The rapid onset of the current after steps to \(-6\) mV from \(-70\) mV was described by a single exponential with a time constant of \(0.07 \pm 0.03\) ms \((N = 7\) somata). The current decayed as a single exponential function with a time constant of \(0.29 \pm 0.08\) ms \((N = 13\) somata). Large Na⁺ currents were not correlated with cell size as measured by linear regression of current vs. cell capacitance (correlation coefficient = 0.30, \(N = 13\) somata), but with the presence of a neurite. Steady-state inactivation was half-maximal at \(-48\) mV \((N = 7\) somata; Fig. 3 A). Steady-state inactivation was evoked by prepulses to a series of increasing potentials for 50 ms before the membrane potential was brought to 0 mV to elicit the Na⁺ current. Recovery from inactivation followed a single exponential function with a mean time to half-maximal activation of \(1.7 \pm 0.4\) ms \((N = 3\) somata) at room temperature (Fig. 3 B). Recovery from inactivation was measured using a two-pulse protocol in which a series of paired pulses to 0 mV from a holding potential of \(-70\) mV were separated by an increasing interval of time.

**Figure 2.** The Na⁺ current. *(Top)* Transient inward currents elicited by depolarizing voltage steps *(inset)*. Leak currents were not subtracted from these records. *(Bottom)* The I-V relationship of this current. Activation began between \(-38\) and \(-30\) mV. The peak current occurred at 0-10 mV. Pipette: 200 CsCl patch solution. Bath: saline plus 4 mM CoCl₂, 4 mM CdCl₂, and 10 mM TEA.
Ca++ Current

A sustained inward current (N = 44 somata) was isolated when Cs+ replaced K+ in the patch solution and 0.1 μM TTX was added to the bath (Fig. 4). This current, which washed out within minutes after breakthrough into the whole-cell configuration, was carried by either Ca++, Ba++, or Sr++ and was absent when either Co++ or

**Figure 3.** Properties of inactivation of the Na+ current. (A) Steady-state inactivation (h) of the Na+ current elicited by 50-ms prepulses to the potentials depicted on the abscissa. Shown are the means and standard deviations from four cells. Small standard deviations fall within circles marking the data points. (B) Recovery from inactivation as evoked by a paired-pulse protocol. The plot denotes the amplitude of the Na+ current evoked by the second pulse relative to the first vs. the interval between the two pulses. These data were fit by a single exponential function with a time constant of 1.7 ms. Solutions as in Fig. 2.

**Figure 4.** The Ca++ current. (Top) Sustained inward currents carried by Sr++ were elicited by depolarizing voltage steps (inset). Leak currents were subtracted from these records. (Bottom) The I-V relationship of the current from the same soma. Activation began at ~ -40 mV. Peak current occurred at ~ -10 mV. Pipette: 150 CsCl patch solution plus 2 mM adenosine triphosphate, 0.2 mM guanosine triphosphate. Bath: saline, with 20 mM SrCl₂ substituted for 13 mM CaCl₂ and 7 mM MgCl₂, plus 0.1 μM TTX.
Cd++, or both, was added to the bath at a concentration of 4–10 mM \((N = 65\) somata). When carried by Ca++ \((N = 4\) somata), the current decayed more rapidly (Fig. 5), suggesting that a Ca++-dependent process is involved in the inactivation of this current. These observations identified this current as a Ca++ current. Like the Na+ current, this current activated between -40 and -30 mV. The washout of this current was not suppressed by 2 mM adenosine triphosphate and 0.2 mM guanosine triphosphate \((N = 5\) somata).

**Ca++-activated K+ Current**

With 5 mM tetraethylammonium (TEA) and 0.1 \(\mu\)M TTX in the bath, a very large outward current was apparent (Fig. 6; \(N=12\) somata). Like the Ca++ current, this current disappeared within minutes after breakthrough into the whole-cell configu-
ration. This washout was not suppressed \((N = 3\) somata) by 1 mM dibutyryl 3',5'-cyclic adenosine monophosphate (Chad and Eckert, 1986). The current was absent if 4 mM Co\(^{++}\) or Cd\(^{++}\) was added to the bath \((N = 55\) somata) or if internal K\(^{+}\) was replaced by Cs\(^{+}\) or Na\(^{+}\) \((N = 64\) somata). These observations identify this as a Ca\(^{++}\)-activated K\(^{+}\) current. This current was not blocked by 20 mM TEA applied internally \((N = 2\) somata) or 5 mM TEA applied externally \((N = 6\) somata). This current decayed to varying degrees during voltage steps, apparently because of the inactivation of the Ca\(^{++}\) current and decreasing internal Ca\(^{++}\) concentration, as the single channel underlying the Ca\(^{++}\)-activated K\(^{+}\) current showed no voltage-dependent inactivation (see below).

Delayed Rectifier K\(^{+}\) Current

A delayed outward current was isolated by adding 0.1 \(\mu\)M TTX and 4 mM Co\(^{++}\) or Cd\(^{++}\), or both, to the bath \((n = 55\) somata). This current activated with a delay and inactivated slowly. The current was blocked by 5 mM external TEA \((N = 6\) somata) and was absent when internal K\(^{+}\) was replaced by Cs\(^{+}\) or Na\(^{+}\) \((N = 60\) somata). Collectively, these features identify this current as a delayed rectifier type of K\(^{+}\) current. Inactivation of this current was voltage dependent (Fig. 8). The inactivation elicited by 250-ms prepulses, which were too brief to completely achieve steady-state inactivation, was half-maximal at \(-41\) mV \((N = 4\) somata).

This current was also modulated by at least one mechanism involving a GTP-binding protein. Perfusing somata with normal patch solution plus 20 mM NaF and 10 \(\mu\)M AlCl\(_3\), a mixture that generates AlF\(_4\), which nonselectively activates GTP-binding proteins (Blackmore et al., 1985; Sternweis and Gilman, 1982), reduced the
FIGURE 8. The voltage dependence of inactivation of the delayed rectifier current under conditions where complete steady-state inactivation had not been reached. The plot shows the percent of maximum current evoked by stepping the holding potential to 40 mV, immediately after 250-ms prepulses applied in positive steps of 7.5 mV beginning at -110 mV. Between voltage step protocols the membrane was held at -70 mV. The mean and standard deviation of four cells are plotted. Solutions as in Fig. 6.

delayed rectifier over time (Fig. 9, A vs. C). Analysis of variance showed that the reduction of the current in five somata perfused with this mixture was significantly greater than that of seven somata perfused with normal patch solution ($P < 0.05$). Perfusing somata with normal patch solution containing 1 mM GTP-$\gamma$S, another GTP-binding protein activator, caused similar reductions in the current over time.

FIGURE 9. Effect of agents that activate GTP-binding proteins on the delayed rectifier current. (A) The $I$-$V$ relationships of the delayed rectifier current from an untreated cell showed little suppression over time. Plotted are current amplitudes immediately after breakthrough (circles), 2 min later (triangles), and 6 min later (squares). Solutions as in Fig. 6. (B) The $I$-$V$ relationships of the delayed rectifier current from another cell perfused with GTP-$\gamma$S were successively suppressed over time. Plotted are current amplitudes immediately after breakthrough (circles), 2 min later (triangles), and 5 min later (squares). Solutions as in Fig. 6, except that 0.5 mM GTP-$\gamma$S was added to the patch solution. (C) The $I$-$V$ relationships of the delayed rectifier current from a third cell perfused with AlF$_4^-$ were also suppressed. Plotted are current amplitudes from immediately after membrane breakthrough (circles), 3 min later (triangles), and 6 min later (squares). Solutions as in Fig. 6 except that 20 mM NaF and 0.1 $\mu$M AlCl$_3$ were added to the patch solution.
Analysis of variance showed that the reduction of current in five somata perfused with GTP-γ-S was significantly greater than that of the seven somata perfused with normal patch solution ($P < 0.05$).

**A Ca$^{2+}$-activated K$^+$ Channel**

A large-conductance, Ca$^{2+}$-activated K$^+$ channel was observed in both cell-attached and inside-out patches. In symmetrical 480 mM KCl, its $I-V$ relationship (Fig. 10 A) had a slope conductance of 215 ± 33 pS ($N = 5$ channels). The average permeability ratio of K$^+$ to Na$^+$, calculated from inside-out patches bathed in Na$^+$ patch solution and KCl saline, was 3.9 ± 0.9 ($N = 9$ channels). Increasing the Ca$^{2+}$ concentration bathing the internal face of this channel from $10^{-7}$ to $10^{-6}$ M or $10^{-5}$ M increased the frequency of opening. At a constant Ca$^{2+}$ concentration, depolarization also increased the probability of the channel being in the open state ($P_o$) by increasing the mean open time, with a tendency to decrease the mean closed time as well (Fig. 10 B). The $P_o$ was not observed to decrease with time. In cell-attached patches, this channel was most often observed during depolarizations, and rarely observed at the resting membrane potential. Neither 20 mM TEA, 5 mM 4-aminopyridine, nor 10 mM Cs$^+$ in KCl saline blocked this channel when applied to its intracellular face.

**A Voltage-activated K$^+$ Channel**

Depolarizing voltage steps applied to cell-attached patches often revealed a channel-carrying outward current. Hyperpolarizing prepulses of −30 or −50 mV increased the frequency of channel openings but were not a necessary condition for activation of the channel. With Na$^+$ salines in the patch pipette, the slope conductance for outward currents was 9.7 ± 2.6 pS ($N = 5$ channels). The slope conductance of the
inward current through the cell-attached channel was 49.4 ± 14.8 pS with KCl saline in the patch pipette (N = 5 channels). The inward rectification predicted by these slope conductances was small but apparent in the I-V relationships (Fig. 11, A and B). Reversal potentials (in millivolts from the resting membrane potential) extrapolated from the most linear portions of the I-V relationships were 45 ± 14 mV (N = 4 channels) with KCI saline in the pipette and −105 ± 14 mV (N = 3 channels) with NaCl saline in the pipette. These values suggest a selectivity for K⁺ over Na⁺. The activation of this channel by depolarizing voltage pulses was delayed. For example, the first latencies of a channel activated by depolarizing pulses preceded by 50-mV hyperpolarizing prepulses was 259 ± 147 ms at −25 mV from rest, 55 ± 89 ms at 0 mV from rest, and 27 ± 15 ms at 25 mV from rest. Channel activity sometimes occurred throughout depolarizations lasting as long as 5 s. Steady-state activity was never observed, however, indicating slow, but complete inactivation.

The properties of this channel suggest that it underlies the delayed rectifier current.

A Steady-State Cl⁻ Channel

Also observed in cell-attached patches was a 35 ± 13 pS channel whose reversal potential was near the resting membrane potential (−8.2 mV ± 9.5 mV from rest, N = 9 channels). This channel was relatively common, being found in 26 of 102 patches, and was selective for Cl⁻ over cations. The I-V relationships of inside-out patches with KCl saline in the patch pipette and NaCl saline in the bath (Fig. 12 A and B), gave a mean reversal potential of −2.0 ± 1.8 mV (N = 5 channels). In these inside-out patches the slope conductance was 70 ± 4 pS. In three channels in inside-out patches, bath perfusion with the Cl⁻ channel blocker potassium isothiocyanate
FIGURE 12. A steady-state, Cl⁻-selective channel. (A) Representative recordings of a Cl⁻-channel in an inside-out patch. Pipette: KCl saline. Bath: NaCl saline. Arrows indicate the current level of the closed state. f = 1.5 kHz. (B) The I-V relationship of another Cl⁻-channel in an inside-out patch under the same conditions. The slope of the linear regression fit to these data was 71 pS.

(20 mM) reduced the slope conductance of the chloride channel by 45 and 57%, while the third channel was unaffected.

The Cl⁻ channel was active at all holding potentials tested and its Po was relatively independent of the holding potential (Fig. 13) and of internal Ca ++ concentrations of 0.01 to 13 mM. The distributions of the open dwell times were usually best fit by single exponentials, giving mean open times ranging from 1.1 to 6.9 ms. The distributions of the closed dwell times were better fit by two exponentials, with mean closed times ranging from 1.6 to 3.9 ms. Neither the mean open or closed times showed any consistent dependence on membrane potential.

DISCUSSION

Depolarizing receptor potentials in lobster olfactory receptor cells can be larger than 40 mV and have peak amplitudes lasting hundreds of milliseconds (Schmiedel-Jakob et al., 1989). Our results support the hypothesis that these receptor potentials spread passively from the dendrite and generate Na⁺ action potentials in the axon by depolarizing the cell membrane from an average resting potential of -56 mV, to above threshold, which lies between -45 and -30 mV (Schmiedel-Jakob et al.,

FIGURE 13. Plot of the P₀ of a Cl⁻-channel vs. voltage. The P₀ of this channel displayed little dependence upon voltage in either the cell-attached (filled circles) or the inside-out (open circles) configuration. These data were obtained from the same patch before and after excision. Lines were fit to the data by linear regression.
1989). We found no evidence of a voltage-dependent current activated at potentials between rest and threshold, at which point such a current could increase the effective space constant of the cell (Yoshii et al., 1988). While the $\text{Ca}^{++}$ current would cause a net increase in inward current between $-40$ and $-30 \text{ mV}$ because the larger outward currents are not yet activated, it would have little or no effect on current spread in the critical range of potential between rest and threshold in most cells. Our results therefore suggest that the spread of subthreshold receptor potentials through the soma is passive. Unless the dendrite has voltage-dependent currents not found in the soma, one must assume that the passive membrane properties of the cell are adequate to account for the spread of current. Our observation that the size of the Na$^+$ current correlated not with the size of the soma, but with the presence of a remnant of neurite, suggests that most Na$^+$ channels are located on the neurite. Assuming that the neurites were axons would be consistent with intracellular recordings from intact cells indicating that the axon is the site of generation of action potentials in lobster olfactory receptor cells (Schmiedel-Jakob et al., 1989). Furthermore, the relatively negative potentials at which the Na$^+$ current inactivates suggests that any Na$^+$ channels present in the soma and dendrite would rapidly inactivate during a receptor potential. Trotier (1986) similarly concluded that the axon contains most of the Na$^+$ current based on its infrequent occurrence in isolated salamander olfactory receptor cells.

Because $1 \text{ mM}$ external TEA broadens action potentials and reduces outward rectification in the intact cell (Schmiedel-Jakob et al., 1989), we conclude that the delayed rectifier K$^+$ current participates in both repolarization of the action potential and in the outward rectification of the cell. The $\text{Ca}^{++}$-activated K$^+$ current could also be involved in both these functions because it activates almost as rapidly as a similar current involved in repolarization of the action potential in bullfrog sympathetic neurons (MacDermott and Weight, 1982) and requires more than 100 ms to decay. Predictions about functions of the $\text{Ca}^{++}$-activated K$^+$ current have been difficult to test, however, due to the rapid washout of the $\text{Ca}^{++}$ currents in the whole-cell recording configuration.

Outward rectification produced by the K$^+$ currents may act to expand the range of odor-evoked inward currents capable of eliciting changes in spike frequency. Given that the interval of potential from threshold to saturation of spike frequency is fixed in any one cell, from Ohm's law, the amount of inward current capable of causing potentials that fall within this interval would increase as the K$^+$ currents decrease the membrane resistance. That this occurs is demonstrated by TEA block of the delayed rectifier current; the injection of 100 pA of current into an intact lobster olfactory receptor cell caused a potential change of 23 mV before, and 47 mV after, TEA application (Schmiedel-Jakob et al., 1989).

Unlike amphibian olfactory receptor cells (Trotier, 1986; Firestein and Werblin, 1987; Suzuki, 1987), the somata of lobster olfactory receptor cells appear to lack an A current or other transient outward current. In many neurons, an A current appears to be necessary to allow repetitive spiking over a wide range of frequencies (Connor, 1976). How the lobster olfactory receptor cell accomplishes repetitive spiking over a wide range of frequencies (e.g., Schmitt and Ache, 1979) in the absence of an A current is unclear. Perhaps one or both of the K$^+$ currents sub-
serves this function by slowing the return to threshold after a spike. A Ca\(^{++}\)-activated K\(^+\) current appears to permit repetitive spiking in frog motoneurons (Barrett and Barrett, 1976). Because mouse olfactory receptor cells also appear to lack the K\(^+\) channel underlying the A current (Maue and Dionne, 1987), multiple mechanisms may exist for controlling spike frequency in olfactory receptor cells.

Another difference from vertebrate olfactory receptor cells is that lobster receptor cells appear to lack inward rectifier K\(^+\) channels, which are believed to contribute to the stability of the membrane potential (Trotier, 1986; Maue and Dionne, 1987). This function could be subserved in lobster olfactory receptor cells by the steady-state CI\(^-\) channel. The steady-state CI\(^-\) channel should provide the receptor cell with a constant leak pathway for CI\(^-\), which suggests that the lobster olfactory receptor cell has a passively distributed concentration gradient of CI\(^-\). That the channel’s reversal potential in cell-attached patches was close to the resting membrane potential agrees with this conclusion. The presence of the CI\(^-\) channel may also be partly responsible for the lower input resistance of lobster olfactory receptor cells compared with that of salamander receptor cells (see Introduction). Block of the CI\(^-\) channel by isothiocyanate, which is a permeant anion in some CI\(^-\) channels (Franciolini and Nonner, 1987), agrees with the isothiocyanate block of the voltage-gated CI\(^-\) channel from *Torpedo* electroplax (Tank et al., 1982).

The depression of the delayed rectifier current by agents that activate GTP-binding proteins suggests that ion channels in the lobster olfactory receptor cell, and therefore olfactory sensitivity, could be modulated by hormonal or synaptic input. The effect also illustrates a caveat for the somatic injection of agents intended to act upon transduction mechanisms in neuritic processes. Care must be taken to ensure that such agents have specific actions that occur only in the desired location within the cell.

In conclusion, our results are consistent with the idea that the spread of odor-evoked currents in lobster olfactory receptor cells, as in their more electrotonically compact vertebrate counterparts, is passive. We propose that the Na\(^+\) current, located mostly in the axon, underlies action potentials in lobster olfactory receptor cells, while the Ca\(^{++}\) and K\(^+\) currents help to shape the plateau of the receptor potential, repolarize the membrane, and control repetitive spiking.

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