Mechanisms of Afterhyperpolarization in Lobster Olfactory Receptor Neurons

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Corotto, Frank S. and William C. Michel. Mechanisms of after-hyperpolarization in lobster olfactory receptor neurons. J. Neurophysiol. 80: 1268–1276, 1998. In lobster olfactory receptor neurons (ORNs), depolarizing responses to odorants and current injection are accompanied by the development of an afterhyperpolarization (AHP) that likely contributes to spike-frequency adaptation and that persists for several seconds after termination of the response. A portion of the AHP can be blocked by extracellular application of 5 mM CsCl. At this concentration, CsCl specifically blocks the hyperpolarization-activated current (Ih) in lobster ORNs. This current is likely to be active at rest, where it provides a constant, depolarizing influence. Further depolarization deactivates Ih, thus allowing the cell to be briefly hyperpolarized when that depolarizing influence is removed, thus generating an AHP. Reactivation of Ih would terminate the AHP. The component of the AHP that could not be blocked by Cs+ (the Cs+-insensitive AHP) was accompanied by decreased input resistance, suggesting that this component is generated by increased conductance to an ion with an equilibrium potential more negative than the resting potential. The Cs+-insensitive AHP in current clamp and the underlying current in voltage clamp displayed a reversal potential of approximately –75 mV. Both Eh and EC are predicted to be in this range. Similar results were obtained with the use of a high Cl- pipette solution, although that shifted EC from –72 mV to –13 mV. However, when EC was shifted to more positive or negative values, the reversal potential also shifted accordingly. A role for the Ca2+-mediated K+ current in generating the Cs+-independent AHP was explored by testing cells in current and voltage clamp while blocking Ik(Ca) with Cs+/Co2+-saline. In some cells, the Cs+-independent AHP and its underlying current could be completely and reversibly blocked by Cs+/Co2+-saline, whereas in other cells some fraction of it remained. This indicates that the Cs+-independent AHP results from two K+ currents, one that requires an influx of extracellular Ca2+ and one that does not. Collectively, these findings indicate that AHPs result from three phenomena that occur when lobster ORNs are depolarized: 1) inactivation of the hyperpolarization-activated cation current, 2) activation of a Ca2+-mediated K+ current, and 3) activation of a K+ current that does not require influx of extracellular Ca2+. Roles of these processes in modulating the output of lobster ORNs are discussed.

INTRODUCTION

Many neurons exhibit the phenomenon of spike-frequency adaptation in which action potential frequency decreases despite a tonically maintained depolarizing stimulus (Hille 1992). Adaptation can result from a wide variety of physiological mechanisms, and often several such mechanisms operate simultaneously in a particular neuron. In many cases these mechanisms cause an outward current to develop slowly during a spike train, and it is this hyperpolarizing influence that causes spike frequency to decrease. After removal of the depolarizing stimulus the outward current generally persists and is manifest as an afterhyperpolarization (AHP; also termed posttetanic hyperpolarization) (Hille 1992). If one is interested in determining factors contributing to adaptation in a particular neuron, and one observes AHPs following spike trains in that neuron, then a reasonable approach to studying the mechanism of adaptation is to study the mechanism underlying the AHP.

Lobster olfactory receptor neurons (ORNs) display AHPs (Michel and Ache 1994) and adaptation (Gomez and Atema 1996a); however, the mechanisms responsible for these processes are largely undetermined. In many cells AHPs result from increased activity of an electrogenic Na+-pump (Edman et al. 1987; French 1989; Gestrelius and Grampp 1983; Jansen and Nicholls 1973; Morita et al. 1993; Parker et al. 1996; Thomas 1972). Tetanic firing is accompanied by Na+ influx that increases pump activity and hyperpolarizes the cell, thus causing adaptation. After spiking is terminated the cell exhibits an AHP until the excess Na+ is removed. AHPs can also be brought about by an increased permeability of the cell to an ion, such as K+, with an equilibrium potential negative to rest. This process requires slow kinetics to reduce firing frequency over a period of several seconds and to generate an AHP that may also last several seconds. In some cases the K+ channels themselves display slow kinetics (Marriion 1997). In others the K+ channels are gated by intracellular Ca2+ (Rudy 1988; Sah 1996), which accumulates slowly during firing and is removed slowly afterward. Finally, in some cells a depolarizing cation conductance (Ih) is active at rest, deactivates during the spike train, and then activates slowly after the spike train ends, allowing the cell to be transiently hyperpolarized before the current fully reacts (McCormick and Pape 1990; Womble and Moises 1993). Here we show that in lobster ORNs, AHPs result from Ih(Ca), Ih, and a slow K+ current that was not observed previously in these cells. Collectively these currents are likely to modulate spike activity in lobster cells in part by bringing about adaptation. Adaptation could influence a lobster’s perception of an odor and may affect the animal’s ability to locate the source of an odor (Gomez et al. 1994; Gomez and Atema 1996a,b; Voigt and Atema 1990).

METHODS

Adult lobsters (Panulirus argus) were obtained from the Florida Keys and maintained in 300-l aquaria with recirculating artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH). Animals were fed a diet consisting of fish, shrimp, and squid. The olfactory organ of the lobster is the lateral filament of the first antenna (Ache...
and Derby 1985). This appendage was excised from the animal and cut into pieces 1–2 mm in length. Pieces were then hemisected longitudinally to expose the somata of the olfactory receptor cells. Hemisections were treated enzymatically first with papain (0.25 mg/ml, activated by L-cysteine) for 20 min followed by trypsin (1 mg/ml) for 20 min in Cs\(^{2+}\)-free lobster saline. Hemisections were kept refrigerated in lobster saline until use.

Experiments were performed by placing a hemisected into a two-compartment recording chamber designed to allow rapid application of odorants to the chemosensory dendrites while maintaining the neuronal somata in a separate chamber (Michel et al. 1991). Here, however, the chamber was used mostly for convenience because most experiments did not involve odor application. Whole cell current and voltage-clamp records were made with the use of patch pipettes fashioned from borosilicate glass pulled and polished to a tip diameter of \(\sim 1 \text{ \(\mu\)m}\) and coated with silicone elastomer (Sylgard).

These cells are normally depolarized in the whole cell current-clamp configuration, presumably because they are so small that the seal resistance provides a significant path for ion flow across the membrane, so in most current-clamp experiments sufficient negative current was injected to hold the cells at approximately \(-65 \text{ mV}\). Data were acquired with the use of the Axopatch 200 amplifier (Axon Instruments, Foster City, CA), digitized on-line, and stored on an IBM compatible 486 computer by using pClamp software (Axon Instruments). Data were analyzed primarily with Webfoot software (Salt Lake City, UT) with some analyses performed with Quattro (Borland International, Scotts Valley, CA).

Because the currents studied in voltage clamp were very small in magnitude, series resistance compensation was not applied.

In some cases, the nystatin perforated-patch recording technique was used to minimize perturbation of the cytosolic environment (Horn and Marty 1988). Nystatin forms nonspecific monovalent ion channels, thus allowing electrical access to the cell while minimizing perturbation of its internal environment. Nystatin pipette solution was made by adding 3 \(\mu\)l of nystatin stock solution (1 mg nystatin in 20 \(\mu\)l of dimethyl sulfoxide) and 3 \(\mu\)l of 25% pluronic acid to 1 ml of normal pipette solution. The resulting nystatin pipette solution was briefly sonicated and used within 2 h. By using this method series resistance sometimes exceeded 100 M\(\Omega\), but useful recordings were possible anyway.

The composition of the standard bath saline was as follows (in mM): 457.7 NaCl, 13.4 KCl, 13.6 CaCl\(_2\), 9.8 MgCl\(_2\), 14.1 NaSO\(_4\), 3 N\(_2\)-hydroxyethylpiperazine - N' - 2 - hydroxyethylpiperazine - N'-2 - ethanesulfonic acid (HEPES), 1.9 glucose, and 1.2 NaOH. Saline with 5 mM CsCl was made by adding CsCl to the above. Cs/Co saline was prepared by replacing all CaCl\(_2\) in the regular Cs\(^{+}\) saline with 4 or 8 mM CoCl\(_2\), and enough MgCl\(_2\) to keep the total concentration of divalent cations constant. High and low K\(^{+}\)/Cs\(^{+}\) salines were prepared with the use of 67 and 2.7 mM KCl, respectively, in Cs\(^{+}\) saline with 404.1 and 468.7 mM NaCl to maintain normal osmolality. The composition of the normal pipette solution was as follows (in mM): 30 NaCl, 11 ethylene glycol-bis(\(\beta\)-aminoethyl ether)\(-N,N,N',N'\)-tetraacetic acid (EGTA), 10 N\(_2\)-hydroxyethylpiperazine-N' - 2 - ethanesulfonic acid (HEPES), 2 CaCl\(_2\), 180 K-acetate, 466.3 glucose, and 23.3 KOH. The high Cl\(^{-}\)-pipette solution contained 172.6 mM KCl instead of K-acetate and 30.7 mM KOH. Nominall Ca\(^{2+}\)-free pipette solution was identical to normal pipette solution except that it contained no CaCl\(_2\) and only 1 mM EGTA. Liquid junction potentials were measured with the method of Neher (1992), and all voltage measurements were corrected for these potentials (Barry and Lynch 1991).

Equilibrium potentials were calculated by using concentration of the concentration was <100 mM and activity when concentration was >100 mM. Weast (1964) shows values of ion activity coefficients but only for certain concentrations, so it was necessary to interpolate between his published values. This was accomplished by fitting the data of Weast (1964) to fourth-power polynomial equations (which provided an excellent fit) and with the use of those equations to predict the activity coefficients that corresponded to particular ion concentrations. Even so, the resulting values were only approximations because the activity of an ion depends on the other ions present (Vaughan-Jones and Aiken 1995). Results of these experiments were previously reported in abstract form (Corotto and Michel 1995).

**RESULTS**

Lobster ORNs respond to excitatory odors with bursts of action potentials followed by prolonged AHPs on removal of the odorant (Fig. 1). AHPs could also be elicited through injection of depolarizing current (Fig. 2A), so further studies were performed with the use of this method. A portion of the AHP could be reversibly blocked by application of 5 mM CsCl, but a small component remained (Fig. 2). In lobster ORNs, application of 5 mM CsCl blocks a hyperpolarization-activated cation current (\(I_h\)) (Corotto and Michel 1994); therefore the Cs\(^{+}\)-sensitive component of the AHP results from this current. Presumably \(I_h\) is active at rest, where it maintains a somewhat depolarized resting potential. The current is deactivated by further depolarization, and when that depolarization is removed \(I_h\) would allow a brief hyperpolarization before activating again (see Discussion).

The magnitudes of the Cs\(^{+}\)-sensitive and Cs\(^{+}\)-insensitive components depend both on the duration of a depolarizing stimulus and on its magnitude. Depolarizations evoked by 0.5 s injection of 40 pA elicited Cs\(^{+}\)-sensitive AHPs attributed to deactivation of \(I_h\) but were of insufficient duration to elicit Cs\(^{+}\)-insensitive AHPs (Fig. 3A). When the duration

![FIG. 1. Current-clamp records showing a lobster olfactory receptor neuron (ORN) responding to 0.5-s pulses of a complex odor mixture delivered at 2 frequencies. Bars below the traces correspond to periods of odor presentation. Dotted lines show the resting membrane potential before odor stimulation. Action potentials may be truncated because the data were acquired at a slow acquisition frequency (100 Hz). The odor mixture was a 1,000-fold dilution of a stock solution of a fish food extract (Tetramarin) prepared as previously described (Schmiedel-Jakob et al. 1990).](http://jn.physiology.org/ftp/jn/2008/09968/fig1.gif)
cell resistance) (Barry and Lynch 1991) was determined by measuring the magnitude of the hyperpolarizations evoked by these test pulses and applying Ohm’s law. Thirty-six cells were tested and Cs⁺-insensitive AHPs were detected in 22 of those cells. In those 22 cells \( R_{app} \) dropped from an average of 2.8 ± 1.4 (SD) GΩ before the depolarizing current to an average of 1.4 ± 0.5 GΩ at the peak of the Cs⁺-insensitive AHP and then returned to normal as the magnitude of the Cs⁺-insensitive AHP decreased (see Fig. 4, A and B). Cs⁺-insensitive AHPs and decreases in \( R_{app} \) were observed regardless of whether the depolarization evoked high-frequency full-sized spikes (Fig. 4A), high-frequency spikes that decreased in amplitude, or just small oscillations (Fig. 4B). Of the 14 cells that did not display a measurable Cs⁺-insensitive AHP, three exhibited slow afterdepolarizations (ADPs), and two of these three cells displayed reduced \( R_{app} \) during the ADPs (Fig. 4C). Eleven cells failed to exhibit a measurable Cs⁺-insensitive AHP or ADP, and of these 11 cells 3 displayed measurable decreases in \( R_{app} \) after the depolarizing pulse.

If the Cs⁺-insensitive component of the AHP results from an increased conductance, then the magnitude (and polarity) of the Cs⁺-insensitive AHP should depend on the membrane conductance change(s) caused by the test pulse(s).

Fig. 2. Current-clamp records showing responses of 1 representative lobster ORN to 40 pA of depolarizing current maintained for 5 s. A: bath solution is normal saline. B: normal saline was replaced by Cs⁺ saline. C: Cs⁺ saline was subsequently replaced by normal saline. The initial action potentials are truncated.

Of stimulus application was extended to 5 s both Cs⁺-sensitive and -insensitive components were observed.

With 5-s pulses, as little as 6 pA of current was sufficient to elicit both Cs⁺-sensitive and -insensitive AHPs (Fig. 3B). Larger depolarizing currents evoked larger AHPs, but this was mostly caused by an increased magnitude of the Cs⁺-sensitive component. Although smaller, the magnitude of the Cs⁺-insensitive AHP also depended on the amount of current injected.

To determine if the Cs⁺-insensitive component of the AHP was accompanied by a change in input resistance, cells were bathed in Cs⁺ saline, and short pulses of hyperpolarizing current (–6 or –10 pA) were applied before and after 5-s applications of 40-pA depolarizing current. Apparent input resistance (\( R_{app} \), seal resistance in parallel with whole-cell resistance) (Barry and Lynch 1991) was determined by measuring the magnitude of the hyperpolarizations evoked by these test pulses and applying Ohm’s law. Thirty-six cells were tested and Cs⁺-insensitive AHPs were detected in 22 of those cells. In those 22 cells \( R_{app} \) dropped from an average of 2.8 ± 1.4 (SD) GΩ before the depolarizing current to an average of 1.4 ± 0.5 GΩ at the peak of the Cs⁺-insensitive AHP and then returned to normal as the magnitude of the Cs⁺-insensitive AHP decreased (see Fig. 4, A and B). Cs⁺-insensitive AHPs and decreases in \( R_{app} \) were observed regardless of whether the depolarization evoked high-frequency full-sized spikes (Fig. 4A), high-frequency spikes that decreased in amplitude, or just small oscillations (Fig. 4B). Of the 14 cells that did not display a measurable Cs⁺-insensitive AHP, three exhibited slow afterdepolarizations (ADPs), and two of these three cells displayed reduced \( R_{app} \) during the ADPs (Fig. 4C). Eleven cells failed to exhibit a measurable Cs⁺-insensitive AHP or ADP, and of these 11 cells 3 displayed measurable decreases in \( R_{app} \) after the depolarizing pulse.

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potential, i.e., it should show a reversal potential. This was tested by applying different holding currents and therefore shifting the membrane potential, both before and after applying the depolarizing current. Thus afterpotentials could be imposed on a range of different “resting potentials” (Fig. 5, A–C). When data from 10 cells are plotted together it is evident that cells displayed Cs\(^+\)-insensitive AHPs at normal membrane potentials (−55 to −65 mV), very small AHPs or ADPs in the region of −70 mV, and ADPs at membrane potentials more negative than −75 mV (Fig. 5D). Seven of these cells were tested with the high Cl\(^-\)-pipette solution, which set \(E_K\) to −72 mV and \(E_{Cl}\) to −13 mV. Three of the cells were tested with the normal pipette solution, which set \(E_K\) to −73 mV and \(E_{Cl}\) to −62 mV. Results from both pipette solutions were similar. Linear regression was performed on data from each cell individually to determine each cell’s reversal potential. The average reversal potential of the cells with the normal pipette solution was −75 mV, whereas the average reversal potential for the cells with the high Cl\(^-\)-pipette solution was −66 mV (Table 1). These data indicate that the Cs\(^+\)-insensitive AHP is predominately a result of an increased conductance to K\(^+\).

Because normal spiking was not necessary to observe Cs\(^+\)-insensitive AHPs (e.g., Fig. 4B), it was possible to study the current underlying the Cs\(^+\)-insensitive AHPs in voltage clamp with the use of a tail current protocol (Fig. 5).

**FIG. 5.** A–C: current-clamp record of a cell subjected to injection of 40 pA of current superimposed on different holding currents used to manipulate membrane potential. The initial action potentials are truncated. D: magnitude of AHP or ADP is plotted as a function of membrane potential before injection of depolarizing current.

Tail current traces were fitted to a single exponential function and the initial magnitude of a tail current was determined by predicting the value at its onset and comparing that with either the baseline leak current predicted by the exponential function or the leak currents measured before the 5-s depolarizing step. With Cs\(^+\) saline and the normal pipette solution the average reversal potential of the tail currents was −73 mV (Table 1). When \(E_K\) was shifted to

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**FIG. 4.** Current-clamp records showing the responses of representative lobster ORNs to injection of hyperpolarizing test pulses before and after a 5-s injection of 40 pA of depolarizing current. A: in this cell the depolarization is followed by a Cs\(^+\)-insensitive AHP and a decrease in \(R_{app}\) from 2.9 G\(\Omega\) before the depolarization to 1.6 G\(\Omega\) afterward. Input resistance returned to normal as the Cs\(^+\)-insensitive AHP diminished in amplitude. Conditions: Cs\(^+\) saline on soma, normal pipette solution, perforated-patch technique. B: this cell also shows a Cs\(^+\)-insensitive AHP and decrease in \(R_{app}\) (from 3.5 to 1.2 G\(\Omega\)) but in this case the cell failed to fire action potentials during the depolarization, possibly because of the loss of axon during dissection. Conditions: Cs\(^+\) saline on soma, normal pipette solution, perforated-patch technique. C: this cell shows an afterdepolarizations (ADP) accompanied by a decrease in \(R_{app}\) from 3.6 to 2.3 G\(\Omega\). Conditions: Cs\(^+\) saline on soma, nominally Ca\(^{2+}\)-free pipette solution.
−33 mV with the use of high-K⁺ Cs⁺ saline, the reversal potential was also shifted in the positive direction (Fig. 6 and Table 1). When $E_H$ was shifted to −114 mV with the use of low-K⁺ Cs⁺ saline the reversal potential was shifted in the negative direction (Fig. 6 and Table 1). In contrast, the reversal potential was only slightly affected by setting $E_C$ to −13 mV with the use of the high Cl⁻-pipette solution (Table 1).

Current and voltage-clamp results taken together indicate that the Cs⁺-insensitive AHP results from a transient increase in K⁺ conductance. Reversal potentials were slightly more positive when $E_C$ was set to −13 mV (Table 1), indicating the possible involvement of an increased Cl⁻ conductance as well, although its contribution to the Cs⁺-insensitive AHP would be much smaller than the increased K⁺ conductance.

A Ca²⁺-mediated K⁺ current was described previously in lobster ORNs (McClintock and Ache 1989), so further experiments were aimed at determining whether the increased K⁺ conductance required an influx of extracellular Ca²⁺. This was accomplished by blocking both $I_{C(A)}$ and $I_{K(Ca)}$ with Cs⁺/Co²⁺ saline because Co²⁺ can block both of these currents (McClintock and Ache 1989; personal observations). Changes in $R_{app}$ were measured in four cells first bathed in Cs⁺ saline, then bathed in Cs⁺/Co²⁺ saline, and then returned to Cs⁺ saline or first bathed in Cs⁺/Co²⁺ saline, then Cs⁺ saline, and then Cs⁺/Co²⁺ saline again. In Cs⁺ saline all four of these cells exhibited drops in $R_{app}$ after injection of depolarizing current, and three of them displayed Cs⁺-insensitive AHPs. Cobalt blocked almost all of the decrease in $R_{app}$ in three of these cells (Fig. 7), whereas the fourth cell was unaffected. AHPs were present in two of the three cells in which Co²⁺ reduced the drop in $R_{app}$, and in these two cells the Cs⁺-insensitive AHPs were also reversibly reduced by Co²⁺ treatment. When one examines the changes in $R_{app}$ that occur during AHP in cells held in Cs⁺ saline (47.5 ± 3.4% decrease, SE, $n = 23$) and compares them with changes in $R_{app}$ in cells held in Cs⁺/Co²⁺ saline (21.1 ± 5.1% decrease, SE, $n = 13$), it is clear that the decrease in $R_{app}$ is suppressed but not always eliminated by the presence of Co²⁺. These results demonstrate that both $I_{K(Ca)}$ and another K⁺ current, which has slow kinetics and does not require influx of Ca²⁺, are involved in generating the Cs⁺-insensitive AHP.

Cells were also tested in voltage clamp to see whether there were reversible effects of Cs⁺/Co²⁺ saline on the tail currents that underlie the Cs⁺-insensitive AHP. Two cells were first tested in Cs⁺ saline, then Cs⁺/Co²⁺ saline, and then Cs⁺ saline again. A third cell was tested in Cs⁺/Co²⁺ saline, then Cs⁺ saline, and then Cs⁺/Co²⁺ saline again. In two of these three cells, tail currents were blocked almost completely by Cs⁺/Co²⁺ saline with only tiny tail currents remaining (Fig. 8C). In the third cell Cs⁺/Co²⁺ saline completely blocked the tail currents (Fig. 8, A and B).

**Discussion**

Prevalence of AHPs in lobster olfactory receptor neurons

AHPs appear to be a common feature in the physiology of lobster ORNs. Nearly all lobster ORNs possess $I_h$ (see the prevalence of depolarizing sags described in the results of Corotto and Michel 1994), and it was found here that $I_h$ leads to AHPs. Also, in this study, when 36 cells were examined for changes in input resistance after tetanic firing, and Cs⁺ saline was applied to block $I_h$, 22 displayed Cs⁺-insensitive AHPs. These findings, in conjunction with their frequent observation after odor-induced excitation (Fig. 1) (personal observations) indicate that AHPs are common in lobster ORNs.

Mechanisms of AHPs and adaptation

AHPs in lobster ORNs appear to result from three processes that occur during depolarization: 1) deactivation of $I_h$, 2) activation of $I_{K(Ca)}$, 3) and activation of a tiny, slow K⁺ current that does not require influx of extracellular Ca²⁺. The hyperpolarization-activated cation current is likely to be active at rest, where it provides a constant, depolarizing influence (Corotto and Michel 1994). An excitatory odorant would depolarize the cell and deactivate $I_h$. When excitation ceases the cell would be briefly hyperpolarized because $I_h$ first remains deactivated and its depolarizing influence is removed. Then as $I_h$ slowly activates, the membrane potential would return to its more positive resting value. A hyperpolarization-activated cation current was previously implicated in generating AHPs in thalamocortical relay neurons (McCormick and Pape 1990) and basolateral neurons in the amygdala (Womble and Moises 1993).

Spike-frequency adaptation and AHPs commonly result from the activity of $I_{K(Ca)}$ (Rudy 1988; Sah 1996). This current was described previously in lobster ORNs (McClintock and Ache 1989), but its role in generating AHPs or adaptation was not explored in these cells. One can argue that our normal pipette solution should interfere with normal intracellular Ca²⁺ dynamics; the EGTA could clamp the concentration of intracellular Ca²⁺ at some fixed level. However, we used a variety of recording configurations, including

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<td>External</td>
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Values are means ± SD for number of cells tested. All cells were tested with 5 mM Cs⁺ bathing the soma to block $I_h$. |
a nominally Ca$^{2+}$-free pipette solution, which should allow
free Ca$^{2+}$ concentrations to fluctuate, and the perforated-
patch technique (Fig. 4 B), which should leave intracellular
Ca$^{2+}$ dynamics unperturbed, and similar results were
obtained regardless of the method employed. EGTA is a rela-
tively slow buffer (Hille 1992) and may not affect rapidly
changing Ca$^{2+}$ concentrations in the vicinity of the mem-
brane where Ca$^{2+}$ channels and Ca$^{2+}$-mediated K$^+$ channels
are located. The perforated-patch technique provides the
closest approximation to the in vivo situation, and the fact
that results obtained with this technique were similar to those
obtained with the use of other recording methods argues
strongly that the processes described here also occur in vivo.

A slowly activating K$^+$ current that does not require in-
flux of Ca$^{2+}$ was not described previously in lobster ORNs. This
current is very small, and its tails were barely discernable
in voltage clamp (Fig. 8 C). However, because lobster ORNs have
high resting $R_{\text{app}}$ values, its influence is obvious in
current clamp (Fig. 7 B). Rat ORNs possess a similar cur-
rent, and it may contribute to AHPs and spike-frequency
adaptation in those cells (Lynch and Barry 1991). Similar
currents are not uncommon. Perhaps the most thoroughly
studied example of a slow, Ca$^{2+}$-independent K$^+$ current is
the M current found in the mammalian central and sympa-
thetic nervous systems (Adams 1982; Brown 1983, 1988;
Marrion 1997). Another possible explanation of the results presented here
is that the slow K$^+$ current in lobster ORNs may result from

FIG. 6. Reversal potential for the depolarization-activated currents contribut-
ing to AHPs follows the potassium equilibrium potential. Cells were subjected
to the protocol illustrated. Tail currents were recorded during the 4-s step to a
test potential that followed the 5-s step to −1 mV. A: tail currents recorded from
a cell exposed to the normal extracellular K$^+$ concentration. B: tail currents
recorded from the same cell as in A after the bath was switched to high K$^+$
saline. C: tail currents recorded from a different cell exposed to the normal
concentration of extracellular K$^+$. D: tail currents recorded from the same cell
as in C but after the bath was switched to low K$^+$ saline.

FIG. 7. Current-clamp records of a cell subjected to −6-pA hyperpolar-
izing test pulses before and after injection of 40 pA of depolarizing current.
Action potentials are truncated. A: Cs$^+$ saline is present on soma and
dendrites. The depolarization was followed by a 6-m V Cs$^+$-insensitive AHP
and a 50% drop in $R_{\text{app}}$. B: soma compartment was changed to Cs$^+$/Co$^{2+}$
saline whereas the solution covering the dendrites remained the same. Depo-
larization was followed by a 4-m V Cs$^+$-insensitive AHP and a 25% drop
in $R_{\text{app}}$. C: Cs$^+$/Co$^{2+}$ saline was removed from the soma compartment
and replaced with Cs$^+$ saline. The depolarization was followed by a 7-m V Cs$^+$-
insensitive AHP and a 44% drop in $R_{\text{app}}$. Data were obtained with the
perforated-patch technique.
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AHPs to be generated (Fig. 4B) and because tails of this current were observed in voltage clamp where spikes were prevented (Fig. 8C). A third possibility, not investigated here, is that prolonged depolarization causes release of Ca²⁺ from intracellular stores and this activates $I_{K(Ca)}$ without Ca²⁺ influx.

In many systems, spike-frequency adaptation and AHPs are caused by increased activity of an electrogenic Na⁺ pump (Edman et al. 1987; French 1989; Gestrelius and Grampp 1983; Jansen and Nicholls 1973; Morita et al. 1993; Parker et al. 1996; Thomas 1972). However, if increased pump activity generated AHPs in lobster ORNs they would not be accompanied by decreased $R_{app}$, would not show a reversal potential in voltage clamp, and would not show the equivalent of a reversal potential in current clamp. Therefore it is possible to explain the AHPs described here without having to invoke a role of the Na⁺ pump.

In a number of systems, Na Channel inactivation plays a role in spike-frequency adaptation (Basarsky and French 1991; Edmen et al. 1987; French 1987; Gestrelius and Grampp 1983). This possibility was not directly addressed in the current study. Two observations hint that Na⁺ channel inactivation may be important in adaptation of lobster ORNs. First, spike-frequency adaptation is accompanied by a decrease in spike amplitude, suggesting that some Na⁺ channels fail to recover from inactivation after each spike. Second, we occasionally observed adaptation in cells that do not display AHPs or decreases in $R_{app}$.

Although some cells failed to exhibit AHPs some exhibited slow, depolarizing afterpotentials (ADPs) accompanied by decreases in $R_{app}$. Although some cells failed to exhibit AHPs some exhibited slow, depolarizing afterpotentials (ADPs) accompanied by decreases in $R_{app}$. This occurred at membrane potentials positive of $E_K$ so the ADPs did not result from a “reversal” of AHPs. The occurrence of ADPs may explain the handful of cells described here that exhibited decreased $R_{app}$ after tetanic firing but not AHPs or ADPs. Such cells may possess AHPs and ADPs superimposed on one another. Interestingly, a Na⁺-gated cation channel was recently described in lobster ORNs (Zhairazov and Ache 1997), and such a channel could account for ADPs in these cells. These channels are found on the outer dendrites of lobster ORNs where they are thought to amplify the primary receptor current (Zhairazov et al. 1997).

Effects of AHPs on spiking

AHPs reflect hyperpolarizing influences that develop during tetanic firing, and such influences are likely to cause spike-frequency adaptation. When Cs⁺ is applied to lobster ORNs to block $I_h$ there is no apparent effect on adaptation (unpublished observations). Deactivation of $I_h$ during a spike train would tend to hyperpolarize the cell and thus should reduce firing frequency. However, deactivation of $I_h$ would also increase $R_{app}$, making the cell more responsive to stimuli. Thus the effect of $I_h$ deactivation during tetanic firing may be modest because of the opposing influences of hyperpolarization and increased input resistance.

Although $I_h$ apparently does not profoundly affect firing frequency, it is likely that $I_{K(Ca)}$ and the slow, Ca²⁺-independent K⁺ current do. These hyperpolarizing currents activate during spike trains and are accompanied by decreased $R_{app}$, not increased $R_{app}$ as is the case with $I_h$. With $I_h$ blocked by

![Fig. 8. A: tail currents of a cell held in Cs⁺ saline and subjected to the protocol illustrated. B: same cell as in A, but with Cs⁺/Co²⁺ saline covering its soma. The tail currents block was reversible (not shown). C: different cell held in Cs⁺/Co²⁺ saline (soma and dendrites) in which very small tail currents remain. In all traces data were smoothed by performing a running 11-point average on the data.](http://jn.physiology.org/DownloadedFrom)
$R_{\text{app}}$ can drop very dramatically during high-frequency spiking (Fig. 4A). Determining accurate values of whole cell resistance is difficult in lobster ORNs because the cells are quite small, so seal resistance contributes significantly to its measured value (Barry and Lynch 1991). Cells with higher $R_{\text{app}}$ values presumably have tighter seals, and recordings from such cells would presumably provide the most accurate approximation of the situation in vivo. It is notable therefore that the cells with the highest initial $R_{\text{app}}$ were the cells that exhibited the greatest drops in $R_{\text{app}}$ during the AHP. For example, a cell with a $R_{\text{app}}$ of 7.6 GΩ before being depolarized displayed an $R_{\text{app}}$ of only 1.7 GΩ immediately afterward. Of course this applies to cells with $I_h$ blocked. Without blocking $I_h$ one would expect that $I_h$ would deactivate during a spike train while $K^+$ currents activate. The overall effect would be the development of a hyperpolarization that causes decreased spike frequency and that is accompanied by little change in input resistance.

It is easy to study $I_h$ in lobster ORNs because 5 mM CsCl blocks this current specifically (Corotto and Michel 1994) and appears to have no other confounding effects. Thus it was possible to perform experiments in which $I_h$ was blocked and no obvious effect on firing was observed (unpublished observations). Unfortunately, the other two currents implicated in generating the AHP are not so easy to investigate. Certain classes of Ca$^{2+}$-mediated $K^+$ channels can be blocked by charybdotoxin or apamin, but when $I_{K(Ca)}$ was isolated in lobster ORNs with the use of the method of McClintock and Ache (1989), and these drugs were applied at 500 nM, $I_{K(Ca)}$ persisted undiminished (data not shown). This is not surprising because the particular channels responsible for the slowest AHPs are not sensitive to these drugs (Sah 1996). Blocking $I_{K(Ca)}$ by substituting Ba$^{2+}$ or Sr$^{2+}$ for Ca$^{2+}$ caused membrane conductance to increase ($R_{\text{app}}$ dropped; data not shown), and this in itself would tend to reduce the effects of other conductances, making it impossible to examine the role of $I_{K(Ca)}$ in adaptation with the use of this approach. Blocking $I_{K(Ca)}$ with Co$^{2+}$ saline caused spike amplitude to rapidly shrink during current injection until spikes failed to be generated (not shown but traces resembled Fig. 2C where it is likely that $I_{Ca}$ ‘washed out’). Thus it was not possible to explore the role of $I_{K(Ca)}$ in spike-frequency adaptation because blocking the current profoundly perturbed the normal firing pattern.

**Possible roles of AHSs in neural coding**

AHPs not only reflect processes that may underlie spike-frequency adaptation but may themselves carry important information. For example, in spontaneously active lobster ORNs removal of excitatory odorants is usually accompanied by a short period of time in which spontaneous activity is suppressed (see Fig. 1A in Michel and Ache 1994). This is presumably a result of the AHP, and this suppressed spontaneous activity could constitute a kind of ‘OFF response’ that could provide enhanced information regarding odorant removal. AHPs appear to serve a similar role in the crayfish stretch receptor (Nakajima and Takahashi 1966).

Another case in which AHPs may be important in themselves would be if a lobster ORN were rapidly stimulated with odor pulses that may occur during normal antennule flicking behavior. If a cell were exposed to an odor pulse during an AHP, its response to that odor would presumably be attenuated. This could limit the ability of lobster ORNs to resolve the complex three-dimensional structure of odor plumes, and resolution of that structure may be important in determining the direction and distance of an odor’s source. The ability of lobster ORNs to follow a rapid sequence of odor pulses was explored by Atema and co-workers (Gomez et al. 1994; Voigt and Atema 1990). They find that abilities vary from cell to cell, but in general when a cell is exposed to 100-ms odor pulses at a frequency $>2-4$ Hz, the electrophysiological response cannot remain synchronized with the odor pulses (Gomez et al. 1994). It may be that lobster ORNs are unable to resolve high-frequency application of odorant pulses because these pulses are applied while the cells are exhibiting AHPs. Because it may not be possible to selectively block all of the processes that lead to AHPs it may not be possible to directly determine the roles of these processes in shaping the output of lobster ORNs. Mathematical modeling of lobster ORNs could, however, prove quite helpful in suggesting roles for AHPs and the processes that lead to AHPs in modulating the output of these cells.

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