Hyperpolarization-Activated, Mixed-Cation Current ($I_h$) in Octopus Cells of the Mammalian Cochlear Nucleus

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Bal, Ramazan and Donata Oertel. Hyperpolarization-activated, mixed-cation current ($I_h$) in octopus cells of the mammalian cochlear nucleus. J Neurophysiol 84: 806–817, 2000. Octopus cells in the posteroventral cochlear nucleus of mammals detect the coincidence of synchronous firing in populations of auditory nerve fibers and convey the timing of that coincidence with great temporal precision. Earlier recordings in current clamp have shown that two conductances contribute to the low input resistance and therefore to the ability of octopus cells to encode timing precisely, a low-threshold $K^+$ conductance and a hyperpolarization-activated mixed-cation conductance, $g_h$. The present experiments describe the properties of $g_h$ in octopus cells as they are revealed under voltage clamp with whole-cell, patch recordings. The hyperpolarization-activated current, $I_h$, was blocked by extracellular Cs$^+$ (5 mM) and 4-($N$-ethyl-$N$-phenylamino)-1,2-dimethyl-6-(methylamino) pyridinium chloride (50–100 nM) but not by extracellular Ba$^{2+}$ (2 mM). The reversal potential for $I_h$ in octopus cells under normal physiological conditions was $–38$ mV. Increasing the extracellular potassium concentration from 3 to 12 mM shifted the reversal potential to $–26$ mV; lowering extracellular sodium concentration from 138 to 10 mM shifted the reversal potential to $–77$ mV. These pharmacological and ion substitution experiments show that $I_h$ in octopus cells is a mixed-cation current that resembles $I_h$ in other neurons and in heart muscle cells. Under control conditions when cells were perfused intracellularly with ATP and GTP, $I_h$ had an activation threshold between about $–35$ to $–40$ mV and became fully activated at $–110$ mV. The maximum conductance associated with hyperpolarization voltage steps to $–112$ mV ranged from 87 to 212 nS [150 ± 30 (SD) nS, n = 36]. The voltage dependence of $g_h$ obtained from peak tail currents is fit by a Boltzmann function with a half-activation potential of $–65 ± 3$ mV and a slope factor of 7.7 ± 0.7. This relationship reveals that $g_h$ was activated 41% at the mean resting potential of octopus cells, $–62$ mV, and that at rest $I_h$ contributes a steady inward current of between 0.9 and 2.1 nA. The voltage dependence of $g_h$ was unaffected by the extracellular application of dibutyryl cAMP but was shifted in hyperpolarizing direction, independent of the presence or absence of dibutyryl cAMP, by the removal of intracellular ATP and GTP.

Introduction

In all mammals, octopus cells occupy a distinct region of the dorsal and caudal posteroventral cochlear nucleus, a region in which there is no apparent intermingling with the other cells and which is particularly prominent in humans (Adams 1997; Brawer et al. 1974; Disterhoft 1980; Golding et al. 1995, 1999; Hackney et al. 1990; Morest et al. 1990; Oertel et al. 1990; Osen 1969; Wickesberg et al. 1991, 1994; Willott and Bross 1990). In mice there are $–200$ octopus cells in each cochlear nucleus (Willott and Bross 1990). Each octopus cell has several large, scantily branched dendrites that emerge from the rostral side of the cell body (Osen 1969). The dendrites spread perpendicularly across the paths of auditory nerve fibers in the dorsocaudal posteroventral cochlear nucleus where the tonotopic array of fibers is closely bundled (Golding et al. 1995; Hackney et al. 1990; Oertel et al. 1990; Willott and Bross 1990). A relatively large number of auditory nerve fibers, more than 60 in mice, excite octopus cells through small, bouton terminals (Golding et al. 1995; Willott and Bross 1990). Octopus cells form one of the major ascending pathways from the ventral cochlear nucleus, projecting through the intermediate acoustic stria to terminate contralaterally in the superior parvocellular nucleus and in the ventral nucleus of the lateral lemniscus (Adams 1997; Schofield 1995; Schofield and Cant 1997). The projection to spherical bushy neurons in the ventral nucleus of the lateral lemniscus from octopus cells is through large, calyceal terminals (Adams 1997; Schofield and Cant 1997; Smith et al. 1993; Vater et al. 1997; Warr 1969).

As the anatomical organization suggests, octopus cells are broadly tuned and are relatively insensitive to pure tones in vivo (Godfrey et al. 1975; Rhode and Smith 1986). Octopus cells respond with sharply timed action potentials to clicks, to the onset of tones, and to periodic sounds (Friauf and Ostwald 1988; Godfrey et al. 1975; Rhode and Smith 1986; Rhode et al. 1983; Smith et al. 1993). Timing of the action potential at the onset of the stimulus is sharper than that of any other units in the auditory system (Rhode and Smith 1986). In response to click trains or loud tones at low frequencies, octopus cells can follow with one action potential at every cycle $\leq 1$ kHz (Rhode and Smith 1986).

The unusual biophysical characteristics of octopus cells enable them to detect coincident activation of auditory nerve fibers and convey the timing of that coincidence with temporal fidelity. Current-clamp experiments revealed that two conductances, $g_h$ and a low-threshold potassium conductance, $g_{KDL}$, each of which is partly activated at the resting potential, help to determine how octopus cells fire in response to synaptic activation of the auditory nerve (Golding et al. 1995, 1999). These conductances contribute to the low input resistance and therefore to the ability of octopus cells to encode timing precisely (Bal, Ramazan and Donata Oertel 1995, 1999).
conductances contribute to the resting low input resistance, 6.7 MΩ, which gives octopus cells short time constants (about 200 μs) and short integration times (~1 ms). They also prevent firing in response to asynchronous synaptic inputs (Ferragamo and Oertel 1998). In the present study we describe gₘ quantitatively under voltage clamp. The results show that gₘ in octopus cells generally resembles gₘ in other cells in its biophysical characteristics but that the maximum conductance is unusually large.

METHODS

Intracellular whole-cell patch recordings were made from coronal slices of the brain stem containing the posteroventral cochlear nucleus from mice (ICR strain) of between 18 and 24 days after birth. Immediately after decapitation, the head was immersed in “normal” physiological saline containing (in mM) 138 NaCl, 3 KCl, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgSO₄, 20 NaHCO₃, 3 HEPES, and 10 glucose, saturated with 95% O₂-5% CO₂, pH 7.4, between 25 and 31°C (Golding et al. 1995, 1999). The whole brain was removed and cut coronally at the level of the inferior colliculi. The specimen was mounted, with the colliculus end down, with a cyanoacrylate glue (Superglue) onto a Teflon block. This block was fixed in a bath, which was filled with saline that was continuously oxygenated. Slices 180-μm thick were then cut using an oscillating tissue slicer (Frederick Haer, NewBrunswick, ME). The one to two slices containing the octopus cell area were transferred to a storage chamber containing fresh, oxygenated saline. For recording, a slice was transferred to a recording chamber with 0.3 ml volume in which it was continuously perfused at a rate of about 8 ml/min with saline whose temperature was maintained by a feedback-controlled heater at 33°C.

In many of the voltage-clamp experiments, the extracellular solution was modified to allow gₘ to be isolated. To block voltage-sensitive sodium, potassium, and cyclically activated currents, slices were bathed in a “control” solution containing (in mM) 138 NaCl, 3 KCl, 2.4 CaCl₂, 1.3 MgCl₂, 10 HEPES, 10 glucose, 2 4-aminopyridine (4-AP), 0.001 tetrodotoxin (TTX), and 0.04 6,7-dinitroquinoxaline-2,3-dione (DNQX). In some experiments, 5 mM Cs⁺, 50 mM 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyridinium chloride (ZD7288; Tocris Cookson, UK), α-dendrotoxin (DTX) (Alomone Labs, Israel), or dibutyl cAMP were added to the control solution. Tetraethylammonium chloride (TEA) at 10 mM was made by equimolar substitution for NaCl. Low-sodium solution (10 mM) was made by equimolar substitution of choline chloride for NaCl. High-potassium solution (12 mM) was made by substitution of 9 mM KCl for NaCl. Test solutions were added to the chamber by redirecting the flow of liquid through a system of tubing and valves. Except where stated otherwise, all chemicals were obtained from Sigma.

Patch electrodes (3–8 MΩ) were pulled from borosilicate glass (1.2 mm OD). The pipette solution contained (in mM) 108 potassium gluconate, 9 HEPES, 9 EGTA, 4.5 MgCl₂, 14 phosphocreatine (Tris salt), 4 ATP (Na-salt), and 0.3 GTP (Tris salt); pH was adjusted to 7.4 with KOH (Forcher and Oxford 1985). The results have been corrected for a junction potential of ~12 mV. The addition of ATP, GTP, and phosphocreatine made recordings from octopus cells stable for several hours; recordings in an earlier study using pipettes without these high-energy phosphates lasted for about 30 min (Golding et al. 1999). To assess modulation of gₘ, a modified pipette solution was used in which ATP and GTP were eliminated.

Octopus cells were recorded under visual control. They form bright holes among fascicles of heavily myelinated fibers when visualized using a Zeiss Photomikoskop with a ×63 water-immersion lens under bright-field illumination when the field diaphragm was almost completely closed. Current- and voltage-clamp data were obtained with standard whole-cell patch-clamp techniques using an Axopatch-200A amplifier. Analog records were low-pass-filtered at 5–10 kHz. Current and voltage records were sampled at 10–40 kHz using a Digidata 1200 interface (Axon Instruments, Foster City, CA), fed to a chart recorder, and stored on an IBM-compatible personal computer for further analysis. Stimulus generation, data acquisition, and off-line analysis of digitized data were performed using pClamp software (version 6.03; Axon Instruments). High-resistance seals (>1 GΩ) were obtained before going to the whole-cell configuration. Series resistance and capacitance compensation were applied on-line. Series resistance was always compensated to ≥96%; in most recordings series resistance was compensated between 98 and 99%.

Numerical results are given as means ± SD, with n being the number of cells on which the measurement was made. Significant differences between the groups were evaluated using a paired Student’s t-test.

RESULTS

Initial observations

The results presented here are based on patch-clamp recordings from 184 octopus cells. The octopus cell area, which in mice contains no other cell types, can be visualized in coronal sections in the most caudal and dorsal portion of the ventral cochlear nucleus amid the bundles of myelinated auditory nerve fibers. The identity of octopus cells was confirmed by their characteristic biophysical features (Golding et al. 1995, 1999). The cells had a mean resting potential of −61.8 ± 1.7 mV (n = 135). From a randomly chosen sample of these cells, measurements were made of input resistance, duration, and amplitude of action potential. The mean input resistance, calculated from a linear fit of the negative section of the current-voltage relationship of the peak voltage responses near rest, was found to be 6.7 ± 0.27 mΩ (n = 56). The input resistance measured as the slope of the steady-state voltage changes as a function of current was 3.8 ± 1.8 mΩ (n = 25). The mean time constant for hyperpolarizing responses close to the resting potential was 0.21 ± 0.07 ms (n = 20). The amplitude of action potentials was between 20 and 35 mV with a mean 36 ± 4 mV (n = 20). The mean duration of action potentials measured halfway between the peak and the inflection point was 0.34 ± 0.07 ms (n = 20). The small differences between the properties reported here and those from the same cells in an earlier report (Golding et al. 1999) may have arisen from the inclusion of phosphocreatine and nucleotide triphosphates in the pipette solution.

All the octopus cells tested with the injection of hyperpolarizing current exhibited a depolarizing sag of the membrane potential toward the resting value. The amplitude of the sag increased with the amplitude of hyperpolarizing current. This sag was blocked by the extracellular application of 5 mM Cs⁺ or 50 nM ZD7288 (Fig. 1). Moreover, both blockers caused the resting potential to become hyperpolarized (Cs⁺ by 4.6 ± 1.2 mV, n = 5; ZD7288 by 10.3 ± 3.1 mV, n = 12), indicating that the conductance associated with the sag is active at rest and contributes to the normal resting potential. In the presence of ZD7288, the shape of the action potential was altered. The rise of action potentials occurred more slowly and the height of action potentials measured from the inflection to the peak was higher by ~25%. The width of action potentials at their base increased from 0.31 ± 0.05 to 0.87 ± 0.7 ms. This observation also indicates that the reversal potential for this current is more...
positive than the resting potential, as expected from a mixed cationic hyperpolarization-activated current, \( I_h \) (Golding et al. 1999). The effects of Cs\(^+\) on \( I_h \) were reversible but those of ZD7288 were not. These observations demonstrate that the pharmacological characteristics of \( I_h \) in octopus cells match those of mixed-cation, hyperpolarization-activated currents in other types of neurons and muscle cells (Chen 1997; DiFrancesco 1993; Fu et al. 1997; Khakh and Henderson 1998; Maccaferri and McBain 1996; Mo and Davis 1997; Pape 1996).

**Biophysical properties under voltage clamp**

All octopus cells tested under voltage clamp (\( n = 159 \)) displayed a large, time- and voltage-dependent current when they were hyperpolarized, a current that is the manifestation in voltage clamp of the sag that was described in the preceding text in current clamp. Figure 2A illustrates a typical family of inward currents in response to hyperpolarizing voltage pulses. These inward currents had two components. Immediately after the capacitative transient, an “instantaneous” change in current, \( (I_{INS}) \) was detected. This was followed by a slowly developing inward current whose amplitude and rate of rise depended on the size of the voltage step. Figure 2B is a plot of the amplitude of instantaneous and steady-state currents as a function of voltage. The slope of the instantaneous current-voltage relationship (119 nS) reflected the conductance of the cell at the holding potential immediately before the voltage steps and was strongly dependent on the holding potential. The plot of the steady-state current near the end of the voltage steps was not linear and reflected the activation of \( I_h \). The difference between \( I_{INS} \) and \( I_{SS} \) (Fig. 2B) reflects the current that was activated by the hyperpolarizing step. In octopus cells, \( I_{SS} \) showed no sign of inactivation even with very long voltage pulses (Fig. 2C).

The total current activated by hyperpolarization was consistently large in octopus cells. Its amplitude ranged from \(-6.5\) to \(-15.7\) nA, averaging \(11.1 \pm 2.2\) nA (\( n = 36 \)) at \(-112 \) mV. Some rundown of \( I_h \) (\(-10\%\)) was observed over the course of experiments that typically ran \(~1\) h.
Isolation of \( I_h \)

To study \( I_h \), this current first had to be isolated from other voltage-dependent ionic currents. Earlier studies have revealed the presence of voltage-dependent \( \text{Na}^+ \), \( \text{Ca}^{2+} \), and \( \text{K}^+ \) currents (Golding et al. 1995, 1999). In the present experiments, 0.1 \( \mu \text{M} \) TTX and 2 mM 4-AP were added to block \( \text{Na}^+ \) and \( \text{K}^+ \) currents, respectively. In some experiments which involved depolarizations more than 5 mV from the resting potential, 6 mM 4-AP, 10 mM TEA, and also 0.2 mM \( \text{Cd}^{2+} \) were used to block low-threshold \( I_{\text{K(L)}} \) and high-threshold \( I_{\text{K(H)}} \) \( \text{K}^+ \) currents and \( \text{Ca}^{2+} \) current, respectively.

At the resting potential and in the cell’s physiological voltage range, both \( I_h \) and \( I_{\text{K(L)}} \) are partially activated (Ferragamo and Oertel 1998; Golding et al. 1999). It was crucial therefore to block \( I_{\text{K(L)}} \), whose voltage sensitivity overlaps that of \( I_h \), near the resting potential and to test whether \( I_h \) is affected by those blockers. Two agents have been reported to block \( I_{\text{K(L)}} \): 4-AP and dendrotoxin (Ferragamo and Oertel 1998; Forsythe and Barnes-Davies 1993; Manis and Marx 1991; Rathouz and Trussell 1998). To test whether these drugs affect \( I_h \), octopus cells were subjected to large, hyperpolarizing voltage pulses (−80 to −100 mV), conditions under which \( I_h \) would be expected to be strongly activated and \( I_{\text{K(L)}} \) and other depolarization-sensitive currents would be expected to be minimally activated. Figure 3, A and B, shows that while 2 mM 4-AP did not significantly affect hyperpolarization-activated currents, 6 mM 4-AP and 100 nM DTX suppressed \( I_h \) by −10%. The current-voltage relationships show that the block of \( I_h \) by these agents is voltage-independent. In the following experiments therefore, estimates of the amplitude of \( I_h \) were made in the presence of only 2 mM 4-AP under conditions in which \( I_h \) was not measurably suppressed. For studying the voltage-dependence of \( I_h \), which required not only hyperpolarization but also depolarization to 20 mV from rest, it was more important to avoid contamination by depolarization-sensitive \( \text{K}^+ \) currents than to have currents of maximum amplitude. For these experiments, 10 mM TEA was added to the control solution to block \( I_{\text{K(IR)}} \), a higher concentration of 4-AP (5–6 mM) was added to suppress \( I_{\text{K(L)}} \), and \( \text{Cd}^{2+} \) was added to suppress the inward \( \text{Ca}^{2+} \) current. Although the amplitude of \( I_h \) was reduced somewhat by both TEA and 4-AP at these concentrations, the voltage-dependence was not measurably affected.

Pharmacological identification of \( I_h \)

To characterize the hyperpolarization-activated current pharmacologically, its sensitivity was tested to extracellularly applied \( \text{Ba}^{2+} \), \( \text{Cs}^+ \), and ZD7288. These drugs differentiate two types of hyperpolarization-activated inward current: \( \text{Cs}^+ \) and \( \text{Ba}^{2+} \) but not ZD7288 block inward rectifiers, \( I_{\text{K(IR)}} \), that are highly selective for potassium ions and that activate relatively quickly. \( \text{Cs}^+ \) and ZD7288 but not \( \text{Ba}^{2+} \) have been shown to block \( I_h \), a current that reflects a hyperpolarization-activated permeability to sodium and potassium ions. Figure 4 shows the results of such tests.

Application of 5 mM \( \text{Cs}^+ \) blocked most of the current activated by hyperpolarization (91.5 ± 1.8%, \( n = 7 \); Fig. 4A). Increasing the concentration to 10 mM \( \text{Cs}^+ \) resulted in a block of 94% (\( n = 2 \)). The effects on \( I_h \) were largely reversible on removal of \( \text{Cs}^+ \).

A bradycardic agent, ZD7288, has also been reported to block \( I_h \) selectively in many types of neurons (Harris and Constanti 1995; Khakh and Henderson 1998; Luthi et al. 1998; Maccarreri and McBain 1996). Figure 4B shows the result of externally applied 50 nM ZD7288 on hyperpolarization-activated currents. There appeared to be a release from block during hyperpolarization manifested by a gradual increase of inward current. Such effects have been also reported in hippocampal neurons (Maccarreri and McBain 1996). ZD7288 blocked inward currents, measured near the beginning of the pulse, by 91 ± 4% (\( n = 3 \)). In octopus cells, as in other cells, the blocking effect developed slowly, taking −10–20 min to become maximal, and could not be reversed with washout periods ≥50 min (Harris and Constanti 1995; Maccarreri and McBain 1996).

These experiments raise the question whether the block by 5 mM \( \text{Cs}^+ \) and ZD7288 is incomplete or whether a portion of the hyperpolarization-activated current was mediated through a different type of conductance. Extracellular application of 2 mM \( \text{Ba}^{2+} \) produced a 13 ± 4% inhibition of \( I_h \) (\( n = 4 \); Fig. 4C). To determine which the \( \text{Cs}^+ \)-insensitive current and \( \text{Ba}^{2+} \)-sensitive currents were different, the sensitivity to \( \text{Ba}^{2+} \) of the residual currents in the presence of \( \text{Cs}^+ \) was tested. Three experiments showed that the 5 mM \( \text{Cs}^+ \)-insensitive, hyperpolarization-activated current was blocked by \( \text{Ba}^{2+} \) by

FIG. 3. \( I_h \) is more strongly affected by \( \alpha \)-dendrotoxin than by 4-aminopyridine (4-AP) at low concentrations. A: to test the effect of \( \alpha \)-dendrotoxin on \( I_h \), the cell was held at a relatively hyperpolarized potential, −72 mV, and stepped to between −77 and −97 mV in 5-mV steps. Left: the slow activation of an inward current that is typical of \( I_h \). Right: currents evoked by the same protocol in the presence of \( \alpha \)-dendrotoxin. \( \alpha \)-Dendrotoxin caused a reduction in the magnitude of \( I_h \) at the steady-state at potentials so hyperpolarized that depolarization-sensitive currents are not expected to be activated. B: to test the effect of 4-AP, a similar experiment was done using the same voltage protocol. At 2 mM 4-AP, \( I_h \) was only reduced by 9.3 ± 1.5% on average. C: plots of steady-state current as a function of voltage of traces in A in control conditions (a) and in the presence of \( \alpha \)-dendrotoxin (b). Right: plots of steady-state current as a function of voltage of the traces in B in control conditions (a), in the presence 2 mM 4-AP (b), and 6 mM 4-AP (c).
only 4% (n = 3), indicating that the block by Ba\textsuperscript{2+} in these experiments was nonspecific and that <1% of the hyperpolarization-activated current could have been \( I_{\text{Kr}} \). The interpretation that the residual current in the presence of 5 mM Cs\textsuperscript{+} represents incomplete blocking of \( I_h \) is strengthened by the finding that a larger proportion (94%) of the hyperpolarization-activated current is blocked by a larger concentration of Cs\textsuperscript{+} (10 mM). We conclude that the hyperpolarization-activated current can be attributed to \( I_h \).

**Kinetics of activation and deactivation**

The kinetics of activation of \( I_h \) was examined by fitting the activation phase of the current with exponentials. The first 7 ms of the current trace at the beginning and after the end of a voltage pulse can include the capacitative transient and was therefore ignored for the exponential fitting (Solomon and Nerbonne 1993a). The rise of the current after this period was well described by double-exponential processes (Fig. 5, A and B). Both the fast and slow time constants of activation, \( \tau_{\text{fast}} \) and \( \tau_{\text{slow}} \), were voltage dependent with \( \tau_{\text{fast}} \) = 44 ± 6 ms and \( \tau_{\text{slow}} \) = 181 ± 39 ms at −77 mV and decreasing to \( \tau_{\text{fast}} \) = 16 ± 3 ms and \( \tau_{\text{slow}} \) = 84 ± 20 ms at −107 mV (n = 8). This decrease in time constants at large step potentials was statistically significant (\( P \leq 0.001 \)). Figure 5B shows the mean values of activation time constants, \( \tau_{\text{fast}} \) and \( \tau_{\text{slow}} \), plotted as a function of voltage.

The rate of deactivation of the inward current was studied by depolarizing the membrane from a hyperpolarized holding potential, −102 mV (Fig. 5C). The current traces were best fitted with a single-exponential function, which varied as a function of voltage. In six experiments, the time constants were 126 ± 15 ms at −62 mV and 178 ± 33 ms at −87 mV.

**Voltage-dependence of activation**

To examine the voltage dependence of \( I_h \) required that the current be isolated not only at hyperpolarized potentials but also in the voltage range depolarized from rest. \( I_h \) was therefore measured under conditions when depolarization-sensitive currents were blocked by 10 mM TEA, 5 mM 4-AP, 250 \( \mu \)M Cd\textsuperscript{2+}, and 1 \( \mu \)M TTX but under which \( I_h \) was also slightly blocked. \( I_h \) was isolated by its Cs\textsuperscript{+} sensitivity (data not shown). This measurement revealed that \( I_h \) has an activation threshold between about −45 and −50 mV, and that it is maximally activated at voltages more hyperpolarized than about −90 mV (n = 10).

A second method for determining the voltage-dependence of \( I_h \) comes from measuring the peak tail currents at a voltage that is as much as possible within the activation range of \( I_h \) but outside the activation range of other voltage-sensitive currents. This method is illustrated in Fig. 6. The cell’s voltage was stepped from −62 mV to a range of voltages between −25 and −115 and then repolarized to −77 mV. The amplitude of the tail current when the voltage was stepped to −77 mV reflected the relative magnitude of the conductance change caused by the previous step to variable voltages. This tail current reflects a deactivation when the voltage is stepped from more hyperpolarizing potentials to −77 mV and activation of \( I_h \) when the voltage is stepped from more depolarizing potentials to −77 mV. The family of tail currents shown in Fig. 6A shows clusters of traces superimposed both at the top and bottom. Those at the top represent the activation of \( I_h \) at −77 mV from voltage steps more depolarized than the threshold of \( I_h \); from these potentials activation of \( I_h \) with hyperpolarization to −77 mV activated a current of constant magnitude. The cluster of current traces at the bottom represents the deactivation of...
respectively. The amplitude of tail currents reveals the voltage-dependence of activation of $g_h$. A: the potential was stepped from $-62 \text{ mV}$ to voltages between $-25$ and $-115 \text{ mV}$ before a test pulse to $-77 \text{ mV}$. The amplitudes of the tail currents were used to measure the conductance at the end of the previous voltage pulse. The recording was made in 10 mM TEA and 6 mM 4-AP and 0.2 mM Cd$^{2+}$ to minimize contamination of tail currents with other voltage-sensitive currents. B: the steady-state activation curve extracted from tail currents in 6 cells. —, the Boltzmann fit with $V_{\text{half}}$ of $-66 \text{ mV}$ and $k$ of 7 mV to the average measurements. The threshold for activation of the conductance was between $-35$ and $-40 \text{ mV}$. The resting conductance is indicated by $-$.

currents from hyperpolarizations that produce maximal activation. The relative amplitude of tail currents, measured immediately after the relaxation of the capacitative transient, are plotted in Fig. 6B. The amplitude of tail currents was normalized to the maximum current levels obtained after the most negative prepulse, $-115$ and then plotted as a function of step potential. This plot shows the voltage-sensitivity of $g_h$ as the fraction of maximal activation. This relationship is well fitted by the Boltzmann relationship

$$I/I_{\text{max}} = 1/(1 + \exp[(V - V_{\text{half}})/k])$$

where $I$ is the amplitude of the tail current evoked from the hyperpolarizing step, $I_{\text{max}}$ is the maximal tail current, $V$ is the step membrane potential, $V_{\text{half}}$ is the half activation threshold, and $k$ is the slope factor. Figure 6B shows the result of such a fit for the normalized averages of tail currents from six experiments. Curve-fitting to the Boltzmann relationship yielded $V_{\text{half}}$ and $k$ of $-65.2 \pm 3.2$ and $7.7 \pm 0.7$ mV ($n = 10$), respectively.

The average maximum conductance associated with $I_h$ can be derived. From the measurement of currents in responses to voltage pulses to $-112 \text{ mV}$, the maximum conductances were calculated to range from 87 to 212 nS and averaged $150 \pm 30$ nS ($n = 36$).

From these results it is possible to determine $g_h$ and $I_h$ at the resting potential of octopus cells. At $-62 \text{ mV}$, the mean resting potential of octopus cells, 41% of the maximum conductance is activated (Fig. 6B). Therefore at rest $g_h$ ranged from 36 to 87 nS, with an average of $62 \pm 12$ nS. With a reversal potential of $-38 \text{ mV}$, $I_h$ contributes a steady inward current of between 0.9 and 2.1 nA at the resting potential.

Reversal potential of $I_h$

The reversal potential for $I_h$ was calculated from currents measured below $-60 \text{ mV}$, in a voltage range in which depolarization-sensitive currents are minimally activated (Banks et al. 1993). Chord conductances were plotted from measurements of the instantaneous current. The slopes of the current-voltage relationships of the instantaneous current reflect the ohmic relationship of the conductance of the cell at the previous holding potential. The slopes of the plots thus vary, depending on the degree of activation of $I_h$ at the holding potential, and intersect at the reversal potential of $I_h$, where there is no driving force. An underlying assumption for these experiments is that $I_h$ is the sole or major conductance activated at the three holding potentials under the present conditions. Two observations support that assumption. The first is that the plots are linear; the second is that all three plots intersect at the same point. For the traces in Figs. 7A, the intersection of the chord conductances that reflects the reversal potential of $I_h$ was $-39 \text{ mV}$ (Fig. 7B). The mean reversal potential of $I_h$ measured in five cells was $-38 \pm 2 \text{ mV}$.

![FIG. 6. Amplitude of tail currents reveals the voltage-dependence of activation of $g_h$. A: the potential was stepped from $-62 \text{ mV}$ to voltages between $-25$ and $-115 \text{ mV}$ before a test pulse to $-77 \text{ mV}$. The amplitudes of the tail currents were used to measure the conductance at the end of the previous voltage pulse. The recording was made in 10 mM TEA and 6 mM 4-AP and 0.2 mM Cd$^{2+}$ to minimize contamination of tail currents with other voltage-sensitive currents. B: the steady-state activation curve extracted from tail currents in 6 cells. —, the Boltzmann fit with $V_{\text{half}}$ of $-66 \text{ mV}$ and $k$ of 7 mV to the average measurements. The threshold for activation of the conductance was between $-35$ and $-40 \text{ mV}$. The resting conductance is indicated by $-$.

![FIG. 7. Measurement of the reversal potential for $I_h$ in 1 cell in control saline. A: $I_h$ was activated to differing degrees by holding the voltage at $-72$, $-82$, and $-92 \text{ mV}$. The symbols above the current traces indicate the time at which the current was measured and correspond to current-voltage plots in B. B: chord conductances were plotted from measurements of the instantaneous current. —, the extrapolated linear fit to the data points. $E_m$, the voltage at the intersection of the 3 lines and an estimate of the reversal potential of $I_h$, $-39 \text{ mV}$.](http://jn.physiology.org/)

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the amplitude of the voltage clamp

Assessment of the voltage clamp

In a class of neurons with low input resistance, the possibility that voltage is not well controlled in portions of the cell at locations distant from the electrode must be considered. In the present series of experiments, two observations suggest that space-clamping is adequate for the study of \( I_h \). One observation is that it was possible to use instantaneous currents to measure reversal potentials from the convergence of chord conductances at a single point (Figs. 7B and 8, C and D). If voltage pulses produced voltage changes that were much smaller in the dendrites than at the cell body, chord conductances measured from instantaneous currents would be expected to deviate from linearity and not to convergence at a single point. The observations illustrated in Figs. 7 and 8 show that the chord conductances are linear and that they do converge at a single value. The second piece of evidence that space clamping is adequate comes from the comparison of reversal potentials and permeability ratios under varying conditions. If space clamping is adequate, the measured reversal potential would be expected to be a consistent function of the concentration gradients of permeable ions. We tested this condition by comparing permeability ratios assuming that only \( Na^+ \) and \( K^+ \) are involved, that their movement is independent and that the electric field in the membrane is constant, using the Goldman-Hodgkin-Katz equation. The permeability ratio, \( P_{Na}/P_{K} \), was calculated under control conditions, when the extracellular \( K^+ \) concentration was high, and when the extracellular \( Na^+ \) concentration was low using the equation:

\[
E_{rev} = 60 \log \frac{(P_{Na})([Na^+])_i + (P_{K})([K^+])}{(P_{Na})([Na^+]) + (P_{K})([K^+])}
\]

The permeability ratios, \( P_{Na}/P_{K} \), in control, high-\( K^+ \), and low-\( Na^+ \) conditions were \( 0.16 \pm 0.02 \) (\( n = 5 \)), \( 0.22 \pm 0.02 \) (\( n = 3 \)), and \( 0.29 \pm 0.05 \) (\( n = 4 \)), respectively. These values did not vary systematically with reversal potential and they are not statistically different from one another (1-way ANOVA, \( P < 0.001 \)).

Modulation of the voltage sensitivity

Modulation of \( g_h \) through G-protein-coupled receptors and cAMP is characteristic of this type of conductance (Pape 1996). We tested whether \( g_h \) in octopus cells was modulated by cAMP by comparing the voltage-sensitivity measured from the tail currents before and after the extracellular application of dibutyryl cAMP in four cells. No significant difference was observed. When no ATP or GTP was included in the intracellular pipette solution, the average \( V_{1/2} \) was shifted in the hyperpolarizing direction. In comparison with measurements in the presence of ATP and GTP where \( V_{1/2} \) was \(-66 \) mV, in the absence of ATM and GTP, \( V_{1/2} \) was \(-72 \) mV and shifted further with time; \( V_{1/2} \) shifted to \(-81.6 \) mV over 12 min (\( n = 6 \)). In the absence of ATP, the amplitude of \( I_h \) gradually decreased. Rundown was on average 40%/h (\( n = 8 \)). The

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**Ionic nature of \( I_h \)**

The measured reversal potential for \( I_h \), \(-38 \) mV, is consistent with the conclusion that this is a mixed-cation current. We tested this conclusion directly by varying extracellular concentrations of \( K^+ \) and \( Na^+ \), ions that have been shown to contribute to \( I_h \) in other preparations.

Increasing the extracellular \( K^+ \) concentration from 3 to 12 mM increased the amplitude of \( I_h \) in all cells tested (Fig. 8A). The reversal potential for \( I_h \) shifted in the depolarizing direction to \(-25.9 \pm 1.2 \) mV (\( n = 3 \); Fig. 8C). The increase in amplitude of \( I_h \) resulted from an increase in the driving force through the conductance that underlies \( I_h \). The shift in the reversal potential was 20 mV/10-fold change in extracellular \( K^+ \) concentration. Reduction of the extracellular \( Na^+ \) concentration from 138 to 10 mM caused \( I_h \) to decrease (Fig. 8B). The reversal potential of \( I_h \) shifted in the hyperpolarizing direction to \(-77 \pm 3.5 \) mV (\( n = 4 \); Fig. 8D). The hyperpolarizing shift of the reversal potential caused the decrease in \( I_h \) by decreasing the driving force that acts on this conductance. The shift in reversal potential was 35 mV/10-fold change extracellular \( Na^+ \) concentration. These results indicate that \( Na^+ \) ions as well as \( K^+ \) ions are the charge carriers for \( I_h \) in octopus cells.

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**Fig. 8.** The amplitude and reversal potential of \( I_h \) depend on the extracellular \( K^+ \) and \( Na^+ \) concentrations. A: the amplitude of \( I_h \) varied with extracellular \( K^+ \) concentration. Superimposed current traces were evoked by hyperpolarizing voltage pulses varying from \(-52 \) to \(-97 \) mV in 5-mV steps from a holding potential of \(-52 \) mV. Left and right: measurements in the control solution that contained 3 mM K\(^+\). Middle: measurements when the extracellular \( K^+ \) concentration was raised to 12 mM. B: the amplitude of \( I_h \) also varied with extracellular \( Na^+ \) concentration. This was revealed by activating \( I_h \) from a holding potential of \(-52 \) mV with hyperpolarizing voltage steps from \(-52 \) to \(-112 \) mV in 5-mV increments before, during, and after changing the extracellular \( Na^+ \) concentration from 138 to 10 mM. The amplitude of \( I_h \) decreased and the tail current was reversed from inward to outward when \( Na^+ \) was lowered. C: the reversal potential became more positive when the extracellular \( K^+ \) concentration was raised. Plots of the instantaneous current as a function of voltage of steps from holding potentials (\( V_h \)) \(-72 \), \(-82 \), and \(-92 \) mV in the presence of 12 mM extracellular \( K^+ \). The intersection of extrapolated chord conductances provides an estimated reversal potential of \(-25 \) mV. D: plots of the instantaneous current as a function of voltage from measurements at different holding potentials, \(-72 \), \(-82 \), and \(-92 \) mV in the presence of 10 mM \( Na^+ \) intersect at the estimated reversal potential of \(-74 \) mV. The 4 panels are derived from experiments on different cells.
rundown and shift was similar in cells bathed in 1 mM dibutyryl cAMP \((n = 6)\) and in the absence of a cAMP analogue \((n = 2)\). The rundown and hyperpolarizing shift in the activation curve are therefore independent of cAMP.

**Discussion**

The present experiments have provided a description of the hyperpolarization-activated mixed-cation conductance of octopus cells in the posteroventral cochlear nucleus. This study shows that while the \(g_h\) in octopus cells resembles \(g_h\) in other types of cells in its biophysical and pharmacological properties, two characteristics of \(g_h\) in octopus cells are notable: the maximum conductance activated by hyperpolarization is large, ranging between 87 and 212 nS, averaging 150 ± 30 nS, and a large proportion of the maximum conductance is activated at rest. At the average resting potential of octopus cells, \(-62 \text{ mV}, 41\%\) of the maximum conductance is activated. Therefore at rest, at the average resting potential of octopus cells, a large proportion of the maximum conductance is activated at

\[ V \]

which is not the role of \(I_h\) in octopus cells, which are characterized by their inability to fire rhythmically (Golding et al. 1995, 1999). The interplay between \(I_{K(L)}\) and \(I_h\) would, however, be expected to shape synaptic responses to activation of the auditory nerve.

\(I_h\) contributes to regulating the “gain” of synaptic responses in octopus cells. Octopus cells detect synchronous firing among its inputs (Golding et al. 1995). Individual auditory nerve inputs contribute a synaptic current; the amplitude of the response to that synaptic current is determined by the input conductance of octopus cells. The present results show that \(g_h\) contributes \(-41\%\) of the input conductance. In addition to its contribution to the input conductance of octopus cells, \(g_h\) also affects the activation of \(g_{K(L)}\) by affecting the membrane potential near rest.

In hippocampal neurons, the greater density and lower voltage range of activation of \(I_h\) in the dendrites relative to the soma affects both the temporal summation of synaptic inputs and also the effectiveness of the back-propagation of voltage into dendrites (Magee 1998). It is not known whether \(g_h\) is expressed nonuniformly in octopus cells, but the possibility that \(g_h\) affects the pattern of temporal and spatial summation of auditory nerve inputs in octopus cells is an intriguing one in cells that encode the timing of auditory nerve inputs with precision (Godfrey et al. 1975; Golding et al. 1995; Rhode and Smith 1986; Rhode et al. 1983).

Two classes of hyperpolarization-sensitive conductances

Two distinct classes of hyperpolarization-sensitive conductances have been described and cloned. One underlies a rectifying \(K^+\) current and has been called \(I_{KIR}\); the other underlies a mixed-cation current and has been called \(I_h\), \(I_h\) or \(I_{h+}\), the heart, or the “pacemaker current” on the basis of the role it has been shown to play in the heart and in thalamic and cortical neurons. These conductances are mediated through two distinct classes of ion channels. The subunits of ion channels that underlie \(I_{KIR}\) are distantly related to the family of depolarization-sensitive \(K^+\) channels; their rectification arises from a voltage-dependent block by polyamines and \(Mg^{2+}\) (Kubo et al. 1993; Nichols and Lopatin 1997; Nichols et al. 1994). \(I_h\) arises through ion channels whose molecular structure resembles depolarization-sensitive \(K^+\) channels more closely (Biel et al. 1999; Ishii et al. 1999; Ludwig et al. 1998; Santoro et al. 1998; Seifert et al. 1999). This current is characterized by its sensitivity to hyperpolarization, by being carried by both \(K^+\) and \(Na^+\), and by being regulated by cyclic nucleotides (Pape 1996). Four families of ion channels have been described that mediate \(I_h\) with differing rates of activation and differing sensitivity to cyclic nucleotides, termed HCN (for hyperpolarization-activated and cyclic nucleotide-gated channels) (Clapham 1998; Ludwig et al. 1998, 1999; Santoro and Tibbs 1999; Santoro et al. 1998; Seifert et al. 1999). The two classes of hyperpolarization-sensitive currents, \(I_h\) and \(I_{KIR}\), can be distinguished pharmacologically. While \(I_{KIR}\) is sensitive to extracellular cations including \(Cs^+\) and \(Ba^{2+}\) (Kubo et al. 1993), \(I_h\) is sensitive to the bradycardiac agent, ZD7288, but relatively insensitive to \(Ba^{2+}\) (BoSmith et al. 1993; Gasparini and DiFrancesco 1997; Khakh and Henderson 1998; Lüthi et al. 1998;
Reversal potential and ionic basis of $I_h$

The reversal potential measured for $I_h$ in octopus cells was $-38$ mV under control conditions and depended on the extracellular concentrations of both Na$^+$ and K$^+$, indicating that $I_h$ in octopus cells is carried by both cations. These findings confirm the conclusion based on current-clamp experiments (Golding et al. 1999). The present findings are comparable to those reported in other cells. Measurements of reversal potentials of $I_h$ in other cells measured under similar but not identical conditions were similar, ranging from $-30$ to $-44$ mV (Banks et al. 1993; Chen 1997; Maccabery and McBain 1996; McCormick and Pape 1990b; Mo and Davis 1997). The permeability ratio, $P_{Na}/P_{K}$, for $I_h$ in octopus cells was estimated from the Goldman-Hodgkin-Katz equation to be between 0.16 and 0.29, depending on the ionic conditions. These values lie within the range measured in other types of cells between 0.2 and 0.4 (Hestrin 1987; Magee 1998; Maricq and Korenbrot 1990; Solomon and Nerboune 1993b).

Voltage sensitivity of $I_h$

Under control physiological conditions, the activation curve for $g_h$ derived from tail current measurements in octopus cells revealed an activation threshold of about $-35$ to $-40$ mV and full activation at voltages more hyperpolarized than $-100$ mV. Fitting the steady-state activation curve with the Boltzmann relationship showed that the $V_{1/2}$ and slope factor, $k$, of $-65.2 \pm 3.17$ and $7.7 \pm 0.7$ mV, respectively. A comparison of $V_{1/2}$ in some of the cells in which it has been measured is given in Fig. 9. This comparison reveals that $V_{1/2}$ in octopus cells lies at a relatively depolarized potential relative to other cells in which it has been measured. It is the large amplitude of the maximal $g_h$ together with the relatively depolarized voltage range of activation that leads to the large $g_h$ in the physiological voltage range of octopus cells.

The measurement of voltage sensitivity depends on the conditions under which the experiments were done. It is characteristic of $g_h$ that its voltage sensitivity is regulated by neurotransmitters through intracellular messengers (Cathala and Paupeard-Trisch 1999; DiFrancesco and Tortora 1991; Ingram and Williams 1996; Ludwig et al. 1998; McCormick and Pape 1990b; Pape 1996; Pape and McCormick 1989; Santoro and Tibbs 1999; Santoro et al. 1998; Spain et al. 1987; van Ginneken and Giles 1991; Womble and Moises 1993).

Proportionality-activated, mixed-cation currents have been described in many neuronal and nonneuronal cells (Pape 1996). In general, the properties of $I_h$ in octopus cells are similar to those of $I_h$ recorded in other cells including primary auditory neurons (Chen 1997; Mo and Davis 1997) brain stem auditory neurons (Banks et al. 1993; Chen 1997; Fu et al. 1997), nonauditory neurons (Chen 1997; Mo and Davis 1997) brain stem (DiFrancesco and Tortora 1991; Ludwig et al. 1998; McCormick and Pape 1989b; Pape 1996; Pape and McCormick 1989; Santoro and Tibbs 1999; Santoro et al. 1998; Spain et al. 1987; van Ginneken and Giles 1991; Womble and Moises 1993).
Tokimasa and Akasu 1990; van Ginneken and Giles 1991). Perhaps it is not surprising that in octopus cells, where \( V_{\text{half}} \) lies at a relatively depolarized level even in the absence of the application of cAMP and its analogues, dibutyryl cAMP had no measurable effect. The shift of \( V_{\text{half}} \) in the hyperpolarizing direction by the removal of intracellular nucleotide triphosphates, independent of cAMP and accompanied by rundown that was observed in octopus cells, has also been reported in the heart and in bullfrog sympathetic neurons (Di Francesco and Mangoni 1994; Tokimasa and Akasu 1990).

The question arises to what extent the activation values measured with patch-clamping and intracellular perfusion reflect the function of \( g_h \) in octopus cells in situ. Two lines of evidence indicate that \( g_h \) is strongly activated at rest under physiological conditions. The first is that the prominence of \( g_h \) has been documented in recordings made with sharp microelectrodes (Golding et al. 1995). These recordings reveal properties that are similar to those recorded in current clamp in the present recordings. The second line of evidence is that the properties of octopus cells revealed both in sharp-electrode recordings and whole-cell patch recordings are consistent with their responses to sound (Godfrey et al. 1975; Golding et al. 1995; Rhode and Smith 1986).

Amplitude of \( I_h \)

In octopus cells, \( g_h \) and \( I_h \) are larger than in other neuronal cell types in which they have been measured. To make a comparison of amplitude of \( g_h \) across different types of cells, \( g_h \) at \(-100 \text{ mV}\) and the voltage at which the conductance is activated half-maximally are graphed in Fig. 9. At \(-100 \text{ mV}\) \( g_h \) is 99\% activated in octopus cells, averaging 150 nS. It is two orders of magnitude larger than in many cells and it is at least twice as large as the largest conductance measured in any other group of cells. The combination of a relatively large conductance and relatively depolarized voltage range of activation gives octopus cells an unusually large \( g_h \) in the physiological voltage range. Interestingly, \( g_h \) is also particularly large and the half-maximal voltage of activation is relatively depolarized in another auditory brain stem nucleus, the medial nucleus of the trapezoid body (Banks et al. 1993). Comparison between cells is necessarily crude because measurements were made under conditions that differed in the temperature and the composition of extracellular and intracellular solutions as well as in the species in which they have been recorded. It is likely that the states of modulation also differed in different studies. Comparisons in amplitude and voltage of half-maximal activation are further hampered by the fact that the rates of activation of \( I_h \) are in some cases so slow that it is not practical to measure activation in the steady state. Crude though the comparison may be, it shows that \( g_h \) in octopus cells is unusually large.

Rate of activation

The activation phase of \( I_h \) in octopus cells was best described by the sum of two voltage-dependent exponentials in the tens and hundreds of milliseconds, respectively. The rate of deactivation was described by a single exponential with a time constant in the hundreds of milliseconds (Fig. 5). These rates are comparable to the fastest rates of activation and deactivation observed in other types of cells. The rates of activation that have been described vary over a wide range. While in some cells, including most neurons, \( I_h \) is fully activated within 1 s, in other cells, including thalamic neurons and cardiac cells, \( I_h \) is not fully activated even after several seconds (Ludwig et al. 1999; Santoro and Tibbs 1999; Seifert et al. 1999; Solomon et al. 1993). These differences probably reflect differences in the molecular composition of the ion channels. Four members of the HCN gene family have been cloned, one of which (HCN4) has particularly slow rates of activation when measured in an expression system and is strongly expressed in the heart and thalamus (Ludwig et al. 1999; Santoro and Tibbs 1999). Our results suggest that the slow forms HCN2 and HCN4 are probably not strongly expressed in octopus cells, but at present it is not possible to know which of the other classes of subunits would be expected to be prominent.

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