Voltage-Dependent Conductances in Cephalopod Primary Sensory Hair Cells

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Chrachri, Abdesslam and Roddy Williamson. Voltage-dependent conductances in primary sensory hair cells. J. Neurophysiol. 78: 3125–3132, 1997. Cephalopods, such as sepia, squid, and octopus, show a well-developed and sophisticated control of balance particularly during prey capture and escape behaviors. There are two separate areas of sensory epithilum in cephalopod statocysts, a macula/statolith system, which detects linear accelerations (gravity), and a crista/cupula system, which detects rotational movements. The aim of this study is to characterize the ionic conductances in the basolateral membrane of primary sensory hair cells. These were studied using a whole cell patch-clamp technique, which allowed us to identify five ionic conductances in the isolated primary hair cells; an inward sodium current, an inward calcium current, and three potassium outward currents. These outward currents were distinguishable on the basis of their voltage-dependence and pharmacological sensitivities. First, a transient outward current (Iₒ) was elicited by depolarizing voltage steps from a holding potential of −60 mV, was inactivated by holding the cell at −40 mV, and was blocked by 4-aminopyridine. A second, voltage-sensitive, outward current with a sustained time course was identified. This current was not blocked by 4-aminopyridine nor inactivated at a holding potential of −40 mV and hence could be separated from Iₒ using these protocols. A third outward current that depended on Ca²⁺ entry for its activation was detected, this current was identified by its sensitivity to Ca²⁺ channel blockers such as Co²⁺ or Cd²⁺ and by the N-shaped profile of its current-voltage curve. Inward currents were studied using cesium aspartate solution in the pipette to block the outward currents. Two inward currents were observed in the primary sensory hair cells. A fast transient inward current, which is presumably responsible for spike generation. This inward current appeared as a rapidly activating inward current; this was strongly voltage dependent. Three lines of evidence suggest that this fast transient inward current is a Na⁺ current (Iₙa). First, it was blocked by tetrodotoxin (TTX); second, it also was blocked by Na⁺-free saline; and third, it was inactivated when primary hair cells were held at a potential more than −40 mV. The sustained inward current was not affected by TTX and was increased in amplitude 5 min after equimolar Ba²⁺ replaced Ca²⁺ as a charge carrier. This inward current also was blocked after external application of 2 mmol/l Co²⁺ or Cd²⁺. Furthermore, this current was reduced significantly in a dose-dependent manner by nifedipine, suggesting that it is an L-type Ca²⁺ current (IₖCa).

INTRODUCTION

Mechanosensory hair cells are present in almost all animal species and often are associated with ancillary structures to form specific sense organs, e.g., organs that detect sound, gravity, or body movements (e.g., Platt 1984; Wiederhold 1976). In general, mechanosensory hair cells have apical filaments, often a bundle of cilia, which, when stressed or deflected, induce the opening of ion channels that lead to a change in the membrane potential of the cell (e.g., French 1988). Such mechanosensory hair cells can be divided into two basic types, primary sensory hair cells, which have a centripetally running axon extending from the base of the cell, and secondary sensory hair cells, which have no axon of their own but that make synaptic contact with an afferent neuron. The former type occurs in invertebrates and may be the more primitive form (Jorgensen 1989), whereas the latter is almost exclusive to vertebrates (although see following text). Most studies of the basic mechanisms that underlie hair cell function, i.e., the mechanoelectric transduction process or the ionic basis of the responses, have focused on the secondary sensory hair cells (Corey and Assad 1992; Torre et al. 1995) with very little work on the operation of primary hair cells (French 1988, Wiederhold et al. 1989).

This paper provides the first comprehensive study of the voltage-sensitive ionic conductances present in a primary sensory hair cell. Using dissociated cells, we characterize five separate conductances in the primary sensory hair cells, three outward currents and two inward currents. We also used sharp microelectrodes to examine how these conductances are activated in cells in situ within the intact receptor epithelium. This is important because the cells under these conditions can show varying strengths of electrical coupling, and we therefore need to examine the likely functional significance of the conductances under normal conditions.

By using primary sensory hair cells from the statocysts of cephalopod mollusks, we can directly compare our results with those obtained previously from equivalent secondary sensory hair cells (Williamson 1995a), for cephalopods are unique in having both primary and secondary sensory hair cells present in the same sense organ, the statocyst (Budemann 1990). In addition, the statocysts have strong functional parallels with their vertebrate analogue, the semicircular canal system (Williamson 1990, 1995b; Williamson and Budemann 1985a,b), and so it is also of interest to compare our results with those already obtained from vertebrate sensory hair cells (e.g., Housley et al. 1989; Hudspeth and Lewis 1988; Rennie and Ashmore 1991).

METHODS

Squid, Loligo forbesi, and octopus, Eledone cirrhosa, of both sexes were used in this study. Animals were caught in the Plymouth area and held in aquaria at temperature of 12–18°C.

In situ preparation

For the intracellular recordings, we used mainly squid preparations as the cristae in these statocysts have a thicker and thus more...
stable cartilage base (Chrachri and Williamson 1993). Briefly, the animal was killed, and the paired statocysts and surrounding cranial cartilage were dissected free. Each statocyst was opened carefully to keep the anterior transverse crista and the longitudinal crista segments and their nerves undamaged. The tissue was pinned out in a silicone elastomer (Sylgard)-lined dish such that the primary hair cells were accessible to microelectrodes penetration.

**Dissociated cell preparation**

For dissociated cell preparations, we used both squid and octopus preparations; no differences were found in the responses of the cells, and so these are reported together in RESULTS. As before, the animal was killed, and the statocyst and the surrounding cartilage dissected free and unopened from the cranial cartilage. The statocysts then were transferred to a calcium-free artificial sea water (ASW), and both statocysts opened by a sagittal section along the medial length of the cranial cartilage. Opened statocysts were incubated for 3–4 h in 8 mg/ml protease (Nagarse, Sigma) in calcium-free ASW. Different enzymes (e.g., papain, trypsin, and collagenase) and various combinations of time and temperature were tried, but the best yields of viable hair cells were obtained with the above protocol. After careful washing with fresh ASW, the sensory epithelium within each statocyst was removed by aspiration with a fire-polished pipette, and then, with a gentle trituration, the sensory hair cells were dissociated. The cells then were transferred to the recording chamber and viewed with a Nikon inverted microscope.

**Identification of primary hair cells**

The sensory epithelia in coleoid cephalopod statocysts comprises three main elements (Budelmann et al. 1987; Williamson 1995a). First, the sensory hair cells, which uniquely are of two types in cephalopods: primary hair cells with an apical ciliary bundle and an axonal process extending from the cell’s base (Fig. 4A), and secondary hair cells, also with an apical ciliary bundle but no axonal process extending from the cell’s base. Second, there are the first-order afferent neurons; these have no apical ciliary bundle, but have an elongated or a rounded cell body and a clear axonal process. Finally, there are the terminals of the efferent inputs from the brain; in the present study, these efferents were not studied. In addition, there are numerous nonsensory supporting cells (Budelmann et al. 1987).

**Recording apparatus**

Intracellular recordings from primary sensory hair cells in the nondissociated squid statocysts were performed using sharp microelectrodes made from filamented Borosilicate glass capillaries (Clark Electromedical GC-150F), filled with 3 M KC1 and having tip resistances of 20–40 MΩ. A conventional bridge circuit was used for recording and passing current pulses.

Ionic currents from freshly dissociated primary sensory hair cells were investigated under voltage clamp using the patch-clamp technique in the whole cell configuration (Hamill et al. 1981). Membrane currents were recorded using an Axopatch 200A amplifier, low-pass filtered at 5 kHz. Whole cell pipettes, made from filamented Soda glass capillaries (Intarcia, 1.5 mm OD × 0.86 mm ID), had resistances of 2–6 MΩ when filled with potassium or cesium aspartate. Series resistance was compensated electronically (usually to >90%). Thus voltage errors of only a few millivolts occurred at peak current levels. Liquid junction potentials were measured using the AgCl-pellet as a reference electrode and found to be <4 mV with potassium as the pipette internal solution and <5 mV with cesium as the internal solution. Pulse generation and data acquisition were performed with a CED 1401 computer-controlled laboratory interface, using the Patch software suite (Cambridge Electronic Design, United Kingdom).

**Solution and drugs**

The artificial sea water contained (in mmol/l) 430 NaCl, 10 KCl, 10 CaCl$_2$, 50 MgCl$_2$, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) buffer at pH 7.6, osmolarity = 997 mosM. Other solutions were made by equimolar substitution of this basic formula. Ca$^{2+}$ was replaced with Ba$^{2+}$ to enhance the calcium current. For the calcium-free ASW, magnesium was substituted for calcium.

Patch pipettes were filled with a solution containing (in mmol$^{-1}$) 500 K-aspartate, 10 NaCl, 4 MgCl$_2$, 3 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, and 20 HEPES, titrated with KOH to a pH of 7.4, osmolarity = 870 mosM to study outward currents or CsOH to a pH of 7.4 to block outward currents and therefore to study inward currents.

Pharmacological agents (all from Sigma) were used to block ionic currents selectively. These included tetrodotoxin (TTX), tetraethylammonium (TEA$^+$), 4-aminopyridine (4-AP), cesium (Cs$^+$), cobalt (Co$^{2+}$), and nifedipine (dissolved in absolute ethanol to make 5 mmol/l stock solutions and stored at 5°C in the dark). Experiments with nifedipine were carried out in dim light to prevent photo-oxidation.

**RESULTS**

Intracellular recordings with conventional sharp microelectrodes

The primary sensory hair cells (PH) in squid statocysts are located on the dorsal side of both the anterior transverse and the longitudinal crista segments (Budelmann 1990; Chrachri and Williamson 1993). When these sensory hair cells were impaled with single microelectrodes under visual control, they had membrane resting potentials around $-61 \pm 3$ mV (mean ± SD; $n = 25$) and usually displayed a low level of spontaneous repetitive firing of action potentials (Fig. 1A). Single action potentials also could be elicited either by direct electrical stimulation of the cell’s axon in the statocyst crista nerve, which induced an antidromic action potential (Fig. 1B), or by intracellular injection of brief
depolarizing current pulses (Fig. 1C). The amplitude of these action potentials was around 30 ± 10 mV (n = 25).

The addition of 1 μmol/l TTX to the bath blocked the generation of action potentials (Fig. 1D), while further increasing the current stimulus now failed to elicit any subsequent action potentials. These findings indicate that the spike is TTX sensitive and that the rising phase of the action potential is most likely to be carried by Na⁺. Substitution of choline⁺ for Na⁺ in the external medium also blocked the generation of action potentials.

Bath application of a solution containing only half the regular concentration of CaCl₂ (Fig. 2A), 5 μmol/l nifedipine (Fig. 2B) or 5 mmol/l CoCl₂ (not shown) reduced the size of the action potentials in these primary sensory hair cells, implying that Ca²⁺ also may contribute to action potential in these cells. This was investigated further using whole cell patch-clamp technique (see following text).

Bath application of 5 mmol/l 4-AP caused a clear prolongation of the action potential in these primary sensory hair cells (Fig. 3), had little effect on the rising phase of the action potential, and caused a dose-dependent slowing in the repolarization phase of the action potential. 4-AP is known to block A type voltage-activated potassium currents in some preparations (Thompson 1977), and so this result is consistent with the presence of an A-type potassium current in these cells, which may be involved in repolarization of the membrane potential.

Overall voltage-clamp responses

In addition to the conventional intracellular recordings, more definitive evidence for the ionic basis of the regenerative activity, repolarization, and afterhyperpolarization was obtained from whole cell current recordings. No differences were observed in the currents detected in squid and octopus primary sensory hair cells and so these are here reported together.

Under initial voltage-clamp conditions, depolarizing pulses from a holding potential of −60 mV elicited two main categories of currents, a large outward current and a smaller inward current, both of which had initial transient components (Fig. 4, B and C).

Whole cell outward currents recorded with patch electrodes

The large outward current observed in these sensory primary hair cells at depolarized potentials (Fig. 4B) was studied using potassium aspartate solution in the pipette and usually 1 μmol/l TTX and 2 mmol/l Co²⁺ in the external solution to block inward currents. The outward current was suppressed substantially by adding Ba²⁺ to the bath and presumably was carried by K⁺ or at least included a large component of K⁺ current. This overall current could be separated into two components using either voltage step protocols or pharmacological agents (e.g., Connor and Stevens 1971a). First, if the cell was held at a membrane potential of −40 mV, instead of −60 mV, the initial large transient current (Fig. 5A) was not observed during the applied voltage steps (Fig. 5B). Computer subtraction of the remaining current from the original current revealed the characteristics of the inactivated current (Fig. 5, C and D). Thus at a holding potential of −40 mV, the large transient outward current was inactivated, leaving a sustained current (Fig. 5B). Similarly, the addition of 4 mmol/l 4-AP to the exter-
A. CHRACHRI AND R. WILLIAMSON

FIG. 5. Separation of the fast transient potassium outward current (A current) in an isolated primary sensory hair cells using voltage steps from different holding potentials. A: whole cell outward current in response to membrane depolarizations to voltage steps from a holding potential of $-60 \text{ mV}$. Total outward current is composed of a transient outward current and a sustained outward current. B: whole cell outward current in response to membrane depolarizations to the same voltage steps as in A, but this time from a holding potential of $-40 \text{ mV}$. $I_t$ largely is inactivated, leaving only the delayed rectifier, $I_{K}$. C: computer subtraction of B and A to show the isolated $I_t$ current (●), and the isolated $I_{K}$ current in C (▲). D: $I-V$ plots of the instantaneous currents 5 ms after the start of the voltage steps for the whole cell outward current (●), the mainly $I_{K}$ current in B (▲), and the isolated $I_{K}$ current in C (▲). The current-voltage relationships for these currents are shown in Fig. 6B.

These results are consistent with the presence of two separate outward currents, which, on the basis of their kinetics, $I-V$ curves, and pharmacological sensitivities, can be identified as similar to the delayed rectifier ($I_{K}$) and the A current ($I_{A}$) as previously reported in other preparations (e.g., see Adams et al. 1980; Connor and Stevens 1971a,b; Thompson 1977).

As shown in Fig. 7, the delayed rectifier current seen in these primary hair cells could be reduced in a dose dependent manner by bath application of TEA. However, the TEA concentration necessary to reduce this current (250 mmol/l) for a 50% reduction) was high compared with that used to block the delayed rectifier in other cell types (e.g., Griguer et al. 1993; Rudy 1988). Ten percent of cells tested, did not show $I_{K}$, and unfortunately, washing with fresh ASW did not consistently produce a full recovery from the effects of TEA or 4-AP.

Under conditions where calcium currents were not blocked, a third component of the outward current could be seen in ~25% of the cells tested (Fig. 8A). This current was insensitive to TEA but could be blocked by the external

FIG. 6. Separation of the fast transient potassium outward current (A current) in the primary sensory hair cells by 4-AP. A: whole cell outward current in response to membrane depolarization to a voltage step of $+70 \text{ mV}$ from a holding potential of $-60 \text{ mV}$. Total outward current is composed of a transient outward current and a sustained outward current (control). Bath application of 4 mmol/l 4-AP suppresses the transient outward current, leaving only the sustained outward current (4 mM 4-AP). B: $I-V$ plots of the instantaneous currents 5 ms after the start of the voltage steps for the whole cell outward current (●), the mainly $I_{K}$ current (▲). C: Co$^{2+}$-sensitive current obtained by subtracting series B from series A. D: $I-V$ plots for the whole cell currents before (●) and after 4 mmol/l 4-AP. E: Co$^{2+}$-sensitive current activation curve obtained by subtracting series B from series A.
Inward currents were studied using cesium aspartate in inactivated progressively by setting the holding potentials at 60 mV before (control) and after bath application of 2 μmol/l Co2+ ions (Fig. 8B). The I-V curve (Fig. 8D) for the whole current displayed an N-shaped curve, with a peak around +40 mV. This peak can be seen in isolation when the current in the presence of a calcium channel blocker (Fig. 8B) is subtracted from the whole current (Fig. 8A) revealing a separate peak current at around +30 mV (Fig. 8E). This current also could be abolished by 5 μmol/l apamin (data not shown), a relatively specific blocker for Ca2+-dependent potassium currents and is similar to the one reported by Meech and Standen (1975) in Helix neurons.

Whole cell inward currents recorded with patch electrodes

Inward currents were studied using cesium aspartate in the pipette internal solution to block the outward K+ currents described above. Depolarizing pulses of 100-ms duration, from a holding potential of −60 mV, were used to set the membrane potential at voltages ranging from −50 to +60 mV with 10-mV increments. Under these conditions, two inward currents were observed in primary hair cells (Fig. 9). First was a large, rapidly inactivating transient current (Fig. 9A), which could be blocked by the addition of 2 μmol/l TTX to the bath, leaving only a small sustained inward current (Fig. 9B). TTX is a known blocker of sodium currents in many different types of cells (Hille 1992). The I-V plot (Fig. 9D) of the TTX-sensitive current isolated from the total current by computer subtraction (Fig. 9C) showed that the current appeared at potentials more positive than −50 mV, peaked around +10 mV, and then decreased. Filled circles illustrates the TTX-sensitive inward current.

Three lines of evidence suggest that this fast transient inward current is a Na+ current (INa). First, it was blocked by TTX (Fig. 9B); second, it was abolished in Na+-free saline (not shown); and third, this inward current could be inactivated progressively by setting the holding potentials at values more positive than −40 mV. Taken together with the data from the in situ recordings, it is probable that this sodium current is responsible for the rising phase of the spike generation.

The sustained inward current (Fig. 9B) was not affected either by TTX or Na+-free saline. The amplitude of this inward current could be increased by the substitution of barium for calcium in the external solution (or by the simple addition of 2 mmol/l Ba2+ to the bath; data not shown). The inward current was activated rapidly by voltage steps more positive than −40 mV and was maintained throughout the voltage step with no sign of inactivation. The current achieved a maximum for test steps to around +10 mV and then decreased. This sustained inward current could be blocked by the addition of Co2+ (Fig. 10) or Cd2+ (not shown) to the external solution at a concentration of 2 mmol/l. Furthermore, this sustained inward current was reduced significantly in the presence of nifedipine (Fig. 11), implying that this is probably an L-type Ca2+ current (ICa).

DISCUSSION

This report provides the first identification and characterization of the range of voltage-dependent ionic conductances...
present in invertebrate primary sensory hair cells. These hair cells are probably ancestral to the secondary sensory hair cells found in vertebrates (Jorgensen 1989) and differ from their vertebrate counterparts in a number of significant respects e.g., the mechanoelectric transduction process is based on kinocilia not stereocilia (Williamson 1990), and these invertebrate hair cells do not make synaptic contact with a primary afferent neuron in the periphery but have an axon that carries their information directly to the CNS. Nevertheless, we have found a range of five voltage-sensitive ionic currents in cephalopod primary sensory hair cells that are similar to currents previously reported in vertebrate secondary sensory hair cells.

$K^+$ currents

Two outward potassium currents, a delayed rectifier ($I_K$) and an A current ($I_A$) were identified in the cephalopod primary sensory hair cells on the basis of their kinetics and pharmacological sensitivities (Figs. 5–7). Such currents are present in many other cell types, from cephalopods (e.g., Llano and Bookman 1986; Lucero et al. 1992; Williamson 1995a), from other invertebrates (e.g., Connor and Stevens 1971a,b; Thompson 1977), and from vertebrates (e.g., Rudy 1988), and usually are considered to contribute to the repolarization of the cell after, for example, an action potential. This view is supported in the present study by the observed increase in action potential duration in the presence of pharmacological blockers for these currents (e.g., Fig. 3).

The low sensitivity of $I_K$ to TEA observed in the primary sensory hair cells is not unusual for cephalopod cells, for example, Williamson (1995a) reported a similar response in secondary sensory hair cells and Tasaki and Hagiwara (1957) found that even 250 mmol/l external TEA had little effect on $I_K$ in the squid giant axon. Both $I_K$ and $I_A$ have been identified in vertebrate sensory hair cells (e.g., Griguer et al. 1993; Lang and Correia 1989), however, a large $I_A$ has been found in hair cells of the chick’s basilar papilla, and auditory end-organ (Murrow 1994), with much less representation in hair cells from auditory systems.

In the present study, we observed a third outward current (Fig. 8), a calcium-dependent potassium current ($I_{KCa}$), which has not been previously identified in invertebrate hair cells but has been seen in vertebrate sensory hair cells (e.g., Fuchs 1992; Masetto et al. 1994). There are two types of calcium-activated potassium currents; some that are sensitive to apamin and have a small single-channel conductance (SK) and a low affinity to Ca$^{2+}$ (Art et al. 1995) and others that are insensitive to apamin but can be blocked by charybdotoxin. They have a larger single-channel conductance (BK) and a higher affinity to Ca$^{2+}$ (Art et al. 1995). In isolated cephalopod hair cells, the calcium-activated potassium currents we have recorded seem to resemble to the SK channels because of their sensitivity to apamin, which may suggest that cephalopod hair cells use SK channels instead of BK channels to modulate voltage-gated calcium influx.

Although other studies have indicated that this current also can be involved in membrane repolarization after an action potential (Adams et al. 1982; Blatz and Magleby 1987; Takahashi 1990), we did not find that blocking this current prolonged the action potential in cephalopod hair cells (Fig. 2), and it is therefore unlikely to play this role here. This kind of observation also has been made for other cell types (Chandler et al. 1994; Nishimura et al. 1989; Schwindt and Crill 1981).

Na$^+$ current

In all cases where the axon was still present on the dissociated primary sensory hair cells, we observed a rapidly activating and inactivating inward current, which we have identified as carried by sodium ions ($I_{Na}$) (Fig. 9). Because this current was not observed in dissociated cells without an axon, it is likely that the membrane channels responsible for this $I_{Na}$ are restricted to the axon and that they are involved in the production of the axonal regenerative action potential observed in these cells (Fig. 1). A current with similar kinetics and pharmacological sensitivity has been identified previously in a variety of cephalopod and non-cephalopod of cell types (e.g., Armstrong et al. 1973; Bullock and Schauf 1979; Lasater 1986; Lo and Shragger 1981; Neher 1971) and shown to be associated with the action potential (e.g., Chrachri 1995; Kuffler and Eyzaguirre 1955). There are, however, only a few reports of such a current in vertebrate sensory hair cells (Evans and Fuchs 1987; Sugihara and Furukawa 1989); but these vertebrate hair cells do not have axons, and so the current must serve another function, perhaps, as suggested by Evans and Fuchs (1987), to synchronize the outputs from many cells. $I_{Na}$ has been reported in cephalopod secondary sensory hair cells, which like their vertebrate counterparts have no axons, but here it has been associated with the presence of long basal processes that extend to other hair cells and that may carry action potentials (Williamson 1995a).

Ca$^{2+}$ current

The present study identified a second inward current, carried by calcium ions, in cephalopod primary sensory hair cells (Figs. 10 and 11). On the basis of its kinetics, ion specificity, and pharmacological sensitivity, this inward current ($I_{Ca}$) is similar to the high-voltage activated calcium current, or L-type calcium current, previously seen in a variety of other cell types (Carbone and Lux 1984; Hagiwara and Byerly 1981). No low-voltage–activated calcium current, or T-type current (Nowycky et al. 1985), was detected in the cephalopod primary sensory hair cells, although it has been found in other cephalopod cells (A. Chrachri, unpublished data). L-type calcium currents have been reported in vertebrate sensory hair cells (e.g., Fuchs et al. 1990), where the current activates at membrane potentials around −40 mV, more negative than the L-type calcium current found in nerve cell bodies because the calcium influx in the hair cells must be capable of regulating transmitter release for small voltage deflections about the resting potential. This difference in activation characteristics also can be seen in cephalopod hair cells, where the activation potential is much lower in the primary sensory hair cells (around −20 mV, Fig. 10), which have no transmitter release from the cell body, than in the secondary sensory hair cells (around −40 mV) (Williamson 1995a), which do have a chemical synapse, or one close to, the cell body.
The reduction in action potential amplitude in cephalopod primary sensory hair cells seen in the presence of calcium channel blockers (Fig. 2), together with the rapid activation kinetics of $I_{Ca}$, implies that calcium also is involved in this process, as found in other sensory cells and neurons (e.g., Harada and Takahashi 1983; Hounsgaard and Mintz 1988; Viana et al. 1993), although whether this involvement is restricted to the axonal action potential or occurs only close to the cell body, for the primary sensory hair cells, cannot yet be determined. However, it is surprising that no residual action potential was observed in the presence of TTX (Fig. 1D), as part of the action potential seems to be due to a calcium influx (Fig. 2A). It may be that calcium also can alter cell excitability as well as acting as a charge carrier. With the exception of $I_{Na}$, all other currents were observed, regardless of whether an axonal process was present or not, on the dissociated cells; the ion channels responsible for these currents therefore must be located on or close to the cell bodies.

**Comparisons with other invertebrate sensory hair cells**

Although the transduction process in a number of other invertebrate mechanosensory cell types has been studied, e.g., arthropod mechanosensilla (French 1992; Lemmnnitz and Wolf 1990) and gastropod mollusks primary hair cells (Alkon and Bak 1973), the only membrane currents characterized have been voltage-insensitive chloride and potassium currents in mechanosensory neurons isolated from the cockroach antennal chordotonal organ (Stockbridge et al. 1990). The present work therefore represents a significant step forward in identifying the basic processes involved in invertebrate hair cell operation. The ionic conductances involved in the mechanoelectric transduction process have not yet been identified in invertebrate sensory hair cells but, because the response to positive stimulation is a depolarization of the cell (e.g., Wiederhold 1976; Williamson 1990), a non-specific cation channel may be involved. This depolarization will spread into the cell body and activate voltage-dependent calcium and sodium channels, which will increase the depolarization and result in action potentials in the axon. However, voltage- and calcium-dependent potassium conductances also will be activated and these then will act to repolarize the cell.

With respect to the cephalopod secondary sensory hair cells, both primary and secondary sensory cells show a similar spectrum of currents, although the sodium conductance is much less common in the secondary sensory hair cells, occurring only where basal processes are present, and, as yet, no calcium-activated potassium conductance has been reported for these cells (Williamson 1995a). Such similarities might be expected if, as proposed by Jorgensen (1989), secondary sensory hair cells evolved from primary sensory hair cells, although the evolutionary pressure leading to this development and the advantage to the animal of having both cells types is not yet understood.

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