Potassium Currents in Isolated Statocyst Neurons and RPeD1 in the Pond Snail, Lymnaea stagnalis

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Sakakibara, Manabu, Futoshi Okuda, Kazutoku Nomura, Kenji Watanabe, Hongxu Meng, Tetsuro Horikoshi, and Ken Lukowiak. Potassium currents in isolated statocyst neurons and RPeD1 in the pond snail, Lymnaea stagnalis. J Neurophysiol 94: 3884–3892, 2005. First published August 10, 2005; doi:10.1152/jn.01163.2004. To begin to determine the underlying neural mechanisms of memory formation, we studied two different cell types that play important roles in different forms of associative learning in Lymnaea. Statocyst neurons (hair cells) mediate classical conditioning, whereas RPeD1 is a site of memory formation induced by operant conditioning of aerial respiration. Because potassium (K+) channels play a critical role in neuronal excitability, we initiated studies on these channels in the aforementioned neurons. Three distinct K+ currents are expressed in the soma of both the hair cells and RPeD1. In hair cells and RPeD1, there is a fast activating and rapidly inactivating 4-aminopyridine (4-AP)-sensitive A current (IA), a tetraethyl ammonium (TEA)-sensitive delayed rectifying current, which exhibits slow inactivation kinetics (ICaK), and a TEA- and 4-AP-insensitive Ca2+-dependent current (ICa,Ca). In hair cells, the activation voltage of IA; its half-maximal steady-state activation voltage and its half-maximal steady-state inactivation were at more depolarized levels than in RPeD1. The time constant of recovery from IA inactivation was slightly faster in hair cells. IA in hair cells is also smaller in amplitude than in RPeD1 and is activated at more depolarized potentials. In like manner, ICaK is smaller in hair cells and is activated at more depolarized potentials than in RPeD1.

INRODUCTION

Lymnaea is a well-established model system for studying the underlying neural and molecular mechanisms of adaptive behaviors and the snail has the capacity to be both classically and operantly conditioned (Benjamin et al. 2000; Lukowiak et al. 2003b). Previously we demonstrated that Lymnaea can be classically conditioned by pairing a flash of light (the conditional stimulus, CS) with orbital rotation (the unconditional stimulus, UCS) (Sakakibara et al. 1998). Photic stimulation, the CS, initially does not elicit a withdrawal response, whereas the rotational stimulus, the UCS, does. However, after 30-paired CS–UCS presentations, the CS elicits a robust withdrawal response (i.e., they learned and formed memory). We further found that the visual information for this learned behavior is mediated exclusively by ocular photoreceptors and not by the dermal photoreceptors (Ono et al. 2002; Tsubata et al. 2003).

An important homeostatic behavior in Lymnaea, aerial respiratory behavior, is capable of being operantly conditioned (Lukowiak et al. 1996, 2003a). A three-neuron central pattern generator (CPG), which is both necessary and sufficient, drives this behavior (Syed et al. 1990, 1992). In the operant conditioning procedure, snails associatively learn not to perform aerial respiration as a result of the contingent presentation of a tactile stimulus to their respiratory orifice each time they attempt to breathe aerially. Neural correlates of this operant conditioning have been demonstrated in RPeD1, the CPG neuron that initiates aerial respiratory behavior (Spencer et al. 1999, 2002).

We have recently characterized some of the basic physiological and morphological properties of Lymnaea photoreceptors and statocyst neurons (Sakakibara et al. 2005). Briefly we found that one type of photoreceptor, the T-type neuron, sends its primary axon out into the cerebral ganglion into the vicinity of the endings of the statocyst neurons (the cells that are activated by the rotational stimulus). Medial and rostral statocyst neurons are depolarized by photo-stimulation of the eye in a Ca2+-dependent manner, suggestive of a synapse between the T-type photoreceptors and the statocyst neurons. We now direct our attention to a more-thorough investigation of the biophysical properties of the ionic currents of statocyst neurons and compare these with currents found in RPeD1, a neuron known to be a necessary site for the formation of long-term memory (LTM) during operant conditioning (Sangha et al. 2003a; Scheibenstock et al. 2002). Here we examine K+ channels in these two neuronal types. We choose to initially concentrate on K+ channels because they play a critical role in neuronal excitability. Neural correlates of learning and memory formation in Lymnaea have been shown to involve changes in cell excitability. Because altering the activity of K+ channels can lead to either increased or decreased neuronal excitability, they are an excellent place to begin our studies. Moreover, their opening, or gating is governed by remarkably diverse mechanisms (e.g., ligand binding, mechanical changes, membrane voltage, and pH) (MacKinnon 2003). Our data are vital if we are to begin to be able to characterize the neuronal loci and the molecular events causal to learning and its consolidation into LTM.

Statocyst neurons (i.e., hair cells) form part of the statocyst organ in mollusks (Detwiler and Alkon 1973; Wiederhold...
respectively. In *Lymnaea*, a pair of statocysts are located in the left and right pedal ganglia. The neuritic processes of the hair cells project from the statocysts to the cerebral ganglia (Sakakibara et al. 2005). Within the statocyst are 13 spherical hair cells ranging in size from 30 to 60 μm in diameter. Hair cells bear ~100 kinocilia and microvilli (Janse et al. 1988), in contrast to vertebrate hair cells, which may have a single kinocilium and 50–100 stereocilia, depending on the species (Hudspeth and Jacobs 1979).

RPeD1 is one of the largest identified neurons in *Lymnaea* (110–150 μm in diameter) and is located in the right pedal ganglion immediately adjacent to the statocyst. RPeD1 uses dopamine as one of its neurotransmitters and is the cell that initiates rhythmogenesis in the three-neuron CPG that drives aerial respiratory behavior (Syed et al. 1990).

Here we present the kinetics and pharmacologic description of the voltage-, time-, and Ca\(^{2+}\)-dependent outward K\(^+\) currents in both hair cells and RPeD1 of *Lymnaea*. These currents may play important and different roles in ultimately mediating learning and the formation of LTM in *Lymnaea*.

**METHODS**

**Animals**

Laboratory-reared fresh water pond snails, *Lymnaea stagnalis*, originally derived from stocks obtained from Vrije Universiteit (Amsterdam) were used. The snails used in these experiments had a shell length between 18 and 20 mm and were maintained at 20°C in well-aerated pond-water, on a 12-h light:12-h dark cycle (0800–2000). They were fed ad libitum cabbage, lettuce, and goldfish pellets.

**Chemicals and solutions**

Solutions used and their chemical compositions (in mM) were 1) *Lymnaea* saline: 51.3 NaCl, 1.7 KCl, 5.0 MgCl\(_2\), 1.5 CaCl\(_2\), and 5.0 HEPES, pH 7.9–8.1 (NaOH), 2) external solution for K\(^+\) current recordings: 51.3 tetra-methyl-ammonium (TMA), 1.7 KCl, 0–4 CaCl\(_2\), 2–6 MgCl\(_2\), 10 HEPES, pH 7.8–8.0 (KOH), and 3) pipette solution for K\(^+\) current recordings: 60 K-glucosone, 1 CaCl\(_2\), 2 MgCl\(_2\), 10 HEPES, 5 ethyleneglycol-bis-(β-amino-ethylether)N,N\(^{-}\)-tetra-acetic acid (EGTA), pH7.4 (KOH). For elimination of voltage-dependent potassium currents, 5 mM 4-aminopyridine (4-AP) and/or 40 mM TEA were added to inactivate the fast activating current and the delayed rectifying K\(^+\) current, respectively.

**Electrophysiology**

Experiments were performed at room temperature (20°C) on isolated (i.e., without the primary neurite [axon]) statocyst hair cells and the RPeD1 neuron. The circumesophageal ganglia were dissected out in *Lymnaea* saline and incubated with protease (type XIV, Sigma Chemical, St. Louis, MO) solution (1 mg/ml) for 18 min at 20°C to loosen the connective tissue sheath surrounding the statocyst. After rinsing thoroughly with *Lymnaea* saline, a statocyst and a RPeD1 neuron were mechanically isolated with fine forceps. The isolated statocyst or RPeD1 was then transferred to a glass slide. The whole cell voltage-clamp recording method of Hamill et al. (1981) was used to record K\(^+\) and Ca\(^{2+}\) currents. We used an EPC7 amplifier (Listelectronic, Darmstadt-Eberstadt, West Germany) with a recording electrode fabricated from a glass capillary (No. 5964; AM-Systems, Carlsborg, WA) and pulled on an electrode puller (P-2000, Sutter Instrument, Novato, CA). Data were recorded on a storage oscilloscope (DCS-7020, Kenwood, Tokyo, Japan) and on a PC-AT compatible personal computer digitized at a sampling rate of 10 kHz via an interface board (Digidata 1200, Axon Instruments, Union City, CA) controlled by pCLAMP (Axon Instruments). Passive leakage and capacitive currents were subtracted by the p/4 method, i.e., currents were averaged from four successive recordings to the same clamping potential. The sum of currents elicited by four hyperpolarizing pulses from the holding potential with an amplitude one-fourth of the test pulse was added to the test pulse induced current.

Data were analyzed with the clampfit of pCLAMP, and curve fits were performed by the least-squares method with Origin (Microcal Software, Northampton, MA). Unless indicated, data are presented as means ± SD. The steady-state activation (\(m_a\)) and inactivation (\(h_a\)) properties of the currents in relation to voltage were described by the following Boltzmann equations where

\[
m_a = \frac{1}{1 + \exp \left( \frac{V_m - V_{1/2}}{K_m} \right)}
\]

\[
h_a = \frac{1}{1 + \exp \left( \frac{V_m - V_{1/2}}{K_h} \right)}
\]

where \(V_m\) is the membrane voltage, \(V_{1/2}\) denotes the half-activation or inactivation voltage, and \(K\) is the slope factor.

To characterize ionic currents, the membrane was usually held at –80 mV and stepped down to –100 mV for 20 ms for inactivation of the steady-state K\(^+\) current and stepped up from –80 to 70 mV for 150 ms in 10-mV steps.

**RESULTS**

Figure 1A shows a representative photograph of *Lymnaea* statocyst neurons (i.e., hair cells) surrounded by statoconia and a recording electrode. Figure 1B shows a typical whole cell voltage-clamp record of a *Lymnaea* hair cell and RPeD1 in the presence of Na\(^+\) in the external bathing medium. The fast inward current, carried by Na\(^+\), is followed by a prolonged outward current in both hair cells and RPeD1.

**Voltage-dependent K\(^+\) currents**

An inward, transient, voltage-dependent sodium current was apparent in somata of both hair cells and RPeD1 in normal *Lymnaea* saline (Fig. 1B). We therefore replaced the NaCl in the *Lymnaea* recording solution with equimolar tetra-methyl-ammonium (TMA) to create an “Na\(^+\)-free *Lymnaea* solution” to better investigate outward K\(^+\) currents.

From a holding potential of –80 mV in the Na\(^+\)-free *Lymnaea* solution an outward current was activated at ~10 mV in hair cells and at ~30 mV in RPeD1 (Fig. 2). In hair cells, the transient outward current peaked at ~25 ms after the onset of the depolarization step and was followed by a fast inactivation component and then a sustained component. The sustained component plotted in Fig. 2 was measured 100 ms after the onset of the depolarizing step. The I-V relationships for both the transient and the sustained outward currents in hair cells and RPeD1 are also plotted in Fig. 2. The transient fast component in the hair cells became less apparent at depolarizing steps to +60 mV or greater. In RPeD1, the transient current was apparent at all depolarizing steps and became larger with increasing depolarizing steps. Unlike the situation in hair cells where the inactivating component of the transient...
The current was not observed above +40 mV, the inactivating current in RPeD1 was apparent at all tested voltage steps. The currents evoked in RPeD1 were also of a much larger amplitude than in the statocyst neurons.

One of the components of the outward potassium current found in a wide variety of neurons is the so-called A current \( (I_A) \), which is characterized by its fast activation followed by a rapid inactivation (Alkon et al. 1984; Sakakibara et al. 1993;...
Another characteristic of $I_A$ is its sensitivity to the K-channel blocker 4-AP. We therefore subjected both hair cells and RPeD1 to a 4-AP challenge. That is, we attempted to pharmacologically isolate $I_A$ by superfusing the neurons with 5 mM 4-AP-containing solution (Fig. 3). Addition of 4-AP to the external solution rapidly resulted in the removal of the fast transient component of the whole cell outward potassium current and a reduction in its amplitude. To obtain $I_A$ we subtracted the waveforms obtained in the 4-AP-containing Na$^+$-free Lymnaea solution from those of Na$^+$-free Lymnaea solution. The family of current traces is illustrated for hair cells in Fig. 3A and for RPeD1 in Fig. 3B. The IV relationships for the 4-AP-sensitive current (i.e., $I_A$) for both hair cells and RPeD1 are plotted in Fig. 3B.
After the pharmacological isolation of $I_A$, we then superfused an external solution containing both 4-AP and 50 mM TEA over hair cells and RPeD1, respectively. The addition of TEA substantially reduced the sustained outward current remaining after the application of 4-AP (Fig. 3A). This remaining current (Fig. 3A) is termed $I_{Ca-K}$. The TEA sensitivity of the sustained current is indicative of the delayed rectifier $K^+$ current ($I_{KV}$). Moreover, subtraction of the TEA resistant current waveforms (i.e., $I_{Ca-K}$) from the waveforms obtained before TEA application yield $I_{KV}$ (Fig. 3A). The $I-V$ relationships for the TEA-sensitive current for both hair cells and RPeD1 are plotted in Fig. 3C. The data shown in Fig. 3A were depicted from the recordings of one hair cell. The family of currents in Fig. 3, B and C, were the examples of $I_A$ and $I_{KV}$, respectively, observed in RPeD1 neurons and in hair cells.

From the $I-V$ relationship plots for $I_A$ and $I_{KV}$ for hair cells and RPeD1, we can draw the following conclusions: $I_A$ in hair cells is smaller in amplitude (by a factor of 3) than RPeD1 and is activated at more depolarized potentials than RPeD1 (−10 mV in hair cells compared with −60 mV in RPeD1). In a similar manner, $I_{KV}$ is different between the two cell types. In hair cells, it is smaller (by a factor of 1.5) in amplitude and is activated at more depolarized potentials than RPeD1 (+10 vs. −30 mV).

**Reversal potential of the outward currents**

To further support our conclusion that we were dealing with an outward $K^+$ current, we assessed the tail currents of the TEA-sensitive waveforms at various membrane potentials to determine the reversal potential of the sustained component of the outward current. These data are plotted in Fig. 4. Shown are traces of the TEA-sensitive current ($I_{KV}$) evoked from a holding potential of −40 mV. An outward current is then elicited by depolarizing the cell to +40 mV for 200 ms. At the end of this pulse, the membrane potential is repolarized to different, less depolarized potentials in 20-mV increments between +20 and −120 mV, and the tail currents were examined. The current traces from a RPeD1 neuron and a single hair cell are shown in Fig. 4 as well as the instantaneous current-voltage relationship ($n = 4$ for each cell type). The calculated reversal potential of the hair cells is −70 mV, whereas that for RPeD1 is −78 mV.

**Properties of the transient outward $K^+$ currents**

We also investigated the differences in $I_A$ between the hair cells and RPeD1 in another manner, one without having to resort to pharmacological blockers, and thus avoiding possible side effects of TEA. We therefore examined the steady-state inactivation of $I_A$. The steady-state inactivation was investigated by application of a 200-ms conditioning pulse to various potentials between −120 to 60 mV, followed by a test pulse to 20 mV for 100 ms as shown in the protocol drawn at the far right of Fig. 5A, top. The best fitted half-maximal steady state activation voltage ($V_{1/2}$) of the hair cell was 6.5 mV, whereas it was −18.1 mV for RPeD1 as shown by $m_e$ in Fig. 5A. The best fitted slope factor ($k_m$) of the hair cell and RPeD1 was 20.0 and 7.26, respectively.

The half-maximal steady-state inactivation curve denoted by $h_e$ in Fig. 5A was −30.0 mV for hair cells, whereas it was −69.2 mV for RPeD1. The slope factor for hair cells and RPeD1 was 11.0 and 8.8, respectively. Thus there were easily discernible differences in $I_A$ between hair cells and RPeD1.

Based on these and the data presented in Fig. 3, we conclude that $I_A$ magnitude is smaller and less easily activated in hair cells than in RPeD1. As previously demonstrated, the contribution of $I_A$ was smaller, more easily saturated, and less easily activated in hair cells than RPeD1.

Recovery from inactivation of $I_A$ in both hair cells and RPeD1 was also investigated. We determined the recovery time by presenting twin identical command voltages from −80 to 20 mV of 150-ms duration with varying the interstimulus interval, $T$, between the pulses as shown in protocol diagrammed in Fig. 5B. We plotted the amplitude ratio of the two current transients versus the duration of the inter stimulus duration.
FIG. 5. Dynamical properties of $I_A$. A: steady-state activation, $m_\infty$, and inactivation, $h_\infty$, curves of $I_A$ current were fitted with a Boltzmann equation. The half-activation voltage was 19 and −9 mV in hair cell and RPeD1, respectively. Inset: examples of current traces elicited to generate the inactivation curve in hair cell. The cell was held at −80 mV and stepped to conditioning potentials varying from −100 to 40 mV for 200 ms followed by a test pulse of 20 mV for 100 ms. The current was normalized against the noninactivating component evoked by the test potential. B: recovery from inactivation of $I_A$ was measured after currents were elicited by twin pulses with varying interstimulus intervals. The stimulus interval was plotted against the amplitude ratio of the 2 current transients. ■, hair cell; ○, RPeD1 in A and B.
Recovery occurred exponentially, with a time constant of 12 and 15 ms in hair cells and RPeD1 neurons, respectively.

**DISCUSSION**

We set out to explore the similarities and differences in ionic currents in two distinctive types of neurons: statocyst hair cells and RPeD1. Both neuronal cell types play important and necessary roles in the acquisition of associative learning and its consolidation into LTM. As such, an understanding of the “base-line” of the biophysical properties of these neurons is important, as it will eventually allow us to come to an understanding of how different forms of associative learning (i.e., classical vs. operant conditioning) are mediated either similarly or differently at the cellular level and then how these changes are consolidated into LTM. We therefore chose to first study K⁺ channel activity, as reflected by changes in various K⁺ currents, because of their known ability to regulate neuronal excitability, before examining other currents.

The hair cells are sensory neurons that mediate the vestibular sense (Wiedenhold 1974), whereas RPeD1 is an interneuron that is a member of the three-neuron CPG that drives aerial respiratory behavior (Luksowski 1991; Syed et al. 1990). Activity in RPeD1 initiates rhythmogenesis, and RPeD1 has been shown to be a necessary site for LTM formation of associative learning, its reconsolidation and extinction (Sangha et al. 2003a,b; Scheibenstock et al. 2002; Syed et al. 1990, 1992). Hair cells in *Lymnaea*, as their counterparts in *Hermissenda*, have been implicated to play a major role in classical conditioning of the withdrawal response to a photic stimulus after pairing of a photic stimulus with a rotational stimulus (Sakakibara et al. 2005; Tsubata et al. 2003). Here we have begun an investigation of the biophysical properties of some of the ionic currents of both statocyst neurons and RPeD1, cells that are hypothesized to play major and possibly causal roles in mediating learning and memory formation in *Lymnaea*. Data such as those we have obtained here are necessary if we are to begin to gain a better understanding of the underlying causes of learning and the consolidation of learning into long-lasting memory. Our study is the first that we know of where the K⁺ currents of two distinctive cell types that are thought to play key roles in two different forms of learning (classical vs. operant conditioning) and memory have been compared and contrasted in some detail. Knowing their similarities and their differences may enable us to better explore how learning and memory are mediated in this model system.

We found in both these types of *Lymnaea* neurons the “typical” complement of inward and outward currents including an inward Na⁺ current and three K⁺ outward currents. In this present series of experiments, we have concentrated most of our efforts delineating the three different K⁺ currents as these K⁺ currents play a key role in determining neuronal excitability. Future studies will explore similarities and differences in Ca²⁺ currents between these two cell types.

**Comparison of hair cell currents in two mollusks**

We first need to compare the family of currents found in *Lymnaea* hair cells with those found previously in the longer studied hair cells from *Hermissenda*. We found that the family of currents in *Lymnaea* hair cells differ qualitatively and quantitatively from the family of currents in *Hermissenda* hair cells. For example, while a voltage-gated Na⁺ current was found in the soma of *Lymnaea* hair cells; *Hermissenda* hair cells lack this voltage-gated Na⁺ current. In both *Lymnaea* and *Hermissenda* hair cells, three voltage-dependent K⁺ currents (Iₐ, Iᵥ, Iᵥ,K) were found. However, there were quantitative differences between the values of each of these currents between *Lymnaea* and *Hermissenda* hair cells.

Yamoah (1997) found that Iₐ was partially activated at the normal resting membrane potential (RMP) of −50 mV, whereas in *Lymnaea*, this same current was only activated at −10 mV. Similar differences were also found when the half-maximum steady-state activation and inactivation values were compared. The half activation and inactivation voltage in *Hermissenda* was −31 and −51 mV, respectively, whereas in *Lymnaea*, the half activation and inactivation voltage was 7 and −30 mV, respectively. The activation dynamics of Iₐ were described by time constant (τᵥ); we obtained a value of 12 ms in *Lymnaea* hair cells, whereas in *Hermissenda* hair cells, the value ranged from 2.3 to 10 ms. As a further comparison between the Iₐ current in *Hermissenda* versus *Lymnaea*, the half activation and inactivation voltage at 10 mV was 1.0 nA compared with a value of 1.2 nA in *Hermissenda* hair cells. Thus while hair cells in both mollusks signal among other things, gravitational orientation, and while both are similarly located within the central ring ganglia, there are major differences in the magnitude and operation of Iₐ. We are not certain why the qualitative and quantitative differences exist, but one possibility is that these differences exist as a result of differences in “their lifestyles” (marine vs. freshwater; carnivorous vs. herbivorous).

**Comparison of the voltage-gated K⁺ currents in hair cells and RPeD1**

As just mentioned, there are three different outward K⁺ currents (Iₐ, Iᵥ,K, and Iᵥ,K) in *Lymnaea* hair cells, and the same three currents were found in RPeD1. However, Iₐ makes a far greater contribution to the outward current profile in RPeD1 than it does in the hair cells. We arrived at this conclusion based on the following findings. In RPeD1, Iₐ was activated at −50 mV (in hair cells only weakly activated at 10 mV), its half-maximal steady-state activation voltage was −18 mV (at 7 mV in hair cells), whereas its half-maximal steady-state inactivation was −69 mV (at −30 mV in hair cells). Furthermore, at a clamping potential of 10 mV the magnitude of Iₐ in RPeD1 was 5.9 nA compared with 1 nA in hair cells. In other *Lymnaea* interneurons, in the giant cerebral cells (CGCs), for example (Staras et al. 2002), Iₐ was activated at approximately −60 mV and showed steady-state inactivation with more depolarized holding potential. Moreover, it was completely inactivated at holding potentials more positive than −40 mV. In VD4, on the other hand (Barnes et al. 1994), Iₐ was not measurable at voltages more positive than −40 mV, and its inactivation was complete in a cell held more positive than −30 mV. Thus Iₐ in *Lymnaea* interneurons located in three different ganglia (cerebral, pedal, and visceral) possesses
relatively similar properties and that are distinctly different from the properties exhibited by $I_{Na}$ in the sensory hair cells.

A large $I_{Na}$ in a neuron serves to limit the neuron’s excitability. Thus altering $I_{Na}$, for example by making it smaller, would allow a neuron to be more active for a given input. Alternatively, increasing $I_{Na}$ would cause to a cell to be less active. Thus, this current is an attractive one from the standpoint of being able to modify the excitability of a neuron. Previous data in Hermissenda suggest that $I_{Na}$ is modifiable by conditioning (Alkon et al. 1985). Whether $I_{Na}$ is modifiable in RPeD1, VD4, or the CGCs as a result of associative learning and the subsequent formation (i.e., the consolidation process) of LTM remains to be determined.

$I_{KV}$ in hair cells and RPeD1

The second K$^+$ current that we investigated in the hair cells and RPeD1 was $I_{KV}$. The delayed rectifying current, $I_{KV}$ was activated from $\sim 10$ mV in the Lymnaea hair cell and was activated $\sim 30$ mV in RPeD1. In Hermissenda hair cells, $I_{KV}$ was apparent from $\sim 20$ mV, and the half activation voltage of $I_{KV}$ was 8 mV (Yamoah 1997). On the other hand, $I_{KV}$ in CGC of Lymnaea was activated from $\sim 47$ mV and the half activation voltage was 12 mV (Staras et al. 2002). Comparing these values leads us to conclude that this current is not easily activated in Lymnaea hair cells and this current is larger in RPeD1 than in Hermissenda hair cells but it is smaller than in the CGCs. Because this is the current that determines in large measure the duration of the action potential and the magnitude of hyperpolarization following the cessation of the action potential, its modulation as a result of learning-induced release of neurotransmitters and/or neuromodulators, for example, could significantly alter synaptic transmission and/or neuron excitability.

$Ca^{2+}$-activated K$^+$ current: $I_{Ca-k}$

The properties of this current were similar in all neurons we investigated as well as in Hermissenda hair cells and other Lymnaea neurons. Modulation of this current as a result of associative learning or by the release of specific neuromodulators (Staras et al. 2002) could lead to alterations in oscillations of the “resting” membrane potential (Yamoah 1997). We will in the future investigate whether in Lymnaea hair cells or RPeD1 significant changes occur as a result of classical or operant conditioning.

Voltage-gated Na$^+$ current ($I_{Na}$)

The currents that we have described here from the hair cells and RPeD1 are similar to the currents described previously in Lymnaea neurons. Thus for example, the description of currents for an interneuron, the CGCs that play a key role in mediating adaptive feeding behaviors are not substantially different from those described here (Staras et al. 2002). The CGC neurons are thought to play an important modulatory role in the neural networks that control feeding and play a key role in mediating forms of associative learning and memory of feeding-related behaviors (Benjamin et al. 2000; Kojima et al. 1997; Nakamura et al. 1999).

Our voltage-clamp experiments show that $I_{Na}$ plays a key role in the generation of action potentials much as in most other neurons studied (Kiss 2003). Overall the Na$^+$ currents in the hair cells and RPeD1 had similar characteristics. We did not attempt, as have others, to determine the extent to which this current is blocked by TTX and other inward current blockers (Barnes et al. 1994; Onizuka et al. 2004; Staras et al. 2002). Gilly et al. (1997) and Staras et al. (2002) further report a second type of Na$^+$ current in spontaneously active molluscan neurons such as the CGCs and RPeD1 of Lymnaea. This is a slowly inactivating sodium current and is hypothesized to lead to the appearance of spontaneous action potentials. This other Na$^+$ current studied by Staras et al. (2002) was a low-threshold persistent current that could be activated by relatively small depolarizing inputs to the cells even when they were hyperpolarized. This current would lead to increased spiking activity of the cells, which may be an important and necessary role for these cells in adaptive feeding behaviors. Future studies will determine whether this current is present in the hair cells and whether in RPeD1 it plays a role in the establishment of LTM. Because we used “axotomized” RPeD1’s (i.e., the primary axon was crushed close to the somata of RPeD1 in all experiments) to effectively space clamp the neuron, it is possible that the low-threshold persistent current (and others) may play a substantial and important role in vivo preparations. Because RPeD1 becomes more inactive as a result of operant conditioning (McComb et al. 2003; Spencer et al. 1999, 2002), it is possible that this current is made less effective with operant conditioning.

Role of currents in learning and memory

The intrinsic membrane properties of hair cells, for example $I_{Na}$, are hypothesized to change as a result of classical conditioning. Hair cells receive input from T-type photoreceptors from the eye (Sakakibara et al. 2005). As a result of pairing of the photic stimulus (the conditional stimulus, CS) with a rotational stimulus (the unconditional stimulus, UCS) snails learn to withdraw to a photo stimulus. Thus we expect that following learning and the formation of memory hair cells will be more excitable to photic stimulation. A possible means of increasing the excitability of the hair cells is to decrease the contribution made by $I_{Na}$. That is, as $I_{Na}$ can serve to limit spiking, a decrease in $I_{Na}$ could result in increased excitability. This preliminary hypothesis will be tested.

RPeD1 as a site of LTM formation for the learned decrease in aerial respiratory activity that occurs as a result of operant conditioning. A neural correlate of LTM has been described in RPeD1 when trained versus untrained snails were examined. RPeD1 was significantly less excitable and less likely to elicit respiratory rhythmogenesis when depolarized. Because $I_{Na}$ in RPeD1 makes a large contribution to the total outward K$^+$ current, a simple, testable hypothesis would be that $I_{Na}$ is increased in RPeD1 as a result of operant conditioning training and thus RPeD1 would be less excitable.

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