Output Sign Switching in Molluscan Neurons is Mediated by a Novel Voltage-Dependent Sodium Current

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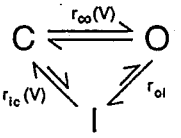
Abstract

We found neurons that can change the sign of their response to hyperpolarizing inputs depending on the amplitude of that input. After small hyperpolarizations, neurons showed a transient depolarization or post-inhibitory-rebound (PIR) while larger hyperpolarizing inputs lead to a post-inhibitory hyperpolarization (PIH). PIR is sometimes sufficient to produce an action potential while PIH prevents spiking. Voltage-clamp and pharmacological data indicate that PIH is mediated by the potassium current I_A. PIR is mediated by a novel sodium current that we call I_B, which has an unusual voltage-dependence for activation. Small hyperpolarizations appear to remove inactivation from I_B channels and this leads to PIR upon repolarization of the membrane as the channels open and once again inactivate. Interestingly, larger hyperpolarizations do not result in PIR upon repolarization even when I_A is not present or is blocked pharmacologically. Voltage clamp experiments suggest that I_B channels enter a second closed state during large hyperpolarizations. The transition from the second closed state back to the first is slow compared to the double transition from the first closed state to the open state to the inactivated state so that no current can be detected. I_B is the first known voltage-dependent current whose activation depends on conditioning at a holding potential within a voltage window.

Introduction

Neurons use voltage-dependent ion channels to integrate synaptic inputs, to produce action potentials, to control repetitive firing, and to stabilize membrane potential (Llinas, 1988; Hille, 1992). Most voltage-dependent channels can be realistically modeled with three-state kinetic models that include a closed state (C), an open state (O), and an inactivated state (I). Transitions between states depend on a rate constant (r) that can be voltage (V) dependent:



(1)

Only forward rate constants are shown, but the distribution of states also depends on the backward rate constants.

In this general model, channels rarely go from closed to inactivated or from inactivated to open (but see e.g. Horn et al. 1981). At resting potential, most voltage-dependent channels are closed. They open when the membrane is depolarized, and opening is followed by inactivation. Inactivation is then removed by repolarization of the membrane back to resting potential. There are exceptions: 1) some channels do not inactivate so the model may be simplified to include only closed and open states, 2) the voltage dependence can be reversed so that hyperpolarizations cause channel opening, and 3) some channels are open and/or inactivated at resting potential. Nevertheless, this general kinetic scheme (1) or a simplified version of it can describe all known voltage-dependent currents, at least to a first approximation (e.g. Destexhe et al. 1994).

Two well known voltage-dependent currents are the fast sodium current, I_{Na}, and the delayed rectifier potassium current, I_K, (Hodgkin & Huxley, 1952). The rising phase of the action potential is caused by opening of I_{Na} channels while the falling phase is produced by inactivation of I_{Na} and the opening of I_K channels. I_K channels do not have an inactivated state according to the original description.

We take the approach of Hodgkin and Huxley (1952) and others (Kandel and Tauc, 1966; Connor and Stevens, 1971a,b; Meech, 1974) who used large, identifiable molluscan neurons to identify ion-selective currents underlying active properties of neurons. Experiments were done primarily on S-cells in isolated brain preparations of *Tritonia diomedea* (Getting, 1976). S-cells are thought to be the primary afferents triggering the *Tritonia* escape swimming behavior.

Output Sign Switching

We studied the response of S-cells to hyperpolarizing inputs applied through an intracellular electrode. Small negative currents caused the usual hyperpolarization followed

by a depolarizing sag back toward resting potential (Figure 1). When the current pulse was terminated, there was a post-inhibitory rebound (PIR) that sometimes resulted in an action potential. Slightly larger negative current pulses produced hyperpolarization, but no depolarizing sag or PIR. Finally, still larger hyperpolarizing pulses produced either no sag or a very small hyperpolarizing sag that was followed by a post-inhibitory hyperpolarization (PIH). PIH can prevent spiking and has also been called delayed excitation for this reason (Getting, 1983). Thus, these neurons change the sign of their response to a hyperpolarizing input, from depolarizing to hyperpolarizing, as the amplitude of the input increases.

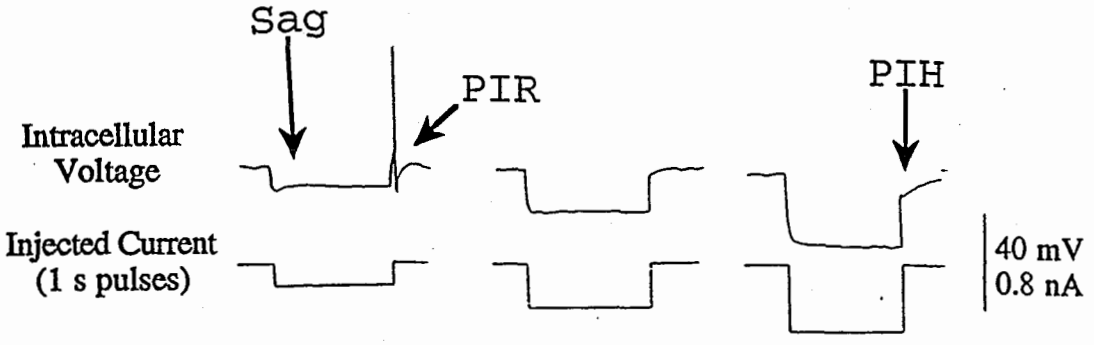


Figure 1. Sign switching to hyperpolarizing pulses

The currents that underlie PIR and PIH can be observed under voltage clamp (Figure 2). When a neuron is clamped at resting potential and stepped down 10 - 15 mV, an inward current develops slowly during the voltage step and then is fully activated when the membrane is stepped back to rest. In contrast, larger hyperpolarizing steps do not result in inward current activation. Instead, an outward current is activated when the membrane is stepped back to rest. To our knowledge, this is the first report of sign-switching in a neuron depending on the amplitude of its input.

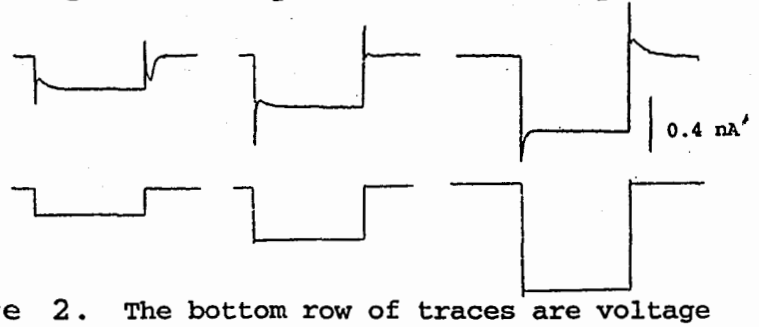


Figure 2. The bottom row of traces are voltage commands starting from a holding potential of -40 mV and stepping down -15, -30, and -60 mV from left to right. The top traces are applied current. Note the slowly developing inward current during the -15 mV step and the large inward current at the termination of the step. In contrast, note the large outward current at the termination of the -60 mV step.

PIH is Mediated by I_A

PIH has been observed in many neurons (e.g. Harris-Warrick et al. 1995), including *Tritonia* swimming neural network neurons (Getting, 1983). In each case, PIH appears to be mediated by an A current, I_A, similar to the one described by Connor and Stevens (1971). The activation curve of I_A is shifted to more hyperpolarized levels compared to I_K, and I_A is often inactivated at rest (see Salkoff et al. 1992 for a review). I_A is blocked by low concentrations of 4-aminopyridine (4 AP) in most neurons including those of *Tritonia* (Thompson, 1977). Thus, we tested to see if the transient outward current produced by large hyperpolarizing voltage steps in S-cells could be blocked by 4 AP (Figure 3). The channels appeared to be blocked in the open position, which is consistent with Thompson's report of a slowing of the inactivation time constant.

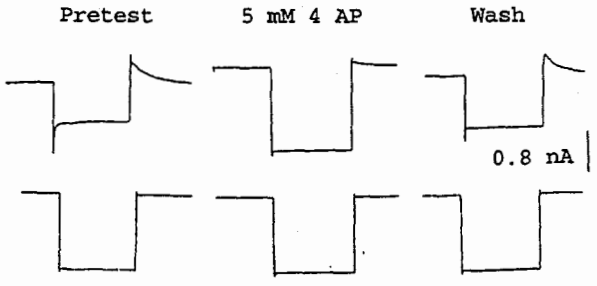


Figure 3. Bottom traces again are voltage commands: holding potential was -40 mV and the step was -60 mV. The large outward current transiently activated at the end of the current pulse was blocked by 4 AP.

We also measured the activation and inactivation properties of the outward current and found them to be similar to those of I_A. Interestingly, the steady-state inactivation gating parameter (see Hille, 1992), which is a sigmoidal function of membrane voltage, is shifted to the left compared to other Tritonia neurons (see also Connor and Stevens, 1971). Thus, these neurons must reach a more hyperpolarized level before inactivation can be removed compared to I_A in other *Tritonia* neurons (see discussion below). Nevertheless, all pharmacological and voltage-clamp data indicate that PIH is produced by I_A in S-cells.

A Novel Sodium Current for PIR, I_B

A mixed cation current activated by hyperpolarization (DiFrancesco, 1986) called I_h is thought to be the primary voltage-dependent current underlying PIR in vertebrate (e.g. Johnson and Getting, 1991) and invertebrate (e.g. Angstadt & Calabrese, 1989; Harris-Warrick et al. 1995) neurons. I_h is also known to produce a sag back toward resting potential

during a hyperpolarizing current pulse, not unlike the sag shown in Figure 1. Thus, we first tested to see if PIR in S-cells was mediated by I_h.

The pharmacological signature of I_h is blockage by low concentrations of Cs^+. However, the current underlying PIR in *Tritonia* S-cells was stable even in relatively high concentrations of Cs^+ (Figure 4a). Furthermore, I_h channels are opened by hyperpolarization while the current underlying PIR in S-cells was only fully activated after repolarization of the membrane following the hyperpolarizing step (Figures 2 & 4). Thus, PIR is not mediated by I_h in these neurons.

The reason the PIR current in S-cells produces a depolarizing sag is because the underlying current is taken from a level where it is completely inactivated (around rest) to a level where it is only partially inactivated. Inactivating currents that have a steady-state activation range are called window currents because there is a window of voltage where they are at least partially activated but not completely inactivated. Thus, the PIR current in S-cells is a window current.

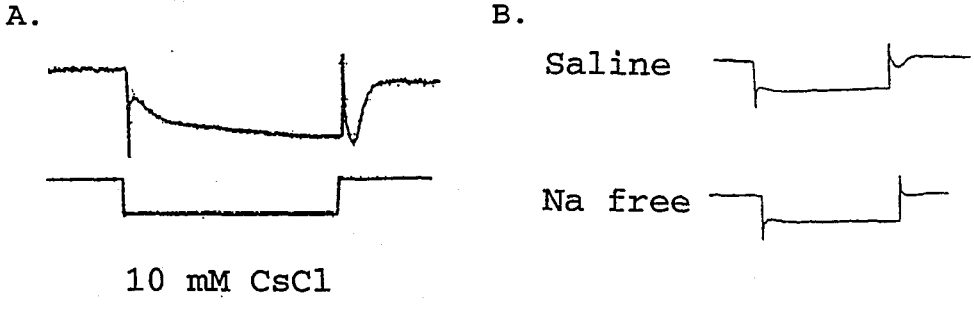
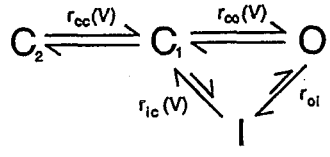


Figure 4. (A) I_B is activated in 10 mM Cs+. Holding potential was -35 mV and the step was -10 mV. The voltage step lasted for one second here and elsewhere. I_B inactivates after 150 - 200 ms. (B) I_B was abolished in Na+ free saline (only current traces are shown; hold = -40, step = -10).

Another mechanism for PIR is called anode-break excitation (ABE), which occurs when there are some I_{Na} channels that are inactivated at resting potential. When a neuron is hyperpolarized, I_{Na} channels go from inactivated to closed. When the membrane is repolarized, channels open, and the membrane is depolarized until channels inactivate. The PIR current studied here does appear to be carried by Na+ (Figure 4b). However, I_{Na} cannot explain the sag back toward resting potential during the hyperpolarizing current pulse. The kinetics of the current underlying PIR in S-cells are also much slower than I_{Na} kinetics.

There is another property of the PIR current in these neurons that differentiates it not only from I_{Na}, but from all other voltage-dependent currents described thus far. *Large hyperpolarizations do not result in the activation of the PIR current upon repolarization after the current step* Neither the partial activation during the sag nor the full activation upon repolarization is observed when a large hyperpolarizing step is given. This is not due to a masking of the PIR current by I_A because it is true even when I_A is blocked pharmacologically or is not present. (We have made recordings from other *Tritonia* neurons that do not have an appreciable I_A, but do have the PIR current.)

We call the PIR current I_B since it has not been identified previously. I_B is the first voltage-dependent current described whose activation depends on conditioning within a voltage window. At potentials around resting potential or just above, most I_B channels are inactivated. Small hyperpolarizations move the channels out of the inactivated state into a combination of open and closed states. In order to explain the behavior of I_B in during large hyperpolarizing inputs, we propose the following model:



(2)

Channels enter a second closed state during large hyperpolarizations, which prevents steady-state activation (the sag in Figure 1). The transition from the second closed state back to the first would be slow compared to the double transition from the first closed state to the open state to the inactivated state. Thus, when the neuron is repolarized after a large hyperpolarizing step, only a small number of channels are in the open state at any time. This model makes certain predictions that could be tested under voltage clamp conditions. However, the most direct tests would come from single channel recording.

Discussion

The behavioral significance of sign-switching by S-cells in *Tritonia* is not known, but it is possible to speculate because so much is known about these neurons (Getting, 1976). During the swimming behavior, S-cells receive strong, periodic hyperpolarizing inputs. S-cells are afferents, and this eliminates feedback from the periphery during swimming. PIH (and by the same argument I_A) may help to keep neurons hyperpolarized between waves of input.

S-cells fire at a high rate compared to other neurons in the *Tritonia* brain when a stimulus is presented in their receptive fields. At the recording electrode, the undershoot of S-cell action potentials reaches the appropriate voltage

to de-inactivate I_B but not to cause channels to enter the second closed state. Activation of I_B should then reduce the time to the next action potential and thus contribute to high frequency firing.

I_A and I_B are complementary currents de-inactivated by different amounts of hyperpolarization: I_B just below resting potential and I_A at lower voltages. (I_B channels are also de-inactivated at lower voltages but they enter a second closed state according to the model (2).) The I_A described in the present study is de-inactivated at more negative voltages than the original I_A described in *Tritonia* neurons (Connor and Stevens, 1971), and this reinforces the separation of these two currents. How these neurons regulate I_A currents depending on cell type or presence of I_B is not known.

The novelty of the observation that I_B activation depends on conditioning within a voltage window raises the question of whether this phenomenon can be explained by some other combination of known currents and/or dendritic geometries. There were no differences in I_B when recorded in dissociated cell bodies suggesting that morphological explanations will not explain it. Indeed, we cannot even hypothesize a combination of geometries and currents that could produce this behavior. Nevertheless, we are exploring this possibility with computer models. Again, single-channel recordings to confirm I_B's properties would be helpful.

Unfortunately, we have not yet been able to determine the reversal potential of I_B because of contamination from I_{Na}. I_B appears to be a sodium current because it is abolished in sodium-free saline (Figure 4B) but is not affected by calcium-free or potassium-free saline. We hope to use some of the new sodium channel blockers recently isolated from mollusc-hunting conus snails to dissect out I_B (e.g. Hasson et al. 1995).

Sensitization in *Tritonia* includes an increase in the excitability of S-cells, perhaps due to the release of serotonin from dorsal swim interneurons (Brown, 1994). It will be of interest to determine if I_B plays a role in the sensitization memory trace.

Most voltage-dependent ion channels are highly conserved (e.g. Salkoff et al. 1992). We have already observed I_B in *Pleurobranchaea californica* and *Clione limacina* indicating that I_B is present throughout the opisthobranch molluscs. We next want to determine its relationship to slow sodium currents in vertebrate neurons.

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