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Two Kinds of Transient Outward Currents, I_A and I_{Adepol}, in F76 and D1 Soma Membranes of the Subesophageal Ganglia of *Helix aspersa*

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Abstract. Transient outward currents were characterized with twin electrode voltage clamp techniques in isolated F76 and D1 neuronal membranes (soma only) of *Helix aspersa* subesophageal ganglia. In this study, in addition to the transient outward current (A-current, I_A) described by Connor and Stevens (1971*b*), another fast outward current, referred to as I_{Adepol} here, is described for the first time. This is similar to the current component characterized in *Aplysia* (Furukawa, Kandel & Pfaffinger, 1992). The separation of these two current components was based on activation and steady-state inactivation curves, holding potentials and sensitivity to 4-aminopyridine (4-AP). In contrast to I_A, I_{Adepol} did not require hyperpolarizing conditioning pulses to remove inactivation; it was evoked from a holding potential of −40 mV, at which I_A is completely inactivated. I_{Adepol} shows noticeable activation at around −5 mV, whereas I_A activates at around −50 mV. The time courses of I_{Adepol} activation and inactivation were similar but slower than I_A. It was found that I_{Adepol} was more sensitive than I_A to 4-AP. 4-AP at a concentration of 1 mM blocked I_{Adepol} completely, whereas 5–6 mM 4-AP was needed to block I_A completely. This current is potentially very important because it may, like other A currents, regulate firing frequency but notably, it does not require a period of hyperpolarization to be active.

Key words: Transient outward currents — A-current — Potassium current — Soma membranes — Electrophysiology — In vitro

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Introduction

The transient outward current (A-current, I_A) generated by depolarization after a conditioning hyperpolarization was first characterized in molluscan neurons (Hagiwara, Kusano & Saito, 1961) and was later named I_A by Connor and Stevens (1971*b*). This current has subsequently been demonstrated in many other invertebrate and vertebrate preparations. The A-current is known to regulate firing frequency (Connor & Stevens, 1971*a*), set resting potential (Bal & Oertel, 2000) and also modulate synaptic transmission (Daut, 1973). A wide diversity in transient outward potassium currents is known between cells of the same class within an animal population and even between classes of cells in the same animal at the level of macro and single channel recordings (Salkoff, 1983; Iverson et al., 1988; Premack, Thompson & Coombs-Hahn, 1989; Serrano & Getting, 1989; Baker & Salkoff, 1990), suggesting that there are subtypes of transient outward current.

Different ion channels can be distinguished from each other in their ionic selectivity, pharmacology, voltage and time dependence and modulation by intracellular messengers (Serrano & Getting, 1989). Accordingly, I_A-like fast outward currents have been described. In cultured embryonic mouse hippocampal neurons two types of fast outward potassium current, I_A and D-current (Wu & Barish, 1992) are known; in *Aplysia*, three types of early transient potassium current, two of which are similar to conventional A-current were characterized (Furukawa et al., 1992). This study investigates, using kinetic and pharmacological techniques, whether there are potassium A-type currents in the soma membranes of *Helix aspersa* that do not require hyperpolarization to be active. Some of this work has been published in abstract form (Bal et al., 1997).

Materials and Methods

The preparation and electrophysiological techniques have been described in detail elsewhere (Bal et al., 2000). Briefly, the experiments were carried out in isolated F76 and D1 neurons (soma only) of *Helix aspersa* subesophageal ganglia (Blades Biological, UK). No enzymes were used for isolation. No differences were found between the current and firing pattern of these two cells and data from both have thus been pooled.

Solutions

Normal Ringer solution had the following composition (in mM): NaCl, 80; KCl, 4; $MgCl_2$, 5; $CaCl_2$, 10; glucose, 10; HEPES, 5, as described by Taylor (1987) (all these chemicals were obtained from BDH and were AnalaR grade). The pH of the Ringer solutions was adjusted with TRISMA base (Sigma) to 7.4. In some experiments, Na^+ ions were replaced on an equimolar basis by choline chloride or in some cases mannitol (Sigma) or a mixture of N-methyl D-glucamine and D-glutamic acid (Sigma) to suppress the sodium inward current. Ca^{2+} ions were replaced by Co^{2+} on an equimolar basis.

Recording Techniques and Equipment

Voltage-clamp experiments were made using twin electrode voltage-clamp techniques. Both microelectrodes were filled with 3 M KCl and had tip resistances of 1–6 Mohm. Both current and voltage-clamp experiments were performed using an Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA). All recordings were performed at room temperature (20–24°C).

Generated data were filtered at 2000 Hz with a two-channel low pass variable filter (Kemo). Current and voltage records were sampled at 16 KHz and were digitized online using a 16-bit A/D converter (Tucker Davis-Technologies, TDT) and stored for further analyses. At the end of each experiment, the tip potential was measured. If it was greater than 5 mV, the data were then discarded. Leak currents were obtained by blocking all known ionic currents. Leak currents were digitally subtracted from the data presented.

Activation and Inactivation Curves

The voltage dependence for inactivation was determined by using different holding potentials between −30 and −110 mV and then stepping to 0 mV for I_A. For I_{Adepol}, it was determined using holding potentials between 0 and −40 mV, and then stepping to a fixed potential, +70 mV. The relationship for activation was investigated with a holding membrane potential of −40 mV for I_{Adepol} and of −100 mV for I_A and then stepping to progressively more positive potentials. The data points for the steady-state inactivation curves were fitted using a Boltzman function of the form:

$$G/G_{max} = [1+\exp(V_{Half} - V_{step})/K]^{-1.....}, \quad (1)$$

in which G_{Max} is the maximum conductance; V_{Half} is the voltage at which the conductance is half inactivated and K is the slope of inactivation curve. Similarly, activation curves were fitted using Boltzman function of the form:

$$G/G_{max} = [1+\exp(V_{step} - V_{Half})/K]^{-1.....}, \quad (2)$$

where G_{Max} is the maximum conductance, V_{Half}, the voltage at which the conductance is half activated, K is the slope factor.

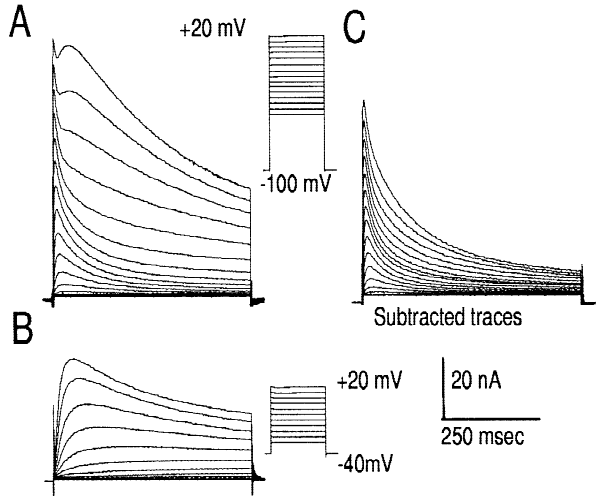

Fig. 1. Isolation method to obtain the A-current. The current traces in *A* and *B* were elicited by the voltage protocols shown in the insets. Note that the holding potential was −100 mV in *A* and −40 mV in *B.* The current traces in *B* were subtracted from the ones in *A;* the subtracted traces are shown in panel *C.* The current traces were obtained in Ca-free and Na-free Ringer.

Numerical results are given as mean ± SE with n being the number of cells on which the measurement was made. Significant differences between the groups were evaluated using a paired t test.

Results

The data reported here was collected from 45 isolated F76 and D1 neurons. One of the characteristics of the conventional A-current (I_A) is that it is activated when depolarized to positive voltages from holding potentials more negative than −40 mV (Fig. 1) (Connor & Stevens, 1971*b;* Neher, 1971; Thompson, 1977). However in the present study, a fast outward current followed by a slow outward rectifier current (I_{Kdr}) was observed when the neurons were depolarized to relatively positive potentials, namely voltages positive to +5 mV, from −40 (Fig. 2) or even more positive holding potentials. This current was found to be sensitive to 4-AP (Figs. 2 and 3). This result suggests the existence of another fast outward current separate from the A-current and this here was termed "I_{Adepol}" gating at more depolarized potentials in a manner similar to the one seen in *Aplysia* (Furukawa et al., 1992).

Isolation of I_A and I_{Adepol}

To eliminate sodium and calcium inward currents and slowly developing calcium-sensitive slow outward currents ($I_{K(C)}$) Na-free, Ca-free Ringer solution was used (NaCl was replaced by choline chloride and $CaCl_2$ by $CoCl_2$). Isolation of the A-current from the remaining

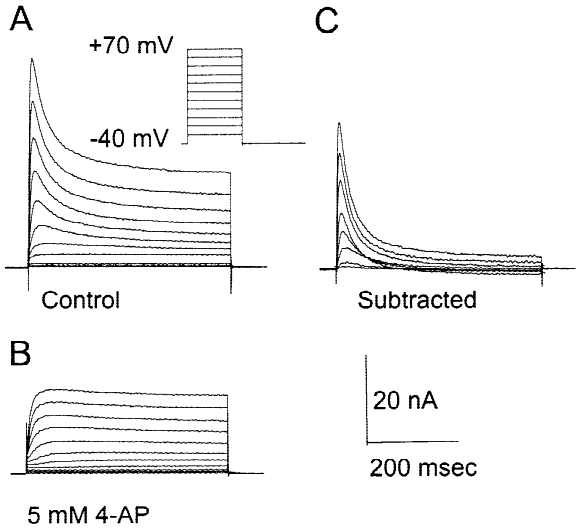

Fig. 2. Measurement of I_{Adepol}. (*A*) I_{Adepol} was evoked by depolarizing the neuron to a range of potentials between −30 and +70 mV in 10 mV steps from −40 mV holding membrane potential in Ca-free, Na-free Ringer and in the presence of 50 mM TEA (the voltage records are shown in the insert). (*B*) The fast component was reduced upon application of 4-AP (5 mM). I_{Adepol} was isolated by subtracting the traces obtained after 4-AP (*B*) from those before 4-AP. Thus the subtracted traces in panel *C* show a 4-AP sensitive current from −40 mV holding potential, I_{Adepol}. This current is a fast component like the transient outward current, the A-current.

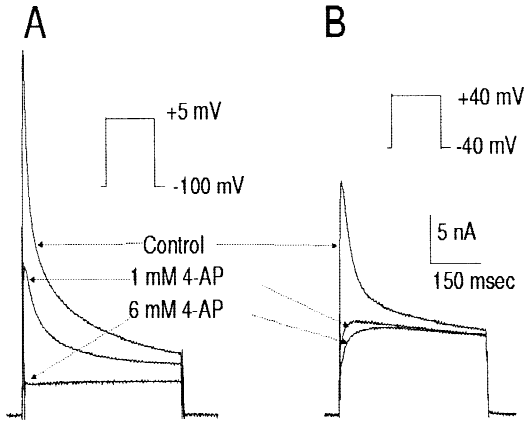

Fig. 3. Effect of 4-AP on I_A and I_{Adepol}. I_A was evoked by depolarizing the neuron to +5 (*A*) and I_{Adepol} was evoked by depolarizing the neuron to +40 mV from −40 mV (*B*) in control (Na-free, Ca-free Ringer and 40 mM TEA), 1 and 6 mM concentration of 4-AP. The voltage records are shown in the insets. Note that 1 mM 4-AP blocked I_{Adepol} in panel *B* completely, whereas it reduced the A-current by about 60%. A complete blockage was observed following the application of 6 mM 4-AP.

outward currents was based on a subtraction method. I_A is obtained by subtracting the traces elicited by depolarizing to a range of potentials from −40 mV holding potential from the traces elicited by depolarizing to the same potentials from −100 mV holding potential. At −40 mV, I_A is completely inactivated, whereas at −100 mV inactivation of I_A is completely removed (Fig. 1). Isolation of I_{Adepol} was achieved using both pharmacological and subtraction approaches together. In the presence of TEA (40–50 mM), which is regarded as a suppresser of I_{Kdr}, (Thompson, 1977; Bardoni & Belluzzi, 1993), the neuron was depolarized from −40 mV holding potential to a range of potentials between −30 and +70 mV in 10 mV steps (Fig. 2*A* and *B*). As with the A-current, this current was found to be blocked by 4-aminopyridine. Therefore 5 mM 4-AP was used to segregate I_{Adepol} from the leak and the partially blocked slow outward current seen in the presence of 40–50 mM TEA (Fig. 2).

Pharmacology of I_{Adepol}

4-Aminopyridine is regarded as a selective I_A channel blocker in vertebrate and invertebrate neurons (Thompson, 1977; Gustafson et al., 1982; Herman & Gorman, 1986). In this study it was found that application of 5–6 mM 4-AP blocked 98 ± 0.6% (n = 10) of the transient current elicited from −100 mV holding potential, leaving only the slow components very nearly intact. This indicates that 4-AP blocks I_{Adepol} as well as the A-current. But the degree of the sensitivity of these fast currents to 4-AP was different; the A-current was less sensitive to 1 mM 4-AP, whereas I_{Adepol} was found to be sensitive enough to be completely blocked by this dose of 4-AP. Application of 1 mM 4-AP blocked 61 ± 2.8% (n = 5) of the A-current, whereas it blocked 96 ± 1% of I_{Adepol} (n = 5). The effect of 4-AP on I_A and I_{Adepol} is shown in Fig. 3. The block of transient outward currents was seemingly voltage independent in these cells.

Activation and Inactivation of I_A and I_{Adepol}

I_A-activated at −51 ± 1 mV (n = 33) (Fig. 7*A*) and the peak amplitude of I_A clearly increased when stepped to more depolarized potentials. The activation of I_A was rapid. The order of the A-current activation kinetics was determined by fitting the rising phase of the current traces with exponentials; 1–4 exponentials were tested. It was reasonably well described by a single exponential process. I_A reaches a peak with mean activation time constants, ranging between 4.6 ± 1.3 (n = 6) msec at −35 mV and 0.8 ± 0.12 (n = 6) msec at +65 mV.

The falling phase of the I_A traces elicited by depolarizing to voltages negative to about −10 mV were best described by one exponential, whereas for those elicited by depolarizing to voltages more positive than −10 mV, a second exponential had to be introduced (Fig. 5*A*). It is likely that the second exponential introduced might reflect activation of a slower outward current, possibly

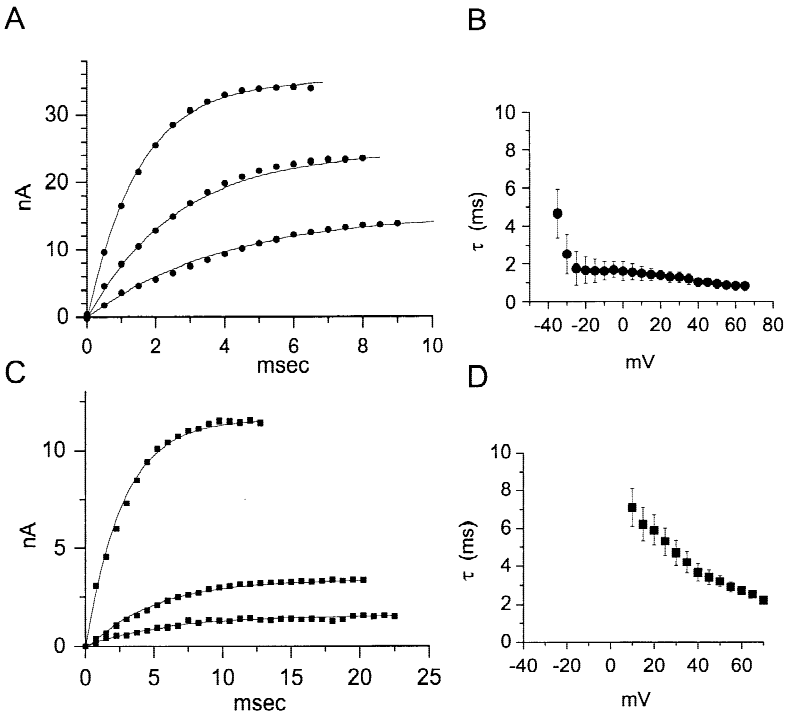

Fig. 4. Voltage-dependence of activation time constants for I_A and I_{Adepol}. (*A*) Analysis of the I_A activation kinetics. The current traces were elicited by clamping the neuron at −30, −15 and 10 mV from −100 mV holding potential. The fitting procedure with one exponential resulted in time constants of 4.0, 2.6 and 1.5 msec, respectively. The continuous lines represent the best fit of the current rising phase. (*B*) Voltage dependence of activation time constants for I_A. The time constants obtained from the fitting procedure were plotted as a function of step voltages. The data points represent the mean value from 6 neurons. (*C*) Analysis of the I_{Adepol} activation kinetics. The continuous line represents the best fit of the current rising phase with one exponential (I_{Adepol} tracings were evoked by depolarizing pulses to +10, +20 and +40 mV). The fitting procedure with one exponential resulted in time constants of 6.1, 4.9 and 2.7 msec, respectively. (*D*) Voltage dependence of activation time constants for I_{Adepol}. The time constants obtained from the fitting procedure was plotted as a function of step voltages. The data points represent the mean value from 6 neurons.

I_{Adepol}. The amplitudes for both fast and slow components were comparable.

The *I–V* relationship for I_{Adepol} revealed that I_{Adepol} activated at −5 ± 2.1 mV. This is about 50 mV more positive than that for I_A activation. I_{Adepol} rose to a peak amplitude quickly but more slowly than I_A (Figs. 2 and 4). The magnitude of I_{Adepol} increased with more depolarizing potentials. The rising phase was best fitted with one exponential with voltage-dependent time constants, decreasing when stepping to more depolarizing potentials. The time constants of activation were 5.9 ± 0.8 msec and 2.7 ± 0.2 msec for step voltages to +20 and +60 mV, respectively (Fig. 4*B*) ($n = 5$). After reaching a peak, the current decayed to an increasing nonzero amplitude, revealing either a residual, noninactivating component of this outward current or another slow outward current activation. The decay of I_{Adepol} was well described by one exponential at depolarized voltages close to the resting, whereas two exponentials were required at more depolarized potentials, at about +35 mV and at more positive potentials (Fig. 5*B*). The slow and fast time constants of the inactivation of I_{Adepol} were not strongly voltage dependent (Fig. 4*B*). The amplitudes for both fast and slow components were also comparable.

Reversal Potential of I_A and I_{Adepol}

The reversal potentials of the I_A and I_{Adepol} currents were determined using a double pulse protocol; the A-current was evoked by depolarizing to 0 mV for 10 msec from a holding potential of −100 mV and then repolarizing to a range of potentials between −10 and −100 mV for 100 msec (Fig. 6*Aa*). I_{Adepol} was activated from a holding potential of −40 mV by depolarizing to +50 mV for 10 msec and then stepping to a range of potentials between −100 and −30 mV (Fig. 6*Ba*). Subsequently the magnitude of the tail current was examined just after the transient capacitance settled. The peak amplitude of the tail currents was measured and *I–V* relationships were plotted (Fig. 6*Ab* (●) and *Bb* (●) for I_A and I_{Adepol}, respectively). The reversal potentials determined from these relationships were −66.2 ± 0.6 mV ($n = 12$) and −65.4 ± 1.6 mV ($n = 3$) for I_A and I_{Adepol}, respectively. The reversal potential is very close to the theoretical potassium equilibrium potential, about −75 mV, calculated from the measured internal free-K^+ concentration with K^+-sensitive microelectrodes (Alvarez-Leefmans, Gamino & Rink, 1984). This indicates that these currents are largely, if not exclusively, carried by potassium ions.

The selectivities of the I_A (Fig. 6*A*) and I_{Adepol} (Fig. 6*B*) channels to potassium ions were further investigated by changing the extracellular potassium concentration to 1, 10, 25 and 50 mM for I_A and to 1 and 25 mM for I_{Adepol} compared with control of 4 mM. The reversal potentials of the tail currents were −78.5 ± 0.9, −52.1 ± 0.8, −34.5 ± 1.4, and −18.1 ± 1.5 ($n = 5$) mV, respectively at 1 mM 10, 25 and 50 mM $[K^+]_O$ for I_A. The measured reversal potentials for I_{Adepol}, −78.7 ± 1.3 mV ($n = 3$) and −35.7 ± 2.1 mV ($n = 3$) at 1 mM and 25 mM $[K^+]_O$, respectively (Fig. 6*Ac* and 6*Bc*). The slope of the curves of E_{rev} *vs.* log $[K]_O$ were about 40 mV/log unit when all the data

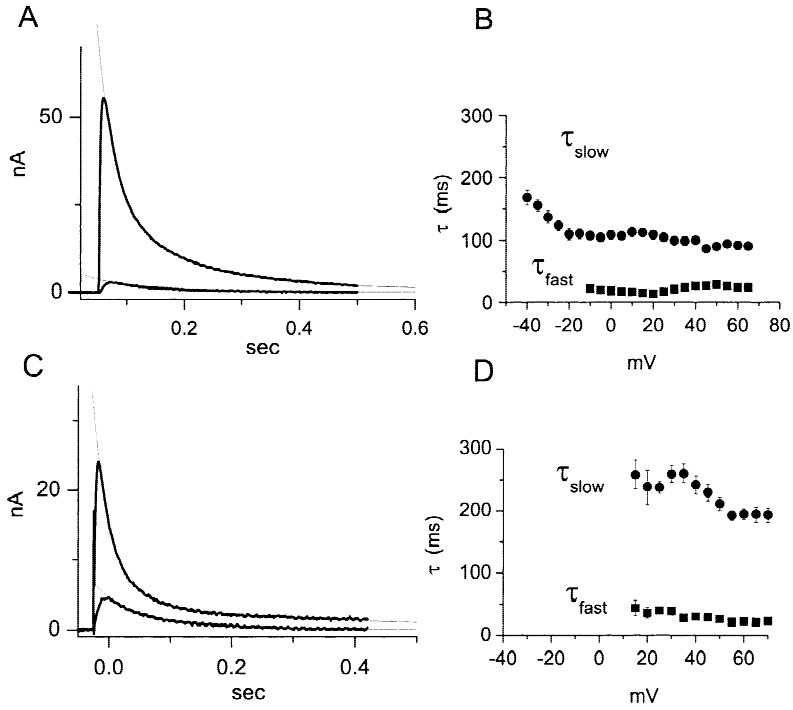

Fig. 5. Voltage-dependence of inactivation time constants for I_A and I_{Adepol}. (*A*) The cell was held at −100 mV and depolarized to −25 and +50 mV. The falling phase of the A-current trace evoked by stepping to −20 mV was fitted by a single exponential and that of the other traces was best described by two exponentials (thin lines). (*B*) The slow (●) and fast time constants (■) of I_A decay are plotted as a function of step potentials. Each point is the mean from 5 experiments. (*C*) I_{Adepol} traces were obtained by stepping to 0 and +70 mV from −40 mV holding potential. The inactivation of I_{Adepol} evoked by depolarizing to 0 mV was best described by one exponential, whereas the other evoked by depolarizing to +70 mV was described by two exponential (thin lines). (*D*) The slow (●) and fast time constants (■) of I_{Adepol} decay are plotted as a function of step potentials. Each point is the mean from 5 experiments.

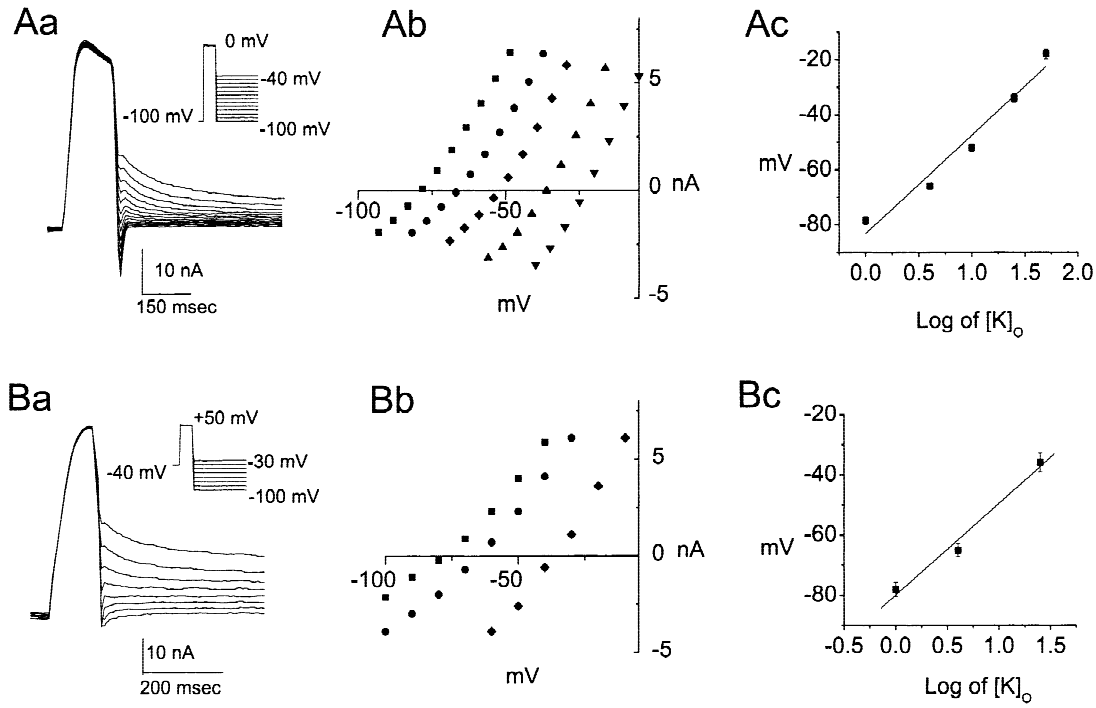

Fig. 6. Measurement of reversal potential for I_A and I_{Adepol}. The reversal potential of I_A and I_{Adepol} was measured from the tail current analysis, using the voltage protocol shown in the insets and representative samples of the current traces are shown in *Aa* and *Ba*, respectively in two different cells. In panel *Ab*, representative current voltage relationships are shown for I_A tail currents in the presence 1 mM (■), 4 mM (●), 10 mM (♦), 25 mM (▲) and 50 mM $[K]_O$ (▼) and in panel *Bb*, for I_{Adepol} tail currents in the presence of 1 mM (■), 4 mM (●) and 25 mM potassium concentrations (♦). The reversal potentials for the I_A and I_{Adepol} tail currents were dependent on $[K]_O$. *Ac*: The reversal potentials for I_A at 1, 4, 10, 25 and 50 mM $[K]_O$ were plotted *vs.* corresponding $[K]_O$ on a semilogarithmic scale. The straight line shows the linear fit ($r = 0.984$). (*Bc*) The reversal potentials for I_{Adepol} at 1, 4 and 25 mM potassium chloride concentration were plotted *vs.* corresponding potassium concentration on a semilogarithmic scale. The straight line shows the linear fit ($r = 0.986$). The points are means from 6 experiments for each.

points were included. However, for I_A, when the data points for only three concentrations, 10, 25, 50 mM $[K]_O$ were included, the slope is about 50 mV/log unit, which is closer to 58 mV/log unit as expected for a K^+-selective channel. The deviation is less when $[K]_O$ is increased and it is bigger when $[K]_O$ is decreased. Since the experiments were carried out in isolated F76 and D1 neurons (soma only), the deviation from the Nernst equation cannot be explained by imperfect space clamping. Cl^- contamination may be the best explanation for this deviation. As the experiments were carried out using microelectrodes having tip resistances of 1–6 MΩ, filled with 3 M KCl, chloride ion leak into the cell is very probable. Wu and Oertel (1986) showed that impalement of sharp electrodes of over 100 MΩ of tip resistance rapidly changed the chloride equilibrium potential, E_{Cl} from about −70 to about −20 mV in ventral cochlear nucleus cells. A similar depolarizing shift in E_{Cl} in F76 and D1 of *Helix aspersa* could be the reason for a significant deviation in 1 mM $[K]_O$ compared to negligible deviations at 10, 25 and 50 mM $[K]_O$. Also other ions might possibly be contributing as nonspecific leak currents, for example, Ca^{2+}: although Ca^{2+} was replaced by Co^{2+}, it is still possible that Ca^{2+} could stick on the exterior surface of the cell and then could leak nonspecifically into cells. Kuffler et al. (1966) in glial cells showed that the reversal potential *vs.* $[K]_O$ data are well described by the Nernst equation. However when lower $[K]_O$ than normal was used, a striking deviation from the Nernst equation occurs, which confirms our data. One mM $[K]_O$ might be an extreme condition for these cells.

In all the experiments thus far, choline was substituted for sodium ions. The possibility that I_{Adepol} might be a choline-activated current was investigated. Extracellular sodium was replaced by mannitol in three experiments and a mixture of N-methyl D-glucamine and D-glutamic acid in another three experiments. In all these experiments, I_{Adepol} was still observed, suggesting that I_{Adepol} is not a ligand-gated current, but the evidence presented here suggest that it appears to be a distinct voltage-dependent current on the kinetic grounds stated.

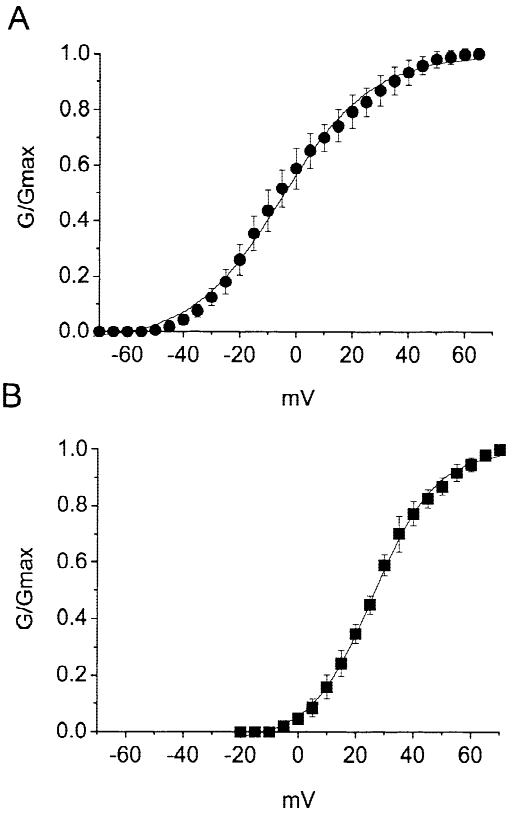

Fig. 7. Voltage dependence of steady-state activation of I_A (*A*) and I_{Adepol} conductance (*B*). Activation of I_A and I_{Adepol} were studied with the same protocols given in Figs. 1 and 2, respectively. The exponential decay of each I_A and I_{Adepol} traces was extrapolated to the time of pulse onset, thus resulting in a measure of the steady-state activation. The values were converted into conductance and then plotted as a function of step voltages. The data points for activation were fitted with the Boltzman Eq. (2) (the continuous lines) with half activation membrane potentials at −5.8 ± 2.5 and +26.4 ± 2.9 mV and slope factors of mV 16.7 ± 0.7 and 10.8 ± 1.6 mV for I_A (●) and I_{Adepol} (■), respectively. Each point is the mean from 8 experiments for I_A and 5 experiments for I_{Adepol}.

Voltage Dependence of Activation and Inactivation

The voltage dependencies of I_A and I_{Adepol} were determined by applying a series of depolarizing test pulses from a holding potential of −100 and −40 mV, respectively ($n = 6$). It is assumed that the macroscopic A-current is shaped by both activation and inactivation processes, starting at zero time when depolarized. Therefore the amplitude of the steady-state activation of I_A and I_{Adepol} at each test pulse was obtained by extrapolating the exponential fits to the falling phase of the current traces back to zero time. The corresponding amplitudes at time zero were taken (Thompson, 1977). This procedure was carried out to obtain the current that can be attained in the absence of inactivation. However the extrapolated current amplitude was usually little different from the observed peak current amplitude. The amplitudes of each corresponding current amplitude were then expressed as a fraction of that obtained by the most positive test potential and these values were plotted as a function of test voltages (Fig. 7*A* and 7*B* for I_{Adepol} I_A, respectively). The steady-state *I–V* relationships for both I_A and I_{Adepol} as a function of voltage can be described by a Boltzman function (Eq. 2) under the assumptions that each of these channel has stable open or closed conformational states, that the total conductance is the summation of the individual channels and that the probability of a channel being in the open configuration is determined

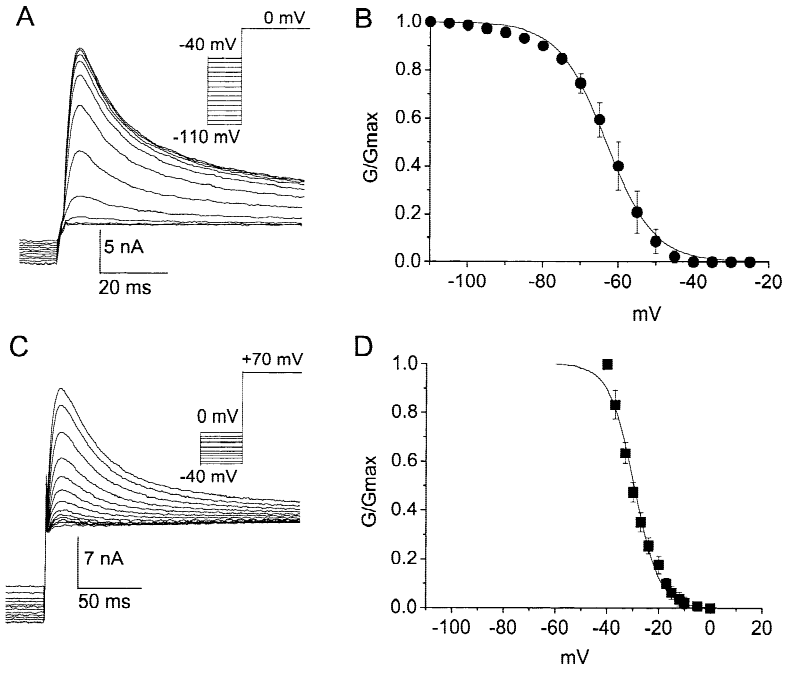

Fig. 8. Voltage dependence of steady-state inactivation of I_A (*A*) and I_{Adepol} (*B*). (*A*) The A-current records were evoked with the protocol shown in the insets. The maximum peak current amplitude was obtained after a conditioning holding potential to −100 mV, whereas at −40 mV holding potential no I_A current was obtained. (*B*) The steady-state inactivation curve was extracted by plotting the peak A-current as a fraction of the maximum current obtained as a function of the conditioning holding potential (●). The data points have been fitted with a Boltzman function (1) (continuous lines), giving a half inactivation membrane potential of −63.2 ± 1.2 mV with a slope factor of 6.3 ± 0.3 mV ($n = 12$). (*C*) Inactivation of I_{Adepol} was studied using the protocol given in the inset. The current traces in *A* and *C* were recorded from two different neurons. (*D*) The steady-state inactivation curve for I_A was constructed in the same way as for I_A (■). The *smooth line* is the Boltzman function having a midpoint of −29.5 ± 1.6 mV and a slope factor of 4.9 ± 1.3 mV ($n = 5$). Each point is the mean from 5 experiments.

by the Boltzman distribution. This fitting analysis yielded a V_{Half} for activation at −5.8 ± 2.5 mV and a slope factor of 16.7 ± 0.7 ($n = 8$) mV for I_A and a V_{Half} for activation at 26.4 ± 2.9 mV and a slope factor of 10.8 ± 1.6 mV ($n = 5$) for I_{Adepol} (continuous lines in Fig. 7*B*).

The number of I_A and I_{Adepol} channels that are ready to activate is dependent on the holding potential. At resting potentials of about −55 mV, I_A channels are largely inactivated, the more negative the resting potential, the less I_A channels are inactivated. To determine the relationships between steady-state inactivation and membrane voltage, a depolarizing test pulse was preceded by different conditioning holding potentials. At more depolarized holding potentials the amplitude of the currents decreased. The amplitudes of the peak currents were measured at each depolarizing step and these were normalized with respect to the largest current evoked from the most hyperpolarizing holding potential (Fig. 8). These normalized values were plotted as a function of holding potentials. Complete inactivations of I_A and I_{Adepol} were observed at about −45 and 0 mV, respectively. Inactivation of I_A channels was completely removed at about −100 mV. However, complete removal of inactivation of I_{Adepol} channels could not be investigated, since it was impossible to use more hyperpolarized conditioning potentials than −40 mV, at more negative potentials of which I_A contaminated I_{Adepol}. It is probable that the more hyperpolarizing preconditioning pulses would remove more inactivation of these channels similar to I_A. The relationships can be described by a Boltzman function (Eq. 1). The V_{Half} and K values are −63.2 ± 1 and 6.3 ± 0.3 mV ($n = 12$) for I_A, and −29.5 ± 1.6 and 4.9 ± 1.3 mV ($n = 5$) for I_{Adepol}, respectively.

Discussion

Are There Two Types of Transient Outward Currents in the Soma of F76 and D1?

Clear evidence has been presented which suggests that membranes of isolated F76 and D1 neurons (soma only) of *Helix aspersa* subesophageal ganglia have two fast outward currents: I_A and a novel type of fast outward current (I_{Adepol}). These two fast outward currents differ in their voltage dependencies of activation and inactivation and in their sensitivities to 4-AP. The A-current results here are similar to the A-current described by Connor and Stevens (1971*b*) and Neher (1971) with the following properties: (i) it shows noticeable activation at potentials close to resting and inactivates rapidly (Figs. 7*A*, 4*A* and *B*); (ii) it is completely inactivated at −40 mV holding potential and it is relieved from inactivation at −100 mV (Figs. 7*A* and 8*A*); (iii) it is blocked by 4-AP (Fig. 3). These properties of the A-current described in this study are generally very similar to those of most invertebrate and vertebrate neuronal A-currents (Connor & Stevens, 1971*b;* Neher, 1971; Thompson, 1977; Rudy, 1988; Buchholtz et al., 1991; Bardoni & Belluzzi, 1993). However, in contrast to the conventional I_A, I_{Adepol} has the following properties: (i) it shows noticeable activation at about −5 mV (Fig. 7*B*); (ii) it is completely inactivated at around 0 mV (Fig. 8*B*); (iii) similar to I_A, it is blocked by 4-AP, but complete blockage is obtained with lower doses at around 1 mM (Fig. 3); (iv) it has fast activation and inactivation time constants but they are not as fast as those of I_A (Fig. 4*C* and *D*). This current

has not been reported before in these neurons, presumably because it shows noticeable activation at considerable depolarizing potentials compared to I_A. The activation time constant of I_{Adepol} has a strong voltage dependency with shorter time constants at more depolarizing potentials whereas that of I_A does not seem to be voltage dependent overall; it is voltage dependent only at voltages close to the resting potential.

This current, I_{Adepol} is probably carried by potassium ions, since the tail currents reversed at around −65 mV, which is close to the theoretical potassium reversal potential (Fig. 6*Bb*). The possibility that it is a ligand-gated current, activated by choline, is excluded because it was still present in the absence of choline. I_{Adepol} described here in the membranes of F76 and D1 neurons is similar to that described in R2 and R15 neurons of *Aplysia californica* (Furukawa et al., 1992). They have some common properties; both have very similar activation and inactivation time courses; both are blocked by 1 mM 4-AP and their steady-state inactivation curves have similar voltage relationships. However I_{Adepol} in *Aplysia* starts to activate at relatively less depolarizing potentials.

Functional Implications

The amplitude of the I_{Adepol} was about 17% of the I_A component at a membrane potential of +35 mV. But it is important to note that this ratio, 17% is obtained when a holding potential of −40 mV was used for recording I_{Adepol}. It is highly likely that at more hyperpolarizing holding potentials, more of the I_{Adepol} channels in the soma may be relieved from inactivation (Fig. 8*D*) and therefore the proportion of I_{Adepol} may well be much higher than this value. So, I_{Adepol} may be functionally very important because at normal resting soma membrane potentials, of −47 mV for D1 and −50 mV for F76 neurons, I_A is nearly completely inactivated. During the after-hyperpolarization phase following an action potential, I_A inactivation was removed by up to 35% of the total A-conductance, but I_{Adepol} will be active. It is conceivable that inhibitory postsynaptic potentials (IPSPs) are required to remove the inactivation of I_A channels in order for them to function, whereas I_{Adepol} can function without a need for a period of hyperpolarization. This soma current, I_{Adepol} may be involved in the functions attributed to the A-current, such as regulating firing frequency, but in this case without the need for hyperpolarization of the soma membrane.

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