Calcium-Dependent Inactivation of a Potassium Current in the *Aplysia* Neuron R15

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The endogenously bursting pacemaker neuron R15 of *Aplysia* exhibits an inwardly rectifying K^+ current (I_R) that was shown previously to be enhanced by various neurotransmitters via the intracellular second messenger, cyclic AMP (Drummond et al., 1980; Benson and Levitan, 1983; Levitan et al., 1987). Here we present evidence that Ca^{2+} influx, either caused by spontaneous bursting activity or elicited by depolarizing voltage-clamp pulses, causes a large, long-lasting inactivation of I_R . The ionic current inactivated by bursts is identified as I_R by several criteria: it activates steeply at membrane potentials more negative than the K^+ equilibrium potential, has very fast kinetics, is reduced by lowering external K^+ from 10 to 2 mM, and is blocked by adding 1 mM Ba^{2+} , 10 mM Cs^+ , or 5 mM Rb^+ to the bathing medium. The peak inactivation of I_R is delayed following a single burst of spikes in R15, such that I_R decreases maximally by about 20% after 60–90 sec, and then recovers gradually over more than 10 min. The inactivation caused by many bursts of spikes can reduce I_R to less than 50% of its initial amplitude. The delay in onset and slow time course of recovery from inactivation of I_R suggest that a complex biochemical mechanism underlies the effect of Ca^{2+} on I_R .

The effect of depolarization on I_R is due specifically to the influx and intracellular accumulation of Ca^{2+} . Depolarizing voltage-clamp pulses are maximally effective at reducing I_R when they elicit a large influx of Ca^{2+} , while pulses approaching the Ca^{2+} equilibrium potential have little effect. The effect of depolarizing pulses on I_R is blocked by removing Ca^{2+} from the bathing medium and adding 3 mM Mn^{2+} to block Ca^{2+} channels, or by intracellular injection of the Ca^{2+} chelator EGTA. The results indicate that changes in intracellular Ca^{2+} occurring during normal bursting activity in R15 cause a profound inactivation of I_R . Hence, I_R is modulated in opposite directions by 2 intracellular messengers in the same neuron; it is enhanced by intracellular cAMP and it is inactivated by intracellular Ca^{2+} .

Calcium ions regulate a diverse assortment of ionic currents in a wide variety of cells. Intracellular Ca^{2+} is known to activate K^+ (for review, see Meech, 1978; Latorre et al., 1984), Cl^- (Barish, 1983), and nonselective cation currents (Colquhoun et al., 1981; Maruyama and Peterson, 1982; Yellen, 1982; Kramer

and Zucker, 1985a). In addition, internal Ca^{2+} causes the inactivation of Ca^{2+} currents (for review, see Eckert and Chad, 1984). This diverse assortment of Ca^{2+} -regulated currents enables Ca^{2+} to play a central role in controlling the electrophysiology of cells.

Ca^{2+} -regulated ionic currents have been studied in great detail in *Aplysia* bursting pacemaker neurons. Regulation of ionic currents by changes in intracellular Ca^{2+} is essential for generating the oscillation of membrane potential that drives bursting pacemaker activity (Gorman et al., 1982). There are 3 Ca^{2+} -regulated currents previously identified in these cells: (1) Ca^{2+} -activated K^+ current ($I_{K(Ca)}$) (Gorman and Hermann, 1979; Meech, 1979), (2) Ca^{2+} -activated nonselective cation current ($I_{Ca(Ca)}$) (Kramer and Zucker, 1985a), and (3) voltage-gated Ca^{2+} current (I_{Ca}), which is inactivated by Ca^{2+} (Eckert and Tillotson, 1981; Kramer and Zucker, 1985b). The Ca^{2+} that enters the neuron during a burst of action potentials accumulates near the membrane and acts quickly to activate or inactivate these 3 ionic currents. Following the burst, the magnitude of each of these currents returns to its initial level with distinct kinetics as the elevated intracellular Ca^{2+} concentration returns to normal. Hence the Ca^{2+} -dependent regulation of these 3 currents determines the timing of bursting pacemaker activity.

The subject of this paper is the identification of an additional effect of intracellular Ca^{2+} on an ionic current in the *Aplysia* neuron R15. Neuron R15 exhibits an inwardly (“anomalously”) rectifying K^+ current (I_R) that activates with hyperpolarization (Benson and Levitan, 1983). Normally, the membrane potential of R15 does not become sufficiently hyperpolarized to activate a large amount of I_R . However, the neurotransmitter serotonin (5-HT) (Benson and Levitan, 1983; Lotshaw et al., 1986) and the neuropeptide egg-laying hormone (ELH) (Levitan et al., 1987) cause a large enhancement of I_R in cell R15. There is convincing evidence that the effects of these transmitters are mediated by an increase in the level of cAMP in R15 (Drummond et al., 1980; Levitan et al., 1987). Activation of adenylate cyclase, inhibition of phosphodiesterase, and application of membrane-permeable cAMP analogs all lead to an increase in I_R (Levitan and Adams, 1981). In addition, application of both 5-HT (Levitan and Drummond, 1980) and ELH (Levitan et al., 1987) causes an increase in the content of cAMP in single R15 cells. The enhancement of I_R by cAMP results in an augmentation of the hyperpolarizing phase of bursting pacemaker activity.

In this paper we present evidence for a novel action of intracellular Ca^{2+} : The influx and intracellular accumulation of Ca^{2+} leads to the prolonged inactivation of the inwardly rectifying K^+ current in neuron R15. Hence I_R is an ionic current that is modulated in opposite ways by 2 intracellular messengers, Ca^{2+}

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and cAMP. In the following paper (Kramer et al., 1988), the biochemical mechanism of the regulation of I_R by intracellular Ca^{2+} is examined. A preliminary report of this work has appeared previously (Kramer and Levitan, 1986).

Materials and Methods

Aplysia californica (200–400 gm), obtained from Alacritty Marine Biologicals (Redondo Beach, CA) and maintained at 16°C, were used in all experiments. The abdominal ganglion was removed and pinned to a Sylgard base of a plastic chamber with a volume of 1 ml. The ganglion was desheathed, and R15 was impaled with 2 intracellular microelectrodes (2–5 M Ω) filled with 0.5 M K_2SO_4 for conventional 2-electrode voltage clamping.

Ganglia were bathed in saline consisting of 420 mM NaCl, 100 mM $MgCl_2$, 10 mM KCl, 10 mM $CaCl_2$, and 10 mM NaHEPES (pH 7.5). This medium contains 50 mM more $MgCl_2$ and 70 mM less NaCl than were used previously (i.e., Kramer and Zucker, 1985a) in order to minimize spontaneous synaptic inputs onto cell R15. In addition, 0.5 mM hexamethonium (Sigma) was sometimes added to block spontaneous cholinergic inputs. The effect of Ca^{2+} influx on I_R was essentially unaffected by the use of high Mg^{2+} or hexamethonium. Nominally Ca^{2+} -free saline contained no added $CaCl_2$ and 3 mM $MnCl_2$. Low- K^+ saline contained 2 mM KCl. Ganglia were continuously superfused with saline solutions (21–23°C) at 2–3 ml/min.

The 2-electrode voltage-clamp arrangement used was as described in Kramer and Zucker (1985a). Membrane current was measured with a virtual ground circuit. Signals were low-pass-filtered with an active filter with a corner frequency of 100 Hz. EGTA was injected iontophoretically into cells through a third thin-wall glass microelectrode filled with 0.25 M K_2EGTA . Ions were injected under voltage-clamp so that the current used for the injection did not cause a change in the membrane potential. Injections were accomplished with a constant-current iontophoresis unit (Model 160, W-P Instruments), while the current was monitored with an isolation amplifier circuit.

Results

Effect of bursting activity on membrane conductance

Ca^{2+} influx caused either by spontaneous bursting activity or controlled depolarization under voltage-clamp has effects on $I_{K(Ca)}$, $I_{CaT(Ca)}$, and I_{Ca} in *Aplysia* bursting neurons, as described previously (Gorman and Hermann, 1979; Eckert and Tillotson, 1981; Adams and Levitan, 1985; Kramer and Zucker, 1985a, b). In addition, Ca^{2+} influx regulates a distinct ionic current that activates upon hyperpolarization of the membrane potential in cell R15. Figure 1 shows the effect of bursting activity on membrane currents (top) elicited by applying 40 mV hyperpolarizing voltage pulses (bottom) from a holding potential of -90 mV. The cell had been held at -90 mV for more than 15 min to allow the membrane conductance to reach steady state. At the arrow in Figure 1A, the cell was released from voltage-clamp and allowed to fire a spontaneous burst of 12 action potentials. The cell was then voltage-clamped to -90 mV once again, and the hyperpolarizing pulses were resumed. After the burst of spikes there is a decrease in the inward current generated by the hyperpolarizing pulses; hence there is a decrease in the membrane conductance. The effect on the membrane conductance does not appear to be immediate, but develops gradually after termination of the burst, such that the membrane conductance is smallest 60–90 sec after the burst. In Figure 1A, the steady-state membrane conductance decreased from 1 μS before the burst to 0.75 μS 90 sec after the burst.

The effect of bursts on the membrane conductance is more dramatic if multiple bursts are allowed to occur. In Figure 1B the cell was released from voltage-clamp, and a total of 45 spontaneous bursts occurred over a period of 400 sec. The result is a decrease in the membrane conductance from 1 μS before

the bursts to 0.38 μS immediately after the bursts. It should be noted that, following this long period of bursting, there is no delay in the conductance decrease, but instead the conductance is minimal immediately after the series of bursts and rises steadily as the cell remains hyperpolarized under voltage-clamp. The lack of an apparent delay suggests that the process that regulates the membrane conductance reaches steady state during the long period of bursting activity.

The time course of the decrease in membrane conductance following 1, 3, or 10 bursts of spikes is shown in Figure 2. In each case the conductance was allowed to reach steady state by holding the cell at -75 mV for at least 25 min before the bursts were allowed to occur. The decrease in membrane conductance caused by bursting activity takes more than 10 min to recover fully after a single burst of spikes, and lasts even longer following 3 or 10 bursts. The fraction of the resting membrane conductance blocked by bursts increases with additional bursts, such that a single burst blocks 12–26% of the conductance ($n = 9$), while a train of 10 bursts blocks 36–50% ($n = 4$) of the resting membrane conductance. The long time course of recovery and the additive effect of bursts on the membrane conductance suggest that the factor responsible for reducing the membrane conductance accumulates over many bursts. There is once again a delay of about 60–90 sec between the end of the bursts and the maximal decrease of the membrane conductance. The fraction of the total conductance decrease that is delayed in onset diminishes as additional bursts are added such that, after 10 bursts, the membrane conductance 15 sec after the bursts is nearly the same as that 90 sec after the bursts. This again suggests that the process responsible for decreasing the membrane conductance approaches steady state after repeated bursts of action potentials.

Ionic basis of the conductance decrease

Voltage-clamp studies of R15 cells have shown that most of the ionic current at membrane potentials more hyperpolarized than -75 mV is due to I_R (Benson and Levitan, 1983). In order to test whether I_R is affected by bursting activity as is the current shown above, ionic substitution experiments and specific pharmacological blockers of I_R were employed. The voltage-clamp pulse paradigm illustrated in Figure 3A was used in these experiments. The cell was held at -75 mV and 200–400 msec depolarizing and hyperpolarizing voltage pulses were applied in order to measure membrane currents and construct a steady-state current–voltage (I – V) curve. A train of five 200 msec depolarizing pulses to $+20$ mV was applied to simulate a burst of action potentials.

The simulated burst has prolonged effects on 2 distinct ionic currents in R15. First, the simulated burst causes a long-lasting reduction (inactivation) of an inward current that appears upon hyperpolarization of the membrane potential negative to -75 mV (Fig. 3B). This current activates so rapidly (within 2 msec) after a voltage step that it appears without a delay after the decay of the capacitive current. Hence we could not distinguish the hyperpolarization-induced current from the instantaneous leakage conductance of the membrane unless pharmacological blockers were used (see below). The rapid kinetics characteristic of this current are typical of I_R found in other systems (e.g., Hagiwara and Takahashi, 1974). R15 cells that were damaged during the course of an experiment exhibited a time-dependent component of the hyperpolarization-induced inward current, probably due to a slow hyperpolarization-gated Cl^- current

Figure 1. Effect of bursting activity on membrane conductance in cell R15. *A*, Membrane currents elicited by repeated 400 msec hyperpolarizing voltage pulses from -90 to -130 mV. At the arrow, the cell was released from clamp and fired a burst of spikes. Membrane current decreases in amplitude, with a delay, after the cell is once again voltage-clamped. *B*, Large decrease in the amplitude of membrane currents after multiple bursts of spikes. Note the lack of delay in onset of the effect. Voltage pulses are the same as in part *A*. Dotted lines in *A* and *B* denote the membrane current level before the burst(s).

(Chesnoy-Marchais, 1983; Lotshaw et al., 1986). Cells that exhibited this current were not used in this study. The simulated burst also causes the reduction (inactivation) of a second voltage-gated inward current in R15. This current appears upon depolarization of the membrane potential positive to -75 mV, and exhibits slow kinetics (Fig. 3C). This slow inward current has previously been identified as a subthreshold voltage-gated Ca^{2+} current (Eckert and Lux, 1976; Gorman et al., 1982; Kramer and Zucker, 1985b).

The 2 ionic currents partially inactivated by the simulated burst can be seen clearly in the I-V curve of Figure 4A. The simulated burst causes a decrease in the inward current and

reduces the slope of the I-V curve at membrane potentials more negative than -75 mV, as expected when I_R is decreased by the burst. Application of 5-HT (Benson and Levitan, 1983) or ELH (Levitan et al., 1987) increases the slope of the I-V curve negative to -75 mV by increasing I_R . The K^+ equilibrium potential is near -75 mV in *Aplysia* neurons in normal saline (Coyer et al., 1983); hence a decrease in the K^+ conductance at membrane potentials more negative than -75 mV should result in an outward shift in current. It is therefore likely that the burst causes an inactivation of I_R . The burst also causes a decrease in a second inward current that activates with depolarization at membrane potentials positive to -75 mV. Molluscan bursting neurons, including R15, are known to have a voltage-gated subthreshold Ca^{2+} current that activates between -75 and -30 mV (Eckert and Lux, 1976; Gorman et al., 1982; Kramer and Zucker, 1985b). The regenerative activation of this current underlies the depolarizing pacemaker wave that initiates bursts of spikes. The subthreshold Ca^{2+} current is known to be inactivated by Ca^{2+} influx that occurs during bursts (Kramer and Zucker, 1985b). Hence the decrease in the inward current at potentials positive to -75 mV probably represents Ca^{2+} -dependent inactivation of the Ca^{2+} current (Eckert and Tillotson, 1981). The amplitude of the Ca^{2+} current, the magnitude of its inactivation, and the time course of the recovery from inactivation were quite variable in different R15 cells. Hence, the inactivation of the Ca^{2+} current is not always apparent 90 sec after the burst (e.g., Fig. 7A).

If the current affected by the burst negative to -75 mV is due to I_R , then the effect of the burst on this region of the I-V curve should be diminished in the presence of agents that reduce or block I_R . In cell R15 (Benson and Levitan, 1983), as well as in other systems (Hagiwara and Takahashi, 1974; Sakmann and Trube, 1984), I_R is greatly reduced by lowering the external K^+ concentration. This is because the activation of I_R is shifted as the K^+ equilibrium potential is shifted to more hyperpolarized

Figure 2. Time course of the conductance decrease following bursts of spikes in R15. The membrane conductance between -75 and -115 mV was measured at various times after 1 (squares), 3 (circles), or 10 (triangles) bursts of spikes. Percentage conductance decrease is the difference between the conductance after and before the bursts, divided by the conductance before the bursts.

potentials, and because the conductance of I_R channels is dependent on the external K^+ concentration. Figure 4*B* shows the effect of low external K^+ (2 mM) on the I-V curve and on its modulation by a burst. The conductance decreased by the burst is shifted such that it now activates at membrane potentials negative to -105 mV, in accord with the expected shift of the K^+ equilibrium potential to -110 mV in 2 mM K^+ . Furthermore, both the membrane conductance negative to -105 mV and the inactivation of the conductance are decreased in 2 mM K^+ . The inactivation of the Ca^{2+} conductance at depolarized potentials (more positive than -75 mV) is still apparent in 2 mM K^+ .

I_R is blocked by low concentrations of various cations, including Ba^{2+} , Cs^+ , and Rb^+ in cell R15 (Benson and Levitan, 1983), and in other systems (Hagiwara et al., 1978; Standen and Stanfield, 1978, 1980). The effect of Ba^{2+} on the I-V curve, and on its modulation by a burst, is shown in Figure 5. In this R15

neuron the simulated burst resulted in a large long-lasting decrease in the slope of the I-V curve negative to -75 mV, while the effect on the inward Ca^{2+} current was small and recovered almost completely within 90 sec (Fig. 5*A*). In the presence of 1 mM Ba^{2+} , the inward current negative to -75 mV was greatly reduced, and the effect of the burst on this region of the I-V curve was almost completely blocked (Fig. 5*B*). In contrast, Ba^{2+} caused a 10% enhancement in the inward Ca^{2+} current and a small enhancement of Ca^{2+} -dependent tail currents that occur after depolarizing voltage-clamp pulses (data not shown). In each of 4 experiments with 1 mM Ba^{2+} , the effect of the burst on the conductance negative to -75 mV was reduced by at least 85%. In other experiments, using 10 mM Cs^+ ($n = 2$) or 5 mM Rb^+ ($n = 2$), the effect of the burst was also reduced each time by more than 85%. Hence the conductance affected by the burst has the expected characteristics of I_R : it activates very rapidly

Figure 4. Effect of the simulated burst on the I-V curve of R15 in normal saline (*A*) and in low- K^+ (2 mM) saline (*B*). In normal saline, 2 inward currents are decreased after the burst, one appearing negative to -75 mV and one appearing positive to -75 mV. In low- K^+ saline, the conductance decrease in the hyperpolarized range is shifted to potentials more negative than -110 mV, while the conductance decrease in the depolarized range (positive to -75 mV) is not greatly affected. I-V curves were obtained as in Figure 3, 15 sec before and 90 sec after the burst. In this and other figures, "steady-state" currents were measured at the end of each 400 msec pulse and are plotted with respect to pulse potential.

Figure 5. Effect of Ba²⁺ on the burst-induced changes in the I-V curve. *A*, Effect of the burst on the I-V curve in normal saline. *B*, Effect of the burst on the I-V curve in saline containing 1 mM Ba²⁺. I-V curves were obtained 15 sec before and 90 sec after the burst, as in Figure 3. *C*, Time course of the effect of the simulated burst in normal saline (squares) and in saline containing 1 mM Ba²⁺ (circles). Note that 1 mM Ba²⁺ does not reveal any transient (90 sec) conductance increase induced by the burst.

upon hyperpolarization at potentials more negative than the K⁺ equilibrium potential; it is diminished by low external K⁺; and it is blocked by low concentrations of specific blocking cations.

Once we learned the identity of the ionic current responsible for the long-lasting conductance decrease, we wondered about the cause of the apparent delay in its inactivation following a burst. The apparent delay may result from a slowly developing process that reduces I_R, or it may result from a large transient conductance increase that obscures the conductance decrease during the initial 90 sec after a burst. We again used Ba²⁺ as a tool in distinguishing between these 2 possibilities. If a burst indeed elicits a 90 sec conductance increase, then this should be revealed by adding 1 mM Ba²⁺, which completely blocks I_R, but which we found to have little effect on other Ca²⁺-regulated currents at this low concentration. Figure 5C shows the time course of the effect of the burst on I_R in normal saline and in 1 mM Ba²⁺ saline. The time course of the effect of the simulated burst on I_R is similar to the time course of the effect of a natural burst on I_R (see Fig. 2). After addition of Ba²⁺, the effect of the simulated burst on the membrane conductance is greatly reduced at all times after the burst. There is no transient conductance increase revealed by the addition of Ba²⁺. Hence, the delay in the inactivation of I_R after a burst seems to be authentic.

Inactivation of I_R is Ca²⁺-dependent

Bursts of action potentials in cell R15 elicit a large influx of Ca²⁺

(Gorman and Thomas, 1978), which regulates various ionic currents, including inactivating the Ca²⁺ current (Eckert and Tillotson, 1981; Kramer and Zucker, 1985b). Therefore, it seemed likely that the long-lasting inactivation of I_R caused by bursts also results from Ca²⁺ influx. The following experiments were designed to test this hypothesis.

In order to test whether Ca²⁺ influx is required for I_R inactivation, the amplitude of the depolarizing pulses that comprise a simulated burst was varied. Depolarizing voltage-clamp pulses large enough to approach the Ca²⁺ equilibrium potential (estimated at about +150 mV; Hagiwara and Byerly, 1981) should elicit little Ca²⁺ influx. Figure 6A shows the effect of a burst of depolarizing pulses to either +30 mV or to +100 mV on I_R elicited by repeated pulses from -70 to -110 mV. The burst of pulses to +30 mV caused a considerable inactivation of I_R, while the burst to +100 caused no noticeable inactivation in this R15 cell. The graph of Figure 6B shows the relationship between I_R inactivation and pulse potential in a more representative R15 cell. Inactivation of I_R is maximal at +20 mV and declines as the pulse potential is made more depolarized. Similar results were obtained from 4 R15 cells. The bell-shaped relationship between pulse potential and I_R inactivation is characteristic of cellular processes that are caused by Ca²⁺ influx, such as synaptic release of neurotransmitter (Katz and Miledi, 1967), activation of I_{K(Ca)} (Meech and Standen, 1975), and inactivation of voltage-gated Ca²⁺ current (Eckert and Tillotson, 1981).

Figure 6. Effect of pulse potential on inactivation of I_R . *A*, A burst of 400 msec depolarizing pulses to +30 causes a large inactivation of I_R , while a burst of pulses to +100 mV causes little change in I_R . I_R was elicited by repeated 400 msec pulses to -105 mV from a holding potential of -75 mV. *Broken line* signifies the basal level of I_R before the bursts. *B*, Relationship between pulse potential of the simulated burst and decrease in membrane current measured between -75 and -115 mV. Membrane current was measured 15 sec before and 90 sec after the burst, as in Figure 3.

Ca^{2+} influx can also be prevented by completely removing Ca^{2+} from the bathing medium or adding divalent cation blockers of Ca^{2+} channels, such as Co^{2+} or Mn^{2+} . Unfortunately, concentrations of Co^{2+} or Mn^{2+} sufficient to completely block voltage-gated Ca^{2+} current (i.e., 10–20 mM) also block I_R (unpublished observations). However, we found that saline containing no added Ca^{2+} and 3 mM Mn^{2+} greatly reduces the voltage-gated inward current activated at membrane potentials more positive than -75 mV without substantially reducing I_R . The effect of normal and Ca^{2+} -free 3 mM Mn^{2+} saline on I_R inactivation is shown in Figure 7. In this cell, and in 3 other R15 cells, a single burst in Ca^{2+} -free saline elicited no detectable inactivation of I_R .

Ca^{2+} -dependent processes can also be blocked by loading cells with a Ca^{2+} chelator, such as EGTA. Figure 8 shows the effect of a burst on the I-V curve before (Fig. 8*A*) and after (Fig. 8*B*) iontophoretic injection of EGTA into cell R15. EGTA injection nearly completely blocks the inactivation of I_R caused by the burst. The injection also results in an 18% increase in the basal I_R , suggesting that EGTA removes some pre-existing inactivation

of I_R by lowering the resting Ca^{2+} concentration. In addition, EGTA injection eliminates the effect of the burst on the voltage-gated Ca^{2+} current activated positive to -75 mV, presumably by preventing Ca^{2+} -dependent inactivation of the Ca^{2+} current. In each of 3 other R15 cells, EGTA injection reduced the effect of the burst on I_R by at least 75%.

Discussion

The results presented in this paper demonstrate that I_R , an inwardly rectifying K^+ current in cell R15, is inactivated by the influx and accumulation of Ca^{2+} . Moreover, the inactivation of I_R is generated by the physiological influx of Ca^{2+} during spontaneous bursting pacemaker activity. Hence, inactivation of I_R is another process that can be added to the extensive list of cellular events regulated by physiological changes in intracellular Ca^{2+} .

The Ca^{2+} -dependent regulation of a variety of K^+ currents is well known. There are at least 2 distinct Ca^{2+} -activated K^+ currents, studied in several preparations including molluscan neu-

Figure 7. Inactivation of I_R is blocked in Ca^{2+} -free saline. *A*, Effect of the burst on the I-V curve in normal saline. *B*, Effect of the burst on the I-V curve in saline containing no added Ca^{2+} and 3 mM Mn^{2+} . I-V curves were obtained 15 sec before and 90 sec after the burst, as in Figure 3.

Figure 8. Inactivation of I_R is blocked by EGTA injection. *A*, Effect of the burst on the I-V curve in normal saline. *B*, Effect of the burst 20 min after iontophoretic injection of EGTA into cell R15. I-V curves were obtained 15 sec before and 90 sec after the burst, as in Figure 3.

rons, that can be distinguished on the basis of their Ca^{2+} sensitivity, voltage-dependence, single-channel conductance, and sensitivity to block by pharmacological agents (Latorre et al., 1984; Blatz and Magleby, 1986; Jones and Adams, 1987). In addition, Ca^{2+} activates a fast, transient K^+ current in *Drosophila* (Salkoff, 1983), similar to I_A of molluscan neurons (see D. J. Adams et al., 1980). The K^+ current studied in this report is distinct from all of these K^+ currents in 2 important respects: (1) I_R activates upon hyperpolarization rather than depolarization, and (2) I_R is inactivated rather than activated by internal Ca^{2+} . Ca^{2+} -dependent inactivation of K^+ current does not appear to be unique to *Aplysia* neurons, however; there have been recent reports of Ca^{2+} inactivating K^+ currents in human T lymphocytes (Bregestovski et al., 1986), and in *Limulus* (Chinn and Lisman, 1984) and *Hermisenda* (Alkon et al., 1984) photoreceptors. In addition, high concentrations of internal Ca^{2+} (1 mM and above) block conduction of K^+ through the pore of certain Ca^{2+} -activated K^+ channels (Vergara and Latorre, 1983).

The Ca^{2+} -dependent inactivation of I_R is remarkably long-lasting. The effect of a single burst of spikes causes an inactivation of I_R that persists for over 10 min, much longer than the duration of the intracellular Ca^{2+} transient generated by a burst of spikes in R15 (i.e., 15–30 sec; Gorman and Thomas, 1978). Thus, the recovery of the intracellular Ca^{2+} transient is not rate-limiting for the recovery of I_R from inactivation, suggesting that Ca^{2+} does not regulate I_R directly, but through an intermediate process. In addition, the inactivation of I_R lasts much longer than the average period of bursts in R15 (i.e., 15–30 sec). Hence, the Ca^{2+} that enters during bursts of action potentials appears to set the steady-state level of I_R over long time periods, rather than to cause oscillations of the current from one burst to the next. In contrast, the subthreshold Ca^{2+} current of *Aplysia* bursting neurons does oscillate during the bursting rhythm, in part because of Ca^{2+} -dependent inactivation (Kramer and Zucker, 1985b). It is interesting to note that Ca^{2+} -dependent inactivation of I_R is an intrinsic cellular mechanism that operates over a time course of many minutes. Such a long-term action is often thought to be in the realm of processes regulated by extracellular neuromodulatory substances.

Functional significance

What are the functional implications of I_R and of its Ca^{2+} -dependent inactivation in cell R15? The activation of I_R is steeply

voltage-dependent, gating strongly with hyperpolarization negative to -75 mV, such that very little I_R is present within the voltage range that R15 traverses during normal bursting pacemaker activity (i.e., -55 to -35 mV, excluding action potentials). For example, in an active cell the amplitude of I_R is less than 1 nA at -55 mV, if the current is measured immediately after the cell is voltage-clamped (unpublished observations). Hence, in the absence of neuromodulators, it is unlikely that I_R plays a major role either in generating the bursting activity or as a background current in setting the amplitude of the membrane potential oscillation.

The importance of I_R is greater when R15 is exposed to certain modulatory neurotransmitters. I_R is enhanced by 5-HT (Benson and Levitan, 1983) and ELH (Levitan et al., 1987), both of which induce an augmentation of both the depolarized bursting phase and the hyperpolarized interburst phase of pacemaker activity in R15. In the presence of 5-HT or ELH, I_R appears to be large enough to provide a considerable background current, sufficient to drive the membrane potential toward the K^+ equilibrium potential during the interburst hyperpolarization. The voltage-dependent activation of I_R may act to amplify the interburst hyperpolarization, as well as other hyperpolarizing stimuli (e.g., inhibitory synaptic potentials) by causing a regenerative hyperpolarization. Indeed, recent results have demonstrated that intracellular injection of hyperpolarizing current pulses causes a long-lasting inhibition of R15 in the presence of an enhanced I_R (Levitan and Levitan, 1988). We suggest that Ca^{2+} influx during bursting pacemaker activity limits the effects of modulators such as 5-HT and ELH on I_R by causing Ca^{2+} -dependent inactivation of I_R . Hence, the duration and amplitude of modulatory effects on I_R may be lessened by Ca^{2+} -dependent inactivation (see Kramer et al., 1988).

Conversely, if Ca^{2+} influx into cell R15 is reduced, then the effects of transmitters that increase I_R may be accentuated. There is a synaptic input ("input III") onto R15 that both increases I_R and causes a large decrease in the subthreshold Ca^{2+} current (W. B. Adams et al., 1980). Activation of input III causes an extremely long-lasting (e.g., 45 min) inhibition of bursting activity in R15 (Parnas and Strumwasser, 1974). There is the intriguing possibility that the long-term inhibition is in part due to a decrease in Ca^{2+} -dependent inactivation of I_R . This possibility needs to be tested in further experiments.

The long time course and the apparent delay in the onset of

Ca^{2+} -dependent inactivation suggest that a complex biochemical mechanism may be involved in the mechanism of Ca^{2+} action on I_R . Since I_R is enhanced by cAMP, one possibility is that Ca^{2+} exerts its effect on I_R by interacting with the cAMP metabolic cascade. The following paper (Kramer et al., 1988) addresses this issue.

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