VOLTAGE-DEPENDENT CALCIUM AND CALCIUM-ACTIVATED POTASSIUM CURRENTS OF A MOLLUSCAN PHOTORECEPTOR

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ABSTRACT Two-microelectrode voltage clamp studies were performed on the somata of *Hermissenda* Type B photoreceptors that had been isolated by axotomy from all synaptic interaction as well as any impulse-generating (i.e., active) membrane. In the presence of 2–10 mM 4-aminopyridine (4-AP) and 100 mM tetraethylammonium ion (TEA), which eliminated two previously described voltage-dependent potassium currents (I_A and the delayed rectifier), a voltage-dependent outward current was apparent in the steady state responses to command voltage steps more positive than −40 mV (absolute). This current increased with increasing external Ca^{++}. The magnitude of the outward current decreased and an inward current became apparent following EGTA injection. Substitution of external Ba^{++} for Ca^{++} also made the inward current more apparent. This inward current, which was almost eliminated after being exposed for ~5 min to a solution in which external Ca^{++} was replaced with Cd^{++}, was maximally activated at ~0 mV. Elevation of external potassium allowed the calcium ($I_{Ca^{++}}$) and calcium-dependent K^+ (I_C) currents to be substantially separated. Command pulses to 0 mV elicited maximal $I_{Ca^{++}}$ but no I_C because no K^+ currents flowed at their new reversal potential (0 mV) in 300 mM K^+. At a holding potential of −60 mV, which was now more negative than the potassium equilibrium potential, E_{K^+}, in 300 mM K^+, I_C appeared as an inward tail current after positive command steps. The voltage dependence of $I_{Ca^{++}}$ was demonstrated with positive steps in 100 mM Ba^{++}, 4-AP, and TEA. Other data indicated that in 10 mM Ca^{++}, I_C underwent pronounced and prolonged inactivation whereas $I_{Ca^{++}}$ did not. When the photoreceptor was stimulated with a light step (with the membrane potential held at −60 mV), there was also a prolonged inactivation of I_C. In elevated external Ca^{++}, $I_{Ca^{++}}$ also showed similar inactivation. These data suggest that I_C may undergo prolonged inactivation due to a direct effect of elevated intracellular Ca^{++}, as was previously shown for a voltage-dependent potassium current, I_A. These results are discussed in relation to the production of training-induced changes of membrane currents on retention days of associative learning.

INTRODUCTION

The neural systems that mediate associative learning behavior (Alkon, 1974; Crow and Alkon, 1978) of the nudibranch mollusc *Hermissenda crassicornis* have now been studied in depth (Alkon, 1976, 1980; Tabata and Alkon, 1982; Goh and Alkon, 1984). These neural systems include the five photoreceptors in each of the two eyes, the thirteen hair cells in each of the two vestibular organs (called statocysts), the optic ganglion cells, identified interneurons, and motorneurons. The three Type B photoreceptors can be distingusihed from the two Type A photoreceptors in each eye by position (the Type B cells are dorsal and posterior, i.e., away from the lens), morphology, smaller impulse amplitude and other electrophysiologic characteristics, and greater sensitivity to light (Alkon and Fuortes, 1972; Alkon, 1980). Membrane conductances of the Type B photoreceptors have received close attention because these cells have been shown to play a casual role in the acquisition and retention of this learning (Alkon et al., 1982*a*; Farley and Alkon, 1982; West et al., 1982; Farley et al., 1983). Previous work (Alkon, 1979) indicates that three ionic currents occur during the depolarizing response of the Type B cell to light: (*a*) an inward sodium current, (*b*) a voltage-dependent inward calcium current, and (*c*) a voltage-dependent outward calcium-dependent potassium current (see also Grossman et al., 1982). Two voltage-dependent outward currents have also been observed in darkness (Shoukimas and Alkon, 1980; Alkon et al., 1982*a*, *b*): (*a*) an early, rapidly inactivating potassium current (I_A) and (*b*) a delayed potassium current. Elevated intracellular calcium, accompanying prolonged Type B cell depolarization and augmented by light paired with this depolarization, has also been implicated as a factor in the production of the long-term reduction of I_A measured during associative training and retention of the associatively learned behavior (Alkon et al., 1982*b*).

Recently, other observations pointed to the change of at least one additional Type B membrane current other than the change of I_A during retention. These observations (Farley and Alkon, 1983; Forman et al., 1984; Farley et al., 1984), together with past reports (West et al., 1982), suggest that long-term changes occur that involve membrane currents other than I_A. In addition, differential absorption changes of iontophoresed Arsenazo III complexed with calcium indicate that a rise of intracellular calcium is caused by depolarizing the Type B photoreceptor in the dark, which is consistent with a small strictly voltage-dependent calcium conductance (Conner and Alkon, 1982, 1984). A much larger rise of intracellular calcium (in the absence of a pH change) was measured during the Type B depolarizing response to light, which is consistent with a light- and voltage-dependent elevation of intracellular calcium (Connor and Alkon, 1984).

The data reported here were collected with these objectives: (*a*) to characterize the calcium and calcium-dependent potassium currents in darkness, (*b*) to determine their activation and inactivation properties particularly with long time courses (e.g., minutes), and (*c*) to assess the possibility that prolonged elevation of intracellular calcium might directly reduce the calcium-dependent potassium current (as it does the I_A) and thus further contribute to the changes in the Type B cells correlated with acquisition and retention of associative learning.

METHODS

Cell Preparation

All experiments were performed on Type B cell somata that had been isolated (as previously described in Alkon, 1979) by axotomy from all synaptic interaction as well as from any impulse-generating (i.e., active) membrane. Preparations were immobilized on a glass slide by the weight of stainless steel pins whose ends were embedded in Vaseline (cf., Alkon, 1975). Prior to impalement with microelectrodes, a thin connective tissue sheath was digested by incubation in Protease (Type VIII, Sigma Chemical Co., St. Louis, MO) solution (1 mg/ml) for 6–10 min at 22°C.

Voltage Clamp

Voltage clamp was effected by the insertion of two microelectrodes filled with 3 M KCl, made from thick-walled capillary glass, #6020 (A-M Systems, Inc., Everett, WA). The microelectrode used to inject current had a resistance of 10–15 MΩ. The microelectrode used for measuring the intracellular voltage had a resistance of 20–25 MΩ. A current-to-voltage converter was used to ground (via a silver/silver chloride wire) the perfusion chamber and to measure membrane current. The capacitative transient of the voltage clamp current records settled within 5–12 ms. Command voltage steps occurred with a rise time of ~0.25 ms.

The resting potential measured after either microelectrode was inserted tended to range between 40 and 55 mV. Cells were rejected that had resting potentials <35 mV when measured by the first microelectrode. Cells whose resting potentials were <30 mV when measured by two microelectrodes were also rejected, as were cells whose resting potentials differed by >10 mV when recorded by the two microelectrodes. Holding currents for a resting potential of −60 mV were typically between −0.5 and −2.0 nA. Cells were not accepted when such holding currents were greater than −5.0 nA when 10 mM K^+ was in the bathing medium.

Experimental Conditions

Cells maintained at 20–22°C were bathed in artificial seawater (ASW) with the following composition: 430 mM, Na^+; 10 mM, K^+; 50 mM, Mg^{++}; 10 mM, Ca^{++}; 10 mM, Tris buffer (pH 7.4). In test solutions, Na^+ was lowered to 50 mM and tetramethylammonium ion (TMA) was added to other ions to give approximately equal osmolarity (870–890 mosmol). TMA was not added when external potassium was elevated to 300 mM and Ca^{++} was not included when 10 mM Ba^{++} or 10 mM Cd^{++} was used. The solution containing 300 mM K^+, 100 mM Ca^{++}, and 100 mM tetraethylammonium ion (TEA) was somewhat hyperosmotic (~1,000 mosmol). Solutions in the perfusion chamber (with a volume of ~1.0 ml) were exchanged by 7–10 sequential washes, using at least 10.0 ml of each test solution. EGTA electrodes were filled with 1.0 M Na_4 EGTA, and the pH was adjusted to 7.4 with NaOH.

Measurements

Data were quantitated from records made on a Brush chart recorder (Gould Inc., Instruments Div., Cleveland, OH). Settling time was checked on a storage oscilloscope (Tektronic, Inc., Beaverton, OR). All potentials are given in absolute terms (i.e., inside of the cell is negative with respect to outside); outward currents are represented as having positive value. Leak currents for each test potential were obtained by extrapolation using a current-voltage relation that was established using small positive and negative potential changes (e.g., ±10 mV, ±20 mV) from a holding potential of −60 mV. Currents recorded under voltage clamp at each test potential were then corrected by subtracting the appropriate extrapolated leak current value. The extrapolated leak current values for large (≥50 mV) positive voltage commands agreed well with values measured for equal commands of opposite sign (i.e., negative). All treatment conditions were repeated for Type B cells that had been obtained from at least four different animals. The results presented are typical of those consistently obtained with such repetition.

RESULTS

Isolation of Calcium and Calcium-dependent Potassium Currents

The two previously identified voltage-dependent potassium currents, I_A (Hagiwara and Saito, 1959; Connor and Stevens, 1971; Neher, 1971; Thompson, 1977) and the delayed rectifier (Neher and Lux, 1972) were eliminated (see Shoukimas and Alkon, 1980) by superfusing with 10 or 15 mM 4-aminopyridine (4-AP) and 100 mM TEA, respectively. Use of 2–5 mM 4-AP also eliminated I_A after ~2 min incubation. In the presence of 4-AP and TEA, a voltage-dependent outward current (corrected for leak) was apparent (Fig. 1) in the steady state responses to command voltage steps more positive than −40 mV (absolute). The magnitude of this outward current increased when the external Ca^{++} concentration in the bathing medium was increased from 10 to 100 mM.

The magnitude of the outward current progressively decreased while a sustained inward current became apparent with repeated injections of EGTA into the Type B cell (Fig. 2). These results were consistent with the interpretation that the inward current was carried by calcium ions (Tillotson and Horn, 1978; Adams and Gage, 1979; Hagiwara and Byerly, 1981) and the outward current was carried by potassium ions to which the membrane became more permeable because the intracellular calcium concen-

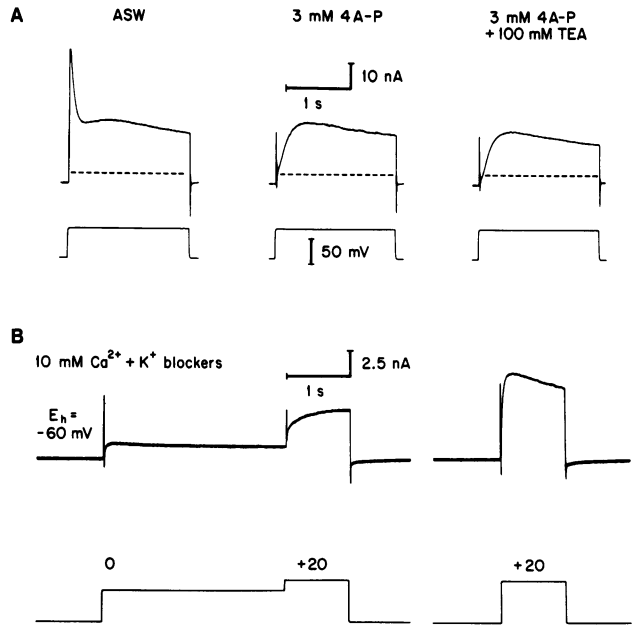

FIGURE 1 (*A*) Voltage-dependent outward currents across the membrane of the isolated Type B cell soma are shown. The bathing solutions are, from *left* to *right*, ASW, 3 mM 4-AP added to ASW, and 4-AP and 100 mM TEA added to ASW. Note that addition of 4-AP and TEA removes only a small portion of the late outward current elicited by command to 0 mV from a holding potential of −60 mV. The dashed lines indicate the level of the non–voltage-dependent or leak current. (*B*) Voltage-dependent outward current that occurs in the presence of 4-AP and TEA is shown. Voltage-dependent activation of outward calcium-dependent K^+ current (I_C) in a Type B photoreceptor is demonstrated, as well as the reduction by a prepulse depolarization. Current (*top*) and voltage (*bottom*) records from a voltage-clamp experiment illustrate that a 3-s depolarization to 0 mV elicits a small net outward current (*top left*), followed by a larger outward current when the membrane is stepped to +20 mV (*top middle*). The prepulse depolarization reduced the outward current by ~40% compared with that normally evoked by an 80 mV step from −60 to +20 mV (*top right*) and slowed the rise time as well.

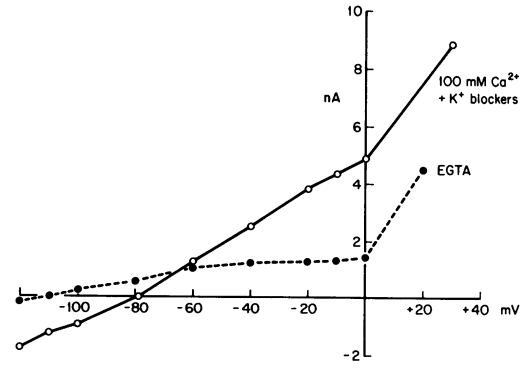

FIGURE 2 The reduction of I_C in a Type B photoreceptor by intracellular iontophoresis of the calcium chelator EGTA is demonstrated. The steady state current-voltage plot indicates the appearance of the outward current at potentials more depolarized than −20 mV and the reduction of this current by EGTA. Note that the non–voltage-dependent current (leak current) is also reduced by EGTA injection, suggesting that I_C contributes to this current.

tration increases (Meech, 1974; Eckert and Tillotson, 1978; Meech, 1978; Connor, 1979). Some experiments were conducted to test the validity of this interpretation (Connor, 1979).

Substitution of barium for calcium in the external bathing medium (in the presence of 4-AP and TEA) made the voltage-dependent inward current more apparent by decreasing the steady state outward current. The sustained time course of this inward current also became more apparent with barium substitution (Fig. 3), as well as after EGTA injection. When substitution of barium for calcium was preceded by thorough washing with O Ca^{++}-ASW, the inward current showed no decrease from its maximum amplitude, i.e., it was entirely sustained. This inward current was almost eliminated after ~5 min exposure to a bathing solution in which 10 mM Cd^{++} had been substituted for Ca^{++} (Fig. 3). The current-voltage relation measured at steady state, then began to approximate linearity in the presence of 4-AP, TEA, Cd^{++}, and O Ca^{++} (Figs. 3, 4). The magnitude of the inward current increased with increasing external Ba^{++} in the bathing medium. An estimate of the voltage-dependent Ba^{++} current (and, therefore, to some extent an estimate of the voltage-dependent Ca^{++} current) could be made from plots such as the one in Fig. 4 after correcting for leak current. This plot demonstrates a negative slope current-voltage relation for potentials up to 0 mV. The ascending, linear region of the relation crosses the zero current axis at approximately +45 mV the voltage that is only an approximation of the barium (calcium) current reversal potential.

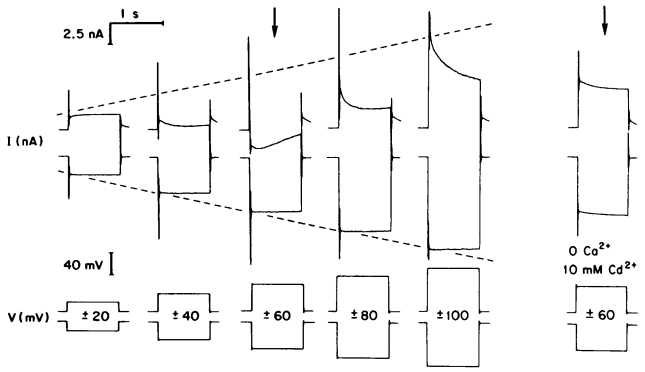

FIGURE 3 Records of voltage-dependent inward current present in a Type B photoreceptor are shown under conditions of 100 mM external Ba^{++} and K^+ current blockers (10 mM 4-AP, 100 mM TEA). From a holding potential of −60 mV, successive command steps in multiples of 20 mV reveal activation of an inward current at potentials more positive than −40 mV. This current increases nonlinearly, reaching its peak value at 0 mV (left arrow) and diminishes thereafter. Note that at 0 mV (absolute) the inward current decreases substantially from its maximum amplitude. Such a decrease was eliminated when substitution of Ba^{++} for Ca^{++} in the ASW was preceded by thorough washing with O Ca^{++}-ASW. From a holding potential of −60 mV, successive commands to potentials less than −60 mV elicit a non–voltage-dependent leak current, indicated by the lower dashed lines. The upper dashed line is extrapolated from potentials less than or equal to −50 mV. Note that when 10 mM Cd^{++} is substituted for Ca^{++} in the external bathing medium the current elicited by the command to 0 mV (absolute) begins to approach the extrapolated leak level (indicated by the dashed line).

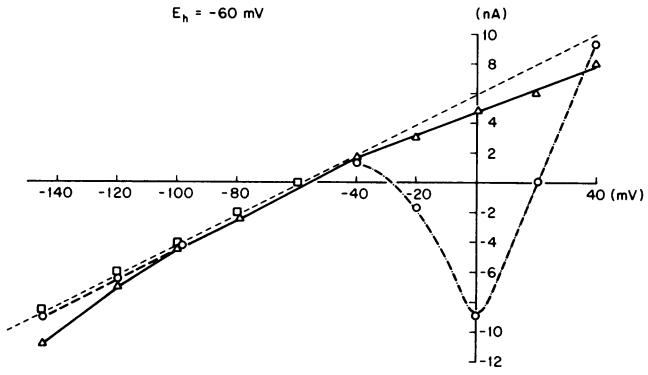

FIGURE 4 The steady state current-voltage plot of the voltage-dependent inward current present in a Type B photoreceptor is shown (same cell as in Fig. 3). Under conditions of high external Ba^{++} (100 mM) and blockade of the voltage-dependent K^+ currents (circles), the current is inward over the range of −40 to +40 mV (absolute), reaching its peak at ~0 mV. Removal of Ba^{++} and Ca^{++} from the bath, and addition of the calcium-channel blocker Cd (10 mM) after 5 min substantially reduced the inward current at all levels of membrane potential (triangles). The residual deviations from the extrapolated leak current values (squares) may be caused by incomplete block of the inward current.

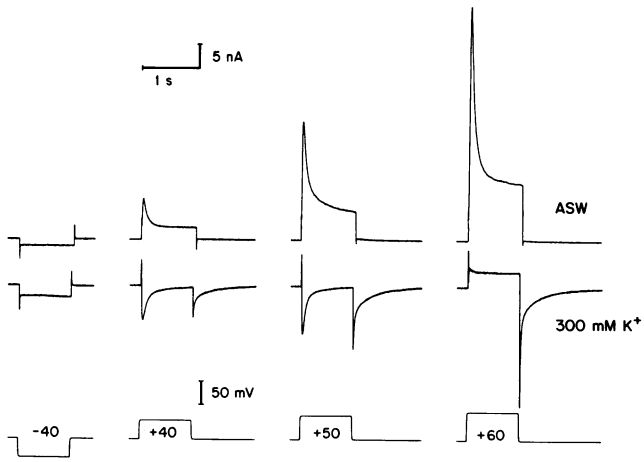

FIGURE 5 The effects of elevated external potassium on the voltage-dependent outward K^+ current are demonstrated. Changing external potassium from 10 mM (upper row of records) to 300 mM (lower row) reverses (in the absence of any pharmacologic blockers) I_A and the delayed outward K^+ currents elicited by command steps to −20 mV, −10 mV, and eliminates these currents at 0 mV from a holding potential of −60 mV. Note that the leak current (indicated by command to −80 mV) has increased. Note also the appearance in 300 mM K^+ of inward tail currents following commands greater than −20 mV. These results are consistent with the establishment of a new equilibrium for K^+ flux a little below 0 mV in 300 mM K^+. In the last panel the I_A current becomes slightly outward so that I_C is also outward, thus obscuring the absolute magnitude of $I_{Ca^{++}}$, which can only be estimated after leak correction at the E_{K^+}.

Separation of Calcium and Calcium-dependent K^+ Currents

External potassium was elevated from 10 to 100 mM and then to 300 mM to characterize the calcium current in the absence of the calcium-dependent K^+ current (as well as I_A and the delayed rectifier, blocked with 4-AP and TEA). Our objective was to make the K^+ current reversal potential (the potential at which no I_C current flows) sufficiently positive to allow substantial activation of the calcium current.

Elevation of External Potassium without 4-AP and TEA. As an indicator of the effect of these solution changes on the potassium equilibrium potential (E_{K^+}), I_A was first measured with increasing potassium in the absence of 4-AP and TEA. In 300 mM external K^+ the resting potential became 55–60 mV more positive than its previous resting level of −60 mV; in 100 mM external K^+ the resting potential became 25–30 mV more positive. This failure of the Type B photoreceptor soma membrane potential to show a Nernstian dependence on external potassium has been previously observed and suggests that other membrane conductance(s) affects the resting potential. We also cannot exclude the possibility that there was an incomplete exchange of $[K^+]_0$ because diffusion occurred in a restricted extracellular space. Also, in 300 mM K^+ a small reversed I_A (i.e., now inward) appeared at command voltages (−30, −20, and −10 mV) which in 10 mM K^+ elicited outward I_A currents (Fig. 5). The reversed I_A current in 300 mM K^+ disappeared (Fig. 5) at the new K^+ equilibrium potential (~0 mV). An inward tail current for a step to −60 mV following a conditioning step to 0 mV became apparent only in elevated external potassium. In 100 mM K^+ the inward tail current reversed at approximately −30 mV, and in 300 mM K^+, at ~0 mV. This appearance of an inward tail current in elevated external K^+ presumably reflects the increased driving force for a net inward current (carried by K^+ ions) because of a more positive value for E_{K^+}.

Elevation of External Potassium with 4-AP and TEA. The inward tail currents also appeared in the presence of 4-AP and TEA when external potassium was increased to 100 and 300 mM. They showed the same reversal potentials as were observed without 4-AP and TEA, and thus also implicate K^+ ions as the charge carriers present during the inward tail current. These reversal potentials were obtained with 10 mM Ca^{++} or 10 mM Ba^{++} in the external bathing medium (Fig. 6, *A* and *B*). Also interesting was that in 4-AP and TEA the magnitude of the tail currents (measured at one holding potential, e.g., −60 mV) increased with increasingly positive command pulses, but more negative than 0 mV, and then began to decrease with positive command pulses more positive than 0 mV. This inflection at 0 mV for the magnitude of tail currents elicited by various command steps was paralleled by a similar inflection for the magnitude of inward currents measured during these command steps.

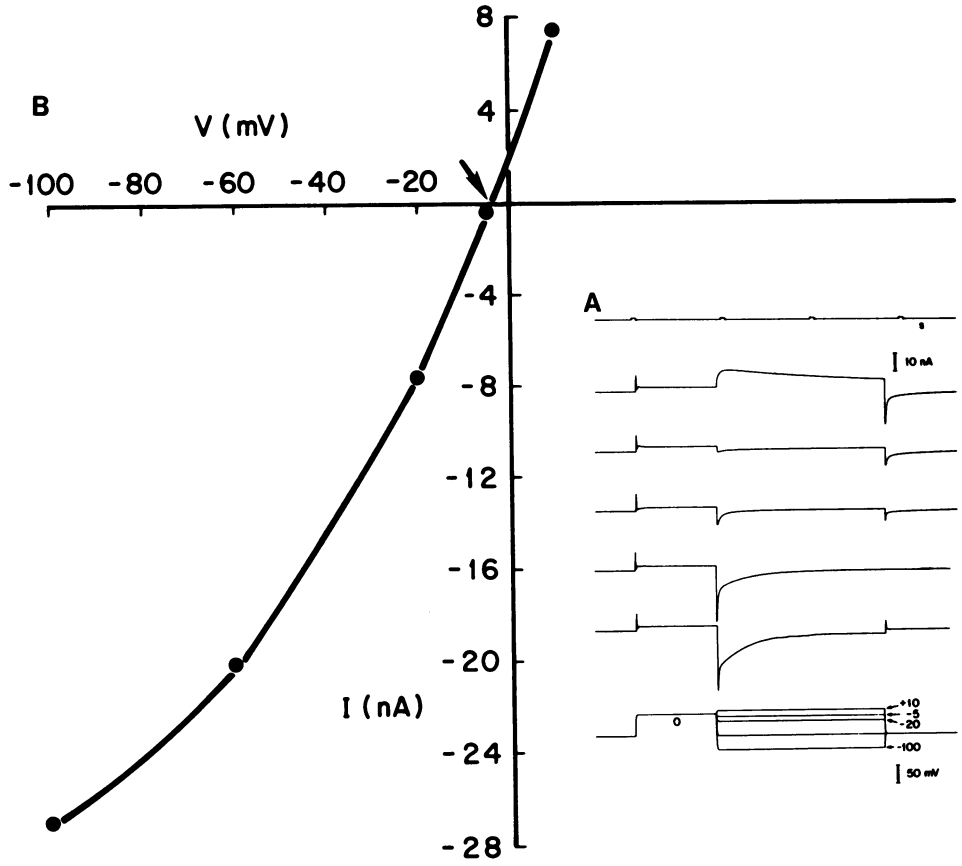

FIGURE 6 (*A*) The voltage dependence of inward tail currents is demonstrated. An inward tail current follows depolarization to −20, −10 (see top record on left), and 0 mV in the presence of 300 mM K^+. Step commands were given (with a 10-μs delay) following a conditioning depolarization to 0 mV. Below 0 mV the inward tail current increases with increasingly negative command steps. Above 0 mV, the tail current become increasingly positive (i.e., more outward). (*B*) Current-voltage plot showing voltage-dependence of tail currents such as those shown in *A*. Step commands were given following a conditioning depolarization to +10 mV. The reversal potential for the inward tail current was ~0 mV. With the settling time of the voltage clamp used here, tail current magnitudes can only be compared for ≥15 ms following the offset of the test depolarization.

These observations were interpreted as follows. Elevation of external potassium allowed substantial separation of the calcium and calcium-dependent K^+ currents. Command pulses to 0 mV elicited approximately maximal voltage-dependent calcium currents but no calcium-dependent K^+ current because K^+ currents did not flow at what was in 300 mM K^+ their new reversal potential (0 mV). At the cessation of the command step, when the membrane potential returned to the holding level of −60 mV, the calcium-dependent K^+ current was still activated. At the holding potential of −60 mV, which was now more negative than the potassium reversal potential, the calcium-dependent K^+ current appeared as an inward current. Note that substitution of Ba^{++} for external calcium (without prior washing in O Ca^{++}-ASW) greatly reduced but did not entirely eliminate this inward tail current. This last observation was consistent with the interpretation that much if not all of the inward tail current (in 300 mM K^+, 4-AP, and TEA) is carried by a Ca^{++}-dependent flux of K^+ ions.

Activation-Inactivation of the Calcium Conductance and the Calcium-dependent Potassium Conductance

During a prolonged depolarizing command step to 0 mV (absolute), the outward calcium-dependent K^+ current (I_C) reached its maximum within 2 s and then diminished during the sustained depolarization. Thus I_C appeared to show significant inactivation during depolarization. Conditioning depolarizing command steps markedly reduced the I_C elicited by test depolarizing pulses in normal K^+ (Figs. 1, 7, 8). This reduction was long lasting, often taking 2–3 min to completely reverse. Similarly, prolonged depolarization or repetitive presentations of test command steps caused a rapid and prolonged reduction of I_C when measured as an inward tail current in 300 mM K^+ (Fig. 7). Conditioning hyperpolarizing command steps had little effect on the I_C elicited by test depolarizing pulses. A very slight enhancement was sometimes suggested (Fig. 7). The relative magnitude of inward tail currents could only be

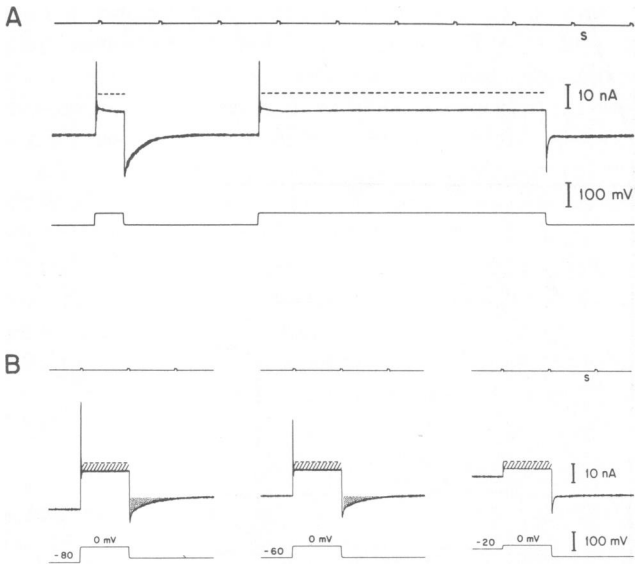

FIGURE 7 (*A*) Inward current and tail currents with brief and prolonged depolarization are shown. An inward current (leak current level is indicated by dashed line) elicited by command to 0 mV, reaches its maximum level within ~300 ms in the presence of 4-AP, TEA, and 300 mM K^+. During a prolonged depolarizing command the inward current shows little or no inactivation. The inward tail current following a brief command to 0 mV is largely eliminated when the depolarization is prolonged. The reversal potential for the inward tail current was determined to be ~0 mV. With the settling time of the voltage clamp used here, tail current magnitudes can only be compared for ≥15 ms following the offset of the test depolarization. (*B*) Effects of conditioning commands on currents elicited by depolarization to 0 mV are shown. 10-s steps to −80 mV and −20 mV preceded a depolarizing step to 0 mV in the presence of 4-AP, TEA, and 300 mM K^+. Note that the inward current (represented by the hashed lines below the leak level, indicated by the dashed lines) is largely unchanged under the three different conditions. The inward tail current, which reversed at ~0 mV, was slightly enhanced by the conditioning step to −80 mV and and almost entirely eliminated by the conditioning step to −20 mV. With the settling time of the voltage clamp used here, tail current magnitudes can only be compared for ≥10 ms following the offset of the test depolarization.

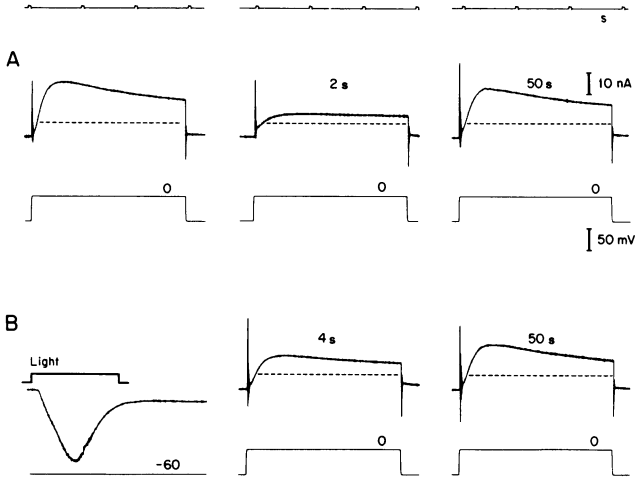

FIGURE 8 Long-lasting inactivation of I_C is demonstrated. Depolarizing command to 0 mV (from holding potential of −60 mV) elicits an outward current in 3 mM 4-AP and 100 mM TEA. (*A*) The maximum current, I_C, is not elicited by subsequent command steps until the interval between commands is ≥1 min. (*B*) Following a light step a delay of ≥1 min is also necessary before a command to 0 mV elicits the maximum I_C. Dashed lines indicate the level of the non–voltage-dependenct or leak current.

assessed at times lasting more than 15 ms following the offset of depolarizing voltage steps, given the 12–15 ms settling time of the two-microelectrode voltage clamp used in this study.

I_C, measured as above in 4-AP and TEA, also showed marked inactivation following light stimulation of the photoreceptor at a holding potential of −60 mV (Fig. 8). This inactivation is most likely a result of a light-induced release of Ca^{++} from intracellular stores (Connor and Alkon, 1982, 1984) since the inactivation decreases following iontophoretic injection of EGTA (Alkon and Sakakibara, 1984). Such release activates and then inactivates I_C (Alkon and Sakakibara, 1984).

Activation and inactivation of $I_{Ca^{++}}$ were assessed in 10 mM Ca^{++}, 300 mM K^+, 4-AP, and TEA. During a prolonged depolarizing command step to 0 mV, at which potential there was little or no I_C, the inward Ca^{++} current reached its maximum within 1 s and remained at or close to this maximal value throughout the subsequent depolarization (Fig. 7 *A*). Thus $I_{Ca^{++}}$ showed little evidence of inactivation during prolonged depolarization. The lack of I_{Ca++} inactivation was also indicated when Ba^{++} was substituted for Ca^{++} in the ASW after thorough washing with O Ca^{++}-ASW. Conditioning depolarizing or hyperpolarizing command steps had little effect on the $I_{Ca^{++}}$ elicited by test depolarizing pulses in 10 mM Ca^{++} or 10 mM Ba^{++} (Fig. 7 *B*). Similarly, repetitive presentation of test command steps had little effect on $I_{Ca^{++}}$.

For all of the measurements described above the leak current, i.e., the non–voltage-dependent current, was assumed to be a linear function of voltage and to be time independent. This is obviously true for small depolarizing voltage changes (≤20 mV) from a holding potential of −60 mV. It is also true over a range of negative voltage steps from −60 to −160 mV (Fig. 3). Extrapolation of leak values at positive potentials (≥30 mV), based on a linear relation derived from current amplitudes in the range −40 to −160 mV was significantly justified by observations following substitution of Cd^{++} for Ba^{++} or Ca^{++} in the external bathing medium. With Cd^{++} the positive voltage changes elicited currents that, after a few minutes, began to approximate the extrapolated leak levels (Figs. 3, 4) with an error ≤20%. That they never exactly matched these extrapolated levels may be a result of the presence of very small additional current(s), for example, one carried by chloride ions. Alternatively, the Cd^{++} itself might be capable to a small degree of substituting for Ca^{++} as a charge carrier across the membrane.

Elevated External Ca^{++}

In 300 mM K^+, 4-AP, and TEA, elevation of external Ca^{++} caused I_C, manifested as an inward tail current, to

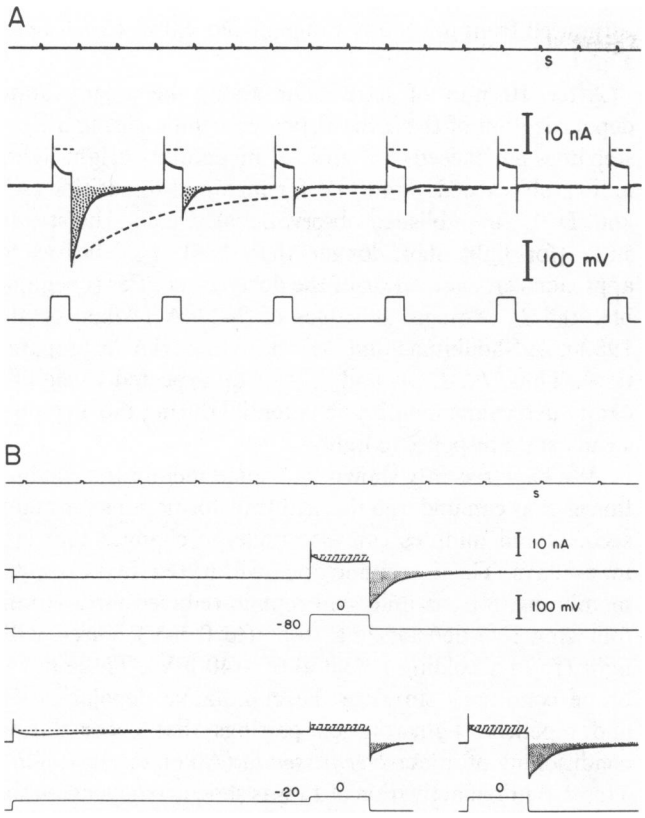

FIGURE 9 (*A*) Inactivation of inward current and inward tail currents with repetitive depolarizations are demonstrated. Depolarizing commands occur at 0.5 Hz in upper record. Inward current (beneath leak current indicated by upper dashed line) inactivates as does the inward tail current (indicated by shaded areas) with repetition of steps to 0 mV in the presence of 100 mM Ca^{++}, 4-AP, TEA, and 300 mM K^+. (*B*) Effects of conditioning commands on currents elicited by depolarization to 0 mV are shown. 5-s steps to −80 mV and −20 mV preceded a depolarizing step to 0 mV in the presence of 100 mM Ca^{++}, 4-AP, TEA, and 300 mM K^+. The inward current (beneath leak current indicated by dashed line) shows clear inactivation during and following the conditioning step to −20 mV. This was also true for the inward tail current following the step to 0 mV that had been preceded by the conditioning step to −20 mV. The inward tail current as well as the inward current during a step to 0 mV were largely unaffected by the conditioning step to −80 mV. A command step to 0 mV without a conditioning step (i.e., held at −60 mV) is shown in the lower right. The reversal potential for the inward tail current was determined to be ~0 mV.

become substantially larger and to show rapid, prolonged inactivation (Fig. 9 *A*). $I_{Ca^{++}}$ during a prolonged depolarizing step to 0 mV now slowly declined (Fig. 9 *B*) from its maximal value (reached within ~400 ms). Similarly, repetitive presentation of test command steps caused clear and progressive reduction of $I_{Ca^{++}}$ (Fig. 9 *A*). Slow decline from a maximal value of clearly inward $I_{Ca^{++}}$ was apparent during command steps to −10 mV as well as to 0 mV (absolute), the new equilibrium potential for K^+ flux in 300 mM K^+. Thus, a slowly activating outward I_C during the command step could not be confused with a slowly inactivating $I_{Ca^{++}}$.

As mentioned above, substitution of 10 mM Ba^{++} for 10 mM Ca^{++} substantially reduced or almost eliminated the inward tail current following a positive command to 0 mV in 300 mM K^+, 4-AP, and TEA. Repetition of such commands, however, did result in a small but slowly increasing inward tail current that required some seconds to decay. It is possible, therefore, that residual Ca^{++} (which remained without prior washing with O Ca^{++}-ASW) activated I_C that did not inactivate because of the low levels of intracellular Ca^{++}. This is consistent with the slow relaxation of the inward current measured in 100 mM Ba^{++}, 4-AP, and TEA (Fig. 3) in response to a command step step to 0 mV. This slow relaxation only occurred when substitution of Ba^{++} for external Ca^{++} was not preceded by thorough washing with O Ca^{++}-ASW.

Voltage Responses

To further assess the roles of $I_{Ca^{++}}$ and I_C in determining the Type B cell's response characteristics, voltage responses were recorded using a variety of ionic concentrations in the external bathing medium and in the presence of different ionic channel blockers. 4-AP (10–15 mM) added to ASW caused increased input resistance (cf., Acosta-Urquidi et al., 1984) and enchanced initial transient and steady state depolarization during a light step. It also enhanced long-lasting depolarization (LLD) following the light step. 4-AP, together with TEA (100 mM), caused even greater changes in the same direction. The Type B photoreceptor showed another change in the presence of 4-AP alone or with TEA. Depolarizing current pulses elicited an impulse that was never present with ASW in the absence of these blockers (Fig. 10). The amplitude of this impulse varied as a function of the concentration of calcium in the bathing medium (Fig. 10). Impulse amplitude did not change when external sodium was lowered to 0. The impulse still appeared and tended to occur repetitively when barium was substituted for calcium. The impulse, as well as the enhanced depolarization during and

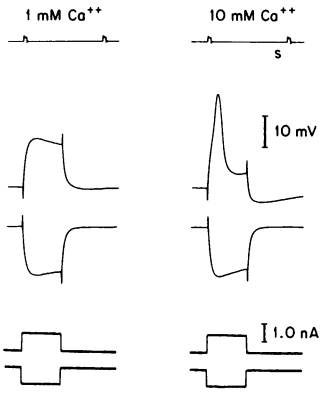

FIGURE 10 Voltage recordings obtained in the presence of K^+ current blockers. A positive current pulse elicits a regenerative depolarizing wave in the presence of 4-AP, TEA, and 10 mM Ca^{++} (*right*). Lowering external Ca^{++} eliminated the wave (*left*) as did the addition of 10 mM Cd^{++} (not shown). Removal of external Na^+ also did not affect the wave (not shown).

following a light step in the presence of the blockers 4-AP and TEA, were eliminated when cadmium was substituted for calcium in the bathing medium.

DISCUSSION

The voltage dependence of the calcium current that flows across the Type B cell soma bears some similarity to that measured for other gastropod somata (Tillotson and Horn, 1978; Adams and Gage, 1979; Hagiwara and Byerly, 1981), as well as presynaptic endings in another mollusc (cephalopod), the squid (Llinás et al., 1981). However, values for the calcium equilibrium potential ($E_{Ca^{++}}$) in *Hermissenda* were less positive than values reported in the past for gastropod neurons (Adams and Gage, 1979; Eckert and Tillotson, 1981). Both an overestimate of the leak current magnitude or some contamination of the voltage-clamp records with residual K^+ current (with 4-AP, TEA, and Ba^{++} substituted for Ca^{++} in the perfusion medium) could cause an underestimate of $E_{Ca^{++}}$ (cf., Thomas and Gorman, 1977; Ahmed and Connor, 1979; Andressen et al., 1979; Hagiwara and Byerly, 1981; Thomas and Meech, 1982). The first possibility seems unlikely, but we cannot discount the second. Based on the results of previous work on the Type B cell (Shoukimas and Alkon, 1980, 1983) and other cells (Hagiwara and Saito, 1959; Connor and Stevens, 1971; Neher, 1971; Neher and Lux, 1972; Thompson, 1977; Connor, 1979), we expect complete or nearly complete block of both the A current and the delayed K^+ current. However, a small amount of unblocked K^+ conductance (due to Ca^{++}-dependent K^+ conductance activated by residual Ca^{++} flux) given that E_{K^+} is about -74 mV in ASW (Shoukimas and Alkon, 1980, 1983) could lead to an appreciable underestimate of the zero current potential at very positive potentials.

The magnitude of the steady state voltage-dependent calcium current ($I_{Ca^{++}}$) described here ranged from 1 to 3 nA at a test potential of -10 mV (absolute). The initial transient depolarization (reaching a maximum value in <1 s) of the Type B photoreceptor response to a light step does not exceed -10 mV (absolute) for moderately bright lights ($\leq 10^4$ ergs/cm^2·s) and rarely exceeds 0 mV (absolute) for intensities approaching the maximal light ($\sim 10^6$ ergs/cm^2·s) encountered by the animal in its natural environment (cf., Alkon and Fuortes, 1972; Alkon, 1976). As determined in another study (Shoukimas and Alkon, 1980, 1983), the predominant currents during the initial depolariziang transient in response to light are I_A and I_{Na^+}. Because the combined magnitude of these currents at -10 mV is in the range of 70–80 nA, $I_{Ca^{++}}$ does not have a substantial effect during the depolarizing transient. Because the calcium-dependent potassium current (I_C) is slower to rise to its peak value, it probably also does not greatly affect the depolarizing transient response to light. The absolute magnitude of I_C at -10 mV was difficult to assess because some blocking effect of TEA (necessary to block the delayed rectifier) cannot be ruled out. Values estimated from this study ranged from 2.0 to 15 nA (e.g., Fig. 1 *A*).

After 10 min of dark adaptation, the steady state depolarization of the Type B photoreceptor during a light step does not exceed +30 mV for moderately bright lights and rarely exceeds +40 mV for maximal intensities (Alkon, D. L., unpublished observations). During the steady state (for light steps longer than 5 s), I_{Na^+} begins to approach zero, activation of the delayed rectifier is negligible, and I_A remains at values of 2–3 nA (Alkon et al., 1982*a*, *b*; Shoukimas and Alkon, manuscript in preparation). Thus, I_A, $I_{Ca^{++}}$, and I_C can be expected to significantly determine membrane potential during the Type B's steady state response to light.

We have recently shown that some membrane conductances that can undergo marked transformations for many seconds and minutes can also undergo changes that last many days. The I_A conductance within the Type B soma membrane, for example, will remain reduced for 2–3 min following five depolarizing steps (to 0 mV) paired with light (from a holding potential of -40 mV). These membrane conditions simulate the cumulative depolarization and repetitive light-rotation pairings that occur during conditioning of intact *Hermissenda* (Alkon et al., 1982*b*). This 2–3 min inactivation of I_A was shown to depend on the concentration of calcium in the external bathing medium. It also can be produced by direct injection of calcium ions under voltage-clamp control of the Type B soma membrane (Alkon et al., 1982*b*). Iontophoresis of a calcium/calmodulin-dependent protein kinase (phosphorylase kinase) markedly enhances and prolongs this calcium-mediated inactivation of I_A (Acosta-Urquidi et al., 1984). It seems reasonable, though not demonstrated, that calcium-mediated inactivation of I_A lasting minutes precedes and ultimately leads to semipermanent I_A reduction. In fact, reduction of I_A has also been shown to last for days after associatively training *Hermissenda* (Alkon et al., 1982*a*).

In the present study I_C, like I_A, remained inactivated following depolarization of the Type B soma membrane. It was also reduced for 1–2 min following light stimulation of a Type B photoreceptor whose membrane potential was maintained at -60 mV under voltage clamp. Intracellular calcium (Ca^{++}) is elevated by depolarization when external calcium flows across the Type B membrane (Connor and Alkon, 1984) through voltage-dependent calcium channels (see also Thomas and Gorman, 1977; Ahmed and Connor, 1979; Andressen et al., 1979; Thomas and Meech, 1982). It is also elevated by light that releases it from intracellular stores (cf., Brown and Blinks, 1974; Brown et al., 1977; Alkon and Sakakibara, 1984). Reduction of I_C across the Type B soma could resemble I_A reduction in its origin. Thus, elevated intracellular calcium would not only activate I_C, it would also directly mediate its short-term inactivation as suggested in earlier studies (Heyer and Lux, 1976; Eckert and Lux, 1977) and possibly its long-

term inactivation. Recently, direct inactivation of I_C by elevated Ca_i^{++} has been implicated for the Type B soma membrane (Alkon and Sakakibara, 1984).

Many different studies have indicated that the input resistance of the Type B soma membrane remains elevated during retention of *Hermissenda* associative learning (Crow and Alkon, 1980; Farley and Alkon, 1982; West et al., 1982; Goh and Alkon, 1984). Persistent reduction of I_C like that previously shown for I_A, could contribute to increased input resistance (and thus enhanced excitability) of a neural membrane as a mechanism for storing associatively learned information. Recently this contribution of semipermanent reduction of I_C to enhanced input resistance has been confirmed for associatively conditioned animals as compared with control animals (Forman et al., 1984; Farley et al., 1984).

In contrast to the observations on other molluscan cells (Tillotson, 1979; Brehm et al., 1980; Eckert and Ewald, 1981; Eckert and Tillotson, 1981; Eckert et al., 1981), elevation of intracellular calcium does not cause inactivation of $I_{Ca^{++}}$ across the Type B soma membrane with normal extracellular Ca^{++} in the bathing medium. Our results indicate that I_C inactivates in the absence of $I_{Ca^{++}}$ inactivation. It should be emphasized, however, that when extracellular calcium was elevated (from 10 to 100 mM), $I_{Ca^{++}}$ showed clear reduction with prolonged depolarization or repeated depolarizing commands. Thus, whether or not $I_{Ca^{++}}$ readily inactivates may simply depend on how elevated the intracellular calcium becomes in different cells. In normal extracellular calcium, intracellular calcium probably never becomes sufficiently elevated to cause Type B $I_{Ca^{++}}$ inactivation. Our results do suggest that elevated intracellular calcium, at different concentrations, can reduce directly four distinct membrane currents: the light-induced sodium current (I_{Na^+}), I_A, I_C, and $I_{Ca^{++}}$.

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