Selective Inhibition of Transient K^+ Current by La^{3+} in Crab Peptide-Secretory Neurons

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Duan, Shumin and Ian M. Cooke. Selective inhibition of transient K^+ current by La^{3+} in crab peptide-secretory neurons. *J. Neurophysiol.* 81: 1848–1855, 1999. Although divalent cations and lanthides are well-known inhibitors of voltage-dependent Ca^{2+} currents (I_{Ca}), their ability to selectively inhibit a voltage-gated K^+ current is less widely documented. We report that La^{3+} inhibits the transient K^+ current (I_A) of crab (*Cardisoma carnifex*) neurosecretory cells at ED_{50} ~5 μM, similar to that blocking I_{Ca}, without effecting the delayed rectifier K^+ current (I_K). Neurons were dissociated from the major crustacean neuroendocrine system, the X-organ-sinus gland, plated in defined medium, and recorded by whole cell patch clamp after 1–2 days in culture. The bath saline included 0.5 μM TTX and 0.5 mM $CdCl_2$ to eliminate inward currents. Responses to depolarizing steps from a holding potential of −40 mV represented primarily I_K. They were unchanged by La^{3+} up to 500 μM. Currents from −80 mV in the presence of 20 mM TEA were shown to represent primarily I_A. La^{3+} (with TEA) reduced I_A and maximum conductance (G_A) by ~10% for 1 μM and another 10% each in 10 and 100 μM La^{3+}. Normalized G_A-V curves were well fit with a single Boltzmann function, with $V_{1/2}$ +4 mV and slope 15 mV in control; $V_{1/2}$ was successively ~15 mV depolarized and slope increased ~2 mV for each of these La^{3+} concentrations. Cd^{2+} (1 mM), Zn^{2+} (200 μM), and Pb^{2+} (100 μM) or removal of saline Mg^{2+} (26 mM) had little or no effect on I_A. Steady-state inactivation showed similar right shifts (from $V_{1/2}$ −39 mV) and slope increases (from 2.5 mV) in 10 and 100 μM La^{3+}. Time to peak I_A was slowed in 10 and 100 μM La^{3+}, whereas curves of normalized time constants of initial decay from peak I_A versus V_c were right-shifted successively ~15 mV for the three La^{3+} concentrations. The observations were fitted by a Woodhull-type model postulating a La^{3+}-selective site that lies 0.26–0.34 of the distance across the membrane electric field, and both block of K^+ movement and interaction with voltage-gating mechanisms; block can be relieved by depolarization and/or outward current. The observation of selective inhibition of I_A by micromolar La^{3+} raises concerns about its use in studies of I_{Ca} to evaluate contamination by outward current.

INTRODUCTION

Lanthanum chloride is widely used as a potent blocker of Ca^{2+} permeation through membranes. It is effective at micromolar concentrations when added to physiological salines having their usual concentrations of divalent cations. It is used to completely block calcium currents (I_{Ca}) to evaluate whether any voltage-dependent outward currents contaminate the currents recorded under regimes designed to isolate I_{Ca} and thus improve the characterization of voltage-dependent I_{Ca}. We found that La^{3+} inhibits the voltage-dependent, rapidly inactivating potassium current (I_A) of crab secretory neurons at concentrations similar to those inhibiting I_{Ca} without affecting the delayed rectifier potassium current (I_K).

Although inhibitory effects of divalent Ca^{2+}-channel blockers on both transient and delayed outward currents were described for a variety of invertebrate (e.g., Thompson 1977) and vertebrate neurons (e.g., Mayer and Sugiyama 1988), there appear to be few reports of selective effects of La^{3+} on I_A at concentrations normally used to block I_{Ca}. Because negative results are rarely reported, it is difficult to know how widely the effects of low concentrations of La^{3+} on potassium currents have been examined. Selective effects of La^{3+} on the I_A component of outward current of rat cerebellar granule cells (Watkins and Mathie 1994) and hippocampal neurons (Talukder and Harrison 1995) point to the possibility that such actions may be more general than is currently appreciated. Except for an abstract (Duan and Cooke 1997), the effects of La^{3+} on potassium currents of crustacean neurons have not to our knowledge been previously reported.

METHODS

Dissection and culturing

The procedures used to dissociate and culture X-organ neurons from the semiterrestrial tropical crab, *Cardisoma carnifex* Herbst, were described in detail elsewhere (Cooke et al. 1989; Grau and Cooke 1992). Briefly, the X-organ with <1 mm of the axon tract was removed from the eye stalk of adult male crabs and agitated in the dark for 1.5 h in a Ca^{2+}/Mg^{2+}-free saline containing 0.1% trypsin (Gibco). A large volume of Ca^{2+}/Mg^{2+}-free saline was then added to retard enzymatic activity, and the cells were dissociated by gentle trituration in a 60-μl drop of culture medium on 35-mm Primaria dishes (Falcon 3801). The dishes were carefully flooded after allowing 1–2 h for the cells to adhere to the substratum. The culture medium consisted of Leibowitz L-15 (Gibco) diluted 1:1 with double-strength crab saline to which D-glucose (120 mM, Fisher), L-glutamine (2 mM, Sigma), and gentamicin (50 mg/ml, Gibco) were added. Cultures were maintained in humidified incubators (Billups-Rothenberg) in the dark at 22–24°C.

Experiments were performed on cultured X-organ somata that were 2–3 days in culture. Cells whose regenerative outgrowth took the form of large, lamellipodial growth cones ("veilers") (Grau and Cooke 1992) and were thus identifiable as containing crustacean hyperglycemic hormone (Keller et al. 1995) were chosen for this study. Before starting electrophysiological recording, the culture dish was rinsed three times, and the medium was replaced with filtered crab saline consisting of (in mM) 440 NaCl, 11 KCl, 13.3 $CaCl_2$, 26 $MgCl_2$, 26 Na_2SO_4, and 10 HEPES, pH 7.4 with NaOH. During the experiments,

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the dish was constantly superfused with crab saline at a rate of ~0.2 ml/min. A self-priming siphon maintained a relatively constant fluid level. Somata were viewed with a Nikon Diaphot inverted microscope with a ×40 objective and Hoffman modulation optics.

La^{3+} application

The use of a miniature Y-tube (Murase et al. 1989) manipulated to within <25 μm of the soma permitted rapid (<100 ms) application of La^{3+}-containing saline to the neuron with minimal dilution or mixing during patch clamping. The solutions to be applied, held in reservoirs higher than the recording bath, are drawn through the arms of the Y, which are of larger diameter tubing (PE 50) than the stem (PE10), by gentle suction; some bath fluid is drawn in through the stem ensuring that no agent is applied unintentionally. A solenoid pinch valve controlled by a Grass S-15 stimulator through which the arm on the suction side of the Y passes is used to stop the suction for a selected duration, permitting gravity flow of the material out of the Y while suction is blocked.

Electrophysiology

Voltage-clamp recordings were obtained in the whole cell patch-clamp configuration with an EPC9 amplifier (Instrutech, NY). Data acquisition, storage, and analysis were performed with HEKA software (Instrutech, NY) run on a Macintosh Centris 650 computer. Correction for leak was achieved with a p/N of four scaled subtractions obtained at zero-current hyperpolarizing command potentials. Signals were filtered with a corner frequency of 2.9 kHz. All experiments were recorded at room temperature (22–26°C). Pipettes used to obtain tight-seal whole cell recordings were pulled from Kimax thin-walled glass capillaries (1.5–1.8 mm OD) on a vertical puller (David Kopf Instruments, TW 150F-4). Pipettes were coated with dental wax to reduce capacitance and fire polished with a microforge (Narishige, model MF-83). Pipettes filled with the intracellular solution and immersed in the bath had resistances ranging from 1.5 to 5 MΩ but typically 1.5 to 3 MΩ. The intracellular solution, unless otherwise noted, was (in mM) 300 KCl, 10 NaCl, 5 Mg-ATP, 5 BAPTA, and 50 HEPES, pH adjusted to 7.4 with KOH. The extracellular solution was crab saline as described previously with 120 mM D-glucose and with 0.5 μM TTX and 0.5 mM $CdCl_2$ to suppress inward currents. The tonicity was adjusted with sucrose to 1,095–1,100 mosm for intracellular solutions and 1,100 mosm for extracellular solutions. After establishing an electrode seal and breaking in, neurons were held at −50 mV, a value close to the usual resting potential and therefore requiring very little current, when recordings were not being made. Series resistance (R_s), was <6 MΩ, typically ~3 MΩ; it was compensated to the maximum possible without causing oscillation, ringing. or overshoot. Larger cells (capacitance >50 pF) could be more completely compensated and were compensated by 85–90%. The few smaller cells recorded could be less fully compensated but were never compensated at <60%. These cells also had smaller currents. The data were not corrected for uncompensated R_s; we estimate that errors in reported V_m caused by R_s are <5 mV. To record voltage-gated currents the potential was stepped from −50 mV to the intended holding potential (V_h) for 300 ms before initiating additional voltage commands.

RESULTS

Isolation of I_A and I_K

In this study, outward currents were examined with a bathing saline containing 0.5 μM TTX and 0.5–1 mM Cd^{2+} to eliminate possible competing inward Na^+ and Ca^{2+} currents (Meyers et al. 1992; Richmond et al. 1995). The presence of TTX increased the size of the initial outward current, whereas addition of Cd^{2+} to TTX-containing saline decreased a dip in the current traces between the early peak and later outward current but did not alter the peak (<15 ms); the amplitude of the late outward current (measured at 90 ms) was reduced by the presence of Cd^{2+} but by <10% (data not shown) as also noted by Meyers et al. (1992).

As previously described for the cultured *C. carnifex* X-organ neurons (Meyers et al. 1992), outward K^+ currents having typical characteristics of I_A and I_K can be readily isolated. Currents representing a Ca^{2+}-activated potassium current were not resolved and if present were very small. The characteristics and current densities of I_A and I_K did not differ systematically among X-organ neurons having different morphologies of outgrowth in culture nor with time in culture (Meyers et al. 1992). Exploiting the rapid inactivation of I_A during depolarization, currents in response to incremented depolarizing voltage commands were recorded from a holding potential (V_h) of −80 mV, at which I_A is fully available for activation (Fig. 1*A*), and again from V_h −40 mV (Fig. 1*B*), at which I_A is almost fully inactivated, leaving responses primarily representing I_K. The responses at corresponding commands from V_h −40 mV were subtracted from those at −80 mV to provide estimates of I_A (Fig. 1*E*).

Because La^{3+} causes voltage shifts of I_A activation and inactivation as detailed subsequently, the use of different holding potentials to isolate the currents was impractical. We thus evaluated the effectiveness, compared with the use of different V_h, of using pharmacological blockers to separate I_K and I_A (Thompson 1977). As for other neurons, 4-aminopyridine (4-AP, 5 mM) effectively and relatively selectively blocked I_A (Fig. 1*C*), whereas TEA (20 mM in the bath) selectively blocked I_K (Fig. 1*D*) and revealed transient outward currents closely matching those obtained by subtraction (Fig. 1*E*). The slightly smaller currents seen in Fig. 1*E* compared with Fig. 1*D* reflect the presence of I_A remaining in responses from V_h −40 mV (Fig. 1*B*). These can be evaluated by comparing Fig. 1*B*, in which a small initial hump is present in the records, with Fig. 1*C* in which 4-AP has more completely blocked I_A.

It will be noted that the currents we attribute as primarily representing I_A, whether observed as the result of subtraction of currents obtained from V_h −40 from those at V_h −80 mV or from V_h −80 in the presence of 20 mM TEA, do not completely inactivate over the duration of depolarizations lasting >100 ms. Whether this residual current, consistently amounting to ~20% of peak I_A, represents ion movement through channels responsible for I_A or for I_K remains undetermined. We have not corrected measurements of I_A for this noninactivating component of current.

Unless otherwise noted, the observations on outward current presented subsequently were obtained in the presence of 0.5 μM TTX, 0.5–1 mM Cd^{2+}, and 20 mM TEA. Under these conditions inward current was not apparent; voltage-dependent outward current will be referred to as I_A.

La^{3+} inhibits I_A but not I_K

Figure 2 shows records of outward currents in saline with TTX and Cd^{2+}, but without TEA (*A*) and after the addition of 100 μM La^{3+} (*B*) recorded from V_h −80 mV and therefore

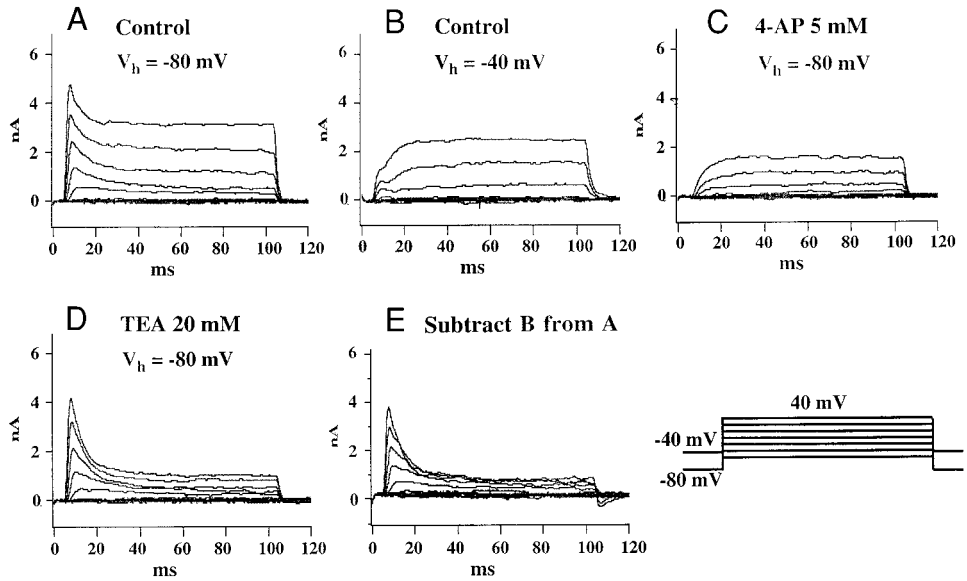

FIG. 1. Separation of outward currents of crab peptidergic neurons in culture. For this and all subsequent figures, *Cardisoma carnifex* X-organ neurons, dissociated and cultured 2–3 days in defined medium, showing a lamellipodial growth cone, were recorded under whole cell patch clamp applied to the soma in crab saline containing 0.5 μM TTX and 0.5–1 mM Cd^{2+} to block I_{Na} and I_{Ca}. Each panel shows superimposed current responses, capacitance, and leak corrected and series resistance compensated >60% to voltage steps from −50 to +40 mV, in 15-mV increments, from the holding potential (V_h) indicated. All records are from the same neuron. *A*: responses to depolarizing commands from V_h −80 mV show a fast, initial peak outward current (I_A), which decays and is followed by sustained current (I_K). *B*: responses from V_h −40 mV show little initial transient current consistent with inactivation of I_A, leaving primarily I_K. *C*: responses from V_h −80 mV in the presence of 4-AP, a selective inhibitor of I_A; comparison with *B* shows the remaining I_K is similar for V_h −80 and −40 mV. *D*: in the presence of TEA, a selective inhibitor of I_K, transient peaks remain (compare with *A*), but late current is reduced. *E*: current remaining in TEA (*D*) closely resembles current calculated by subtraction of the records of *B* from *A*. Except in Fig. 2, subsequent records were obtained from V_h −80 mV in 20 mM TEA except as noted and are considered to represent predominantly I_A.

eliciting both I_A and I_K (*left* panels) and from V_h −40, primarily I_K (*right panels*). A neuron showing relatively large I_K was selected for this example. The early peak evoked from V_h −80 mV representing I_A is absent in La^{3+}, whereas the late and sustained currents (here measured at ~90 ms) are unaffected. In Fig. 2*C*, the measurements of current at 90 ms for control saline from V_h −40 mV are plotted with the observations in La^{3+} from both V_h −40 and −80 mV. The responses are almost superimposable. Concentrations of La^{3+} >0.5 mM were required to obtain a noticeable effect on I_K, seen as a right-shift of the current-voltage relation (data not shown).

Figure 3 presents typical observations on I_A current (isolated by recording in saline with TTX, Cd^{2+}, and TEA) from a veiling neuron exposed to a wide range of La^{3+} concentrations. In Fig. 3*A*, currents recorded in response to steps to +15 mV from V_h −80 mV in control saline (a command evoking ~80% of the maximum response) and saline with 1, 10, and 100 μM La^{3+} are superimposed. The initial outward current peak is reduced, and its activation and inactivation slowed with increasing [La^{3+}]; in 100 μM no peak is seen in response to the +15 mV command, and there is only a non- or slowly inactivating residual current. With more depolarizing commands peaks are observed, as reflected in plots of the conductance giving rise to I_A, G_A versus V_c (Fig. 3, *B* and *C*). As mentioned previously, the responses in La^{3+} at modest V_c lacking an initial peak may represent I_K that is not completely blocked by TEA. Figure 3*B* presents plots of specific conductance (see figure legend) versus command voltage (V_c) for the same neuron (symbols). The data were fitted with a single Boltzmann function as given in the figure legend (Fig. 3*B*, lines).

The major effects of the addition of La^{3+} can be summarized as follows.

1) As seen for the neuron providing the data of Fig. 3*B*, there is a reduction in the maximum I_A current and conductance that could be obtained with depolarizing voltage commands. A summary of all the observations is shown in Fig. 4*A*, which plots the averaged observations of the reduction of maximum conductance by La^{3+} from eight neurons examined. The reduction relative to control saline is nearly linear when plotted against the log [La^{3+}] and amounts to ~30% at 100 μM [La^{3+}].

2) There is a shift of ~15 mV to more depolarized voltages (a right shift) of corresponding points of the I_A-V and G_A-V curves in 1 μM La^{3+} and a further shift of this magnitude with each 10-fold increase of [La^{3+}], as seen in Figs. 3, *B* and *C*, and 4*B* and further described subsequently.

Changes in current amplitude might result from a change in relative permeability or driving force for permeant ions. Tail currents in response to repolarizing commands to a series of voltages after I_A activation were therefore examined to test whether La^{3+} altered the reversal potentials. La^{3+} did not appreciably alter the reversal potential of nearly −80 mV, and thus it can be concluded that it does not alter the selectivity of the channel (data not shown). This value of the reversal potential is in good agreement with the calculated E_K, thus supporting the identification of the current as a K^+ current.

Cd^{2+} (1 mM) had no effect on I_A, whereas Zn^{2+} (200 μM)

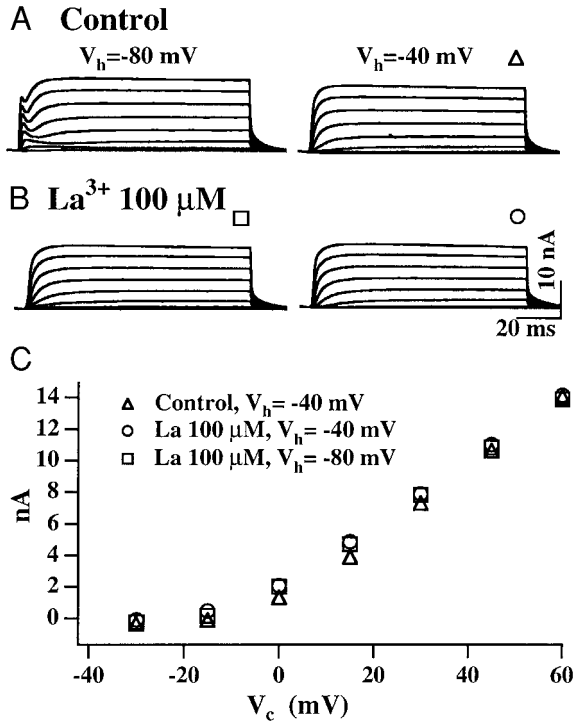

FIG. 2. La^{3+} does not affect I_K. *A*: outward currents recorded as in Fig. 1 (saline with 0.5 μM TTX and 0.5 mM Cd^{2+}, but without TEA). *B*: records from the same neuron after addition of 100 μM La^{3+}. Initial transient (I_A) present in control records from V_h −80 mV is absent. *C*: *I-V* plot of late current (I_K) measured at ~90 ms. Amplitudes of I_K in control records from V_h −40 and those in the presence of La^{3+} from both V_h −40 and −80 mV are indistinguishable. This neuron was chosen for its large I_K (note reduced scale relative to Fig. 1).

and Pb^{2+} (100 μM) produced small (<10 mV) right shifts of the *I-V* curves. Removal of the 26 mM Mg^{2+} present in the normal saline had no effect (data not shown).

La^{3+} effects on I_A activation

To further characterize the effect of La^{3+} in right shifting the voltage dependence of I_A activation, the responses of different neurons tested in control and the [La^{3+}] were normalized with respect to the maximum conductance observed for the particular neuron in each condition. The observations for the normalized G_A-*V* curves averaged at each V_c (n = 7–8) are shown in Fig. 3*C* (symbols, with error bars, ±SE). Each of the normalized curves was fitted with a single Boltzmann function (Fig. 3*C*, lines). The values of the Boltzmann parameters used to fit the averaged normalized G_A-*V* curves are plotted against log [La^{3+}] in Fig. 4*B*. The $V_{1/2}$, a measure of the right shift of the voltage dependence of activation, increased approximately linearly with the log [La^{3+}] from a control value of approximately +4 mV to approximately +49 mV in 100 μM La^{3+}, ~15 mV per 10-fold increase in La^{3+}. Voltage sensitivity was reduced by the presence of La^{3+} as indicated by an increase in the slope factor, from 15 mV in control saline to 22 mV in 100 μM La^{3+}.

La^{3+} effects on I_A steady-state inactivation

The effect of La^{3+} on the voltage dependence of steady-state inactivation was examined with a double-pulse regime as il-

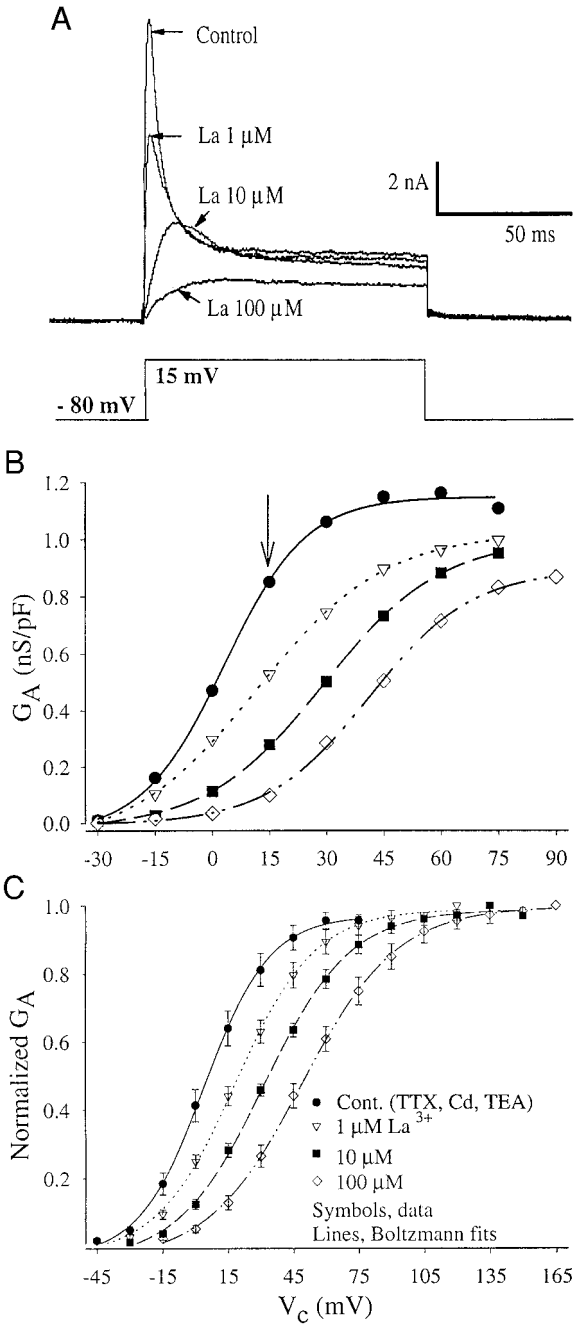

FIG. 3. La^{3+} effects on I_A. *A*: superimposed responses of 1 neuron recorded in saline selective for I_A to depolarizing steps from V_h −80 to +15 mV before and in the presence of La^{3+} at the concentrations indicated. La^{3+} reduces and slows the rise and decay of the transient peak. *B*: specific conductance (G_A) is plotted against voltage (V_c) of the depolarizing step eliciting I_A in control saline and saline with 1, 10, and 100 μM La (symbols, see legend in *C*; arrow indicates points from traces shown in *A*). G_A was calculated as $G_A = [I_A/(V_c - E_K)]/C_m$, where E_K, the calculated Nernst potential for K^+ was −83 mV, and C_m, cell membrane capacitance, was obtained from the autocompensation of the EPC9, for this cell, 138 pF. Lines represent a fit to the data of a single Boltzmann function, $g = G_{Max}/\{1 + \exp[(V_{1/2} - V_c)/s]\}$. Note that maximum conductance is reduced with increasing [La^{3+}] (see also Fig. 4*A*), the *G-V* curves are shifted to more depolarized V_c, and their voltage sensitivity is decreased. *C*: plot of normalized conductance vs. V_c, average ± SE, n = 8 (symbols). Lines represent a fit to the values of a Boltzmann function fitted to the averaged data. The values of $V_{1/2}$ and s increase with increasing [La^{3+}], as seen for specific conductance in *B* (see Fig. 4*B*).

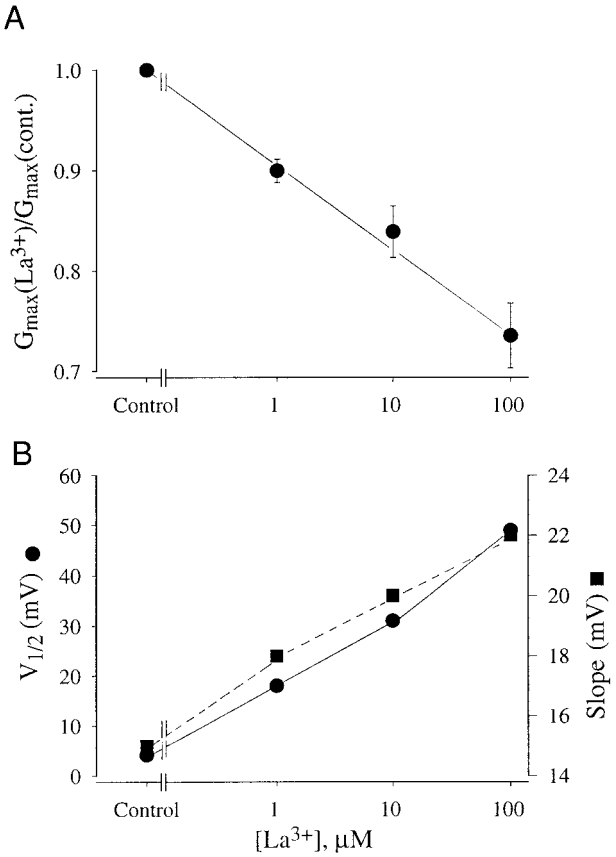

FIG. 4. Summary of La^{3+} effects on I_A activation. *A*: reduction of maximum I_A conductance by La^{3+}. The ratio of the maximum conductance observed in the La^{3+} saline to that in control saline is plotted vs. [La^{3+}] (log scale) (n = 8). *B*: depolarizing shift of Boltzmann parameters of I_A activation by La^{3+}. Values of V_c for eliciting half-maximal conductance and the slope obtained from fits to the averaged, normalized G_A-V curves shown in Fig. 3*C* are plotted against [La^{3+}] (log scale).

lustrated in Fig. 5. A 300-ms hyperpolarizing or depolarizing prepulse (−100 to +20 mV) from V_h −70 mV was followed by a test depolarization chosen to evoke ~80% of the maximal I_A, +20 mV for control saline, +30, +45, and + 60 mV for the 1, 10, and 100 μM La^{3+} salines. Previous studies (Meyers et al. 1992) have shown that 300 ms is ~10 times the maximum duration required to obtain the full extent of a change in steady-state inactivation, regardless of the voltage. A selection of the I_A current traces from a typical experimental series is shown in Fig. 5*A*. In control saline, the responses after prepulses to −70 and −55 mV are superimposable while in saline with 10 μM La^{3+} responses after prepulses to −55 and −40 mV are superimposable. Figure 5*B* plots the fraction of the I_A current in the absence of a prepulse over that after a prepulse versus the prepulse voltage. The prepulses from −100 up to −55 mV were without effect on I_A under any conditions, inactivation reached its maximum (i.e., I_A was minimal) with prepulses to −10 mV in control saline or 1 μM La^{3+}-saline, and more depolarized prepulses were required with increasing [La^{3+}]. There was consistently a residual outward current of ~20% that failed to inactivate. The curves were fitted by a single Boltzmann function, and the parameters are plotted in Fig. 5*C*. These indicate that, although for 1 μM La^{3+} the curves were not shifted, the voltage dependence of steady-state inactivation at 10 and 100 μM La^{3+} is right shifted by ~15 mV for each 10-fold increase in [La^{3+}]. The slope is approximately doubled in 10 μM La^{3+} relative to the control value but not increased by a similar amount at 100 μM.

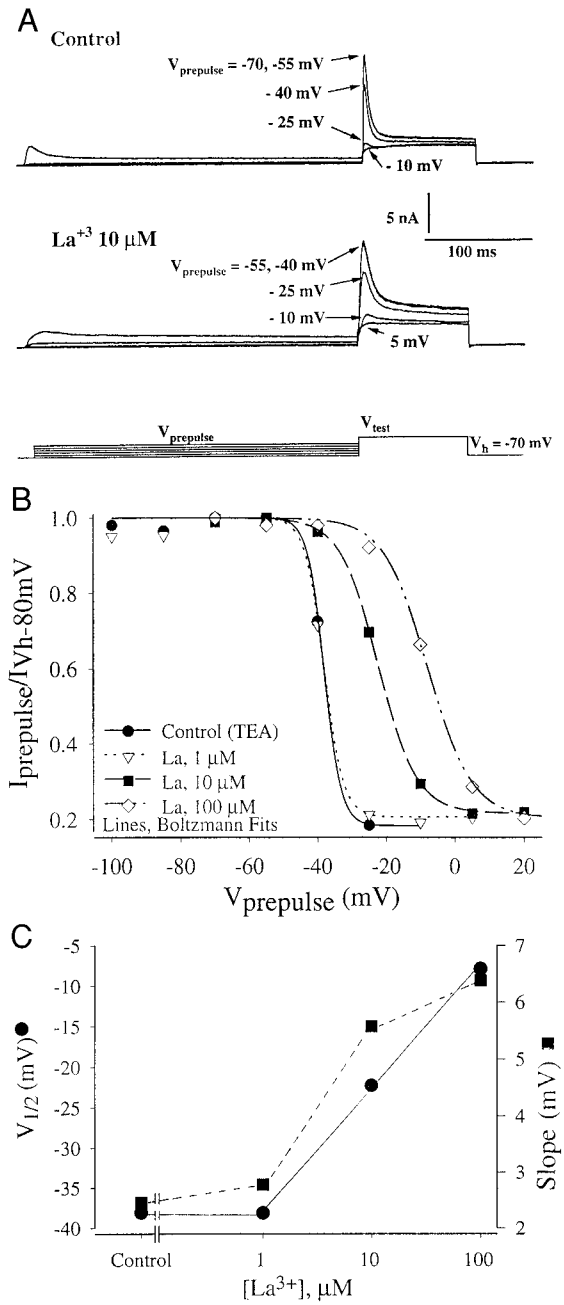

FIG. 5. Effects of La^{3+} on steady state inactivation. *A*: example of records to evaluate effects of prepulse depolarization on I_A. The voltage regime is shown; a 300-ms prepulse (incrementing values from −55 to −10 mV) is followed by a test pulse at V_c eliciting ~80% maximal I_A (+20 mV for control, +30, +45, +60 mV for 1, 10, 100 μM La^{3+} salines). Five superimposed traces are shown in control saline (*top*) and in 10 μM La^{3+}. *B*: fractional reduction of I_A tested after a prepulse compared with I_A in the absence of a prepulse is plotted vs. the prepulse voltage as recorded in control saline and 3 concentrations of La^{3+} (symbols, see legend). The data were fitted with Boltzmann functions (lines). *C*: Boltzmann parameters fitted to the data of *B* are plotted vs. [La^{3+}] (log scale); the parameters show a depolarized shift for 10 and 100 μM La^{3+}.

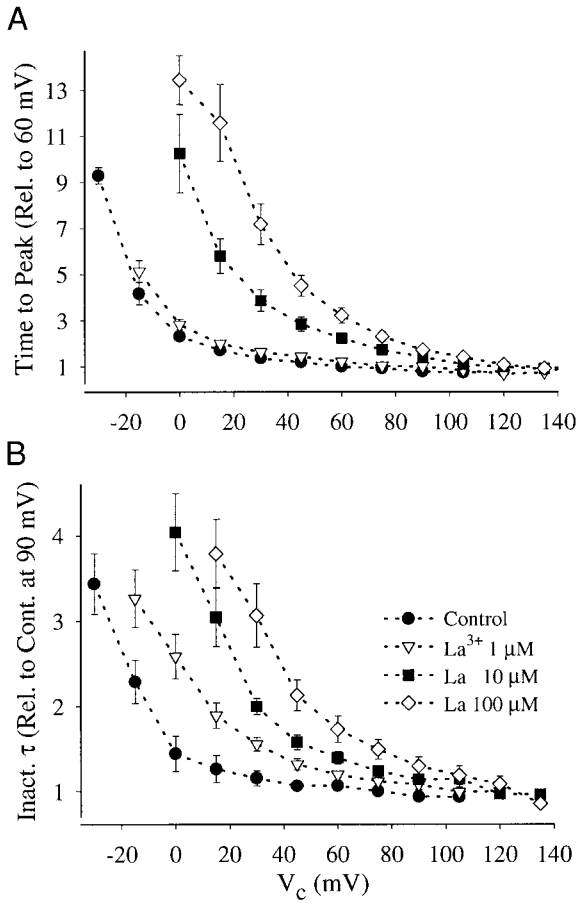

FIG. 6. Effects of La^{3+} on kinetics of I_A activation and fast inactivation. *A*: time to peak is severalfold slower in 10 or 100 μM [La^{3+}] at $V_c < +60$ mV. Time to peak I_A was normalized for each neuron to that in control saline at V_c +60 mV for each V_c. The averaged normalized values ($n = 6$) are plotted vs. V_c for control and 3 concentrations of La^{3+} (see legend). *B*: fast inactivation of I_A is slowed by La^{3+}. The time constants fitted to the initial decay from peak I_A were normalized to that at V_c +90 mV in control saline for each neuron for each V_c in each saline and plotted as in *A*.

La^{3+} effects on I_A kinetics

Inspection of the I_A responses (e.g., Fig. 3*A*) showed that addition of La^{3+} not only reduced the amplitude but slowed activation as reflected by broadened peaks. The time to peak decreased with increasingly depolarized commands and reached a minimum, typically ~3 ms, for depolarizations to more than +45 mV in normal saline. In the analysis shown in Fig. 6*A* to compare different neurons the time to peak for each voltage command is normalized relative to the asymptotic minimum in control saline at +60 mV. The average ($n = 4-8$) for each V_c for all the neurons is plotted versus V_c. Saline with 1 μM La^{3+} showed little effect on the time to peak, whereas higher [La^{3+}] right shifted the curves. The failure to show a right shift of the plot of relative time to peak versus V_c in 1 μM La^{3+} could reflect that G_A is maximal and reduced only ~10% in 1 μM La^{3+} at this V_c. With sufficiently large depolarization, the same asymptotic minimum time to peak as in control saline was obtainable in the La^{3+} salines.

The rate of the fast, initial phase of inactivation that is characteristic of I_A is also slowed by the presence of La^{3+}. The voltage dependence of the rate of inactivation was evaluated by fitting a single exponential to the initial decline of I_A responses to a series of commands. To compare observations from the several neurons, the values of the time constants at each V_c were normalized with respect to the asymptotic minimum observed for the neuron in control saline (+90 mV). The averages of these values ($n = 4-8$) are plotted versus V_c in Fig. 6*B* for the control saline and the three [La^{3+}]. This analysis shows a right shift of the curves with increasing [La^{3+}]. The V_c at which τ is doubled is shifted ~20, 40, and 60 mV more positive for 1, 10, and 100 μM La^{3+} respectively.

DISCUSSION

This study has shown that La^{3+} at micromolar concentrations, in addition to its well-recognized ability to block membrane Ca^{2+} conductances, inhibits the rapidly inactivating K^+ current (I_A) of crab secretory neurons. The inhibition of I_A is selective, as mM concentrations are needed to observe effects on the delayed rectifier K^+ current. The [La^{3+}] needed for half-maximal blocking of I_A is similar to that for blocking the calcium current (I_{Ca}) of these neurons and is between 1 and 10 μM. La^{3+} is the most effective known blocker for the crab I_{Ca}, with Cd^{2+} having nearly equal potency (Richmond et al. 1995). La^{3+} produces a concentration- and voltage-dependent block of I_A; the maximum conductance and current that can be elicited is reduced, and the current- and conductance-voltage relations are shifted to more depolarized values.

The plot of normalized time constants of the decay from peak I_A versus V_c shows a depolarizing shift with increasing [La^{3+}] that is also similar to that for the *G-V* relations (~15 mV/10 × increase in [La^{3+}]). This is consonant with observations linking fast, N-type inactivation to activation (Hoshi et al. 1991; Zagotta and Aldrich 1990). Steady-state inactivation, as observed here with 300-ms prepulses, may also be attributed to N-type, as contrasted with C-type, inactivation. Thus a right shift of the fractional steady-state inactivation of I_A versus prepulse voltage would be expected, as is observed. The lack of a change for the observations in 1 μM La^{3+} may be attributable to the choice of V_c for the test pulse, the more depolarized value used in the La^{3+} saline (+30 rather than +20) having compensated for the ~15 mV right shift of the *G-V* curve relative to control saline for 1 μM La^{3+}.

The prepulse regimes for analysis of steady-state inactivation show a consistent 20% of the outward current that remains under all conditions. This corresponds to the current that is seen to remain at the end of long (>100 ms) depolarizations, whether I_A is obtained by subtraction of records at different V_h or recorded after blocking I_K with TEA (Fig. 1), as for the data considered in this paper. It is unclear whether this should be regarded as representing reactivation of conductance through I_A channels or current through I_K or other channels, a question that would require single-channel recording to resolve. Although this residual conductance was included as a parameter in fitting the Boltzmann parameters to data for steady-state inactivation, no other correction for this possible contamination of I_A with other outward current was made in the analysis.

Our conceptual model of La^{3+} interaction with the I_A channel visualizes repetitive binding and unbinding of a La^{3+} ion at a site part way into the pore channel. The right shift of voltage dependence as well as the voltage-dependent block of current

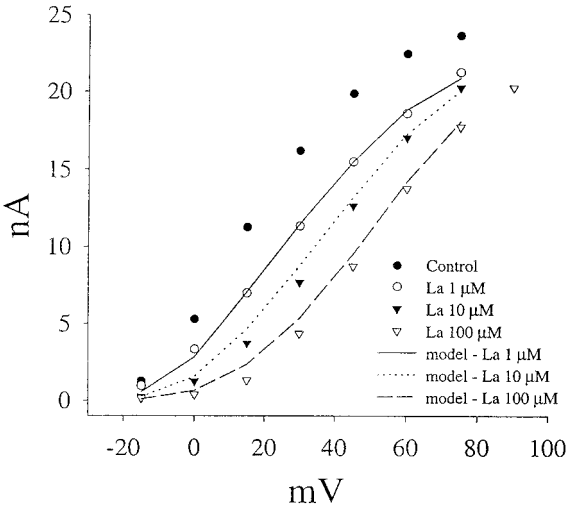

FIG. 7. Observed La^{3+} inhibition of I_A compared with predictions of the Woodhull (1973) model. Data (symbols, see legend) for I_A are from the experiment shown in Fig. 3; lines connect points calculated for each V_c and $[La^{3+}]$ with the equation $I_{model} = I_{control}K_{Vc}/([La^{3+}] + K_{Vc})$, where $K_{Vc} = K_{0\,mV} \exp(z\delta FV_c/RT)$, and z (=3 for La^{3+}), F, R, and T have their usual meaning. The parameters used for 1, 10, and 100 μM La^{3+} for the apparent binding constant for La^{3+} at $V_m = 0$ mV ($K_{0\,mV}$), estimated from the data, were 1.15, 3.1, and 7.8 μM, respectively; the distances of the binding site within the membrane voltage field, δ, were 0.20, 0.28, and 0.34.

is suggested to result from interactions with gating mechanisms when La^{3+} is bound at a specific site and obstruction of the pore by the La^{3+} when present in the channel. The ability of large depolarizing clamps to relieve block might result from the pulse expelling the blocking ion from the pore or the increased electrical driving force on K^+ permitting it to dislodge La^{3+} as it moves outward through the pore.

Such a model is closely analogous to that developed by Woodhull (1973) for block of conductance in Na^+ channels by protons. The assumptions made for development of that model appear reasonable for the case of La^{3+} block of the I_A channel (for an application of this model to La^{3+} block of a barley-root voltage-dependent K^+ current see Wegner et al. 1994). Figure 7 presents fits to a Woodhull model of the data used for Fig. 3. The fit to the observations are best for 1 μM La^{3+} and become poorer with increasing $[La^{3+}]$, especially for modest depolarizing commands. The Woodhull model provides estimates of the apparent affinity of the pore binding site in the absence of a voltage across the membrane and the distance across the electric field of the membrane of the binding site for different concentrations of the blocking ion. The affinities ranged from 1 to 8 μM for the 1- to 100-μM range of $[La^{3+}]$ examined, and distances varied from 0.2 to 0.34 of the membrane field (Fig. 7 legend).

In Woodhull's (1973) observations on $[H^+]$ effects on Na^+ currents, right shift of the voltage dependence was separated from H^+ block and was attributed to change of surface charge. However, an effect of La^{3+} on surface charge (Latorre et al. 1992) seems unlikely for the crab neurons; La^{3+} was effective at micromolar concentration in the presence of a high ionic strength saline and high concentrations of both Ca^{2+} (13 mM) and Mg^{2+} (26 mM). Rather we suggest that changes of the voltage dependence and voltage sensitivity of I_A conductance result from an electrostatic interaction between gating charges and the ion when bound to a selective site near or in the pore. This explanation was proposed in a number of studies of effects of La^{3+} and/or of divalent cations on voltage-gated channels. These include observations in typical vertebrate salines (e.g., Spires and Begenisich 1994; Talukder and Harrison 1995) as well as of squid axons in high ionic-strength saline (Armstrong and Cota 1990; Gilly and Armstrong 1982a,b).

Although there appear to have been a number of studies comparing the effects of divalents on different types of K^+ currents (see Mayer and Sugiyama 1988), relatively few examined the effects of lanthides. Ours is the first full report on crustacean neurons to our knowledge. In cultured hippocampal rat neurons (Talukder and Harrison 1995), La^{3+} differed from the other ions having effects at less than millimolar concentrations, Pb^{3+}, Gd^{3+}, Cd^{2+}, and Zn^{2+}, in its low threshold ($\sim$5 μM) and in producing a marked reduction of maximum I_A current in addition to right shifting the activation and inactivation curves as did the divalents. La^{3+} and the others, unlike Zn^{2+}, which caused a parallel right shift in the activation curves, decreased the voltage sensitivity of the activation curves. La^{3+} (and Pb^{3+}), unlike Cd^{2+} and Zn^{2+}, also inhibited the delayed rectifier current (I_K), although at a much higher concentration (100 μM) in these hippocampal neurons. We report here that unlike in the hippocampal neurons Pb^{3+}, Cd^{2+}, and Zn^{2+} had little effect on the crustacean neurons.

Marked effects of La^{3+} on K^+ currents but much less selectivity for I_A was seen in a study of rat cerebellar granule neurons (Watkins and Mathie 1994). In the cerebellar neurons a separation of La^{3+} effects on activation and inactivation was observed, with inactivation voltage relations being right shifted more than activation so that with appropriate pulse regimes enhanced I_A currents could be elicited. In both cultured rat superior cervical ganglion neurons and embryonic chick sympathetic neurons, 1 μM La^{3+} was seen to enhance transient outward current (Przywara et al. 1992). Although the *I-V* curves appeared to have been shifted to more polarized voltages, data on prepulse effects on steady-state inactivation were not presented. Thus the enhanced I_A may represent the result of a depolarizing shift of the voltage dependence of steady-state inactivation.

Finding apparently different effects of divalents or lanthides on the voltage dependence of activation and fast inactivation raises the question of how independent these processes are. Values of the $V_{1/2}$ and slope for activation and steady-state inactivation differ in all preparations examined, with the value of $V_{1/2}$ usually more polarized and the slope factor smaller (voltage sensitivity greater) for inactivation. As briefly reviewed previously, although some of the di- and trivalent cations produce parallel shifts of activation or inactivation *I-V* curves, in several there are changes in the slope factor as well. The extent of the shifts and of changes in slope factor may differ for activation and steady-state inactivation, as seen in this study. In ours and the experiments discussed previously it was not feasible to analyze the effects of possible rapid time-course interaction between activation and inactivation. From analyses of macroscopic and single-channel currents of a cloned *Drosophila* shaker channel expressed in *Xenopus* oocytes, Zagotta and Aldrich (1990) concluded that inactivation occurs from a transition closed state penultimate to opening. This analysis thus links

inactivation voltage relations and kinetics with those of activation. A more complex scheme involving 15 closed states rather than 5 in the path to opening was later used to model opening kinetics of a mutated shaker A_1 K^+ channel with a truncated N-terminus lacking fast (N-type) inactivation (Schoppa and Sigworth 1998; Zagotta et al. 1994). A similarly detailed model including inactivation has not yet been published to our knowledge. The existence of several genes coding transient K^+ channels as well as a large number of splice variants of these gene products provide a structural basis for finding much variation in the biophysical characteristics of transient K^+ channels and the influence on them of trivalent and divalent cations.

If La^{3+} blocks outward current at concentrations similar to those blocking I_{Ca}, its use to evaluate the presence of residual outward currents in studies of voltage-gated I_{Ca} is invalid. Our work suggests that it is important to also evaluate the effects of La^{3+} on outward currents in the absence of inward currents. It will be of interest to learn how general the selective inhibition of I_A by La^{3+} may be.

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AQ1 Au: Need number with unit.

AQ2 Au: Please edit abstract to 400 characters and spaces.

AQ3 Au: OK to say “decrease in value” instead of “become less good”?

AQ4 Au: “those” what?