

Selective Block of Calcium Current by Lanthanum in Single Bullfrog Atrial Cells

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ABSTRACT A single suction microelectrode voltage-clamp technique was used to study the actions of lanthanum ions (La^{3+}) on ionic currents in single cells isolated from bullfrog right atrium. La^{3+} , added as LaCl_3 , blocked the "slow" inward Ca^{2+} current (I_{Ca}) in a dose-dependent fashion; 10^{-5} M produced complete inhibition. This effect was best fitted by a dose-response curve that was calculated assuming 1:1 binding of La^{3+} to a site having a dissociation constant of 7.5×10^{-7} M. La^{3+} block was reversed (to 90% of control I_{Ca}) following washout and, in the presence of 10^{-5} M La^{3+} , was antagonized by raising the Ca^{2+} concentration from 2.5 to 7.5 mM (I_{Ca} recovered to 56% of the control). However, the latter effect took ~ 1 h to develop. Concentrations of La^{3+} that reduced I_{Ca} by 12–67%, 0.1 – 1.5×10^{-6} M, had no measurable effect upon the voltage dependence of steady state I_{Ca} inactivation, which suggest that at these concentrations there are no significant surface-charge effects of La^{3+} on this gating mechanism. Three additional findings indicate that doses of La^{3+} that blocked I_{Ca} failed to produce non-specific effects: (a) 10^{-5} M La^{3+} had no measurable effect on the time-independent inwardly rectifying current, I_{K1} ; (b) the same concentration had no effect on the kinetics, amplitude, or voltage dependence of a time- and voltage-dependent K^+ current, I_{K} ; and (c) 10^{-4} M La^{3+} did not alter the size of the tetrodotoxin-sensitive inward Na^+ current, I_{Na} , or the voltage dependence of its steady state inactivation. Higher concentrations (0.5–1.0 mM) reduced both I_{K1} and I_{K} , and shifted the steady state activation curve for I_{K} toward more positive potentials, presumably by reducing the external surface potential. Our results suggest that at a concentration of $\leq 10^{-5}$ M, La^{3+} inhibits I_{Ca} selectively by direct blockade of Ca channels rather than by altering the external surface potential. At higher concentrations, La^{3+} exhibits nonspecific effects, including neutralization of negative external surface charge and inhibition of other time- and voltage-dependent ionic currents.

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INTRODUCTION

La^{3+} interacts strongly with Ca-binding sites and therefore affects most membrane transport processes involving Ca^{2+} (dos Remedios, 1981). In cardiac tissues, in addition to inhibiting contraction (Mines, 1910) and blocking the tetrodotoxin-resistant transient inward Ca^{2+} current (Reuter, 1973), La^{3+} binds to negatively charged sites on the sarcolemma (Langer and Frank, 1972; Martinez-Palomo et al., 1973; Bockman et al., 1973), displaces ^{45}Ca from the sarcolemma (Sanborn and Langer, 1970; Langer and Frank, 1972; Meyer et al., 1982a; but see Wendt-Gallitelli and Isenberg, 1985), and may depress activities of both the Na,K-ATPase (Naylor and Harris, 1976) and the Ca-ATPase (Takeo et al., 1979). In addition, Yau and Nakatani (1984) have shown that La^{3+} also inhibits the current generated by an electrogenic Na-Ca exchange process in photoreceptors.

Mechanistic interpretations of the actions of La^{3+} on time- and voltage-dependent ionic currents are often complicated by the ion's ability to significantly modify the negative external surface potential, as originally proposed by A. F. Huxley (Frankenhaeuser and Hodgkin, 1957). This indirect effect manifests itself as a positive shift of the voltage dependence of, e.g., Na and/or K conductance gating parameters (Hodgkin and Huxley, 1952b). La^{3+} has been shown to produce such voltage shifts in lobster axons (Takata et al., 1966; Blaustein and Goldman, 1968), frog myelinated nerve fibers (Vogel, 1974; Hille et al., 1975; Århem, 1980; Brismar, 1980; Neumcke and Stämpfli, 1984), and frog skeletal muscle (Dörrscheidt-Käfer, 1981). However, in addition to these surface-charge effects, La^{3+} is a very potent inhibitor of Ca^{2+} current (I_{Ca}) in squid axons (Baker et al., 1973), *Helix* neurons (Akaike et al., 1978), neuroblastoma cells (Moolenaar and Spector, 1979), and uterine smooth muscle (Anderson et al., 1971); it also blocks voltage-dependent Ca^{2+} entry into rat brain synaptosomes (Nachshen, 1984).

La^{3+} may simultaneously exhibit channel-blocking and surface-charge binding/screening phenomena (Ohmori and Yoshii, 1977; Kostyuk et al., 1982; Wilson et al., 1983). Hence, it is necessary to make a clear distinction between these direct and indirect effects when investigating La^{3+} actions in excitable tissues (cf. McLaughlin et al., 1971).

The present experiments were designed to systematically investigate the actions of La^{3+} on single, isolated cardiac myocytes. Previous work has shown that La^{3+} at relatively high doses has multiple effects on the electrophysiological properties of multicellular cardiac preparations. It blocks I_{Ca} (Katzung et al., 1973; Kass and Tsien, 1975), increases the net outward time-independent plateau current (Kass and Tsien, 1975), increases membrane resistance at the resting potential (Haass, 1975; Hatae, 1982), and produces a positive shift in the voltage-dependent gating mechanism of both the outward plateau current, I_X (Kass and Tsien, 1975), and the pacemaker current, which was originally called $I_{\text{K}2}$ (Tsien, 1974a). La^{3+} may also inhibit electrogenic Na-Ca exchange in multicellular cardiac preparations (Horackova and Vassort, 1979; Reeves and Sutko, 1979; Trospen and Philipson, 1983; Mentrard et al., 1984); however, the evi-

dence on this point is conflicting (see Katzung et al., 1973; Coraboeuf et al., 1981; Jacob et al., 1987). In single cardiac myocytes, 0.1 mM LaCl_3 blocks I_{Ca} and, after 30 min of exposure, may impair the selectivity of Na channels (Wendt-Gallitelli and Isenberg, 1985). La^{3+} also inhibits a current generated by the Na-Ca exchanger (Hume and Uehara, 1986; Mechmann and Pott, 1986; Kimura et al., 1987), as was demonstrated originally in isolated rod outer segments (Yau and Nakatani, 1984).

A general goal of the present study was to find a potent and selective inorganic Ca channel inhibitor. La^{3+} was chosen because of its known potency. We therefore studied its selectivity; i.e., does it block Ca channels directly, or by reducing the negative external surface potential in the vicinity of the channel? Since we were successful in finding a concentration of La^{3+} that blocks I_{Ca} but has no effect upon K^+ currents, it was possible to test whether Ca^{2+} influx (through Ca channels) during the plateau is essential to activate the outward K^+ current that repolarizes the action potential.

METHODS

Cell Isolation and Maintenance

The procedure for isolating single cells was very similar to that used previously (Hume and Giles, 1981). The heart was removed from an adult bullfrog (*Rana catesbeiana*) and, after the right atrium had been separated from the sinus venosus, the atrium was placed in a small flask containing 5 ml of Ca-free Ringer's solution, 0.13% crude bacterial collagenase (type I, *Clostridium histolyticum*, 200 U/mg; Sigma Chemical Co., St. Louis, MO), and 0.08% bovine pancreatic trypsin (type III, dialyzed, lyophilized, and salt-free, 10,100 U/mg protein; Sigma Chemical Co.). This solution was stirred continuously for 45 min at room temperature and then replaced with 5 ml of Ca-free Ringer's containing 0.1% bovine serum albumin (essentially fatty acid free and prepared from fraction V; Sigma Chemical Co.) and stirred for another 5 min. Next, the atrium was incubated in fresh collagenase (0.07% in 5 ml of Ca-free Ringer's solution) for 30 min. After three or four such 30-min cycles, the solution contained a high density of single cells. Aliquots were removed with a Pasteur pipette and placed in a recording chamber for electrophysiological experiments.

Approximately 20 min was allowed for the cells to adhere loosely to the lid of a 35- \times 10-mm tissue-culture dish (type 3001, Falcon Labware Division, Becton, Dickinson and Co., Oxnard, CA). They were then superfused with standard Ringer's solution for \sim 30 min and thereafter with HEPES-buffered Ringer's solution bubbled with 100% O_2 . The cells were viewed with an inverted microscope (Swift Instruments, Inc., San Jose, CA) at a magnification of 600. All experiments, except those in which I_{Na} was measured (see legend to Fig. 6), were performed at room temperature (22–23°C).

Solutions and Drugs

The standard Ringer's solution contained (millimolar): 90.6 NaCl, 20 NaHCO_3 , 2.5 KCl, 2.5 CaCl_2 , 5.0 MgCl_2 , and 10 glucose, and was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). However, to avoid precipitation, all La^{3+} experiments as well as control experiments were performed in a HEPES-buffered Ringer's solution that contained (milli-

molar): 90.6 NaCl, 2.5 KCl, 2.5 CaCl₂, 5.0 MgCl₂, 10 glucose, 20 sucrose, and 5 HEPES. The pH of this solution was titrated to 7.4 with NaOH, and it was bubbled with 100% O₂. LaCl₃ was dissolved in distilled water to make 0.01- or 1.0-mM stock solutions, which were refrigerated and remade every other day. Tetrodotoxin (TTX) was purchased from Sigma Chemical Co.; CdCl₂ was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI; LaCl₃ was purchased from K & K Laboratories, Inc., Plainview, NY; and HEPES was obtained from Research Organics, Inc., Cleveland, OH.

Electrophysiology

Microelectrodes were pulled on a conventional horizontal puller (Industrial Science Associates, Inc., Ridgewood, NY) using 1-mm-o.d. square-bore glass (Glass Co. of America, Bargaintown, NJ), and were back-filled with 1 M potassium gluconate. Acceptable tip resistances ranged from 4 to 6 M Ω . Details of the suction impalement technique for single atrial cells have been described previously (Hume and Giles, 1983) and are similar to those originally reported by Hamill et al. (1981). The experimental setup used for voltage-clamp experiments was similar to that described previously (Hume and Giles, 1983).

The membrane potential was monitored by a high-input impedance amplifier (model 1600, A-M Systems, Everett, WA) and compared with command potentials from an optically isolated voltage-divider circuit that was gated by pulses from a digital clock. Current flow from the clamp amplifier was measured by a differential amplifier as the voltage drop across a 10-M Ω resistor. The voltage-clamp and current amplifiers were designed and constructed by Dr. T. Iwazumi, University of Texas Medical Branch, Galveston, TX. A virtual ground circuit (model 180, W-P Instruments, Inc., New Haven, CT) was used to ground the bathing medium. In the majority of these experiments, no electronic series resistance compensation was used; thus, the amplitudes of I_{Ca} (Figs. 2–4) and I_{Na} (Fig. 6) are likely to have been underestimated because of the relatively long capacitive transients. Moreover, had series resistance increased two- to threefold during voltage-clamp recordings, our 4–6-M Ω electrodes would have produced voltage errors of ≤ 3 mV, given that the maximum I_{Ca} was -170 ± 14 pA. No attempt was made either to cancel or subtract the capacitive transients; however, in protocols in which I_{Na} was recorded, a “supercharging” circuit (Matteson and Armstrong, 1984) was employed to speed charging of the cell capacitance. Before the analysis of I_{Na} data, the capacitive transient was electronically subtracted (see legend to Fig. 6).

Data Analysis

Membrane potentials and currents were amplified and then recorded on FM tape (model 3968, Hewlett-Packard Co., San Diego, CA). A tape speed of 7½ in./s (bandwidth of DC–2.5 kHz) was used to record Ca²⁺ and K⁺ currents, and a speed of 15 in./s was used to record Na⁺ current. The data were later digitized by a digital oscilloscope (model 3001, Norland Corp., Fort Atkinson, WI) and then sent to a PDP 11-70 computer (Digital Equipment Corp., Maynard, MA). Current waveforms were analyzed by the Discrete program (Provencher, 1976), which determines whether a waveform is best fitted by one, two, or three exponential functions and then calculates the time constants and initial (or final) amplitudes of the functions that best fit the data. Two-sample *t* tests were used to determine whether differences between mean values were statistically significant (i.e., $P < 0.05$).

RESULTS

Action Potential Measurements

Action potentials elicited (at 0.2 Hz) from quiescent bullfrog atrial cells were very similar to those recorded previously by Hume and Giles (1981, 1983). The resting potential, overshoot, duration, and maximum upstroke velocity were -90 mV, $+42$ mV, 650 ms, and 48 V/s, respectively (trace 1, Fig. 1). In the presence of 5×10^{-5} M LaCl_3 , continuous recordings first exhibited a marked reduction in the plateau height (trace 2). After the La^{3+} effect had reached a steady state (trace 3), the peak of initial depolarization was depressed slightly, the plateau height was diminished further, and the resting potential was depolarized by ~ 3 mV. Hatae (1982) has recorded similar changes in the action potential configuration when bullfrog ventricular strips were exposed to 5×10^{-4} M La^{3+} for 4–10 min.

To investigate the ionic mechanisms that underlie these changes, we used a single suction microelectrode voltage-clamp technique to measure I_{Ca} , I_{Na} , the

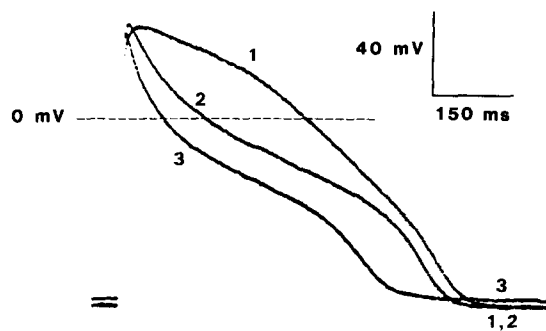


FIGURE 1. Action potentials elicited at 0.2 Hz during bath application of Ringer's solution containing 5×10^{-5} M LaCl_3 . Trace 1 is the control; traces 2 and 3 were obtained 12 and 34 min, respectively, after the addition of La^{3+} . The maximum upstroke velocity was 48 V/s in traces 1 and 2, and 56 V/s in trace 3.

time-independent inwardly rectifying background current carried by K^+ , I_{K1} , and the time- and voltage-dependent outward current carried by K^+ , I_{K} . Some characteristics of each of these currents have been described previously (Hume and Giles, 1983). Detailed studies of I_{K} , the delayed rectifier, and I_{Na} have been published recently (Hume et al., 1986; Clark and Giles, 1987).

 La^{3+} Block of I_{Ca}

In the presence of 3×10^{-6} M TTX, which completely blocks I_{Na} (Hume and Giles, 1983; Clark and Giles, 1987), I_{Ca} could be recorded consistently in normal Ringer's or HEPES Ringer's (Fig. 2 A). At 0 mV, I_{Ca} reached a maximum within 5–7 ms. The apparent reversal potential for I_{Ca} recorded in 2.5 mM Ca^{2+} was $+60$ mV, in agreement with previous data from bullfrog atrial cells (Hume and Giles, 1983) and earlier studies using mammalian ventricular trabeculae (Reuter and Scholz, 1977) or isolated ventricular myocytes (Lee and Tsien, 1982). At potentials more positive than $+60$ mV, a transient outward current was recorded.

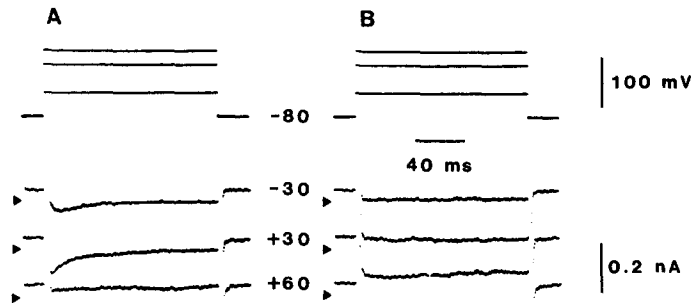


FIGURE 2. I_{Ca} recorded continuously in control conditions and during superfusion with 1.7×10^{-5} M $LaCl_3$; both sets of recordings were obtained in HEPES Ringer's that contained 3×10^{-6} M TTX. (A) Control. (B) 12 min after La^{3+} addition. The numbers between the current records indicate potentials at which the paired traces were elicited. Note the apparent reversal potential at +60 mV and the complete block of I_{Ca} by La^{3+} . A steady outward current, 50 pA in A and 55 pA in B, was applied to maintain the cell at -80 mV; the zero-current levels are indicated by the arrowheads next to each record. The resting potential, or zero-current level, of this cell was -94 mV. Neither leakage corrections nor series resistance compensation was used.

When the effects of $1-2 \times 10^{-5}$ M La^{3+} had reached steady state, complete blockade of I_{Ca} (Fig. 2 B) and cessation of contractile activity were observed consistently. However, the outward shift of current (at +60 mV) that is illustrated in Fig. 2 B was not a consistent finding, since the averaged current recorded from four cells treated with 1×10^{-5} M La^{3+} was not significantly different from that recorded from six controls (see Fig. 4 A and additional results below). Such a shift was probably due to increased leakage at the suction electrode impalement site.

In four experiments, virtually complete reversal of the La^{3+} -induced inhibition of I_{Ca} was obtained. Fig. 3 illustrates one such result, in which La^{3+} ($5 \times$

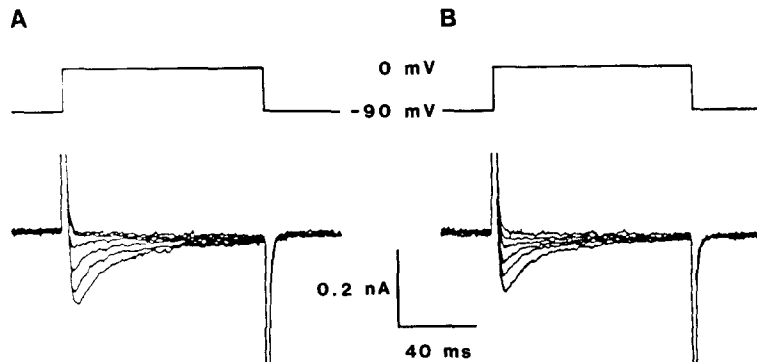


FIGURE 3. Effect of $LaCl_3$ (5×10^{-6} M) on I_{Ca} in the presence of 3×10^{-6} M TTX. Both panels show six superimposed I_{Ca} records, each elicited by a 100-ms depolarizing pulse to 0 mV from the holding potential, -90 mV. A illustrates development of the block; B shows the recovery. The traces were recorded 2.3, 2.5, 2.7, 2.9, 3.1, and 3.3 min after addition of La^{3+} (A) or 3.2, 3.5, 3.8, 4.1, 4.4, and 5.0 min after washout (B).

10^{-6} M) was added to the HEPES-buffered Ringer's. The maximum blocking effect was obtained within 3 min (A); then the La^{3+} -induced block was virtually reversed within 5 min by return to control HEPES Ringer's (B). In other experiments in which La^{3+} was applied in higher concentrations and/or for much longer times, only partial recovery from the block was observed; these effects (e.g., 75% recovery) took ~ 1 h to develop.

In other recovery experiments, La^{3+} was not removed; instead, the Ca^{2+} concentration was increased from 2.5 to 7.5 mM by replacing 5 mM MgCl_2 in the Ringer's with CaCl_2 . Results from these experiments provide evidence that Ca^{2+} and La^{3+} act in a competitive fashion: (a) in 7.5 mM Ca^{2+} , addition of La^{3+} (10^{-5} M) failed to completely block I_{Ca} ; (b) after 1 h of exposure to 7.5 mM Ca^{2+} , cells exhibited $\sim 50\%$ recovery of their (mean) control I_{Ca} . Thus, La^{3+} and Ca^{2+} might compete for the same binding site. This was originally proposed by Henkart and Hagiwara (1976), who obtained data from barnacle muscle. Lansman et al. (1986) have developed this hypothesis further on the basis of measurements of single Ca channel activity in the presence of divalent and trivalent inorganic cations.

As illustrated in Fig. 4 A, La^{3+} produced a dose-dependent reduction of I_{Ca} at potentials between -40 and $+50$ mV, but had no significant effect ($P > 0.05$) upon net current at the apparent reversal potential, $+60$ mV. In cells treated with 1×10^{-5} M La^{3+} , the current at $+60$ mV averaged $+45 \pm 7$ pA (\pm SEM, $n = 4$), whereas in controls it averaged $+32 \pm 13$ pA (\pm SEM, $n = 6$). The lack of a statistically significant effect of La^{3+} at, or very near, the apparent reversal potential is an important finding: (a) it indicates a selective block of I_{Ca} by La^{3+} , and (b) it shows that, on the average, stable impalements could be maintained throughout the protocol without significant changes in leakage current around the suction microelectrode.

The peak values of I_{Ca} at 0 mV were normalized (assuming that 10^{-5} M LaCl_3 produced complete inhibition of I_{Ca}) and plotted against La^{3+} concentration (Fig. 4 B). The mean sizes of I_{Ca} at 0 mV in 10^{-8} M and 10^{-7} M La^{3+} were not significantly different from the control ($P > 0.05$). Dose-response curves were calculated by hand from the Langmuir equation,

$$I_{\text{Ca}}(\text{La}^{3+}) = I_{\text{Ca}}(\text{control}) \left\{ 1 - \frac{1}{1 + (K_m/[\text{La}^{3+}])} \right\}, \quad (1)$$

which assumes 1:1 binding of La^{3+} to a Ca^{2+} -binding site. The value of K_m , the dissociation constant for La^{3+} , that gave the best visual fit to the raw data was 7.5×10^{-7} M (arrow).

La^{3+} Effects on Steady State Inactivation of I_{Ca}

La^{3+} could block I_{Ca} indirectly by screening or binding to negative fixed charges on the cell surface, thereby reducing the external surface potential and, in turn, modulating I_{Ca} . To test this hypothesis, the steady state inactivation relation,

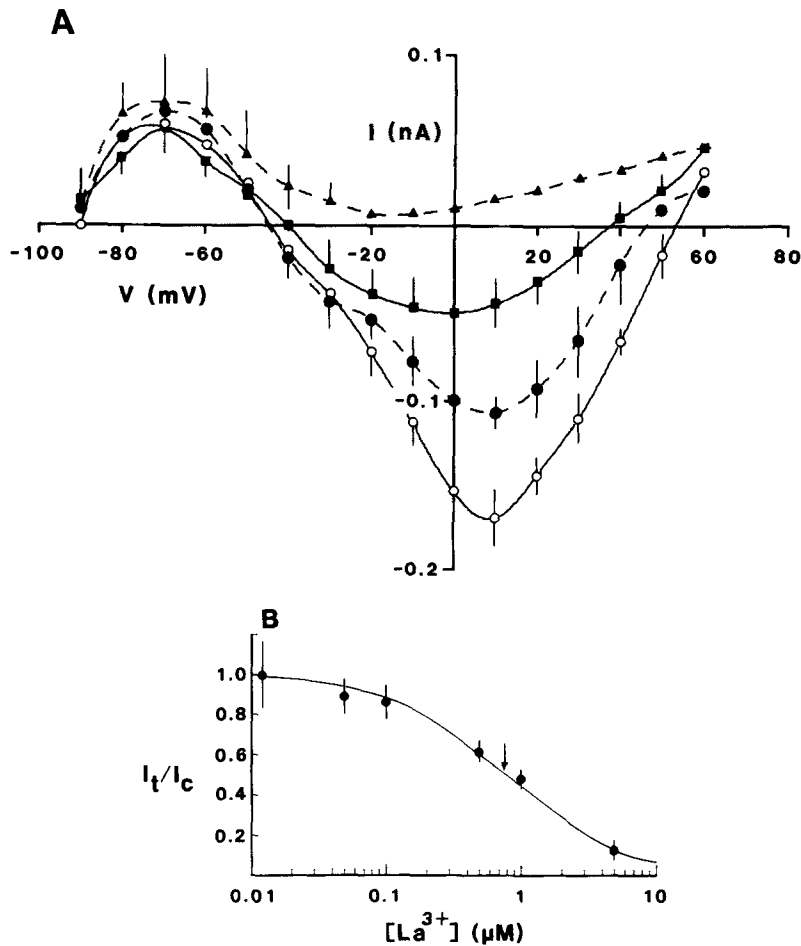


FIGURE 4. (A) I - V relationships for peak I_{Ca} in the presence of 3×10^{-6} M TTX and LaCl_3 . Currents were measured as the maximum inward or minimum outward current from the zero-current level. Each point represents the mean \pm SEM for 8–11 controls (○), 2–3 cells in 10^{-7} M La^{3+} (●), 2–4 cells in 10^{-6} M La^{3+} (■), and 4 or 5 cells in 10^{-5} M La^{3+} (▲). The smooth curves were drawn by eye to fit the mean values. (B) Dose-response curve for the effect of La^{3+} on peak I_{Ca} at 0 mV. I_t and I_c are peak I_{Ca} in the presence and absence, respectively, of La^{3+} . In 10^{-5} M La^{3+} , I_{Ca} was assumed to be zero at each potential. Each point represents the mean \pm SEM for three cells in 10^{-8} M, three cells in 5×10^{-8} M, three cells in 10^{-7} M, four cells in 5×10^{-7} M, four cells in 10^{-6} M, or four cells in 5×10^{-6} M La^{3+} . The smooth curve was calculated from Eq. 1 (text) assuming 1:1 binding and a dissociation constant of 7.5×10^{-7} M (arrow).

$f_{\infty}(V)$, was measured in the presence and absence of effective doses of La^{3+} (cf. Campbell et al., 1983; Kass and Kraft, 1987). If blocking doses of La^{3+} act mainly by neutralizing negative surface charge near the Ca channel, a significant positive shift of the $f_{\infty}(V)$ curve would have been expected (Frankenhaeuser and

Hodgkin, 1957). A double-pulse protocol (Hodgkin and Huxley, 1952a) was used to measure the voltage dependence of I_{Ca} inactivation. A 300-ms prepulse was long enough for inactivation to reach a steady state, but it did not activate a significant amount of I_K .

Examples of I_{Ca} records from this protocol are illustrated in Fig. 5, A and B. In the control (A), a prepulse of -50 mV had no effect upon inward current during P_{II} (trace 1); however, this current was reduced by 48 and 100% (trace 3) following prepulses to -30 and 0 mV, respectively. The small outward current tail during P_{II} (trace 3) may have resulted from the failure of I_K to deactivate completely during the 10-ms delay preceding P_{II} .

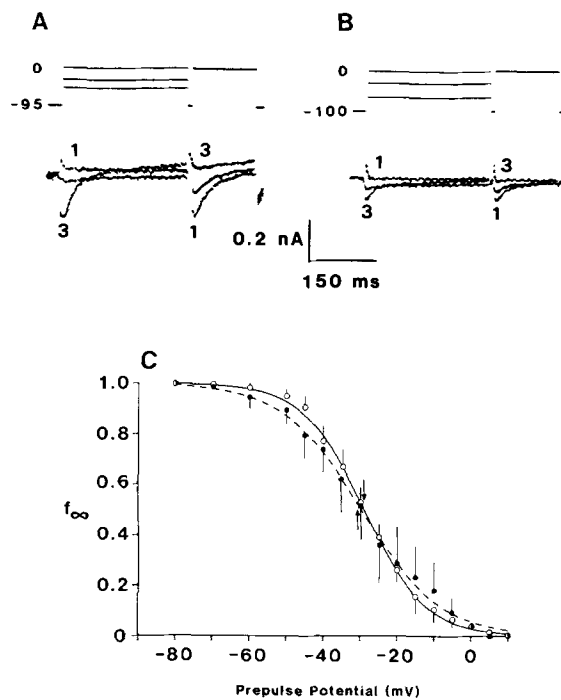


FIGURE 5. Effect of La^{3+} on steady state inactivation of I_{Ca} in the presence of 3×10^{-6} M TTX. (A) I_{Ca} in a control experiment. P_I was 300 ms and was followed by a 10-ms gap at the holding potential; P_{II} was 150 ms. The superimposed I_{Ca} 's were evoked by P_I steps to -50 (trace 1) and -30 and 0 mV (trace 3); P_{II} was 0 mV. (B) I_{Ca} in another cell 20 min after addition of 5×10^{-7} M $LaCl_3$. The P_I potentials were -65 (trace 1) and -30 and 0 mV (trace 3). Complete inactivation was produced by prepulses to 0 mV (trace 3). (C) Averaged results for f_{∞} . Individual values were calculated as $f_{\infty} = [I_{Ca}(P_{II}) \text{ with a prepulse}] / [I_{Ca}(P_{II}) \text{ without a prepulse}]$, where the latter

was the mean of the two values obtained before and after the double pulse. Each point represents the mean \pm SEM for four or five controls (\circ), or for six or seven cells exposed to 10^{-7} – 10^{-6} M La^{3+} (\bullet). The smooth curves were calculated from Eq. 2 (text), and the arrows indicate the potentials at which $f_{\infty} = 0.5$.

The effects of La^{3+} on the inactivation of I_{Ca} in another cell are illustrated in Fig. 5B. In this experiment, 20 min of superfusion with 5×10^{-7} M $LaCl_3$ reduced I_{Ca} to -95 pA (trace 1). I_{Ca} elicited by P_{II} was further diminished by 53 and 100% (trace 3) following inactivating prepulses to -30 and 0 mV, respectively. The close agreement between the two recordings of steady state

inactivation suggests that 5×10^{-7} M La^{3+} had a negligible effect upon the surface charge associated with this gating process. This observation was confirmed by additional experiments in which La^{3+} concentrations up to 1.5×10^{-6} M were employed. In Fig. 5 C, cells were exposed to 10^{-7} M ($n = 1$), 5×10^{-7} M ($n = 2$ or 3), and 1.0 – 1.5×10^{-6} M LaCl_3 ($n = 3$). At 0 mV, these La^{3+} concentrations reduced I_{Ca} by 12, 40, and 57–67%, respectively (Fig. 4 B). The f_{∞} values for these three sets of data were pooled since no one group differed significantly from the others. Individual values for f_{∞} were calculated as $[I_{\text{Ca}}(\text{P}_{\text{II}}) \text{ with a prepulse}] / [I_{\text{Ca}}(\text{P}_{\text{II}}) \text{ without a prepulse}]$. In these experiments, each double-pulse test was preceded and followed by a single test pulse (P_{II}) to 0 mV in order to assess and account for any rundown of I_{Ca} ; when this occurred, the data were rejected. In some cells exposed to positive prepulses (e.g., trace 3, Fig. 5 A), the net current during P_{II} declined to a constant level that was slightly positive to zero. In such cases, this level was assumed to be zero.

The data in Fig. 5 C were fitted by curves calculated from the Boltzmann distribution (Hodgkin and Huxley, 1952a):

$$f_{\infty} = \frac{1}{\{1 + \exp [(V - V_f)/k_f]\}}, \quad (2)$$

where V_f is the membrane potential at which $f_{\infty} = 0.5$, and k_f is a slope factor. For the control cells, $V_f = -29.5$ mV (downward arrow) and $k_f = 8.5$ mV; these values are in good agreement with those obtained by Campbell et al. (1983) in single bullfrog atrial cells as well as by Trautwein et al. (1975), Reuter and Scholz (1977), and Isenberg and Klöckner (1982) in mammalian ventricular preparations. For La^{3+} -treated cells, $V_f = -30.9$ mV (upward arrow) and $k_f = 9.5$ mV. The difference in mean values for f_{∞} for controls and La^{3+} -treated cells was not statistically significant ($P > 0.05$). Hence, the reduction of I_{Ca} by La^{3+} at concentrations between 5×10^{-7} and 1.5×10^{-6} M is unlikely to have been due to an effect of La^{3+} on the surface potential that modulates inactivation.

La^{3+} Effects on Steady State Inactivation of I_{Na}

The results in the previous section have shown that effective doses of La^{3+} do not produce any measurable shifts in the inactivation relation of I_{Ca} . As a further test of the extent to which blocking doses of La^{3+} act in a selective way, we have measured the effects of 10^{-4} M La^{3+} on the steady state inactivation curve for I_{Na} . Since I_{Na} in these and other cardiac cells is much larger and faster than other ionic currents (Hume and Giles, 1983; Clark and Giles, 1987), it was necessary to reduce its size and slow its kinetics by working at a lower temperature (8°C). To obtain the h_{∞} curve, the same protocol as above was employed: P_1 lasted 10 s and the test pulse (P_{II}) followed after a 10-ms gap. CdCl_2 (2.0×10^{-4} M) was added to the HEPES-buffered Ringer's to block I_{Ca} . In the presence of both 2.5 mM CaCl_2 and 5.0 mM MgCl_2 , this concentration of Cd^{2+} would have had little effect upon the h_{∞} curve (Blaustein and Goldman, 1968; Hille et al., 1975). The raw data obtained in response to P_1 pulses ranging from

–55 (smallest trace) to –130 mV (largest trace) are shown in the inset of Fig. 6. The control and La^{3+} data were fitted by Boltzmann distributions (Eq. 2). They demonstrate that La^{3+} at 10^{-4} M produces no significant shift in the voltage dependence of steady state I_{Na} inactivation. These results support the contention that doses of $\text{La}^{3+} \leq 10^{-5}$ M suppress I_{Ca} directly rather than indirectly via an effect upon surface charge.

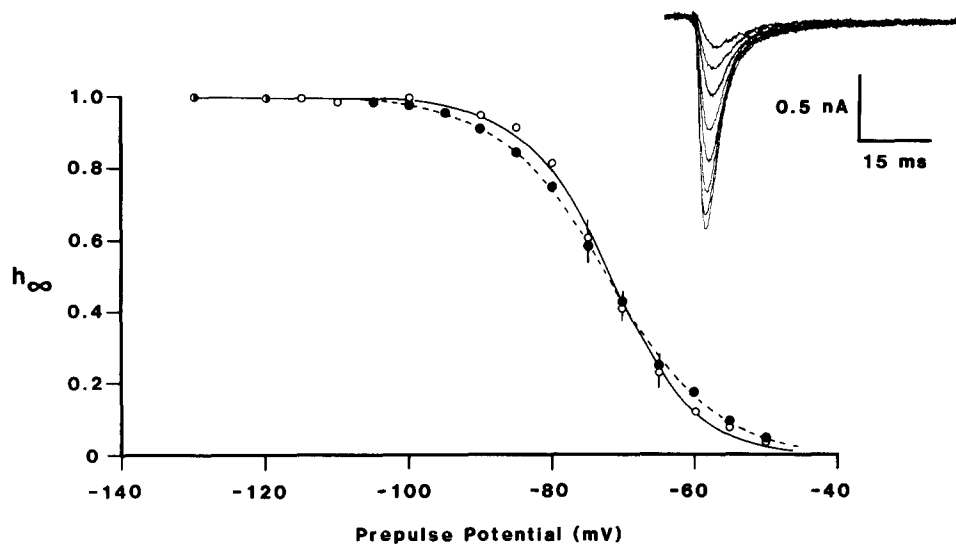


FIGURE 6. Effect of La^{3+} on steady state inactivation of I_{Na} . These experiments were carried out in HEPES-buffered Ringer's at $8.3 \pm 1^\circ\text{C}$; in the control experiments, 2×10^{-4} M CdCl_2 was used to block I_{Ca} . The inset shows raw data from a conventional two-pulse inactivation experiment. Eight superimposed traces were obtained in response to 100-ms test pulses to 0 mV preceded by 10-s prepulses to –55, –60, –65, –70, –75, –80, –90, and –130 mV (largest record). The h_∞ values are means \pm SEM from four controls (\circ) and four cells after addition of 1.0×10^{-4} M LaCl_3 (\bullet). Both sets of data have been fitted by Boltzmann distributions (Eq. 2, text): for the control data, $V_h = -72$ mV and $k_h = 5.2$ mV. After La^{3+} treatment, $V_h = -73$ mV and $k_h = 7.4$ mV. A "supercharging" circuit was used to speed charging and discharging of the cell capacitance and the capacitive transients were removed by subtraction.

La^{3+} Effects on I_{K1}

At potentials negative to –50 mV, a time-independent current that exhibits inward (anomalous) rectification and is carried by K^+ can be recorded consistently in isolated bullfrog atrial cells (Momose et al., 1983). Fig. 7A illustrates this current at potentials between –90 and –120 mV. Neither 1×10^{-5} (during periods of up to 130 min) nor 1.7×10^{-5} M La^{3+} (11 min of superfusion, Fig. 7B) produced any significant change in I_{K1} . Pooled results from four to nine control experiments and two to seven cells exposed to 10^{-5} M LaCl_3 are summarized in Fig. 7C. Since the mean values of I_{K1} in these two groups did not

differ significantly ($P > 0.05$), both sets of data were fitted by eye with a single curve. In contrast, 10^{-3} M La^{3+} reduced I_{K1} significantly ($P < 0.05$ at -120 and -80 mV) in each of three cells. If the two I - V curves are corrected for seal leakage current and one assumes a seal resistance of $1.5 \text{ G}\Omega$, both curves would

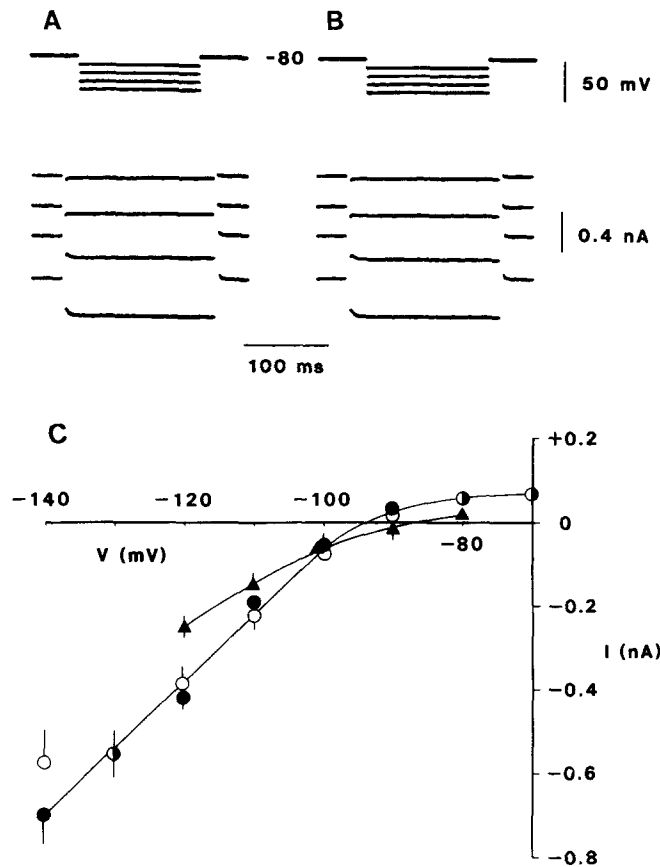


FIGURE 7. Effect of La^{3+} on I_{K1} recorded in the presence of 3×10^{-6} M TTX. (A) Control. (B) 11 min after addition of 1.7×10^{-5} M LaCl_3 . Note the absence of an effect. (C) Mean values (\pm SEM) for four to nine controls (\circ), two to seven cells in 10^{-5} M La^{3+} (\bullet), and three cells in 10^{-3} M La^{3+} (\blacktriangle). All currents were measured from the zero-current level. The points in 10^{-3} M LaCl_3 at -120 and -80 mV are significantly different from the controls. The smooth curves were fitted by eye to the mean values. The holding current was $+50$ pA in A and $+55$ pA in B (same cell as in Fig. 2).

cross the voltage axis at about -100 mV. This is close to the value of E_K that Walker and Ladle (1973) have calculated based upon measurements of intracellular K^+ activity in intact frog atrium. Thus, 10^{-3} M La^{3+} appears to reduce the conductance without affecting the reversal potential for I_{K1} . The small depolarization produced by 5×10^{-5} M La^{3+} (Fig. 1) indicates that I_{K1} might have been reduced by La^{3+} at concentrations $> 1.7 \times 10^{-5}$ M.

La³⁺ Effects on I_K

In these isolated atrial myocytes, long (10-s) depolarizing voltage-clamp steps elicit a slowly rising outward current (Fig. 8, A and B) when the command potential exceeds approximately -30 mV. Although the activation of this current exhibits a sigmoid time course, its decay can be described by a single exponential

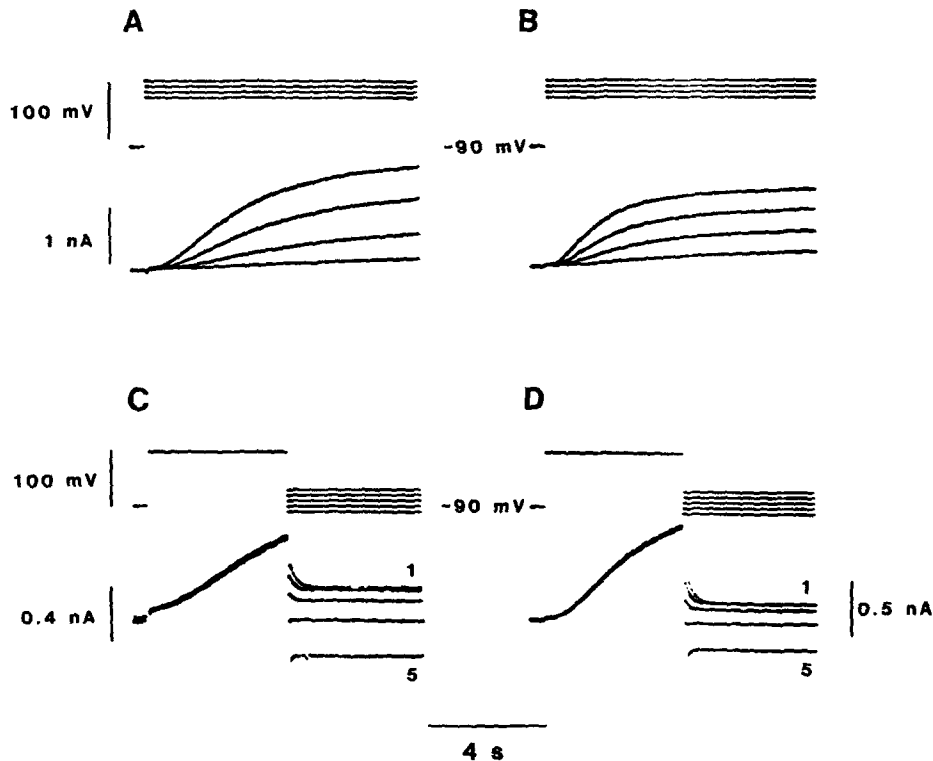


FIGURE 8. Effect of La^{3+} on I_K in the presence of 3×10^{-6} M TTX (A) Activation of control I_K during 10-s depolarizing steps; the holding current was -20 pA. (B) Activation of I_K in another cell, 52 min after addition of 10^{-5} M $LaCl_3$; the holding current was -25 pA. Voltage-clamp steps in A and B were to 0 (smallest I_K), +10, +20, and +30 mV (largest I_K). (C) Control I_K tails following a 5-s prepulse to -10 mV; the holding current was -30 pA. (D) I_K tails in another cell, 58 min after addition of 10^{-5} M $LaCl_3$; the holding current was -25 pA. The I_K tails in C and D were generated at potentials of -60 (trace 1), -70 , -80 , -90 , and -100 mV (trace 5). Note that La^{3+} had little effect upon the activation, deactivation, or reversal potential of I_K .

(Hume et al., 1986). In normal extracellular K^+ (2.5 mM), these current tails reverse at potentials between -90 and -100 mV (Fig. 8, C and D). Hume and Giles (1983) and Hume et al. (1986) recorded similar K^+ currents that reversed between -95 and -100 mV; in addition, their results showed that accumulation of K^+ adjacent to single bullfrog atrial cells is insignificant, even during steady state activation protocols. We therefore have used tests for shifts in the

voltage dependence of I_K as another indicator of the selectivity of effective (I_{Ca} blocking) doses of La^{3+} .

Fig. 8, *B* and *D*, shows that exposure of cells to 10^{-5} M La^{3+} for 52–58 min had no significant effect upon the activation/deactivation kinetics or on the reversal potential of I_K . The former result is confirmed in Fig. 10 *B*. Isochronal I - V relationships (measured at 10 s) are plotted in Fig. 9. The differences in I_K between controls and cells treated for 24–129 min with 5×10^{-6} M $LaCl_3$ were not statistically significant ($P > 0.05$); however, I_K was reduced significantly when cells were exposed to 5×10^{-4} M La^{3+} for 28–48 min. Previous data obtained in calf Purkinje fibers support the idea that this concentration of La^{3+} blocks delayed outward currents, possibly indirectly, by modifying the negative external surface potential (Kass and Tsien, 1975).

To test for such surface-charge effects and to confirm that the surface charge had not been altered by our enzymatic isolation procedure (cf. Barry et al., 1978), we measured the effect of La^{3+} on the voltage dependence of (a) I_K

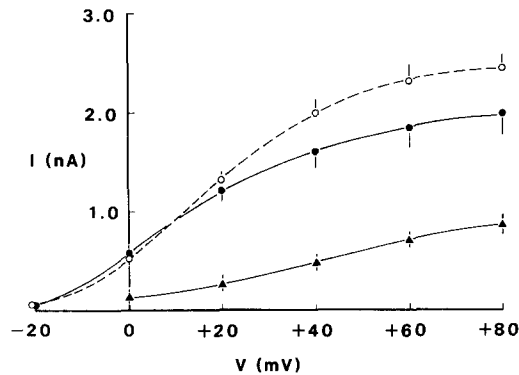


FIGURE 9. Effect of La^{3+} on isochronal (10-s) I - V relationships of I_K . Each point represents the mean \pm SEM for 16–21 controls (\circ), 6 cells in 5×10^{-6} M La^{3+} (\bullet), or 4–7 cells exposed to 5×10^{-4} M La^{3+} (\blacktriangle). The smooth curves were fitted by eye to the mean values.

quasi-steady state activation (n_∞) and (b) the rate constants ($1/\tau_n$) for activation and deactivation of I_K . Fig. 10 *A* illustrates the results of these experiments; I_K tails were recorded following 10-s prepulses to the potentials indicated on the abscissa. A more detailed study of the kinetics of this current (Hume et al., 1986) has shown that the time course of its activation is best fitted by a single-exponential function raised to the second power. Values of n_∞ in Fig. 10 *A* were therefore calculated using the equation:

$$n_\infty = [I_{Ktail}/I_{Ktail(max)}]^{1/2}, \quad (3)$$

where I_{Ktail} represents the magnitude of the current tail elicited at -60 mV. The data were fitted by curves calculated from a Boltzmann distribution,

$$n_\infty = \frac{1}{1 + \exp [(V_n - V)/k_n]}, \quad (4)$$

where V_n is the potential at which $n_\infty = 0.5$ and k_n is a slope factor. In control cells, $V_n = -2.5$ mV and $k_n = 12.5$ mV; for cells exposed to 1×10^{-5} M $LaCl_3$, $V_n = -5.0$ mV and $k_n = 12.0$ mV; and for cells exposed to 5×10^{-4} M $LaCl_3$, $V_n = +11$ mV and $k_n = 16.8$ mV. The differences in n_∞ values between the

controls and cells superfused with 1×10^{-5} M La^{3+} for 24–99 min were not statistically significant ($P > 0.05$); however, superfusion of cells with 5×10^{-4} M La^{3+} for 28–48 min reduced n_{∞} significantly at each potential. The finding that the $n_{\infty}(V)$ curve was shifted by ~ 13 mV toward more positive potentials, with only a small change in slope, suggests that negative external surface charge is intact in this isolated cell preparation, but that the surface potential was reduced markedly by 5×10^{-4} M La^{3+} . In contrast, the $n_{\infty}(V)$ curve was not

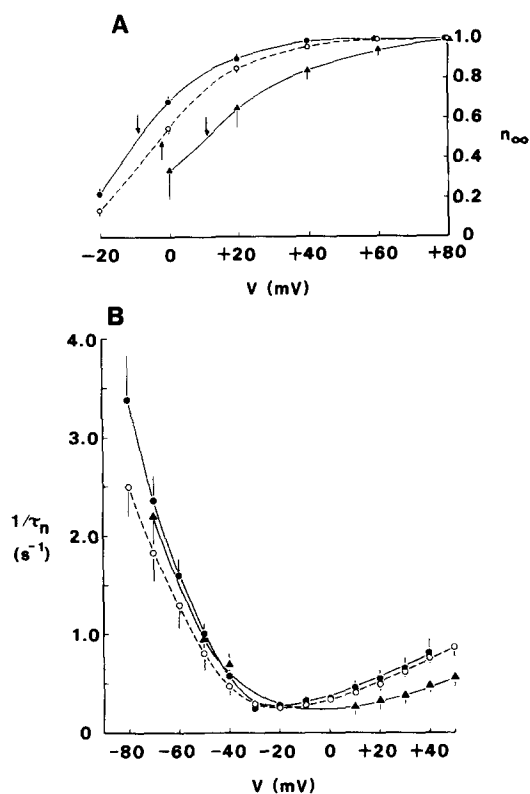


FIGURE 10. Effect of La^{3+} on voltage-dependent gating of I_K . (A) Normalized "quasi"-steady state activation curve for I_K . The amplitude of each tail was measured as the ΔI from its initial value immediately following a 10-s prepulse to its steady state value at -60 mV. Since activation was best fitted by a single-exponential function raised to the second power, individual values for n_{∞} were calculated from Eq. 3 (text). Each point represents the mean \pm SEM for 15–23 controls (\circ), 4 cells exposed to 1×10^{-5} M La^{3+} (\bullet), or 6–8 cells exposed to 5×10^{-4} M La^{3+} (\blacktriangle). The smooth curves were calculated from Eq. 4 (text). The potentials at which $n_{\infty} = 0.5$ (arrows) were: control, -2.5 mV; 1×10^{-5} M La^{3+} , -5.0 mV; 5×10^{-4} M La^{3+} , $+11$ mV. (B) Rate constants for activation (-30 to $+50$ mV) and decay (-80 to -40 mV) of I_K . Each point represents the mean \pm SEM for 4–11 controls (\circ), 3–5 cells exposed to 10^{-5} M La^{3+} (\bullet), or 2–5 cells exposed to 5×10^{-4} M La^{3+} (\blacktriangle). The smooth curves were fitted by eye to the mean values.

affected significantly by 1×10^{-5} M La^{3+} , which suggests that this concentration had no significant surface-charge effect.

These findings are supported by measurements of rate constants for activation and deactivation of I_K in the presence of La^{3+} (Fig. 10 B). In agreement with the results of Hume et al. (1986), the decay of I_K tails following a 5-s prepulse (Fig. 8, C and D) was best fitted by a single exponential, and the activation of I_K during 10-s pulses (Fig. 8, A and B) was best fitted by a single exponential

raised to the second power. Thus, the activation of I_K (-30 to $+50$ mV) was fitted by the equation (Hodgkin and Huxley, 1952b)

$$I_K = I_K(\infty)[1 - \exp(-t/\tau_n)]^2, \quad (5)$$

and deactivation (-80 to -40 mV) by

$$I_K = I_K(0)[\exp(-t/\tau_n)]^2, \quad (6)$$

where $1/\tau_n$ is the rate constant. The data illustrated in Fig. 10 B are mean values \pm SEM of rate constants determined by the Discrete curve-fitting program (Provencher, 1976) and Eqs. 5 and 6. When cells were exposed to 1×10^{-5} M La^{3+} for 24–149 min, no significant effect was observed; however, exposure to 5×10^{-4} M La^{3+} for 12–48 min significantly slowed the activation of I_K at $+30$ and $+40$ mV ($P < 0.02$ and $P < 0.01$, respectively) but not at more negative potentials. This type of asymmetric positive shift of a $1/\tau$ curve has been observed previously in cardiac muscle (Tsien, 1974a) and squid axon (Gilly and Armstrong, 1982).

DISCUSSION

La³⁺ as a Ca Channel Blocker and Ca Antagonist

The major finding of this study is that lanthanum ions block I_{Ca} selectively by binding to a specific site in or near the channel, rather than by neutralizing (screening) negative fixed charges in the vicinity of the channel. The following results support the hypothesis that LaCl_3 , when applied at concentrations of 10^{-7} – 10^{-5} M, blocks Ca channels selectively: (a) a TTX-resistant transient inward current carried predominantly by Ca^{2+} (Hume and Giles, 1983) was blocked by La^{3+} (Figs. 2–4) and (b) the apparent reversal potential ($+60$ mV) was not changed by doses of La^{3+} that blocked $>50\%$ of I_{Ca} (Fig. 4 A).

Our results also suggest that there is a competitive interaction between La^{3+} and Ca^{2+} for a binding site or sites in or near the Ca channel. (a) Mean values of I_{Ca} in the presence of LaCl_3 were best fitted by a dose-response curve calculated on the assumptions of 1:1 binding and a dissociation constant of 7.5×10^{-7} M. (b) Following complete block of I_{Ca} by 10^{-5} M LaCl_3 , partial recovery of I_{Ca} (up to 56% of control after 1 h) could be demonstrated by increasing extracellular Ca^{2+} from 2.5 to 7.5 mM. Such competition has been demonstrated previously, although indirectly, by Belardinelli et al. (1979), who showed that the depressant effects of 4×10^{-4} M $\text{La}(\text{NO}_3)_3$ upon “slow” action potentials in cardiac muscle could be reversed totally by increasing $[\text{Ca}^{2+}]_o$ from 0.5 to 2.5 mM. The original evidence for a binding site within the Ca channel was obtained in barnacle muscle (Hagiwara, 1975; Henkart and Hagiwara, 1976) and *Helix* neurons (Akaike et al., 1978). Dissociation constants for La^{3+} calculated on the assumption that the ion does not move through the channel are 0.33 and 1.15 mM for barnacle muscle and *Helix* neurons, respectively. These values are more than three orders of magnitude greater than the one (7.5×10^{-7} M) calculated from our results. However, it is important to note that both the snail neuron and barnacle muscle measurements were made in much higher extracellular Ca^{2+} concentrations, i.e., in artificial seawater.

Effect of La³⁺ on the Surface Charge of Atrial Myocytes

On the assumption that the negatively charged sites that give rise to surface-charge phenomena have a random and uniform distribution (Kass and Krafte, 1987), surface-charge neutralization by 10^{-5} M La^{3+} cannot account entirely for the observed blockade of I_{Ca} , since this concentration had no significant effect upon the steady state activation of I_{K} (Fig. 10 A). Moreover, a 10-fold-greater concentration of LaCl_3 produced no measurable shift of the h_{∞} curve of I_{Na} , although its slope was reduced slightly (Fig. 6). On the other hand, if the distribution of fixed charges is localized and discrete, as suggested by Brown (1974), Begeisich (1975), and Neumcke and Stämpfli (1984), one might expect the effect of La^{3+} on the Ca channel surface charge to differ from that observed for Na or K channels. Our results do not rule out this possibility. However, concentrations of La^{3+} that reduced I_{Ca} by 12–67% (1×10^{-7} – 1.5×10^{-6} M) had no significant effect upon its steady state inactivation-voltage relationship (Fig. 5).

The fact that La^{3+} failed to affect the activation of I_{K} or the inactivation of I_{Na} or I_{Ca} could be explained simply by a loss of negative surface charge during cell isolation. This possibility has been suggested previously when myocytes were isolated from adult rat hearts by exposure to collagenase and hyaluronidase (Isenberg and Klöckner, 1980) and when cultured cardiac myocytes were isolated with trypsin (Barry et al., 1978). However, if the surface-charge distribution does not differ markedly for each type of channel (see Table II in Gilbert and Ehrenstein, 1984), then the significant positive shift (13 mV) of the I_{K} activation curve recorded when cells were exposed to 5×10^{-4} La^{3+} (Fig. 10 A) would confirm that there is a substantial surface charge in close proximity to all ion channels in these isolated atrial myocytes. The histochemical results of Meyer et al. (1982a, b) show quite clearly that even after bullfrog atrial tissue has been enzymatically dispersed into single cells, La^{3+} still binds to the sarcolemma. Furthermore, their work provides indirect evidence that such La^{3+} binding displaces surface Ca^{2+} in isolated cells and that this effect appears to occur in a spatially uniform way. La^{3+} -induced shifts in voltage-dependent conductance parameters have been observed previously in cardiac Purkinje fibers (Tsien, 1974b; Kass and Tsien, 1975) and in both unmyelinated (Takata et al., 1966; Blaustein and Goldman, 1968) and myelinated axons (Vogel, 1974; Hille et al., 1975; Århem, 1980; Brismar, 1980; Neumcke and Stämpfli, 1984).

It is interesting that 5×10^{-4} M La^{3+} produced an asymmetric positive shift of the I_{K} rate constant vs. voltage curve (Fig. 10 B). Tsien (1974a) observed a similar asymmetric shift for the pacemaker current, $I_{\text{K}2}$, when calf Purkinje fibers were superfused with epinephrine. This asymmetry arose from a greater shift of the deactivation rate coefficient (β_s) than the activation rate coefficient (α_s). Tsien explained these differences on the basis of a discrete surface-charge modification that might produce a nonlinear change in the membrane potential profile (Brown, 1974). Assuming that channel opening and closing are controlled by movements of a charged particle across the membrane, he suggested that α_s and β_s would be affected differently by a nonuniform modification of

surface charge, because the "gating particle" might traverse a different electric field strength depending upon its direction of movement. Gilly and Armstrong (1982) have published further evidence showing that in squid axon, multivalent cations can alter the activation kinetics of a K^+ current by modifying α_n and β_n to different extents.

Direct Effect of La^{3+} on K Channels

The marked reduction of the isochronal I_K - V curve produced by 5×10^{-4} M La^{3+} (Fig. 9) cannot be explained entirely by surface-charge effects. The n_∞ curve for La^{3+} -treated cells approached that for the controls at potentials positive to +20 mV (Fig. 10A), and the reversal potential of I_K was unaffected (compare C and D of Fig. 8). Thus, the reduction of I_K by high doses of $LaCl_3$ was probably caused by a direct effect of La^{3+} on open K channels (for a similar effect on Na channels, see Neumcke and Stämpfli, 1984). However, since the I - V curves in Fig. 9 were measured at 10 s, i.e., not quite at steady state (see Fig. 8, A and B), the slowing of activation produced by La^{3+} (Fig. 10B) also could have accounted for some of the decrease in I_K . In mammalian Purkinje fibers, 5×10^{-4} M $LaCl_3$ reduced the amplitude of plateau current (I_X) tails (Kass and Tsien, 1975). Kass and Tsien attributed this effect to the neutralization of negative external surface charge by La^{3+} .

The decrease in I_{K1} in the presence of 1.0 mM La^{3+} (Fig. 7C) was not analyzed in detail, but similar effects have been observed previously. La^{3+} -induced increases in resting membrane resistance have been observed in guinea pig papillary muscle (Haass, 1975; Ravens, 1975) and bullfrog ventricular muscle (Hatae, 1982). On the other hand, Kass and Tsien (1975) reported that La^{3+} and other "Ca antagonists" increased the time-independent outward K^+ current. They recorded more time-independent current at plateau potentials when Purkinje fibers were treated with 5×10^{-4} M $LaCl_3$. Measurements of I_{K1} at plateau potentials were not done systematically in the present study; however, since La^{3+} -induced changes in outward current at the reversal potential of I_{Ca} were not statistically significant (Fig. 4A), such alterations of I_{K1} at plateau potentials must have been rather small. In agreement with our own results, Pressler et al. (1982) reported that La^{3+} (2×10^{-4} M) had no significant effect on the passive cable properties of isolated canine Purkinje fibers.

Another possible explanation for the reduction of the time-independent background current (Fig. 7C) is an effect of millimolar La^{3+} upon an electrogenic Na-Ca exchanger. Mentrard et al. (1984) have reported that in frog heart cells, 2 mM La^{3+} can block both the outward current produced when extracellular Na^+ is reduced threefold and the inward current produced when extracellular Na^+ is returned to normal. Similarly, Horackova and Vassort (1979) have shown that 3 mM La^{3+} can reduce the outward current and contracture of frog atrial muscle that are induced by application of Na-free (LiCl) Ringer's solution. Coraboeuf et al. (1981), however, were unable to reproduce the latter results (in dog Purkinje fibers), even at concentrations of La^{3+} up to 5 mM.

The Na-Ca exchange process appears to be electrogenic (Horackova and Vassort, 1979; Chapman and Tunstall, 1980; Mentrard et al., 1984), with a stoichi-

ometry of 3:1. Thus, in the steady state, the equilibrium potential for this exchange process is $E_{\text{ex}} = 3E_{\text{Na}} - 2E_{\text{Ca}}$ (Mullins, 1977). If the reversal potential for I_{Na} is +40 mV and the apparent reversal potential for I_{Ca} is +60 mV (Hume and Giles, 1983), then E_{ex} would be 0 mV. An electrogenic Na-Ca exchanger may therefore generate an inward current at potentials negative to -45 mV, and inhibition of it would be manifested by an outward shift of time-independent current at these potentials. Since we observed an inward shift at potentials between -100 and -80 mV (Fig. 7 C), it is unlikely that, under our experimental conditions, this effect of 1 mM La^{3+} was due to block of Na-Ca exchange current.

Ca²⁺ Activation of K Channels in Bullfrog Atrial Cells

Two important observations in the present study are that while 10^{-5} M La^{3+} blocked I_{Ca} completely, it had no significant effect on either I_{K1} , the time-independent current responsible for the resting potential, or I_{K} , a current that initiates repolarization of the action potential in bullfrog atrial cells (Hume and Giles, 1983; Hume et al., 1986). Since I_{K} was not reduced when I_{Ca} was blocked, Ca^{2+} influx (through Ca channels) during the plateau phase does not lead to activation of I_{K} and, hence, indirectly to repolarization of the action potential (see also Hume et al., 1986). Moreover, in bullfrog atrial cells, shortening of the action potential during rapid rates of stimulation (Carmeliet, 1977) is unlikely to result from Ca^{2+} -induced activation of either I_{K} or I_{K1} . Instead, this phenomenon is due to I_{Ca} "repriming" kinetics and the residual activation of I_{K} . The results of de Hemptinne (1971), Giles et al. (1980), and Noble and Shimoni (1981) on frog atrial trabeculae, and Miller and Mörchen (1978) on frog ventricular trabeculae, are consistent with this hypothesis. In contrast to these results, Urata and Goto (1982) have reported that changes in extracellular Ca^{2+} significantly modulate both I_{K1} and I_{K} in frog atrial trabeculae; however, it is possible that these changes were due to the surface-charge effects of Ca^{2+} removal.

Selectivity of La³⁺ as a Ca Channel Blocker

We have shown that La^{3+} at a concentration of 10^{-5} M blocks Ca channels completely but has little, if any, effect upon current flow through inwardly rectifying K channels (I_{K1}) or delayed rectifier K channels (I_{K}). Thus, at concentrations $\leq 10^{-5}$ M, La^{3+} is a specific and selective inhibitor of I_{Ca} . On the other hand, we also have demonstrated that concentrations of $\text{LaCl}_3 \geq 5 \times 10^{-4}$ M have large effects upon both I_{K1} and I_{K} , which indicates that La^{3+} , like other Ca antagonists, becomes nonselective at high concentrations. These data are of interest in the context of statements by Tsien (1983) and Reuter (1983), pointing out the lack of a specific inhibitor for Ca channels compared with the specificity of TTX for Na channels. Our results demonstrate that Ca channels in bullfrog atrial cells can be blocked selectively by La^{3+} . In multicellular cardiac preparations, much greater concentrations of La^{3+} are required to block I_{Ca} .

For example, ~ 0.5 mM was necessary in calf Purkinje fibers (Kass and Tsien, 1975) and pig ventricular trabeculae (Katzung et al., 1973). In addition to blocking the slow inward current (I_{si}), this concentration was found to affect I_{Na} , I_{K2} , I_X , and the net outward time-independent plateau current.

Kass (1982) has shown that nisoldipine is a rather selective blocker of I_{si} in cardiac Purkinje fibers. At concentrations near 10^{-5} M, nisoldipine completely blocked both I_{si} and phasic tension. In addition, nisoldipine reduced the transient outward current, which is activated by intracellular Ca^{2+} (Siegelbaum et al., 1977), but had no measurable effect upon the delayed rectifier (I_X). In contrast, Hume (1985) found that this concentration of nisoldipine reduced the maximum I_K tail by 50% in isolated frog atrial cells, whereas only 10^{-8} M was required to reduce I_{Ca} by 50%. These results suggest that there also might be a range of nisoldipine concentrations that could be used to block I_{Ca} quite selectively.

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