PARADOXICAL ELECTROMECHANICAL EFFECT OF LANTHANUM IONS IN CARDIAC MUSCLE CELLS

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ABSTRACT Although lanthanum ions (La^{+++}) block calcium influx in cardiac cells, they may paradoxically accentuate the sodium-free contracture. We have therefore studied the effects of La^{+++} on the zero sodium response in chick embryonic myocardial cell aggregates. Zero sodium alone causes: (*a*) A maintained contracture; (*b*) Asynchronous localized contractions that are selectively inhibited by caffeine or ryanodine, and presumably reflect release of calcium from the sarcoplasmic reticulum; (*c*) A nonspecific conductance increase that is ascribable to calcium-activated ion channels. Addition of La^{+++} potentiates the sodium-free contracture, and causes similar potentiation of the localized contractions and the conductance increase. All three phenomena occur 5–10-fold faster in 1 mM La^{+++} than in sodium-free fluid alone. In contrast, when La^{+++} is combined with caffeine or ryanodine, the zero sodium response is suppressed. We conclude that the paradoxical effect of La^{+++} on the contracture is not due to calcium influx, but to enhancement, or disinhibition of intracellular calcium release. Relaxation of normal myocardium may involve control of spontaneous calcium release by lanthanum- and sodium-sensitive calcium transport across the surface membrane.

INTRODUCTION

Lanthanum ions (La^{+++}) inhibit the entry of calcium into electrically excitable cells, and thereby abolish a variety of calcium-mediated responses, including synaptic transmission in nerve (1), and contraction in both cardiac (2, 3) and smooth muscle (4). In cardiac muscle, La^{+++} blocks the voltage-sensitive calcium channels, whose activation is necessary for physiologic contractions to occur (5). Furthermore, La^{+++} can block the sodium/calcium exchanger, which mediates calcium entry in sodium-free fluid (6, 7). It is therefore astonishing that, in several published reports, La^{+++} not only fails to inhibit contractures of cardiac muscle in sodium-free fluid, but paradoxically accentuates them (8–10).

Two explanations are commonly given for this paradox. First, it is possible that the effects of La^{+++} on calcium influx are variable, and that in some cases, La^{+++} stimulates calcium influx. Such stimulation has been reported in hepatocytes (11), and isolated cardiac sarcolemmal vesicles (12). A second possibility is that La^{+++} stimulates release of sequestered intracellular calcium.

A number of experimental methods have been developed for deliberately altering intracellular calcium release, and for inferring such alteration from physiological recordings. In digitalis toxicity, spontaneous intracellular calcium release causes a characteristic inward current that is due, at least in part, to calcium-activated inward current channels (13–15). This mechanism also accounts for the membrane conductance increase that occurs when intracellular calcium is increased by removal of external sodium (16–17). Another manifestation of intracellular calcium release are the disorganized local contractions that occur in calcium overload or, to a lesser degree, in normal unstimulated myocardium (18–22). These localized contractions produce fluctuations in resting tension, or light scattering, which can be correlated with similar fluctuations in aequorin luminescence (21–22). All of these fluctuations are accentuated in low external sodium and are abolished by caffeine or ryanodine, which inhibit calcium transport by the sarcoplasmic reticulum (21–22). For these reasons the localized contractions can be taken as indicative of spontaneous intracellular calcium release.

In the present study, we have recorded the contractile response and the accompanying membrane conductance increase in chick embryonic myocardial cell aggregates exposed to sodium-free fluid in the presence and absence of La^{+++}. We find that La^{+++} dramatically accentuates the mechanical response and the conductance increase, even though it prevents calcium entry under similar conditions (7). This paradoxical effect is prevented by caffeine or ryanodine, which abolish the localized contractions in low sodium, and convert the action of La^{+++} from excitatory to inhibitory. We conclude that, in sodium-free fluid, La^{+++} either directly stimulates the sarcoplasmic reticulum to release calcium, or (more likely) that it abrogates an inhibitory effect of the surface membrane on spontaneous calcium release.

METHODS

Chick embryonic ventricular cells were obtained by trypsinization of 9–12 day ventricles as described previously (23). Cells cultured on a hydro-

phobic surface formed spontaneously beating spheroidal aggregates 60–150 μm in diameter. After 3–10 d in culture, cell aggregates were plated onto glass slides, and immersed in physiological saline on the stage of an inverted microscope. The composition of the saline was 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.5 mM dextrose, and 6.0 mM HEPES at pH 7.4 and 37 ± 0.2°C. Sodium-free solutions were prepared by substitution of 146 mM lithium chloride for NaCl and NaH_2PO_4. Ph was buffered with 3mM HEPES, which was neutralized with Tris-base, to give a truly sodium-free mixture. In some experiments, the lithium solution was neutralized with a combination of KOH (which replaced KCl) plus small amounts (<5 mM) of NaOH. Electrical activity was recorded either in current or voltage clamp mode using a Dagan 8100 single-electrode voltage clamp at a sampling frequency of 500 Hz. Adequacy of membrane potential control has been verified previously by independent intracellular electrodes (19). Mechanical activity was recorded by a photodiode placed in the image plane of an eyepiece lens, whose output during synchronized contractions was proportional to the mean displacement of a 30-μm segment of the aggregate's edge (23). Movements confined to smaller regions of the aggregate could also produce light intensity fluctuations (19–20). Aggregates were superfused by a closely apposed polyethelene cannula (280 μm inner diameter), which was connected to a syringe pump. Activation of the pump at a flow rate of 60 μl/min. completely exchanged the surrounding fluid in <1 s. Data were recorded by a Gould-Brush strip chart recorder (Gould, Inc., Cleveland, OH). Membrane currents were normalized to membrane area, which was estimated as $k \pi ab^2$, where a and b are the major and minor hemiaxes of the aggregate (in μm) and k is 4.4×10^{-9} $cm^2/\mu m^3$, a constant derived from the histologic measurements of Nathan and DeHaan (24). Ryanodine was obtained from the Penick Corp., Lyndhurst, NJ.

RESULTS

Paradoxical Mechanical Effects of Lanthanum

The mechanical response of a myocardial cell aggregate to zero sodium is illustrated in Fig. 1 *A*. Abrupt superfusion of a cell aggregate with sodium-free lithium solution (first arrow) produces a sustained contracture, which develops in 20–40 s, and relaxes when external sodium is restored (second arrow). The sustained contracture is accompanied by—and may be comprised of—smaller transient contractions involving limited regions of the aggregate, with no apparent synchrony between them. These localized contractions produce low amplitude fluctuations in the optical signal, which appear and disappear with roughly the same time course as the sustained contracture (Fig. 1 *A* and *B*).

The localized contractions in myocardial cell aggregates have the same principal frequency (about 3/s) as those in native cardiac fibers (18, 22), and may therefore involve the same mechanism. To confirm this, the effects of sodium removal were studied after pretreatment with caffeine and ryanodine, which inhibit calcium transport by the sarcoplasmic reticulum (21–22), and abolish the localized contractions in native cardiac fibers. As seen in Fig. 2 *A*, caffeine (5 mM) and ryanodine (3 μM) abolish the fluctuations in the optical signal without altering the time course of the sustained contracture. When the drug-treated aggregate is observed directly, sodium removal produces a smoothly developing contracture, without localized or asynchronous cell movements. These results have been obtained in five aggregates treated with both caffeine and ryanodine, as well as in aggregates exposed to these agents separately.

If the sodium-free contracture is dependent upon calcium influx, then it ought to be suppressed by La^{+++}. This is particularly true in tissue cultured chick embryonic cardiac cells, where 1 mM La^{+++} has been shown to reduce $^{45}Ca^{++}$ uptake in low sodium by >90% (7). However, we find that 1 mM La^{+++} paradoxically accentuates the mechanical response. In Fig. 2 *B*, the sodium-free contracture and the spontaneous light-intensity fluctuations both become maximal within 4 s, which is 5–10-fold faster than in the absence of La^{+++}. Similar results have

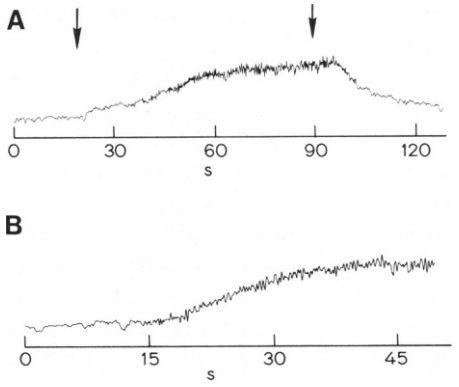

FIGURE 1 Optical recordings of mechanical activity induced by exposure of heart cell aggregates to low sodium. (*A*) Sodium-free lithium solution is applied between the arrows. Sodium removal induces a sustained upward deflection of the optical signal, indicating sustained contracture. There are spontaneous fluctuations (~3/s) due to localized asynchronous mechanical activity. (*B*) Sodium removal is repeated in the same aggregate, and displayed at faster sweep speed. The aggregate is quiescent at the time of sodium removal. However, earlier spontaneous beating had produced deflections about half as large as the contractures.

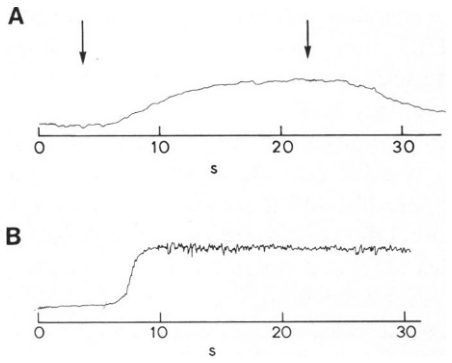

FIGURE 2 Modification of the sodium-free contracture by calcium-transport blockers. (*A*) The cell aggregate is exposed to caffeine (5 mM) plus ryanodine (3 μM) during, and for 60 s prior to sodium removal. The sustained contracture in zero sodium (arrows) develops and decays with the same time course as in Fig. 1, but the optical fluctuations, indicative of localized contractions, are no longer present. (*B*) A different aggregate is exposed to 1 mM La^{+++} during, and for 30 s prior to zero sodium. The contracture and spontaneous fluctuations both develop about 10-fold faster in La^{+++} than in zero sodium alone.

been obtained in seven La^{+++}-treated aggregates studied with the photodiode, and in five studied under direct vision. In the latter group, the mechanical response was exceptionally vigorous compared to that observed in zero sodium alone.

Paradoxical Electrical Effect of Lanthanum

To characterize the La^{+++} effect further, we have studied accompanying changes in transmembrane current and conductance. These observations are pertinent because cardiac cells contain a class of cation channels that open whenever calcium ions are present at the inner surface of the cell membrane (15). Besides mediating phasic inward currents (13, 14, 19), these channels are probably responsible for the sustained conductance increase that occurs in sodium-free solutions (16, 17). The channels are considered non-specific because their reversal potential is near zero, and remains significantly positive to E_K when sodium is replaced by other cations (estimates range from -13 to -60 mV; ref. 14–17).

The effects of sodium removal on electrical activity are illustrated in Fig. 3 *A*, in which the cell aggregate is stimulated by hyperpolarizing current pulses every 500 ms.

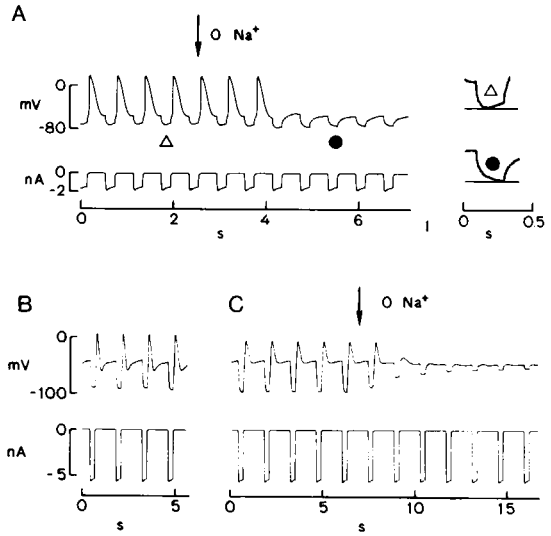

FIGURE 3 Effects of sodium removal on membrane conductance in the presence (*C*), and absence (*A*) of La^{+++}. Top traces are membrane potential and bottom traces membrane current. In each panel, the aggregate is stimulated by hyperpolarizing current pulses, with anode break action potentials occurring between pulses. In *A* sodium removal causes hyperpolarization and cessation of action potentials with little change in membrane conductance. The positive-going pacemaker potential (open triangles) is abolished within 2 s, leaving a passive membrane response (filled circles). Abolition of the pacemaker potential presumably reflects mediation of the pacemaker current by sodium, since there is too little time for exchange of intracellular ions (e.g. potassium loss; ref. 25). The bottom panels are obtained from another aggregate before (*B*) and 45 s after (*C*) exposure to 1 mM La^{+++}. The superfusate is then switched to a low-sodium La^{+++} solution (arrow). While La^{+++} alone decreases membrane slope conductance by 17%, sodium removal in La^{+++} causes a rapid conductance increase. There is little associated change in resting potential.

Sodium removal (arrow) abolishes the pacemaker potential (enlarged traces), and the anode-break action potentials within 2–3 s. Abolition of pacemaking in zero sodium also occurs in mammalian Purkinje fibers (25), where sodium reputedly serves as the charge carrier of the pacemaker current, I_f (26). However, sodium removal in Fig. 3 *A* has little immediate effect on membrane conductance. In contrast, a rapid and drastic conductance increase occurs when sodium is removed in 1 mM La^{+++}. While La^{+++} alone produces a conductance decrease in Fig. 3 *C*, abrupt sodium removal (arrow) then induces a 10-fold conductance increase within 7 s. This conductance increase has little effect on the resting potential (-40 mV), which suggests that the equilibrium potential of the conductance does not differ greatly from this value.

The conductance increase in zero sodium can be measured more precisely under voltage clamp. We have previously shown that, in aggregates clamped in the pacemaker range, replacement of external sodium by Tris or lithium produces an initial outward current (due to interruption of background sodium influx), followed by a slowly developing inward current (17, 19). The inward current is ascribable to calcium-activated nonspecific channels for several reasons: (*a*) It is associated with a conductance increase. (*b*) It reverses at moderate negative potentials. (*c*) It develops with roughly the same time course as the sodium-free contracture. (*d*) It is abolished by 25 mM manganese, which blocks sodium/calcium exchange in heart, and prevents the contracture (17, 27, 28).

The effects of La^{+++} on this current are shown in Fig. 4. In the absence of La^{+++} (Fig. 4 *A* and 5 *A*), the inward current develops exponentially, with a mean time constant of 14.5 ± 5.4 s ($n = 5$). Membrane conductance, measured by brief hyperpolarizing voltage steps, increases ~50% during onset of the current. The inward current and the conductance increase are both accelerated by La^{+++}. In Fig. 4 *B*, the cell aggregate is cycled between -66, -72, and -75 mV every 1,500 ms and superfused, first with La^{+++} alone, and then with sodium-free La^{+++} solution. La^{+++} alone decreases membrane conductance by 30% (not shown). Subsequent removal of sodium (arrow) causes a fourfold conductance increase in the first second, along with an unusually rapid inward current shift. The final conductance increase is also much greater in La^{+++} than in zero sodium alone. Rapid development of inward current in La^{+++} abbreviates the initial outward current markedly, and in some records (e.g. Fig. 4 *B*) outward current is absent. However, the inward current still approaches the steady state exponentially (Fig. 5 *B*) with a significantly shortened time constant of 3.3 ± 1.6 s ($P < 0.001$, $n = 5$).

Abolition of the Paradoxical Effects by Caffeine and Ryanodine

A possible explanation for the paradoxical electrical and mechanical effects of La^{+++} is that, in the presence of

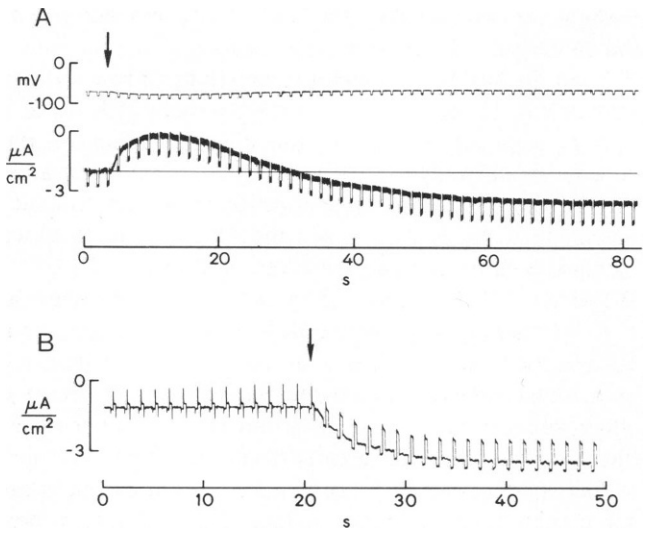

FIGURE 4 Effects of sodium removal and La^{+++} on membrane current. In *A*, the aggregate is clamped at a holding potential of −70 mV, and stepped to −82 mV for 300 ms, every 1.5 s. Abrupt exposure to sodium-free lithium solution (arrow) produces an initial outward current followed by a sustained inward current. The outward current is suppressed by cesium (ref. 17), and may be due to interruption of sodium influx through "I_f" channels. The late inward current is due to a nonspecific conductance increase, thought to be activated by calcium influx. *B* shows a similar experiment in an aggregate pre-treated with 1 mM La^{+++} and cycled between −66, −72, and −75 mV. The conductance increase is much larger, and develops more rapidly, so that no initial outward current is seen. The degree of isopotentiality in La^{+++}-treated aggregates has not been directly measured, but there is no obvious escape from the clamp after hyperpolarization. Aggregate hemiaxes are 65 μm × 50 μm in *A* and 50 μm × 50 μm in *B*.

reduced external sodium, La^{+++} somehow stimulates intracellular calcium release. If this proposal is correct, then the paradoxical effects would be prevented by drugs that inhibit the function of the sarcoplasmic reticulum. Furthermore, if La^{+++} truly blocks calcium influx, then inhibition of the sarcoplasmic reticulum should convert the effect of La^{+++} on the sodium-free contracture from excitatory to inhibitory.

Fig. 6 shows the effects of sodium removal and La^{+++} in a cell aggregate that had been pretreated with caffeine plus ryanodine for 30 min. Sodium removal (Fig. 6 *A*) evokes a smooth, sustained contracture. A similar response had occurred in the same aggregate during five prior sodium-free superfusions. In Fig. 6 *B*, 2 mM La^{+++} has been added for 1 min, followed by a sixth sodium-free superfusion. In contrast to the usual effect of La^{+++}, sodium removal now elicits no mechanical response. Similar results have been obtained in aggregates pretreated with caffeine or ryanodine alone. The effects of ryanodine have also been studied in three aggregates under voltage clamp. In these experiments, the combination of La^{+++} plus ryanodine suppresses the inward current normally produced by sodium removal. This result is similar to that of Sutko and Kenyon (29) who found that treatment of calcium overloaded cardiac fibers with ryanodine abolished the after-contractions and inward currents produced by intracellular calcium release.

DISCUSSION

The experiments reported here show that La^{+++} increases both the speed and magnitude of the contractile response and membrane conductance change in embryonic cardiac cells exposed to zero external sodium. Four observations indicate that this effect is due to stimulation of intracellular calcium release. First, La^{+++} potentiates localized contractions which are known to be indicative of sponta-

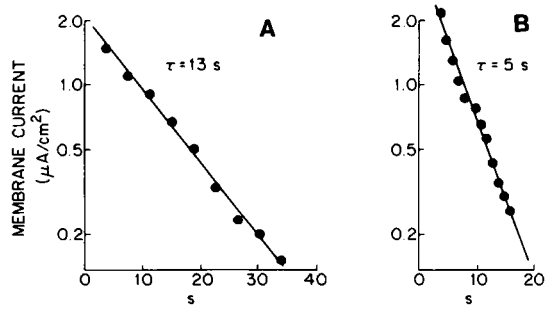

FIGURE 5 Development of the late inward current in the presence (*B*) and absence (*A*) of La^{+++} has been plotted semilogarithmically, for the experiments in Fig. 4. Each plot shows current at the predominant holding potential (−70 mV in *A* and −72 mV in *B*). Similar plots for the other potentials in each experiment are parallel to the ones shown. This indicates that the inward current is due to a single, exponentially developing conductance, whose onset is faster in La^{+++}.

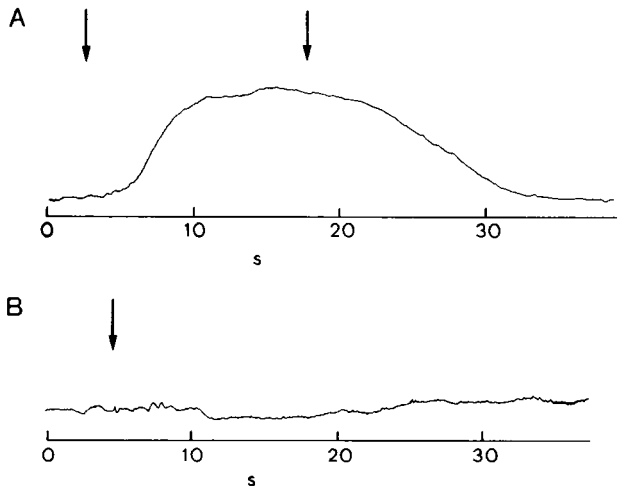

FIGURE 6 Lanthanum abolishes the sodium-free contracture when the sarcoplasmic reticulum is pharmacologically inhibited. In *A*, the cell aggregate has been exposed to caffeine (5 mM) and ryanodine (3 μM) for 30 min, and then superfused with sodium-free lithium solution for the period indicated by arrows. A smooth contracture develops, comparable to that observed during five sodium-free superfusions in the preceding 30 min. In *B*, the same aggregate is exposed to 2 mM La^{+++} for 60 s, and then re-exposed to sodium-free lithium solution (arrow). In contrast to its usual effect, La^{+++} now abolishes the mechanical response.

neous calcium release (18–22). Second, La^{+++} simultaneously potentiates an inward current that is known to be triggered by intracellular calcium release (13–17). Third, La^{+++} does not potentiate the zero sodium response in caffeine or ryanodine. Fourth, since La^{+++} actually blocks the effects of sodium removal in caffeine or ryanodine, it must also block calcium influx. Suppression of calcium influx by La^{+++} has been reported in monolayers of embryonic cardiac cells exposed to low sodium and $^{45}Ca^{++}$ (7). However, this result is not automatically relevant to cell aggregates, since diffusion of La^{+++} is more limited in multicellular preparations (30), and since low concentrations of La^{+++} can have variable effects on calcium influx (11, 12).

Does Lanthanum Directly Stimulate Calcium Release?

How the effects of La^{+++} are explained depends on how the various calcium transport mechanisms are understood to function. The traditional view is that the sarcoplasmic reticulum sequesters and retains calcium during diastole, and releases it in response to a specific physiological trigger. Our results are most extraordinary in this context, since we obtain vigorous calcium-dependent responses under conditions in which both of the putative triggers—membrane depolarization and calcium influx—have been deliberately blocked.

It is possible that La^{+++} itself can substitute for the physiological trigger. Since release of calcium from the SR can be stimulated by calcium (31), one could envision La^{+++} entering the cells in zero sodium, and directly activating calcium release. There are two problems with this explanation. First, lanthanide ions can activate the contractile filaments of muscle cells whose membranes have been destroyed (3, 32). La^{+++} entering an intact cell should therefore produce a contraction that would not be abolished when the sarcoplasmic reticulum is inhibited by drugs. Second, when the distribution of lanthanide ions is studied by electron microscopy, they appear to be restricted to the extracellular space (6, 33, 34). While it is possible that the distribution of La^{+++} is different in sodium-free fluid, there is currently no evidence for this.

Does Lanthanum Dis-inhibit Spontaneous Calcium Release?

An alternative explanation for the La^{+++} effect is to suppose that calcium release is a spontaneous process that requires no trigger, but occurs automatically unless it is suppressed by a mechanism that La^{+++} can block. Our findings are most consistent with this interpretation. As noted above, there is strong evidence that intracellular calcium can trigger its own release from the SR (31). Calcium also appears to be released spontaneously in unstimulated resting cardiac fibers (18). Taken together, these findings suggest that calcium release should be explosively regenerative unless local release is contained by other calcium transport mechanisms.

Two carriers in the sarcolemma are considered important in the maintenance of low cytoplasmic calcium. First, cardiac cells contain an ATP-driven calcium pump similar to that found in erythrocytes (see review by Schatzmann, 35). Second, when cytoplasmic calcium is high, the sodium-calcium exchange can mediate net calcium efflux. Sodium-dependent calcium efflux is fairly insensitive to extracellular La^{+++} (7, 35), presumably because the La^{+++} cannot displace intracellular calcium from its binding site on the carrier. In contrast, the ATP-driven pump is exquisitely sensitive to extracellular La^{+++} (35), and would be totally inhibited at the concentrations used in our experiments. The study of calcium efflux is further complicated by the fact that the two transport mechanisms are redundant. Barry and Smith found that sodium removal alone had no effect on total $^{45}Ca^{++}$ efflux in embryonic cardiac cells, while La^{+++} alone reduced efflux by only 50% (7). For reasons noted above, calcium efflux should decline more dramatically when La^{+++} and sodium-free fluid are applied together.

We propose that combined exposure of cardiac cells to La^{+++} and low sodium abolishes the ability of the sarcolemma to regulate intracellular calcium, and that this leads to explosive intracellular calcium release. The large contracture and conductance increase need not be mediated by La^{+++} itself, but could be produced by the normal intracellular calcium stores, once the restraining influence of the surface membrane on calcium release is withdrawn.

It is possible that La^{+++} has comparable effects on intracellular calcium regulation in other tissues, and that the combination of La^{+++} and low sodium could be used to obtain novel information about the response of calcium-dependent cellular processes to high calcium levels. For example, the conductance increase produced by La^{+++} and low sodium in Fig. 4 *B* is much larger than the total membrane conductance prior to these treatments. Since conductance during the action potential plateau is similar to resting conductance, we infer that most of the calcium-activated channels in the membrane (15) are not utilized during normal action potentials.

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