Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4417–4421, May 1992
Physiology

Modulation of L-type calcium channels by sodium ions

C. WILLIAM BALKE*†‡ AND W. GIL WIER†

Departments of *Medicine and †Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Communicated by V. A. McKusick, January 29, 1992

ABSTRACT **It is universally believed that the removal of external sodium ions is without effect on calcium current. We now report that in enzymatically isolated guinea pig ventricular cells, the replacement of external sodium ions with certain other cations causes a 3- to 6-fold increase in peak L-type calcium current. The increase in current is reversibly blocked by L-type calcium-channel antagonists, not mediated by changes in internal calcium, and is inhibited by intracellular 5′-adenylyl imidodiphosphate, a nonhydrolyzable ATP analogue. The effects of sodium removal (and isoproterenol) were almost completely blocked by intracellular application of a specific (peptide) inhibitor of cAMP-dependent protein kinase. These experiments demonstrate a previously unknown effect of sodium ions to modulate calcium-channel phosphorylation via cAMP-dependent protein kinase.**

L-type Ca^{2+} channels (1) are known to be many times more permeable to Ca^{2+} than to Na^+. In addition, it has been reported that the removal of external Na^+ is entirely without effect on Ca^{2+}-channel currents (1–4). In a recent study (5), however, of excitation-contraction coupling in isolated guinea pig ventricular cells, we incidentally observed that L-type Ca^{2+} current gradually increased after removal of external Na^+. We now demonstrate that the mechanism of this increase may involve Ca^{2+}-channel phosphorylation.

MATERIALS AND METHODS

Cell Isolation. Guinea pig ventricular cells were isolated by an enzymatic dispersion technique previously described in detail (6). Briefly, adult guinea pigs (body weight, 200–300 g) of either sex were heparinized (10 units of heparin per g of body weight, administered i.p.) and anesthetized with sodium pentobarbital (17 mg/100 g of body weight, administered i.p.). While still beating, the heart was excised and rinsed in an oxygenated physiological salt solution (PSS; see below) that was nominally free of Ca^{2+}. The ascending aorta was cannulated to the base of a Langendorff column (height, 1 m) for retrograde perfusion (37°C) of the coronary arteries, first with a nominally Ca^{2+}-free PSS for 6–8 min and next with PSS containing collagenase (Sigma collagenase type I, 4–6 mg/ml) and protease (Sigma protease type XIV, 0.7 mg/ml). The ventricles were then removed, minced, and gently triturated with a large-bore Pasteur pipette in the enzyme solution for an additional 6 min. The solution was filtered through a 200-μm nylon mesh and was centrifuged at low speed (g = 19.70) for 3 min. The pellet was suspended in PSS containing 200 μM Ca^{2+}. After two additional 3-min washes in the same buffer, the cells were stored at room temperature (21–23°C) in PSS containing 1.0 mM Ca^{2+} and used within 6–8 hr of isolation. This procedure produced an average yield of 20–30% of Ca^{2+}-tolerant ventricular myocytes. Only cells that appeared rod-shaped, relaxed, and with visible striations were chosen for experiments.

Control and Test Solutions. The PSS used for cell isolation was composed of 135 mM NaCl, 10 mM dextrose, 10 mM Hepes (Na^+ salt), 4 mM KCl, 0.33 mM NaH_2PO_4, and 1 mM $MgCl_2$ (pH adjusted to 7.3 with NaOH). $CaCl_2$ was added at concentrations appropriate to the stages of cell isolation (see above). The Na^+-containing solution used for control recordings was PSS modified by replacement of 4 mM KCl with 10 mM $CsCl_2$ and by the addition of 1 mM $CaCl_2$. The Na^+-free solution was composed of 140 mM tetraethylammonium chloride (Et_4NCl) obtained by mixing equimolar amounts of tetraethylammonium hydroxide (Et_4NOH) and HCl (both from Sigma), 10 mM dextrose, 10 mM Hepes (Et_4N^+), 10 mM $CsCl_2$, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ (pH adjusted to 7.3 with Et_4NOH). The control pipette solution was Na^+-free and was composed of 120 mM $CsCl_2$, 10 mM EGTA, 1 mM $MgCl_2$, and 4 mM Mg_2ATP (pH adjusted with CsOH to 7.2). Divalent cations ($CaCl_2$ and $BaCl_2$) were added to the Et_4N^+ solution without osmotic compensation. Tetrodotoxin (15–30 μM) was also present in selected experiments. In phosphorylation inhibition experiments (see Fig. 1*f*), 5′-adenylyl imidodiphosphate (AMP-*P*[NH]*P*; Sigma) was dissolved in deionized water and replaced 5 mM Mg_2ATP in the control pipette solution. The protein kinase inhibitor (PKI; 10 μM; synthetic rabbit sequence; Sigma) was included in the pipette solution and allowed to diffuse through the cell for at least 25 min after breaking into the cell and before beginning the experiment. Stock solutions were made of 1 mM nitrendipine in ethanol (made daily and stored in the dark) and 1 mM isoproterenol in Na^+-free PSS.

The volume capacity of the cell bath was 3 ml. At a flow rate of 3–4 ml/min, the exchange of the bath solution was complete within 60 s as evidenced by the rapid disappearance of the inward Na^+ current. In experiments with ion substitutions or drug applications, both control and test solutions were infused at equal flow rates (3–4 ml/min).

Whole-Cell Voltage Clamp. Whole-cell currents were recorded by the method of Hamill *et al.* (7). Cells were placed in a lucite bath on the stage of an inverted Nikon Diaphot microscope and superfused at room temperature (21–23°C) with 2.0 mM Ca^{2+}-containing Tyrode's solution. Fire-polished pipette electrodes were prepared from filament-containing capillary tubes (World Precision Instruments, New Haven, CT) by a horizontal pipette puller (BB-CH, Geneva). The filled pipettes had resistances of 0.5–2.0 MΩ. Prior to cell attachment, the electrode potential was adjusted to a zero current baseline. After formation of a gigaseal, whole-cell voltage clamp was accomplished by rupture of the cell membrane patch by a gentle suction transient. After the membrane currents had stabilized for 5 min, the external solution was changed to the Na^+-free solution in which NaCl was replaced by equimolar Et_4NCl. Membrane currents were measured with a single-electrode continuous-voltage clamp (Axopatch 1C; Axon Instruments, Burlingame, CA). Experimental protocols, data acquisition, and storage were accom-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PKI, protein kinase inhibitor; Et_4N^+, tetraethylammonium ion; AMP-*P*[NH]*P*, 5′-adenylyl imidodiphosphate; NMG, *N*-methyl-D-glucamine; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

‡To whom reprint requests should be addressed.

plished with a custom modified software package (BASICFASTLAB, Indec Systems, Sunnyvale, CA) running on an IBM-AT computer. Current was digitized at 2 kHz with 12-bit resolution (IDA laboratory interface; Indec Systems) and stored on a hard disc for off-line analysis.

L-type Ca^{2+} current was isolated by using a voltage protocol and ionic conditions that minimized the possibilities of either increased inward currents or decreased outward currents from other membrane channels or ion transporters. From a holding potential of −90 mV, peak L-type Ca^{2+} current was evoked after 2 s at a prepulse potential of −50 mV with a 200-ms depolarization to 10 mV every 10 s. Inward Na^+ current was eliminated by a prepulse positive to the potential at which Na^+ channels are inactivated and by equimolar substitution of Na^+ with several different cations. In selected experiments, 0.03 mM tetrodotoxin was added to the external solution to block Na^+ channels. T-type Ca^{2+} channels were also inactivated by the prepulse. K^+ channels were blocked by internal and external Cs^+. Inward current through Ca^{2+}-activated K^+ channels and nonspecific Ca^{2+}-activated channels was largely eliminated by buffering intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) with 10 mM EGTA. Na^+/Ca^{2+} exchange was eliminated by the absence of external and internal Na^+.

Cell capacitance was determined by integrating the current elicited by small hyperpolarizing voltage pulses. The average cell capacitance was 159 ± 51 pF (n = 12). The parallel combination of cell and seal resistance was 2.4–10.2 MΩ. Typically, 80–90% compensation of series resistance could be achieved.

Since the time constant of the capacitive transient was far shorter (average, <0.5 ms) than the time-to-peak L-type current, capacity compensation was rarely necessary and was not routinely done.

RESULTS

Peak L-Type Ca^{2+} Currents in the Presence and Absence of External Na^+. In control experiments (Fig. 1*a*) in solutions containing physiological concentrations of extracellular Na^+ (140 mM), current carried through L-type Ca^{2+} channels declined gradually over the course of 60 min (8). In contrast, after 15–30 min with equimolar replacement of external Na^+ by either organic or inorganic cations including Et_4N^+ (n = 24; Fig. 1 *c* and *e*), *N*-methyl-D-glucamine (NMG, n = 7; not shown), and Cs^+ (n = 11; Fig. 1*b*), peak inward current was increased 3–6 times above the control peak L-type Ca^{2+} current in Na^+-containing solutions. The current always increased gradually, but the time at which the current began to increase after the removal of Na^+ was variable. For example, the increase began after a delay of about 15 min in the experiment of Fig. 1*b*, but it began after much less delay in the experiments of Fig. 1 *c* and *e*. The effect of Na^+ withdrawal on peak L-type Ca^{2+} current was highly reproducible (with Et_4N^+ substitution, n = 24; mean increase in peak L-type Ca^{2+} current ± SD = 361 ± 90% of control, P < 0.0001) and reversible with reexposure to Na^+-containing solutions (Fig. 1 *b* and *c*).

Involvement of Ca^{2+} Channels and Independence from Free Ca^{2+}. The increase in peak inward current was rapidly and reversibly blocked by 0.1 mM Cd^{2+} (Fig. 1*b*) and 10 μM nitrendipine (not shown). Because the increased inward current was blocked by specific L-type Ca^{2+} channel blockers, the removal of external Na^+ is directly or indirectly affecting L-type Ca^{2+} channels. Several plausible hypotheses on the mechanism of this effect involve changes in $[Ca^{2+}]_i$. When Na^+ is present internally and absent externally, undetectable increases in $[Ca^{2+}]_i$ (9) might occur even in the presence of intracellular EGTA in spatially localized unbuffered regions around Ca^{2+}-channel pores as a result of the exchange of internal Na^+ for external Ca^{2+} by the Na^+/Ca^{2+} exchanger (1). For example, when Na^+ is replaced by Cs^+ (Fig. 1*b*), the "tails" of inward current seen with repolarization may represent inward Cs^+ currents through K^+ channels activated by spatially localized increases in $[Ca^{2+}]_i$. These elevations of $[Ca^{2+}]_i$ could increase L-type Ca^{2+} current by activation of Ca^{2+}-dependent membrane-bound enzymes such as adenylate cyclase (10) and phospholipase C (11). It has been reported that elevations in $[Ca^{2+}]_i$ can increase L-type Ca^{2+} current (12–14). However, all of the hypotheses based on a role of Ca^{2+} in the effect of Na^+ removal are effectively eliminated by the observation of the typical increase in current through the L-type Ca^{2+} channel following Na^+ replacement with Et_4N^+ even when Ba^{2+} replaced Ca^{2+} as the charge carrier (Fig. 1*c*). Therefore, the increase in L-type Ca^{2+} current that occurs with Na^+ replacement is not dependent on Ca^{2+} as the charge carrier or on changes in $[Ca^{2+}]_i$.

Effects of Different Molecular Species Used to Replace External Na^+. The possibility that the increase in L-type Ca^{2+} current was a direct consequence of the chemical properties of a particular Na^+ substitute was investigated by replacing external Na^+ with Cs^+, Et_4N^+, NMG, and Li^+. These Na^+ substitutes are chemically distinct from each other and Cs^+, Et_4N^+, and NMG are unable to substitute for Na^+ in physiologic processes. In spite of slight differences in the time of onset and the extent to which other aspects of L-type Ca^{2+} current were affected, equimolar substitution of Na^+ with Cs^+ (Fig. 1*b*), Et_4N^+ (Fig. 1 *c* and *e*), or NMG (not shown) caused a substantial increase in peak L-type Ca^{2+} current. However, an increase in peak L-type Ca^{2+} current was not observed when Na^+ was replaced with Li^+ (Fig. 1*d*) which, as noted above, differs from Cs^+, Et_4N^+, and NMG in its ability to substitute for Na^+ in a number of cellular processes, including permeation of the Na^+ channel (15), Na^+/Ca^{2+} exchange (1), and Na^+/H^+ exchange (16).

Involvement of Adenylate Cyclase. One possible hypothesis for the increase in L-type Ca^{2+} current caused by Na^+ removal would be that known intracellular regulatory processes are involved such as the control of Ca^{2+} channels by adenylate cyclase via cAMP (3, 4, 17, 18). This hypothesis was tested in three ways as described below.

First, this hypothesis predicts that the effects of β-adrenergic stimulation should be occluded, at least partially, by the removal of Na^+. As shown in Fig. 1*e*, β-adrenergic stimulation by maximal concentrations of isoproterenol (1 μM) (19, 20) increased L-type Ca^{2+} current up to 6 times above control in the presence of external Na^+. Within the first 5–10 min after the removal of external Na^+, the sensitivity of peak L-type Ca^{2+} current to isoproterenol was not significantly changed (not shown). However, the extent to which peak L-type Ca^{2+} current could be augmented by exposure to isoproterenol declined after prolonged exposure to Na^+-free external solutions. When isoproterenol (Fig. 1*e*, current 4) was added at the time of nearly maximal L-type Ca^{2+} current caused by Na^+ removal (after at least 10–15 min in Na^+-free external solutions; Fig. 1*e*, current 3), peak L-type Ca^{2+} current was increased by only 15%. In nine control experiments (not shown), the addition of 200 nM isoproterenol to the external solution increased peak L-type current to 339 ± 97% of control, (P < 0.02). This is consistent with the hypothesis that the effect of Na^+ removal might involve some of the same intracellular processes that are involved in the enhancement of L-type Ca^{2+} current via β-adrenergic stimulation (3, 4).

A second prediction of the hypothesis is that block of phosphorylation should also occlude the effect of the removal of Na^+. In support of this, we found that intracellular perfusion with a nonhydrolyzable ATP analogue, AMP-

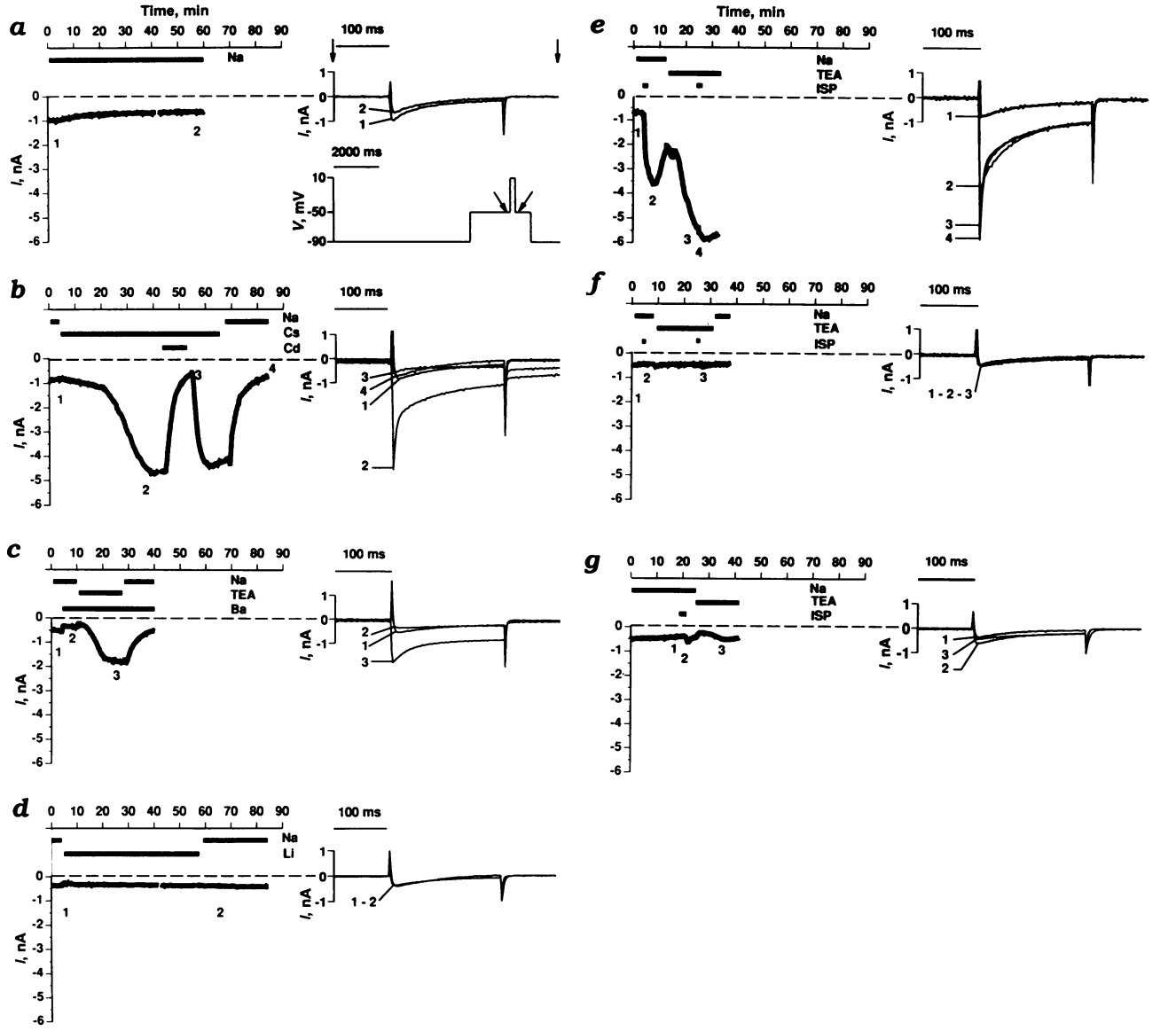

FIG. 1. Effect of Na^+ removal on peak current through L-type Ca^{2+} channels. The time course of peak currents (*Left*) and selected individual current tracings (*Right*), denoted by numbers, are shown. (*a Inset*) Voltage protocol for all experiments. Time scales begin with the time of patch rupture. (*a*) Control currents from a guinea pig ventricular cell superfused with Na^+-containing solutions decreased by 30% (currents 1 and 2) over 60 min. (*b*) Effect of removal of external Na^+ and block of Ca^{2+} channels. After 35 min in solutions where Na^+ was replaced with equimolar concentrations of Cs^+, peak L-type Ca^{2+} current increased 4- to 5-fold (currents 1 and 2). Ca^{2+} channels were reversibly blocked by 0.1 mM Cd^{2+} (currents 2 and 3). After reexposure to Na^+ solutions, peak L-type Ca^{2+} current rapidly decreased to 75% of the control L-type Ca^{2+} current (currents 1 and 4). (*c*) The increase in peak L-current is not Ca^{2+} dependent. With 0.75 mM Ba^{2+} as the charge carrier, peak L-type Ca^{2+} current increased 3-fold when external Na^+ was replaced with equimolar amounts of Et_4N^+ (TEA) (currents 2 and 3). (*d*) When external Na^+ was replaced by equimolar amounts of Li^+, peak L-type Ca^{2+} current was unaffected (currents 1 and 2). (*e*) Effects of isoproterenol. In Na^+ solutions, peak L-type Ca^{2+} current increased 5- to 6-fold (currents 1 and 2) after a brief (1 min) exposure to 1 μM isoproterenol. After equimolar replacement of external Na^+ with Et_4N^+, reexposure to 1 μM isoproterenol near the maximum increase in the peak L-type Ca^{2+} current caused a small 10–15% further increase in peak L-type Ca^{2+} current (currents 3 and 4). (*f*) Low external Na^+ effect is mediated by hydrolysis of ATP. Internal perfusion with AMP-*P*[NH]*P* (nonhydrolyzable ATP analogue whose γ-phosphate is not available for protein kinases) blocked the increase in peak L-type Ca^{2+} current seen both with isoproterenol in Na^+ solutions and with replacement of Na^+ by Et_4N^+ in Na^+-free solutions. (*g*) Inhibition of cAMP-*P*[NH]*P*-dependent protein kinase A by the specific PKI attenuates the increase in peak L-type Ca^{2+} current elicited by isoproterenol and the removal of external Na^+. Experiments were preceded by at least 25 min of internal perfusion with 10 μM PKI.

P[NH]*P* (21), suppressed the increase in L-type Ca^{2+} current elicited by both β-adrenergic agonists (4, 22) and the removal of external Na^+ ($n = 6$; Fig. 1*f*). Therefore, the mechanism by which removal of external Na^+ increases L-type Ca^{2+} current may share ATP-dependent steps with the β-adrenergic enhancement of Ca^{2+}-channel activity.

A third prediction of the hypothesis is that inhibition of protein kinase A should occlude the effects of Na^+ removal. Inhibition of cAMP-dependent protein kinase by the specific PKI peptide (23) attenuated the increases in L-type Ca^{2+} current elicited by both the β-adrenergic response to isoproterenol and the removal of external Na^+ ($n = 5$; Fig. 1*g*). In five experiments with PKI in the pipette solution, the addition of 200 nM isoproterenol to the external solution increased peak L-type Ca^{2+} current to 146 ± 28% (mean ± SD) of control, and removal of external Na^+ increased peak L-type Ca^{2+} current to 105 ± 14% of control (Fig. 1*g*). However, the increases were much greater in the absence of 10 μM PKI; in nine control experiments without PKI in the pipette solution (not shown), the addition of 200 nM isoproterenol to the external solution increased peak L-type current to 339 ± 97% of control ($P < 0.02$), and removal of external

Na^+ increased peak L-type Ca^{2+} current to 361 ± 90% of control ($P < 0.001$). Since the effect of Na^+ removal is blocked by intracellular AMP-*P*[NH]*P* and is attenuated by specific inhibition of protein kinase, the ATP-dependent process probably involves the phosphorylation of Ca^{2+} channels by cAMP-dependent protein kinase.

DISCUSSION

In summary, these experiments show that removal of external Na^+ results in a large reversible increase in current through L-type Ca^{2+}-channels. These results further indicate that the effect is not mediated by Ca^{2+}, is independent of changes in $[Ca^{2+}]_i$, and probably involves ATP-dependent Ca^{2+}-channel phosphorylation via cAMP-dependent protein kinase.

These observations were extremely surprising to us because it is generally believed that L-type Ca^{2+} currents are relatively insensitive to changes in external Na^+. For example, it has been categorically stated in a recent review of Ca^{2+} channels in muscle cells that "the complete removal of external Na^+ does not affect the amplitude of I_{Ca} in mammalian cardiac myocytes" (3). There are several possible explanations why previous investigators have failed to uncover this effect.

In multicellular cardiac preparations, removal of external Na^+ has variously been reported both to decrease and to have no effect on Ca^{2+} current. However, these studies are all subject to the methodological problems that $[Ca^{2+}]_i$ was not controlled and that separation of ionic currents in multicellular preparations was generally inferior to what can be achieved in single cells. For example, Linden and Brooker (24) reported that removal of external Na^+ in guinea pig atria attenuated the increase of cAMP in response to isoproterenol. While these findings could be consistent with our results, they are difficult to interpret because the removal of external Na^+ resulted in the development of spontaneous oscillations and in the loss of a normal force–frequency relationship. These studies, while certainly not without merit, are not as reliable methodologically as present-day experiments on single cells and will not be considered further here (for review of the older literature, see ref. 25).

It seems unlikely that this effect would have been discovered in studies of single Ca^{2+} channels in cell-attached patches. Cells are usually studied in Na^+-free, isotonic KCl in order to "zero" the membrane potential (26, 27). It seems likely that the effect of Na^+ removal would be missed in this case, since the Na^+ may be removed well before establishing a "gigaseal." This would be particularly true when cells are stored in such a medium, as in the recent study of Yue and Marban (28). Furthermore, to increase the magnitude of single-channel current and to prolong the openings, studies are usually done with high concentrations (>20 mM) of Ba^{2+} as the charge carrier and in the presence of Bay K 8644 [methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluromethyl phenyl)-pyridine 5-carboxylate].

In whole-cell recordings of Ca^{2+} current, the lack of reports of this effect is more puzzling, although in some cases there are clear explanations. For example, Bean (29) would have missed effects of Na^+ removal on the whole-cell currents in canine atrial cells because Na^+ was removed at the same time that Ca^{2+} was replaced by high concentrations (110 mM) of Ba^{2+}. Thus, an increase in current would occur simply because of the high concentration of external charge carrier, and possible effects of Na^+ removal alone would be obscured. In Bean's experiments with nearly physiological concentrations of Ca^{2+} as the charge carrier (29), the duration of exposure to Na^+-free external solutions prior to recording Ca^{2+} currents is not mentioned. If the experimental records are obtained early (within the initial 5–10 min) after the change to Na^+-free external solutions, the enhancement of L-type Ca^{2+} current by Na^+ removal could be missed because the increase in Ca^{2+} current can occur after a delay of 10–20 min (Fig. 1*b*). Furthermore, Bean (29) reported that in Na^+-free solutions, 4 μM isoproterenol increased L-type Ca^{2+} current to 200% of control. However, this is a relatively small increase compared with that observed in the presence of external Na^+ (4). In frog ventricular cells, Bean *et al.* (30) reported that in Na^+-free solutions, isoproterenol increased L-type Ca^{2+} current between 6- and 15-fold above control. In the figure shown in ref. 30, 0.5 μM isoproterenol was applied after 3 min, at a time when the effects of Na^+ removal would not have fully developed. Thus, the delay in onset of the increase in L-type Ca^{2+} current after Na^+ replacement may be an important factor that explains why this observation has not been reported by others.

Isenberg and Klockner (31) reported that the Ca^{2+} current, 20 min after Na^+ was substituted by Et_4N^+, exhibited the same amplitude as before substitution. However, intracellular Ca^{2+} was not controlled in their experiments, and the cell went into a transient contracture upon removal of external Na^+. In the absence of intracellular Ca^{2+} buffers, it is impossible to eliminate the possibility that the diminished Ca^{2+} current was a consequence of damage from the contracture rather than from Na^+ removal. Without a continuous recording of Ca^{2+} current, it is impossible to interpret the effects of Na^+-removal on L-type Ca^{2+} channels.

Matsuda and Noma (32) showed that Ca^{2+} currents recorded in Na^+-containing and Na^+-free external solutions were nearly identical and concluded that the contribution of Na^+ to Ca^{2+} current was negligible. Although they stated that "Ca^{2+} current was well maintained for more than 30 minutes," a continuous recording of Ca^{2+} current was not shown, and the time of exposure to Na^+-free solutions was not stated. As noted above in reference to the experiments of Bean (29, 30), it is possible that the Ca^{2+} currents recorded under Na^+-free conditions were obtained during the delay prior to the greatest enhancement of L-type Ca^{2+} current by Na^+ removal. In addition, the voltage protocol used to obtain L-type Ca^{2+} currents in our experiments differed in a potentially important way from the voltage protocol used by Matsuda and Noma (32). Ca^{2+} currents in our experiments were elicited by depolarizing steps from a prepulse potential of −50 mV after being held at −90 mV for 10 sec. The Ca^{2+} currents in their experiments were obtained from a more positive holding potential of −43 mV. In this regard, Schouten and Morad (33) have shown the importance of holding potential on the regulation of Ca^{2+} current in myocytes isolated from frog heart. Peak L-type Ca^{2+} current elicited from a holding potential of −50 mV declined as much as 80% over 20–30 min. In contrast, current did not decline at all when holding potentials negative to −70 mV were used. It is possible that the "rundown" of peak L-type Ca^{2+} current associated with holding potentials that are positive to normal resting membrane potential obscures the increase in Ca^{2+} current caused by the removal of external Na^+.

Harvey *et al.* (34) measured the effect of the removal of external Na^+ on L-type Ca^{2+} currents enhanced by isoproterenol. In control solutions containing external Na^+, 1 μM isoproterenol increased L-type Ca^{2+} current more than 3-fold. During uninterrupted exposure to isoproterenol, subsequent replacement of external Na^+ with Et_4N^+ reduced L-type Ca^{2+} current below control currents obtained in Na^+-containing external solutions. However, as in the experiments of Matsuda and Noma (32), the use of a holding potential (−50 mV) positive to the normal resting membrane potential may obscure observations of increased Ca^{2+} current by the superimposition of a significant component of "rundown" (33).

Yatani and Brown (35) used Na^+-free external solutions to study the effects of rapid β-adrenergic stimulation on L-type Ca^{2+} current in guinea pig myocytes. After 3 min in Na^+-free solutions, 2 μM isoproterenol increased L-type Ca^{2+} current 4- to 5-fold compared with control. As noted above, since their Ca^{2+} currents were obtained early after exposure to Na^+-free conditions, they also would have missed the effect of Na^+ removal on L-type Ca^{2+} current. Thus, the effects of removal of external Na^+ have not been studied as often as it might be thought at first, under the conditions required to make observations of the type illustrated in Fig. 1.

Our results suggest that under physiologic conditions of external Na^+-containing solutions, Na^+ may normally inhibit the phosphorylation of Ca^{2+} channels via adenylate cyclase. Although our experiments do not identify the precise site where Na^+ may exert this inhibiting effect, there are important similarities between our experiments in cardiac cells and several studies using the human platelet model. In isolated membrane preparations, Limbird (36) and others (37) have reported that Na^+ modulates α-adrenergic inhibition of adenylate cyclase. In intact platelets, Connolly and Limbird (38) have demonstrated that Na^+ influences the physiologic processes elicited by α-adrenergic inhibition of adenylate cyclase. When platelets were suspended in Na^+-free solutions, the ability of epinephrine to promote aggregation and secretion via α_2-adrenergic receptors was eliminated. More recent studies have also implicated the importance of Na^+ in modulating inhibition and stimulation of adenylate cyclase in a variety of cell types (39, 40).

While the molecular mechanisms by which sodium modulates adenylate cyclase activity are unknown, these studies suggest that Na^+ may have at least two sites of action (39). In systems that exhibit cation stimulation of adenylate cyclase, Na^+ appears to act directly on the catalytic subunit of the enzyme. In systems that exhibit cation inhibition of adenylate cyclase, Na^+ appears to exert its effect indirectly via pertussis toxin-sensitive guanine nucleotide-binding proteins (36, 39).

To the extent that our observations of the effect of Na^+ on Ca^{2+} current are similar to the biochemical studies on cation inhibition of adenylate cyclase, it is possible to propose the following model for how external Na^+ could modulate cAMP-dependent Ca^{2+} channel phosphorylation in our experiments. It is necessary to assume that when Na^+ is present externally, cells contain Na^+ despite the fact that the solution in the pipette does not contain Na^+. This assumption is supported by the observation of Bielen *et al.* (41) that whole-cell recording in cardiac cells does not always permit effective control of internal Na^+. We also assume that the basal rate of cAMP production probably reflects a balance between stimulatory and inhibitory influences on adenylate cyclase. In the absence of Na^+ in the pipette solution, however, removal of external Na^+ will lower intracellular Na^+. With the fall of intracellular Na^+, inhibition of adenylate cyclase by an inhibitory GTP-binding protein would gradually decrease, resulting in increased cAMP production and increased Ca^{2+}-channel phosphorylation. However, the model must accommodate the fact that intracellular Na^+ falls much more rapidly than the Ca^{2+} current increases. Chapman *et al.* (42) have shown that intracellular Na^+ falls with a half-time of less than 30 sec in the presence of Na^+-free external solutions. This implies that the process inhibited by Na^+ is occurring at a very slow rate.

This research was supported in part by National Institutes of Health Grants 1-K11-HL02466-01 and R01-HL29473 and a Grant-In-Aid from the American Heart Association, Maryland Affiliate.

1. Nilius, B., Hess, P., Lansmen, J. B. & Tsien, R. W. (1985) *Nature (London)* **316,** 443–446.
2. Sheu, S. S. & Blaustein, M. P. (1986) in *The Heart and Cardiovascular System*, eds. Fozzard, H. A., Haber, E., Jennings, R. B., Katz, A. M. & Morgan, H. E. (Raven, New York), pp. 509–535.
3. Pelzer, D., Pelzer, S. & McDonald, T. F. (1990) *Rev. Physiol. Biochem. Pharmacol.* **114,** 107–206.
4. Trautwein, W. & Hescheler, J. (1990) *Annu. Rev. Physiol.* **52,** 257–274.
5. Balke, C. W. & Wier, W. G. (1991) *Circ. Res.* **68,** 899–902.
6. Beuckelmann, D. J. & Wier, W. G. (1988) *J. Physiol. (London)* **405,** 233–255.
7. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. (1981) *Pflügers Arch.* **391,** 85–100.
8. Belles, B., Malécot, C. O., Hescheler, J. & Trautwein, W. (1988) *Pflügers Arch.* **411,** 353–360.
9. Sala, F. & Hernández-Cruz, A. (1990) *Biophys. J.* **57,** 313–324.
10. Rosenberg, G. B. & Storm, D. R. (1987) *J. Biol. Chem.* **262,** 7623–7628.
11. Meyer, T. & Stryer, L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5051–5055.
12. Marban, E. & Tsien, R. W. (1982) *J. Physiol. (London)* **329,** 589–614.
13. Tseng, G. N. (1988) *Circ. Res.* **63,** 468–482.
14. Gurney, A. M., Charnet, P., Pye, J. M. & Nargeot, J. (1989) *Nature (London)* **341,** 65–68.
15. Campbell, D. L., Giles, W. R. & Shibata, E. F. (1988) *J. Physiol. (London)* **403,** 239–266.
16. Lazdunski, M., Frelin, C. & Vigne, P. (1985) *J. Mol. Cell. Cardiol.* **17,** 1029–1042.
17. Cachelin, A. B., de Peyer, J. E., Kokubun, S. & Reuter, H. (1983) *Nature (London)* **304,** 462–464.
18. Reuter, H., Stevens, C. F., Tsien, R. W. & Yellen, G. (1982) *Nature (London)* **297,** 501–504.
19. Kameyama, M., Hofmann, F. & Trautwein, W. (1985) *Pflügers Arch.* **405,** 285–293.
20. Hescheler, J., Nawrath, H., Tang, M. & Trautwein, W. (1988) *J. Physiol. (London)* **397,** 657–670.
21. Yount, R. G. (1975) *Adv. Enzymol.* **34,** 1–56.
22. Trautwein, W., Kameyama, M., Hescheler, J. & Hofmann, F. (1986) in *Membrane Control of Cellular Activity, Progress in Zoology*, ed. Lüttgau, H. Ch. (Fisher, Stuttgart), pp. 163–182.
23. Kameyama, M., Hescheler, J., Hofmann, F. & Trautwein, W. (1986) *Pflügers Arch.* **407,** 123–128.
24. Linden, J. & Brooker, G. (1978) *Science* **199,** 539–541.
25. Corabeouf, E. (1980) in *The Slow Inward Current and Cardiac Arrhythmias*, eds. Zipes, D. P., Bailey, J. C. & Elharrar, V. (Nijhoff, Boston), pp. 25–95.
26. Marty, A. (1981) *Nature (London)* **291,** 497–500.
27. Hess, P., Lansman, J. B. & Tsien, R. W. (1986) *J. Gen. Physiol.* **88,** 293–319.
28. Yue, D. T. & Marban, E. (1990) *J. Gen. Physiol.* **95,** 911–939.
29. Bean, B. P. (1985) *J. Gen. Physiol.* **86,** 1–30.
30. Bean, B. P., Nowycky, M. C. & Tsien, R. W. (1984) *Nature (London)* **307,** 371–375.
31. Isenberg, M. & Klockner, U. (1982) *Pflügers Arch.* **395,** 30–41.
32. Matsuda, H. & Noma, A. (1984) *J. Physiol. (London)* **357,** 553–573.
33. Schouten, V. J. A. & Morad, M. (1989) *Pflügers Arch.* **415,** 1–11.
34. Harvey, R. D., Jurevičius, J. A. & Hume, J. R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6946–6950.
35. Yatani, A. & Brown, A. M. (1989) *Science* **245,** 71–74.
36. Limbird, L. E. (1984) *Am. J. Physiol.* **247,** E59–E68.
37. Steer, M. L. & Wood, A. (1981) *J. Biol. Chem.* **256,** 9990–9993.
38. Connolly, T. M. & Limbird, L. E. (1983) *J. Biol. Chem.* **258,** 3907–3912.
39. Duman, R. S., Terwilliger, R. Z., Nestler, E. J. & Tallman, J. F. (1989) *Biochem. Pharmacol.* **38,** 1909–1914.
40. Watson, R. L., Jacobson, K. L. & Singh, J. C. (1989) *Biochem. Pharmacol.* **38,** 1069–1074.
41. Bielen, F. V., Glitsch, H. G. & Verdonck, F. (1991) *Biochim. Biophys. Acta* **1065,** 269–271.
42. Chapman, R. A., Coray, A. & McCuigan, J. A. S. (1983) *J. Physiol. (London)* **343,** 253–276.