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Intracellular Na^+ modulates the cAMP-dependent regulation of ion channels in the heart

(Cl^- current/Ca^{2+} current/delayed rectifier K^+ current)

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ABSTRACT **The cAMP-dependent regulation of ion channels was studied by using the whole-cell configuration of the patch clamp technique. In isolated cardiac ventricular myocytes, the β-adrenergically regulated Cl^- current (I_{Cl}) exhibited an unusual dependence on Na^+, such that replacement of extracellular Na^+ with compounds such as tetramethylammonium, choline, Tris, or *N*-methyl-D-glucamine resulted in a reduction in current amplitude without changing the reversal potential. Replacement of extracellular Na^+ with tetramethylammonium also reduced the magnitude of the β-adrenergically enhanced Ca^{2+} current and delayed rectifier K^+ current, suggesting that removal of Na^+ was affecting the cAMP pathway that regulates all three currents. Replacement of extracellular Na^+ also reduced I_{Cl} that was stimulated by (*i*) direct activation of adenylate cyclase with forskolin, (*ii*) inhibition of phosphodiesterase with 3-isobutyl-1-methylxanthine, (*iii*) exposure to the membrane-permeable cAMP derivative 8-bromoadenosine 3′,5′-cyclic monophosphate, or (*iv*) direct phosphorylation of the channel with protein kinase A catalytic subunit. This suggests that the Na^+ dependence is at a point beyond the activation of protein kinase A. The Na^+ dependence of I_{Cl} regulation could not be explained by changes in intracellular Ca^{2+}. However, the sensitivity of the I_{Cl} to changes in extracellular Na^+ depended significantly on the intracellular Na^+ concentration, suggesting that intracellular Na^+ plays an important role in the cAMP-dependent regulation of ion channels.**

Autonomic regulation of ion-channel activity is an important mechanism by which cardiac function can be regulated. In the heart it has been shown that β-adrenergic stimulation can modulate the activity of many different ion channels, including those responsible for the high threshold or L-type Ca^{2+} current (I_{Ca}), delayed rectifier K^+ current (I_K), Na^+ current, and pacemaker current (1–6). Recently, it has also been shown that β-adrenergic stimulation also activates a Cl^- conductance (7, 8). The β-adrenergic regulation of all of these channels involves, at least in part, the same cAMP-dependent pathway (see ref. 9 for review). One interesting observation, however, is the fact that the β-adrenergically regulated Cl^- channel exhibits an apparent dependence on extracellular Na^+ (8, 10). When the Cl^- current (I_{Cl}) is activated with the β-adrenergic agonist isoproterenol (Iso), subsequent replacement of extracellular Na^+ results in a decrease in the magnitude of the current. In the present study, we report that the Na^+ dependence of the I_{Cl} is due not to a direct effect of Na^+ on Cl^- channels but rather to an indirect effect on the regulatory pathway, which has important implications concerning the autonomic regulation of ion channels in general.

MATERIALS AND METHODS

Single guinea pig ventricular myocytes were enzymatically dissociated by a previously described method (10). Membrane currents were recorded by using the whole-cell configuration of the patch clamp technique. Cells were dialyzed using 1- to 2-MΩ electrodes filled with an intracellular solution containing 130 mM CsCl, 20 mM tetraethylammonium (TEA) chloride, 5 mM ATP (magnesium salt), 5 mM EGTA, and 5 mM Hepes. Cells were bathed in an extracellular solution containing 140 mM NaCl, 5.4 mM CsCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.5 mM Hepes, and 11 mM glucose. Iso, forskolin (FSK), 3-isobutyl-1-methylxanthine (IBMX), 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP), and protein kinase A catalytic subunit (PKA-CS) were obtained from Sigma. Iso was prepared as an aqueous stock solution. Nisoldipine and FSK were prepared as stock solutions using polyethylene glycol (PEG 400). IBMX was added directly to all solutions. PKA-CS was reconstituted in deionized water containing 0.5 mg of dithiothreitol per ml to prevent rapid loss of activity of the enzyme. This stock solution was then added directly to the intracellular solution. The final concentration of dithiothreitol was <10 nM. Results are reported as the mean ± SE, and statistical differences were determined by paired *t* test unless otherwise noted.

RESULTS

The protocol used to study the I_{Cl} in isolated guinea pig ventricular myocytes is shown in Fig. 1*A*. Under conditions in which the basal level of cAMP is not elevated, the sarcolemma of these cells does not exhibit a measurable Cl^- conductance. Therefore, in the present experiments the I_{Cl} was defined as the difference current that was obtained by subtracting currents recorded before and after stimulation. Furthermore, to eliminate contamination of the difference current by simultaneous changes in conductances regulated by the same pathway, current through K^+ channels was eliminated by exposing the cells to K^+-free intra- and extracellular solutions containing Cs^+ and/or TEA, high-threshold I_{Ca} was blocked by adding either 1 μM nisoldipine or 100 μM Cd^{2+} to the external solution, and Na^+ and low-threshold Ca^{2+} channels were inactivated by using a depolarized holding potential (−30 mV).

In the absence of agonist, very little current could be recorded during voltage clamp steps to membrane potentials between +50 and −120 mV. After exposure of the cell to 1 μM Iso, the I_{Cl} appeared as a time-independent increase of the background conductance (Fig. 1*A*, *Upper Right*). However, when extracellular Na^+ was replaced while in the continued presence of Iso, the magnitude of the I_{Cl} was

Abbreviations: I_{Cl}, Cl^- current; I_{Ca}, Ca^{2+} current; I_K, K^+ current; Iso, isoproterenol; TEA, tetraethylammonium; FSK, forskolin; IBMX, 3-isobutyl-1-methylxanthine; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; PKA-CS, protein kinase A catalytic subunit; TMA, tetramethylammonium; ATP[γS], adenosine 5′-[γ-thio]triphosphate.

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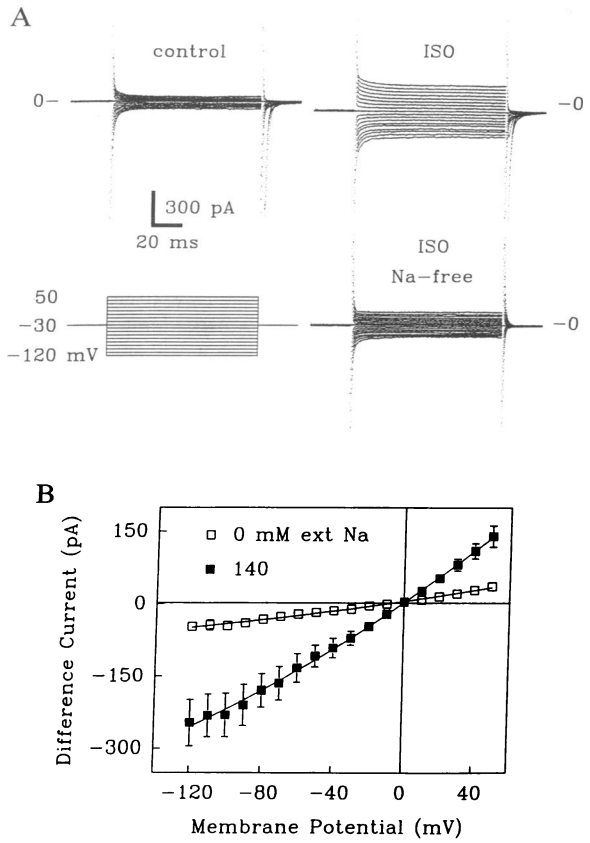

FIG. 1. Na^+ dependence of the Iso-induced I_{Cl}. (*A*) Membrane current recorded in the absence of agonist (*Upper Left*) after exposure to 1 μM Iso (*Upper Right*) and after replacement of extracellular Na^+ with an equimolar concentration of TMA while in the continued presence of Iso (*Lower Right*). Membrane potential was held at −30 mV and stepped to potentials between +50 and −120 mV for 100 ms. (*B*) Current–voltage relationship of Iso-induced difference current recorded in the presence (■) and absence (□) of extracellular Na^+ (140 mM). Current was measured at the end of the 100-ms voltage clamp steps described in *A*. Difference current was obtained by subtracting current recorded under control conditions from current recorded from the same cell after exposure to 1 μM Iso, both before and after replacement of extracellular Na^+ with TMA. Results are expressed as mean ± SE (n = 6).

significantly reduced (Fig. 1*A*, *Lower Right*). Fig. 1*B* illustrates the current–voltage relationship of I_{Cl} before and after removal of extracellular Na^+. In these experiments, the current reversed at the Cl^- equilibrium potential, which was set at 0 mV. While replacing extracellular Na^+ reduced the slope conductance of the current, the reversal potential was not affected. This indicates that the current activated by Iso was not conducted by Na^+; however, it was Na^+ sensitive. The voltage dependence of the Iso-induced I_{Cl} was nearly linear over the voltage range tested, which is due to the fact that symmetrical Cl^- concentrations were used (10). When the current measured between +50 and −50 mV (the most linear portion of the relationship) was fit by linear regression, both before and after replacement of extracellular Na^+, the slope conductance was reduced by 74.9% ± 6.6% (n = 6) after replacement of extracellular Na^+. In the experiments shown, extracellular Na^+ was replaced with an equimolar concentration of tetramethylammonium (TMA). Similar results were also obtained when Na^+ was replaced with either choline, Tris, or *N*-methyl-D-glucamine.

To ascertain whether the Na^+ sensitivity of the I_{Cl} might be due to a specific effect of Na^+ on Cl^- channels or a nonspecific effect on the regulatory pathway, experiments were conducted to discover whether replacement of extracellular Na^+ affected the Iso-enhanced I_{Ca} and the delayed rectifier I_K, which are regulated through the same cAMP-dependent pathway. The solutions used to study the I_{Ca} were the same as those used to study the I_{Cl}, except that the Ca^{2+} channel blocker was left out. It has previously been shown that there is no effect of Na^+ on the Ca^{2+} current under conditions in which I_{Ca} is not stimulated (11), suggesting that there is no direct effect of Na^+ on Ca^{2+} channels. However, in the present study, when the I_{Ca} was enhanced by using Iso (1 μM), subsequent replacement of extracellular Na^+ with TMA appeared to completely reverse the effect of Iso, even though the agonist was still present (Fig. 2). Similar results were also obtained when the Na^+ dependence of the Iso-enhanced delayed rectifier I_K was examined. The solutions used to study this I_K were the same as those used to study the I_{Cl}, except that Cs^+ and TEA were replaced with K^+ in both the intra- and extracellular solutions. After enhancement of I_K with 1 μM Iso, replacement of extracellular Na^+ with TMA resulted in an attenuation of the Iso-enhanced I_K (n = 3; data not shown). The fact that replacement of extracellular Na^+ diminished the β-adrenergic-enhanced I_{Ca} and I_K suggests that the Na^+ sensitivity of I_{Cl} was due not to a direct effect on the channel but rather to an indirect effect on the pathway that regulates all three currents.

To address the possibility that removal of extracellular Na^+ might be affecting the β-adrenergic receptor, the Na^+ sensitivity of I_{Cl} was examined after activation of the current with histamine, an agent also known to elevate cAMP in the heart. It was found that the histamine-induced I_{Cl} exhibited a Na^+ sensitivity similar to the Iso-induced current. This is consistent with the idea that replacement of extracellular Na^+ affects the cAMP-dependent pathway, since histamine-induced changes in both I_{Cl} and I_{Ca} are believed to be mediated through this same second messenger system (12).

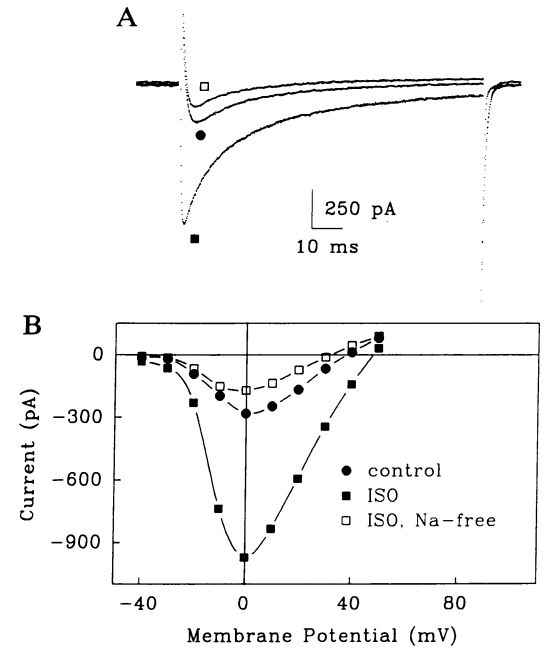

FIG. 2. Na^+ dependence of the Iso-induced I_{Ca}. (*A*) Membrane current recorded in the absence of agonist, after exposure to 1 μM Iso and after replacement of extracellular Na^+ with an equimolar concentration of TMA while in the continued presence of Iso. I_{Ca} was activated by 100-ms voltage clamp steps to 0 mV from a holding potential of −30 mV. (*B*) Current–voltage relationship of the peak inward I_{Ca} elicited during voltage clamp steps to membrane potentials between −40 and +50 mV before exposure to agonist (●), after exposure to 1 μM Iso (■), and after replacement of extracellular Na^+ with TMA while in the continued presence of Iso (□). Same cell as in *A* from a representative experiment (n = 4).

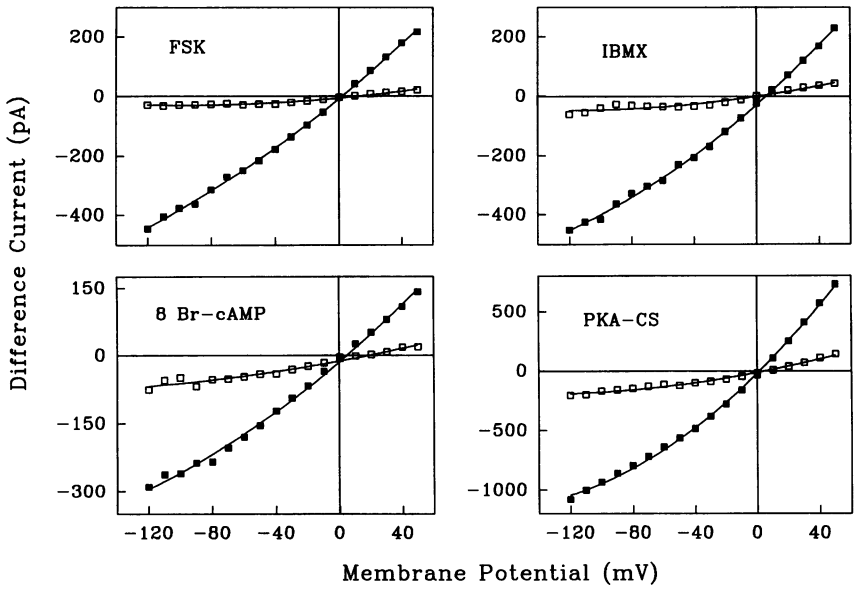

FIG. 3. Na^+ dependence of I_{Cl} activated at various points in the cAMP regulatory pathway. Plots in each graph represent voltage dependence of the I_{Cl} recorded from a cell before (■) and after (□) replacement of extracellular Na^+ (140 mM) with TMA. Membrane current was measured at the end of 100-ms voltage clamp steps to membrane potentials between +50 and −120 mV. I_{Cl} was defined as the difference current, which was obtained by subtracting current recorded before from that recorded after activation of the Cl^- conductance, in both the presence and absence of extracellular Na^+. The data shown are typical of the I_{Cl} activated by extracellular exposure to 1 μM FSK (n = 8), 100 μM IBMX (n = 6), 100 μM 8-Br-cAMP (n = 4), or intracellular dialysis with 50 units of PKA-CS per ml (n = 2).

Assuming that removal of extracellular Na^+ is somehow affecting the cAMP-dependent regulation of ion channels in these myocytes, it should be possible to identify the Na^+-sensitive step by perturbing this pathway at different points and then examining the Na^+ sensitivity of I_{Cl}. Fig. 3 illustrates experiments in which the I_{Cl} was activated by (*i*) increasing intracellular cAMP production by direct activation of adenylate cyclase with FSK (1 μM), (*ii*) decreasing breakdown of endogenous cAMP with the phosphodiesterase inhibitor IBMX (100 μM), (*iii*) exposure to the membrane-permeable cAMP derivative, 8-Br-cAMP (100 μM), or (*iv*) dialysis of the cell with an intracellular solution containing PKA-CS (50 units/ml). In each case, subsequent replacement of extracellular Na^+ resulted in an attenuation of I_{Cl}. This is consistent with the idea that replacing extracellular Na^+ was not affecting the β-adrenergic receptor and indicates that the Na^+-sensitive step in the regulation of the I_{Cl} is at a point beyond the activation of cAMP-dependent PKA. Consistent with this hypothesis was the observation that the Ca^{2+} current enhanced by both 1 μM FSK (n = 4) and 100 μM IBMX (n = 4) exhibited the same Na^+ dependence as the Iso-enhanced I_{Ca}.

The previous results could be explained if Na^+ were affecting the phosphorylation or dephosphorylation of the Cl^- channel. To test this hypothesis, the Na^+ sensitivity of I_{Cl} was examined under conditions in which the current was irreversibly activated. This was accomplished by dialyzing cells with an internal solution in which ATP had been replaced with adenosine 5′-[γ-thio]triphosphate (ATP[γS]). Under these conditions, subsequent stimulation with FSK should result in a dephosphorylation-resistant, thiophosphorylation of the channel (13–15). When the I_{Cl} was then activated (Fig. 4*A*), replacement of extracellular Na^+ did not result in an attenuation of the current, as was previously observed (see Figs. 1 and 3). This result confirms the idea that Na^+ does not have a direct effect on the channel, but instead may be affecting phosphorylation or dephosphorylation of the channel. It is feasible that ATP[γS] could also have been used to synthesize GTP[γS] from endogenous GDP and nucleotide diphosphate kinase. If produced, GTP[γS] would have been expected to result in an irreversible activation of adenylate cyclase and subsequent stimulation of the I_{Cl}. However, this cannot explain the result because activation of adenylate with GTP[γS], as with FSK (see Fig. 3), would not be expected to affect the Na^+ sensitivity of the I_{Cl}.

Removal of extracellular Na^+ would be expected to lead to an increase in intracellular Ca^{2+}, and Ca^{2+}-dependent phosphatases have been shown to play an important role in the regulation of Ca^{2+} channels (16). Even though in all of these experiments the cells were dialyzed with an intracellular solution containing 5 mM EGTA, it is conceivable that in the absence of Na^+/Ca^{2+} exchange there could be a spatial gradient with the Ca^{2+} concentration at the intracellular surface of the membrane being high enough to activate a

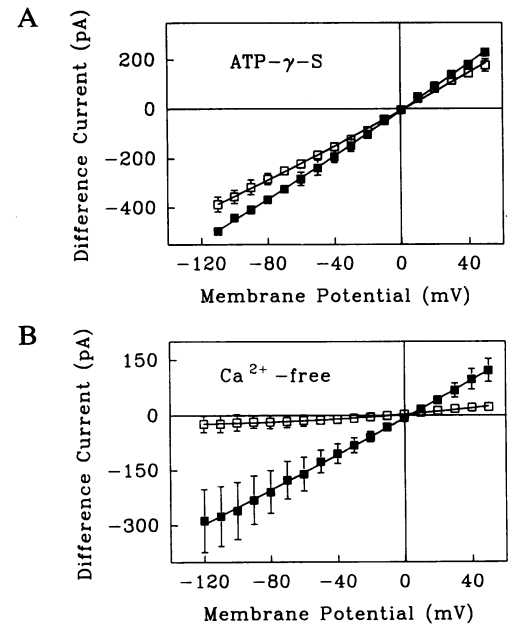

FIG. 4. Voltage dependence of the I_{Cl} recorded before (■) and after (□) replacement of extracellular Na^+ with TMA. (*A*) Na^+ dependence of the I_{Cl} activated under dephosphorylation-resistant conditions. Cells were dialyzed with an intracellular solution in which ATP was replaced with ATP[γS] (5 mM). Results are expressed as mean ± SE (n = 3). (*B*) Na^+ dependence of the I_{Cl} activated under Ca^{2+}-free conditions. In addition to exposing the cells to 1 μM nisoldipine, Ca^{2+} in the extracellular solution was replaced with Mg^{2+}. Furthermore, the cells were exposed to 10 μM ryanodine for 5 min before recording any current in order to eliminate the sarcoplasmic reticulum as a possible source of Ca^{2+}. Results are expressed as mean ± SE (n = 3). In both panels, current was measured at the end of 100-ms voltage clamp steps to membrane potentials between +50 and −120 mV. The I_{Cl} was defined as the difference current obtained by subtracting membrane current recorded before from that recorded after exposure to 1 μM FSK in both the presence and absence of extracellular Na^+.

phosphatase. To address this possibility, experiments were conducted using extracellular solutions in which Ca^{2+} was replaced with Mg^{2+}, and to which ryanodine (10 μM) had been added so that intracellular stores of Ca^{2+} would be depleted. Under these conditions, replacement of extracellular Na^+ would not be expected to result in an increase in intracellular Ca^{2+}, yet when the I_{Cl} was activated, replacement of extracellular Na^+ still resulted in a significant attenuation (Fig. 4*B*), indicating that the Na^+ sensitivity of the I_{Cl} was not mediated through a Ca^{2+}-dependent mechanism.

Although changes in intracellular Ca^{2+} cannot explain the Na^+ dependence of I_{Cl}, another possibility is that the effect seen upon replacement of extracellular Na^+ is due to a change in intracellular Na^+. In all previous experiments, cells were dialyzed with a Na^+-free intracellular solution. However, it is conceivable that when cells are bathed in an extracellular solution containing 140 mM Na^+, there is an inward leak of Na^+ across the sarcolemma, which in this case would result in a spatial gradient with the Na^+ concentration being greatest at the intracellular surface of the membrane. This Na^+ might then facilitate phosphorylation or inhibit dephosphorylation of the Cl^- channel. In cells dialyzed with a Na^+-free intracellular solution (Fig. 5*A*), replacement of extracellular Na^+ with TMA resulted in a 72.1% ± 8.3% (n = 8) reduction of the slope conductance of the FSK-induced I_{Cl}, which was statistically significant ($P < 0.01$). However, when cells were dialyzed with an intracellular solution containing 10 mM Na^+ (Fig. 5*B*), replacing extracellular Na^+ only resulted in a 26.1% ± 5.3% (n = 4) reduction of the FSK-induced Cl^- conductance, which appeared to be a true response, although the change was not statistically significant ($P > 0.05$). Thus, adding only 10 mM Na^+ to the intracellular solution significantly ($P < 0.005$; unpaired t test) diminished the response to replacing extracellular Na^+. This result is consistent with the hypothesis that the Na^+ sensitivity of I_{Cl} is the result of changes in the intracellular Na^+ concentration and that this Na^+ plays an important role in maintaining the channel in an activated or phosphorylated state.

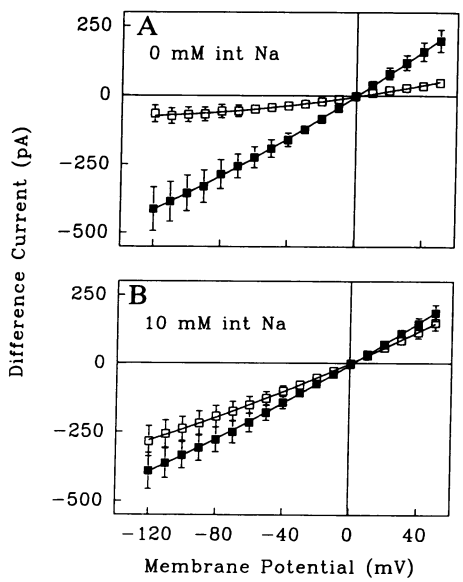

FIG. 5. Role of intracellular Na^+ in Na^+ dependence of the I_{Cl}. (*A*) Na^+ dependence of the I_{Cl} in cells dialyzed with a Na^+-free intracellular solution. Results are expressed as mean ± SE (n = 8). (*B*) Na^+ dependence of the I_{Cl} in cells dialyzed with an internal solution containing 10 mM Na^+ (Na^+ replacing Cs^+). Results are expressed as mean ± SE (n = 4). Each plot illustrates voltage dependence of the I_{Cl} recorded before and after replacement of extracellular Na^+ with TMA. Current was measured at the end of 100-ms voltage clamp steps to membrane potentials between +50 and −120 mV from a holding potential of −30 mV. The I_{Cl} was defined as the difference current obtained by subtracting membrane current recorded before from that recorded after exposure to 1 μM FSK, in both the presence (■) and absence (□) of extracellular Na^+ (140 mM).

DISCUSSION

Even though the cAMP-regulated I_{Cl} found in cardiac myocytes exhibits an unusual dependence on extracellular Na^+, the high degree of selectivity of this conductance pathway for anions over cations has been well established (7, 10), suggesting that, while Na^+ is not acting as current carrier, it does play some role in the regulation of the I_{Cl}. Previously, a pronounced isoprenaline-induced depolarization of the resting membrane potential had been reported in guinea pig ventricular myocytes (17, 18), and it was concluded that this was caused by the activation of a current conducted by Na^+. It is most likely that the catecholamine-induced current responsible for the depolarization was actually the cAMP-dependent I_{Cl} (10) and that the Na^+ dependence of this I_{Cl} led to the conclusion that the isoprenaline-induced current was carried by Na^+. The Cl^--dependent nature of this catecholamine-induced current has been confirmed by Matsuoka *et al.* (19).

In the present study, we found that replacement of extracellular Na^+ affects not only β-adrenergically activated Cl^- channels, but Ca^{2+} and delayed rectifier K^+ channels as well, which is consistent with the idea that replacement of extracellular Na^+ affects a common step in the cAMP-dependent regulation of ion channels in cardiac myocytes. Furthermore, the data presented here also suggest that the effect of replacing extracellular Na^+ is at an intracellular site. However, we observed a reduction of the I_{Cl}, I_{Ca}, and I_K activated by β-adrenergic stimulation after replacement of extracellular Na^+ with ions other than Li^+, suggesting that the effect of replacing Na^+ with Li^+ (20) was the result of a sensitivity to the loss of Na^+ rather than to the presence of Li^+. This is consistent with the later work of Matsuoka *et al.* (19).

In cardiac myocytes, β-adrenergic stimulation of Ca^{2+}, K^+, and Cl^- channels (3, 10, 21) can be antagonized by concurrent muscarinic stimulation. This inhibitory response is believed to be mediated through an inhibitory guanine nucleotide-binding inhibitory protein, G_i, which couples the muscarinic receptor to adenylate cyclase. It is known that Na^+ increases the affinity of acetylcholine for the muscarinic receptor (22), suggesting that Na^+ facilitates the inhibition of adenylate cyclase. Therefore, if removal of Na^+ were affecting G_i, a relief of inhibition and an increased stimulatory effect might be expected. This is the opposite of what was observed.

It is interesting to note that Matsuoka *et al.* (19) found that the magnitude of the Cl^- conductance elicited upon exposure to epinephrine depended on the major cation in the extracellular solution. There was a distinct sequence for the efficacy of cations in the maintenance of Cl^- conductance ($Na^+ > K^+$ and $Rb^+ > Cs$, Li^+, and divalent cations). Our results differ slightly in that we rarely saw a complete reversal of the I_{Cl} when extracellular Na^+ was replaced with TMA, Tris, choline, or *N*-methyl-D-glucamine, even when using intracellular solutions containing no added Na^+.

The exact mechanism of the apparent Na^+ dependence of the cAMP-dependent regulation of the Cl^- and other channels in cardiac myocytes is yet to be determined. The fact that Na^+ appears to affect the phosphorylation of the channel might be explained if Na^+ allosterically affects, either directly or indirectly, the activity of a kinase and/or phosphatase. The fact that there seems to be some selectivity sequence for this modulatory response may reflect the efficacy of the various ions to substitute for Na^+ at a specific site. One possible mechanism to be considered may be related to

previous observations that increasing intracellular Mg^{2+} can reduce the magnitude of both the inward I_{Ca} (23) and the delayed rectifier I_K (24). In the case of the I_{Ca} (23), increasing intracellular Mg^{2+} only decreased I_{Ca} enhanced by cAMP-dependent phosphorylation. Although the mechanisms regulating intracellular Mg^{2+} in cardiac muscle are not well understood, in many types of cells, intracellular Mg^{2+} is believed to be regulated at least in part by a Na^+/Mg^{2+} exchanger that couples the outward movement of Mg^{2+} to the inward movement of Na^+ (25). Therefore, removing extracellular Na^+ may result in an increase in intracellular Mg^{2+}, which might be responsible for the Na^+-dependent phenomena. However, Na^+/Mg^{2+} exchange is believed to work in both directions (26). Therefore, if this were the mechanism underlying the Na^+ dependence of the cAMP-dependent currents, dialyzing cells with 10 mM Na^+ would have been expected to result in a greater increase in intracellular Mg^{2+}, and consequently a greater decrease in current magnitude, upon removal of extracellular Na^+ than would have dialyzing cells with Na^+-free intracellular solution. Yet, the opposite was observed.

The observation that replacement of extracellular Na^+ appears to completely reverse the effect of I_{Ca} stimulation (see Fig. 2) seems to be inconsistent with previous work demonstrating the effects of agonists under Na^+-free conditions (see, for example, refs. 27 and 28). We have also been able to stimulate the I_{Ca} in the absence of extracellular Na^+ (R.D.H. and J.R.H., unpublished observation). The fact that removal of extracellular Na^+ had such a dramatic effect on the Iso-enhanced I_{Ca} could be explained if the magnitude of the response to simulation were reduced in the absence of Na^+. The present study supports the idea that Na^+ may modulate the magnitude or gain of the response to cAMP-dependent stimulation. The magnitude of the response to removal of Na^+ was also most certainly complicated by rundown of the current over the time course of the experiment, since rundown of I_{Ca} is not prevented by cAMP-dependent activation (14). It would be interesting to speculate whether the Na^+ sensitivity of the β-adrenergically enhanced I_{Ca} might explain why this current was originally referred to as the slow-inward (Ca^{2+}, Na^+) current, or I_{si}, and was thought to be carried partially by Na^+. Intrinsic autonomic tone in the multicellular preparations used in early experiments may have maintained a component of the I_{Ca} that would be sensitive to changes in extracellular Na^+.

The most significant finding of the present study is that changes in intracellular Na^+ concentration within the physiologic range may plan an important role in modulating the response of cardiac ion channels to autonomic regulation. This could have important implications concerning the regulation of ion-channel function under conditions in which intracellular Na^+ is altered. One example is the situation in which intracellular Na^+ is elevated during treatment with cardiac glycosides. An increase in intracellular Na^+ could act to amplify the response to stimulation of β-adrenergic receptors. An increased response of the inward I_{Ca} might then explain, at least in part, why contractility is enhanced. However, it remains to be determined exactly how sensitive the cAMP regulation of ion channels is to changes in intracellular Na^+.

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