β-Adrenergic stimulation does not activate Na\(^+\)/Ca\(^{2+}\) exchange current in guinea pig, mouse, and rat ventricular myocytes

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——The effect of β-adrenergic stimulation on cardiac Na\(^+\)/Ca\(^{2+}\) exchange has been controversial. To clarify the effect, we measured Na\(^+\)/Ca\(^{2+}\) exchange current (I\(_{\text{NCX}}\)) in voltage-clamped guinea pig, mouse, and rat ventricular cells. When I\(_{\text{NCX}}\) was defined as a 5 mM Ni\(^{2+}\)-sensitive current in guinea pig ventricular myocytes, 1 μM isoproterenol apparently augmented I\(_{\text{NCX}}\) by ~32%. However, this increase was probably due to contamination of the cAMP-dependent Cl\(^{-}\) current (CFTR-Cl\(^{-}\) current, I\(_{\text{CFTR-Cl}}\)). Because Ni\(^{2+}\) inhibited the activation of I\(_{\text{CFTR-Cl}}\) by 1 μM forskolin with a half-maximum concentration of 0.5 mM under conditions where I\(_{\text{NCX}}\) was suppressed. Five or ten millimolar Ni\(^{2+}\) did not inhibit I\(_{\text{CFTR-Cl}}\) activated by 10 μM forskolin, an activator of adenylyl cyclase, suggesting that Ni\(^{2+}\) acted upstream of adenylyl cyclase in the β-adrenergic signaling pathway. Furthermore, in a low-extra cellular Cl\(^{-}\) bath solution, 1 μM isoproterenol did not significantly alter the amplitude of Ni\(^{2+}\)-sensitive I\(_{\text{NCX}}\) at +50 mV, which is close to the reversal potential of I\(_{\text{CFTR-Cl}}\). No change in I\(_{\text{NCX}}\) amplitude was induced by 10 μM forskolin. When I\(_{\text{NCX}}\) was activated by extracellular Ca\(^{2+}\), it was not significantly affected by 1 μM isoproterenol in guinea pig, mouse, or rat ventricular cells. We concluded that β-adrenergic stimulation does not have significant effects on I\(_{\text{NCX}}\) in guinea pig, mouse, or rat ventricular myocytes.

cystic fibrosis transmembrane conductance regulator; nickel ion exchange protein

THE β-ADRENERGIC RECEPTOR SIGNALING CASCADE IS A PIVOTAL MECHANISM REGULATING CARDIAC CONTRACTILITY, and β-adrenergic receptor agonists such as isoproterenol produce positive inotropy, together with increasing cytosolic Ca\(^{2+}\) transient amplitude and rates of relaxation and intracellular Ca\(^{2+}\) decline. Major mechanisms underlying this positive inotropic action are enhanced activities of the sarcolemmal L-type Ca\(^{2+}\) channel, the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase, and the sarcoplasmic reticulum ryanodine receptor and a decreased myofilament Ca\(^{2+}\) sensitivity (3). However, the question of whether β-adrenergic receptor stimulation upregulates sarcollemmal Na\(^+\)/Ca\(^{2+}\) exchange, which is the main Ca\(^{2+}\) extrusion system of the cardiac myocyte, has proved controversial.

Several groups have reported that the cardiac Na\(^+\)/Ca\(^{2+}\) exchange protein is phosphorylated by PKA (16, 26, 27, 30). The augmentation of exchange activity through the β-adrenergic signaling pathway has also been reported. Linck et al. (19) found that Na\(^+\)-dependent Ca\(^{2+}\) uptake of baby hamster kidney (BHK) cells expressing canine cardiac Na\(^+\)/Ca\(^{2+}\) exchanger 1 (NCX1) was enhanced by 100 μM forskolin, an activator of adenylyl cyclase, by ~20%. In whole cell voltage-clamped guinea pig ventricular myocytes with other major currents inhibited, Perchenet et al. (25) and Zhang et al. (33) demonstrated that β-adrenergic stimulation enhanced Na\(^+\)/Ca\(^{2+}\) exchange current (I\(_{\text{NCX}}\)) by ~25–100%. Ruknudin et al. (26) found that PKA-activating reagents phosphorylated a cardiac isoform of the NCX expressed in Xenopus oocytes and increased both 45Ca\(^{2+}\) uptake (by ~40%) and outward I\(_{\text{NCX}}\) (by >100%). They also observed PKA-dependent enhancement of Na\(^+\)-dependent 45Ca\(^{2+}\) uptake by ~40% in adult rat ventricular cardiomyocytes. Wei et al. (30) also reported that PKA phosphorylated the Na\(^+\)/Ca\(^{2+}\) exchange protein and increased I\(_{\text{NCX}}\) by ~50% in control and by ~100% in failing ventricular cells from pig heart, suggesting that cardiac Na\(^+\)/Ca\(^{2+}\) exchange is hyperphosphorylated in heart failure. To the contrary, no stimulatory effect of β-adrenergic receptor stimulation or PKA on exchange activity was detected in giant membrane patches excised from blebs of guinea pig ventricular cells (5), vesicle preparations of rat hearts (2), guinea pig ventricular cells (20), or BHK cells expressing the dog cardiac NCX1 (13).

The aim of this study was to clarify the effect of β-adrenergic stimulation on the native mammalian NCX. We measured I\(_{\text{NCX}}\) in voltage-clamped ventricular myocytes isolated from guinea pig, mouse, and rat hearts and studied the effect of β-adrenergic stimulation, with particular attention to the contribution of the PKA-activated Cl\(^{-}\) current (CFTR-Cl\(^{-}\) current, I\(_{\text{CFTR-Cl}}\)) to the recorded membrane current. We concluded that β-adrenergic stimulation does not significantly affect I\(_{\text{NCX}}\) in the ventricular cells. When I\(_{\text{NCX}}\) was recorded using a protocol similar to the previous study using Ni\(^{2+}\) (25, 30, 33), a nonselective blocker of the exchanger, the amplitude of Ni\(^{2+}\)-sensitive membrane current apparently increased after the application of isoproterenol, suggesting an increase in I\(_{\text{NCX}}\). However, this potentiation was in fact attributable to an increase in I\(_{\text{CFTR-Cl}}\) contaminating the Ni\(^{2+}\)-sensitive current.

Part of this study was presented at the 48th annual meeting of the Biophysical Society (31).

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METHODS

Cell isolation. Ventricular myocytes were dissociated from guinea pig, mouse, and rat hearts. All procedures were approved by the Animal Research Committee of the Graduate School of Medicine, Kyoto University. The animals were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (>0.1 mg/g body wt). For guinea pig hearts, the thorax was opened under artificial respiration and the aorta was cannulated to start retrograde perfusion of the heart. The heart was quickly excised and mounted on a Langendorff-type perfusion apparatus. The heart was first perfused with a control Tyrode solution and then with a nominally Ca\(^{2+}\)-free control Tyrode solution until the heartbeat stopped, followed by Ca\(^{2+}\)-free Tyrode solution containing 0.4 mg/ml collagenase (type I; Worthington Biochemical) for ∼15 min. Finally, the perfusate was switched to a high-K\(^+\), low-Cl\(^-\) solution. The left ventricle and the septum were diced into ∼10 pieces and shaken gently in the high-K\(^+\), low-Cl\(^-\) solution for ∼5 min. After filtration of the dispersed tissues, the resulting cell suspension was centrifuged (300 rpm, 5 min) and suspended in a modified DMEM solution.

Mouse and rat hearts were perfused with a cell isolation solution containing collagenase (type II, 1 mg/ml; Worthington Biochemical), trypsin (type I, 0.05 mg/ml; Sigma), protease (type XIV, 0.05 mg/ml; Sigma), and 0.2 mM CaCl\(_2\) for 10 min. The left ventricle and the septum were then diced and shaken gently for ∼15 min in the cell isolation solution noted above with BSA (fraction V, 1 mg/ml; Sigma). After filtration, the cell suspension was centrifuged and resuspended in the modified DMEM solution. The myocytes were used for experiments within 8 h.

Electrophysiology. The myocytes were voltage clamped using the whole cell voltage-clamp method with an Axopatch 200B amplifier (Axon Instruments). Current-voltage (I-V) relationships were measured by applying ramp pulses (change in voltage with time = 50 mV/s) as described in our previous study (10). Membrane current was filtered at 1 kHz and digitized at 2 kHz with a 12-bit analog-to-digital converter (ADM 670PCI; Micro Science, Tokyo, Japan), which was controlled using our original software. The ramp voltage pulse was applied every 2 or 6 s as indicated. The holding potential was −40 mV, and the experimental temperature was 36–37°C.

Solutions. The control Tyrode solution contained (in mM) 140 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 0.5 MgCl\(_2\), 0.3 Na\(_2\)HPO\(_4\), 5.5 glucose, and 5.0 HEPES (pH 7.4 with NaOH). The high-K\(^+\), low-Cl\(^-\) solution contained (in mM) 25 KCl, 70 K\(_2\)SO\(_4\), 1.8 CaCl\(_2\), 0.5 MgCl\(_2\), 0.33 Na\(_2\)HPO\(_4\), 25 HEPES, 22 glucose, 1.1-glutamine, and 0.1 EGTA with 0.01 U/ml insulin (pH 7.4 with NaOH). To record \(I_{\text{NCX}}\) or \(I_{\text{CFTR-Cl}}\), K\(^+\) channels, Ca\(^{2+}\) channels and Na\(^-\)K\(^+\) pump were inhibited using tetraethylammonium, Cs\(^+\), Ba\(^{2+}\), nicardipine, and ouabain in a manner similar to methods described in previous studies (10, 22). The compositions of bath and pipette solutions are listed in Table 1. The β-adrenergic receptor signaling cascade was activated by adding 1 μM isoproterenol or 10 μM forskolin to the bath.

In particular, when recording \(I_{\text{CFTR-Cl}}\) (see Fig. 1), bath solution 1 and pipette solution 1 were used. \(I_{\text{NCX}}\) was inhibited by removing Na\(^+\) and Ca\(^{2+}\) from the pipette solution (addition of 10 mM EGTA) and Ca\(^{2+}\) from the bath solution. To record Ni\(^{2+}\)-sensitive \(I_{\text{NCX}}\) (cf. Figs. 2, 4, and 5), bath solution 2 or 3 and pipette solution 2 were used. Free Ca\(^{2+}\) concentration (0.8 μM) was calculated with Win-MAXC (Ref. 4; http://www.stanford.edu/~capton/maxc.html). \(I_{\text{NCX}}\) was defined as the 5 mM Ni\(^{2+}\)-sensitive current recorded under these experimental conditions. For recording extracellular Ca\(^{2+}\)-induced \(I_{\text{NCX}}\), bath solution 4 and pipette solution 3 were used (see Figs. 6 and 7). Free Ca\(^{2+}\) concentration was 0.8 μM. The bath solution was changed from one containing 0.2 mM EGTA and no CaCl\(_2\) to one containing no EGTA and 0.5 (guinea pig and mouse \(I_{\text{NCX}}\)) or 2 (rat \(I_{\text{NCX}}\)) mM Ca\(^{2+}\) with a rapid solution switcher (8, 9). Extracellular Ca\(^{2+}\) was applied for a short period (5 s) every 30 s to avoid intracellular accumulation of Ca\(^{2+}\) and depletion of Na\(^+\) via Na\(^+\)/ Ca\(^{2+}\) exchange.

Statistical analysis. Membrane current was normalized by cell capacitance (pA/pF) and presented as mean (SD). Statistical analysis was performed using one-way repeated-measures ANOVA with the Student-Newman-Keuls test (StatView, SAS Institute). \(P < 0.05\) was considered significant.

RESULTS

Inhibition of isoproterenol-activated \(I_{\text{CFTR-Cl}}\) by Ni\(^{2+}\). In guinea pig ventricular myocytes, β-adrenergic stimulation activates \(I_{\text{CFTR-Cl}}\), whose I-V relationship is similar to that for \(I_{\text{NCX}}\) (1, 12, 21). However, in the previous reports studying the effects of β-adrenergic stimulation on \(I_{\text{NCX}}\) in intact myocytes, activation of \(I_{\text{CFTR-Cl}}\) was not systematically investigated. In this study, we first tested whether Ni\(^{2+}\), which has often been

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TEA, tetraethylammonium.
used for isolating $I_{\text{NCX}}$, affects $I_{\text{CFTR-Cl}}$. In Fig. 1A, $I_{\text{CFTR-Cl}}$ was activated by applying 1 µM isoproterenol, and membrane currents at +50 and −100 mV were plotted against time. The $I-V$ relationship of the isoproterenol-activated current (the difference between $b$ and $a$ in Fig. 1B) showed characteristics of $I_{\text{CFTR-Cl}}$, and the reversal potential ($\sim$34 mV [SD 6] (n = 7)] was close to the calculated equilibrium potential of Cl$^-$ (−33 mV). Under these experimental conditions, $I_{\text{NCX}}$ was effectively inhibited by removing Na$^+$ and Ca$^{2+}$ from the pipette solution (addition of 10 mM EGTA) and by omitting Ca$^{2+}$ from the extracellular solution. Inhibition of $I_{\text{NCX}}$ was confirmed by the finding that 5 mM Ni$^{2+}$ did not attenuate membrane current in the absence of isoproterenol (n = 3, data not shown). In contrast, superfusion of 5 mM Ni$^{2+}$ almost completely and reversibly inhibited $I_{\text{CFTR-Cl}}$ induced by isoproterenol. The half-maximal concentration of Ni$^{2+}$ required to inhibit $I_{\text{CFTR-Cl}}$ was 0.5 mM (Fig. 1C).

It is notable that Ni$^{2+}$ exerted an inhibitory action after a time lag (∼30 s), whereas no delay was observed upon removing Ni$^{2+}$ (see also Fig. 3A). This fact suggests that the Ni$^{2+}$ action was not due to the competition with Cl$^-$ at the channel pore. Indeed, 5 mM (as shown in Fig. 1D; n = 4) or 10 mM Ni$^{2+}$ (n = 4) did not attenuate $I_{\text{CFTR-Cl}}$, which is induced by forskolin, an activator of adenylyl cyclase. Therefore, we concluded that the site of Ni$^{2+}$ action is upstream of adenylyl cyclase in the β-adrenergic signaling cascade, perhaps at the β-adrenergic receptor or related G protein (G$\alpha$). The above experimental findings suggest that if $I_{\text{NCX}}$ is isolated as the Ni$^{2+}$-sensitive current, then $I_{\text{NCX}}$ is overestimated during β-adrenergic stimulation because of contamination of $I_{\text{CFTR-Cl}}$.

Potentiation of Ni$^{2+}$-sensitive current by isoproterenol. The effect of β-adrenergic stimulation on $I_{\text{NCX}}$ was studied with a protocol similar to previous studies by applying 5 mM Ni$^{2+}$ (25, 30, 33) as shown in Fig. 2. Application of 5 mM Ni$^{2+}$ rapidly and reversibly inhibited $I_{\text{NCX}}$ under control conditions. One micromolar isoproterenol increased the membrane conductance such that the amplitude of five millimolar Ni$^{2+}$-sensitive current was larger by 77% in the presence of isoproterenol (d − e in Fig. 2B) than in control (a − b in Fig. 2B).

No significant difference was observed between the reversal potential of the Ni$^{2+}$-sensitive current before and during isoproterenol application. In 29 cells, the degree of isoproterenol activation of the Ni$^{2+}$-sensitive current ranged from −5% to 135% [32% (SD 35)], consistent with previous reports (25, 33). However, it should be noted that Ni$^{2+}$ inhibition in the presence of isoproterenol has both rapid and delayed components in the majority of cells, whereas only the rapid effect is apparent in the control (Fig. 2A).

The time course of the Ni$^{2+}$ action is summarized in Fig. 3. The inhibition of $I_{\text{CFTR-Cl}}$ by Ni$^{2+}$ (Fig. 3A) was characterized by a time lag of ∼30 s on average, and the inhibition time course was variable among cells. No time lag was apparent in the inhibition of $I_{\text{NCX}}$ by Ni$^{2+}$ in the absence of isoproterenol (Fig. 3B). During β-adrenergic stimulation induced by 1 µM isoproterenol, Ni$^{2+}$ rapidly attenuated the membrane current in a manner similar to that of control, followed by a plateau and further inhibition in 19 of 29 cells. The duration of the plateau was close to the observed time lag of Ni$^{2+}$ inhibition of $I_{\text{CFTR-Cl}}$. The delayed component was not clear in the other 10 cells. The amplitude of Ni$^{2+}$-sensitive current during isoproterenol application was significantly larger than that of the control in the group with the delayed component [by 47.4% (SD 35.3); n = 19] but not in the group without the delayed component (Fig. 3D). In the latter case, longer application might be needed to reach a full Ni$^{2+}$ inhibition. The delayed

![Fig. 1](image-url)
component of Ni^2+ action suggested that Ni^2+ also attenuated I_{CFTR-Cl} under this experimental condition.

To decrease the possible contamination of I_{NSC} by I_{CFTR-Cl}, we carried out the same protocol using a lower-Cl− bath solution (bath solution 3) and measured the amplitude of I_{NSC} at a membrane potential near the reversal potential for I_{CFTR-Cl} (Fig. 4). One micromolar isoproterenol did not affect membrane current at +50 mV but increased it at −100 mV (Fig. 4A). Lowering extracellular Cl− concentration shifted the reversal potential of the isoproterenol-induced current to positive potentials (c−b in Fig. 4B) [45 mV (SD 5.5); n = 14], which was clearly different from that of the Ni^2+-sensitive current in control (b−a in Fig. 4B) [−45 mV (SD 3.9); n = 14]. The amplitude of the Ni^2+-sensitive current during isoproterenol application was not significantly different from that in control, when the amplitude was measured at +50 mV (Fig. 4C). The amplitude of the Ni^2+-sensitive current measured at −100 mV tended to be larger in the presence of isoproterenol than that in the control. However, the difference was not statistically significant (data not shown), although isoproterenol significantly increased the membrane current amplitude at −100 mV by 90% (SD 60) (n = 14). The Ni^2+ inhibition of isoproterenol...
action might be weaker under the experimental condition of low extracellular Cl\(^-\).

The effect of \(\beta\)-adrenergic stimulation on \(I_{\text{NCX}}\) was studied further with forskolin (Fig. 5). Because forskolin-activated \(I_{\text{CFTR-Cl}}\) was not attenuated by Ni\(^2+\) (Fig. 1D), the contamination of \(I_{\text{CFTR-Cl}}\) in the recorded Ni\(^2+\)-sensitive \(I_{\text{NCX}}\) should be negligible when adenylate cyclase is activated by forskolin. Ten micromolar forskolin increased membrane conductance, probably because of activation of \(I_{\text{CFTR-Cl}}\) (Fig. 5A). However, the \(I-V\) relationship of Ni\(^2+\)-sensitive \(I_{\text{NCX}}\) during forskolin application was almost identical to control (Fig. 5B), and no statistical significance was found between the amplitude of the Ni\(^2+\)-sensitive current before and during forskolin application (Fig. 5C).

No potentiation of \(I_{\text{NCX}}\) by isoproterenol. The above findings indicated that \(\beta\)-adrenergic receptor stimulation does not increase \(I_{\text{NCX}}\). However, Ni\(^2+\) has multiple nonspecific effects and could conceivably interfere with the detection of an underlying \(\beta\)-adrenergic activation of \(I_{\text{NCX}}\). To rule out this possibility, \(I_{\text{NCX}}\) was isolated as an extracellular Ca\(^2+\)-activated current as previously described (6, 22) instead of using Ni\(^2+\) (Fig. 6). Figure 6A shows 0.5 mM Ca\(^2+\)-induced outward \(I_{\text{NCX}}\) at a holding potential of \(-40\) mV. One micromolar isoproterenol induced an inward shift of the holding current and increased membrane conductance due to activation of \(I_{\text{CFTR-Cl}}\). It should be noted that \(I_{\text{NCX}}\) \((b - a,\) Fig. 6A\) was outward at the membrane potentials examined because of a higher Na\(^+\) concentration in the pipette solution and was clearly different from \(I_{\text{CFTR-Cl}}\) \((c - a,\) Fig. 6A\). Isoproterenol did not change \(I_{\text{NCX}}\) \((d - c,\) Fig. 6A\). Figure 6B shows the time courses of Ca\(^2+\)-induced \(I_{\text{NCX}}\) and membrane current in the absence of extracellular Ca\(^2+\), which reflects \(I_{\text{CFTR-Cl}}\). \(I_{\text{NCX}}\) at the end of isoproterenol application tended to be smaller than the control \((95.5\%\) (SD 13.7) of control, \(n = 10\)), but the difference was not statistically significant (Fig. 6C). We conclude from this and the above-described experiments that \(\beta\)-adrenergic stimulation does not significantly affect \(I_{\text{NCX}}\) in guinea pig ventricular myocytes.

The effect of \(\beta\)-adrenergic stimulation on \(I_{\text{NCX}}\) was studied further in murine and rat ventricular myocytes using the same protocol as described in Fig. 6 and as shown in Fig. 7. In these ventricular cells, 1 \(\mu\)M isoproterenol did not activate \(I_{\text{CFTR-Cl}}\)-consistent with the results in previous studies (18, 24). No significant difference was found between the amplitude of \(I_{\text{NCX}}\) before and during isoproterenol application in either murine or rat myocytes. We concluded that \(\beta\)-adrenergic stimulation has no significant effect on \(I_{\text{NCX}}\) in guinea pig, mouse, or rat ventricular cells.

**DISCUSSION**

We studied the effect of \(\beta\)-adrenergic stimulation on \(I_{\text{NCX}}\) in voltage-clamped guinea pig, mouse, and rat ventricular cells. No significant activation of \(I_{\text{NCX}}\) by \(\beta\)-adrenergic stimulation was detected. When \(I_{\text{NCX}}\) was defined as Ni\(^2+\)-sensitive current in guinea pig ventricular myocytes, it was apparently increased by isoproterenol. However, this augmentation was most likely due to the activation of \(I_{\text{CFTR-Cl}}\), which is also sensitive to Ni\(^2+\). Our results support previous studies (2, 5, 13, 20) and a
Fig. 6. Effect of isoproterenol on the extracellular Ca\(^{2+}\)-induced \(I_{\text{NCX}}\). A, left: membrane current at the holding potential before and during the application of 1 μM isoproterenol. Four consecutive ramp pulses with an interval of 2 s were applied every 30 s. Right: I-V relationships of \(I_{\text{NCX}}\) before (gray trace, b - a) and during (d - c) isoproterenol application and \(I_{\text{CFTR-Cl}}\) (c - a). B: time course of \(I_{\text{NCX}}\) (0 mV, ○) and membrane current recorded in the absence of extracellular Ca\(^{2+}\) (0 mV, □). \(I_{\text{NCX}}\) was determined as the difference current between the membrane current with and without Ca\(^{2+}\). C: comparison of the amplitudes of Ca\(^{2+}\)-induced \(I_{\text{NCX}}\) before (control) and at the end of 1 μM isoproterenol application.

Fig. 7. Effect of isoproterenol on \(I_{\text{NCX}}\) in mouse (A) and rat ventricular cells (B). Left: time course of the Ca\(^{2+}\)-induced \(I_{\text{NCX}}\) (0 mV, ●) and the membrane current without extracellular Ca\(^{2+}\) (0 mV, ○). Center: representative I-V relationships of \(I_{\text{NCX}}\) before (gray trace, a) and at the end (b) of 1 μM isoproterenol application. Right: comparison of the amplitudes of Ca\(^{2+}\)-induced \(I_{\text{NCX}}\) before (control) and at the end of 1 μM isoproterenol. \(I_{\text{NCX}}\) was induced by applying 0.5 mM (A) or 2 mM Ca\(^{2+}\) (B).
recent report of studies of rabbit ventricular cells by Ginsburg and Bers (11).

Using guinea pig ventricular cells, Perchenet et al. (25) and Zhang et al. (33) reported the enhancement of \( \text{IN}_{\text{NCX}} \) by \( \beta \)-adrenergic stimulation. We were able to confirm their experimental results using a similar experimental protocol with \( \text{Ni}^{2+} \) (Fig. 2). However, the apparent enhancement of \( \text{IN}_{\text{NCX}} \) was probably due to the activation of \( I_{\text{CFTR-Cl}} \) in light of the following findings. 1) \( \text{Ni}^{2+} \) inhibited the activation of \( I_{\text{CFTR-Cl}} \) by 1 \( \mu \text{M} \) isoproterenol, with a half-maximum concentration of 0.5 \( \mu \text{M} \), but not activation by 10 \( \mu \text{M} \) forskolin. 2) Forskolin did not augment the amplitude of \( \text{IN}_{\text{NCX}} \). 3) The amplitude of \( \text{IN}_{\text{NCX}} \) was not affected by isoproterenol when measured near the reversal potential of \( I_{\text{CFTR-Cl}} \). 4) Isoproterenol did not significantly affect the extracellular \( \text{Ca}^{2+} \)-induced \( \text{IN}_{\text{NCX}} \). The fact that the \( I-V \) relationships in control and in the presence of both 1 \( \mu \text{M} \) isoproterenol and 10 \( \mu \text{M} \) \( \text{Ni}^{2+} \) were superimposable in the experiments reported by Perchenet et al. (Ref. 25, Fig. 1) suggests that \( I_{\text{CFTR-Cl}} \) was suppressed by \( \text{Ni}^{2+} \) in their experiments. They ruled out the involvement of \( I_{\text{CFTR-Cl}} \) in their study, because 9-anthracencarboxylic acid and glibenclamide did not affect the increases in \( \text{IN}_{\text{NCX}} \) (25). However, the dose of the blockers and the experimental protocols were not clearly stated in their study. We did not study these blockers in the present study, because \( \text{Ni}^{2+} \) clearly attenuated the activation of \( I_{\text{CFTR-Cl}} \) by isoproterenol. One difference between our study and that of Perchenet et al. (25) is the effect of forskolin on \( \text{IN}_{\text{NCX}} \). Although they found an enhancement of \( \text{Ni}^{2+} \)-sensitive \( \text{IN}_{\text{NCX}} \) by forskolin, we failed to detect this. The reason for this discrepancy is not yet clear. However, our finding is consistent with the view that the apparent activation of \( \text{IN}_{\text{NCX}} \) was due to the contamination of \( I_{\text{CFTR-Cl}} \); because \( I_{\text{CFTR-Cl}} \) activated by forskolin was not attenuated by \( \text{Ni}^{2+} \) (Fig. 1D).

We reported that isoproterenol enhanced \( \text{IN}_{\text{NCX}} \) (5 mM \( \text{Ni}^{2+} \)-sensitive current) in pig heart by \( \approx 500 \% \). If \( I_{\text{CFTR-Cl}} \) is also activated by \( \beta \)-adrenergic stimulation in pig ventricular cells, the increase in \( \text{IN}_{\text{NCX}} \) is likely to be overestimated. In mouse and rat ventricular myocytes, we could not detect significant effects of isoproterenol on \( \text{IN}_{\text{NCX}} \) and isoproterenol did not activate a membrane current similar to \( I_{\text{CFTR-Cl}} \). The lack of isoproterenol-activated Cl\(^{-}\) current in mouse and rat ventricular cells is consistent with previous reports (18, 24). In these myocytes, agonists to purinergic receptors activate the Cl\(^{-}\) current (17, 29, 32).

The cardiac Na\(^+\)/Ca\(^{2+}\) exchange protein may indeed be phosphorylated by PKA (16, 26, 27), and its activity may be enhanced by PKA in heterogeneous expression systems (19, 26). However, the enhancement might be due to secondary modulation of Na\(^+\) or Ca\(^{2+}\)-dependent regulation (14, 15) because intracellular Na\(^+\) and Ca\(^{2+}\) were not tightly controlled in these experiments. Further study is needed to clarify the differences between the results in heterogeneous expression systems and those in native cells. To the contrary, the current through the amphibian cardiac NCX, which has a potential nucleotide-binding site (a Walker A motif), seems to be attenuated by the activation of the \( \beta \)-adrenergic receptor (7, 13).

\( \text{Ni}^{2+} \) appears to act upstream of adenylyl cyclase in the \( \beta \)-adrenergic signaling pathway. We (21, 23) and Tareen et al. (28) previously reported that extracellular Na\(^+\) is pivotal for the action of isoproterenol on both \( I_{\text{CFTR-Cl}} \) and L-type Ca\(^{2+}\) current. Replacement of extracellular Na\(^+\) with other cations attenuated the stimulatory action of isoproterenol but did not affect \( I_{\text{CFTR-Cl}} \) activated by forskolin or cAMP (28) as is the case with \( \text{Ni}^{2+} \). The underlying mechanisms of \( \text{Ni}^{2+} \) action and the removal of extracellular Na\(^+\) in \( \beta \)-adrenergic signaling may be similar, although the precise mechanism is not clear at present.

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