Biochemical and Biophysical Research Communications 432 (2013) 46–51

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Nickel inhibits β-1 adrenoceptor mediated activation of cardiac CFTR chloride channels

Palash P. Barman, Hongwei Cheng, Jules C. Hancox *, Andrew F. James *

Cardiovascular Research Laboratories, School of Physiology & Pharmacology and Bristol Heart Institute, University of Bristol, Bristol BS8 1TD, UK

ARTICLE INFO

Article history:
Received 22 January 2013
Available online 31 January 2013

Keywords:
Rabbit cardiomyocytes
PKA-dependent Cl^- current
CFTR
CFTR-inhibitor
Nickel
Ni^{2+}

ABSTRACT

Cardiac ventricular myocytes exhibit a protein kinase A-dependent Cl^- current ($I_{Cl.PKA}$) mediated by the cystic fibrosis transmembrane conductance regulator (CFTR). There is conflicting evidence regarding the ability of the divalent cation nickel (Ni^{2+}), which has been used widely *in vitro* in the study of other cardiac ionic conductances, to inhibit $I_{Cl.PKA}$. Here the action of Ni^{2+} on $I_{Cl.PKA}$ activated by β-adrenergic stimulation has been elucidated. Whole-cell patch-clamp recordings were made from rabbit isolated ventricular myocytes. Externally applied Ni^{2+} blocked $I_{Cl.PKA}$ activated by 1 μM isoprenaline with a log IC_{50} (M) of -4.107 ± 0.075 ($IC_{50} = 78.1$ μM) at +100 mV and -4.322 ± 0.107 ($IC_{50} = 47.6$ μM) at −100 mV. Thus, the block of $I_{Cl.PKA}$ by Ni^{2+} was not strongly voltage dependent. Ni^{2+} applied internally via the patch-pipette was ineffective at inhibiting isoprenaline-activated $I_{Cl.PKA}$, but in the same experiments the current was suppressed by external Ni^{2+} application, indicative of an external site of Ni^{2+} action. In the presence of 1 μM atenolol isoprenaline was ineffective at activating $I_{Cl.PKA}$, but in the presence of the β2-adrenoceptor inhibitor ICI 118,551 isoprenaline still activated Ni^{2+}-sensitive $I_{Cl.PKA}$. Collectively, these data demonstrate that Ni^{2+} ions produce marked inhibition of β1-adrenoceptor activated ventricular $I_{Cl.PKA}$ at submillimolar [Ni^{2+}]: an action that is likely to involve an interaction between Ni^{2+} and β1-adrenoceptors. The concentration-dependence for $I_{Cl.PKA}$ inhibition seen here indicates the potential for confounding effects on $I_{Cl.PKA}$ to occur even at comparatively low Ni^{2+} concentrations, when Ni^{2+} is used to study other cardiac ionic currents under conditions of β-adrenergic agonism.

© 2013 Elsevier Inc. Open access under CC BY-NC-ND license.

1. Introduction

A number of distinct chloride conductances have been identified that may contribute to the normal and pathological function of cardiac myocytes [1,2]. These include swelling-activated Cl^- current [1,2], Ca^{2+}-activated Cl^- current [1,2], anionic background current [3,4] and cAMP/PKA-activated Cl^- current ($I_{Cl.PKA}$) activated by β-adrenergic agonists [2,5,6]. The channels that carry $I_{Cl.PKA}$ are mediated by a cardiac isoform of the cystic fibrosis transmembrane conductance regulator protein (CFTR: [2,7–9]). Sympathetic activation of $I_{Cl.PKA}$ may act to counter the effects of β-adrenergic stimulation of L-type calcium current ($I_{Ca,L}$); consequently $I_{Cl.PKA}$ may contribute to the rate-dependent shortening of ventricular action potentials [10,11]. However, the direct measurement of $I_{Cl.PKA}$ from cardiac cells and of its modulation of action potentials under physiological recording conditions is confounded by a lack of potent and selective pharmacological CFTR inhibitors [2]. Consequently, cardiac $I_{Cl.PKA}$ is usually studied under 'selective' recording conditions, with other overlapping conductances inhibited.

The *in vitro* study of β-adrenergic modulation of some other cardiac ionic conductances is facilitated by the availability of selective pharmacological inhibitors [12,13], which in principle allows these to be separated from β-adrenoceptor activation of $I_{Cl.PKA}$. However, this is not necessarily the case for all the ion currents of cardiac myocytes. The electrogenic Na^+–Ca^{2+} exchanger (NCX) is present throughout the heart and plays an important role in Ca^{2+} ion handling and in shaping cardiac action potentials [14,15]. Similar to $I_{Cl.PKA}$, cardiac NCX current (I_{NCX}) is difficult to study under normal physiological conditions due to a lack of NCX-selective pharmacology. Direct measurements of I_{NCX} have therefore tended to involve the inhibition of overlapping voltage and time-dependent conductances and I_{NCX} measurement as current sensitive to millimolar concentrations of nickel ions (Ni^{2+}) [16–18]. Selective measurement conditions for cardiac I_{NCX} exclude overlapping $I_{Cl.PKA}$ in the absence of PKA stimulation, but in the presence of such stimulation there is potential for both currents to be activated [15,19,20]. The results from some studies are suggestive that the use of Ni^{2+} to study I_{NCX} under conditions of β-adrenergic agonism may be complicated by an inhibitory effect of Ni^{2+} on β-adrenoceptor activated $I_{Cl.PKA}$ [19,20], although other data appear inconsistent with this possibility [21]. The present study was therefore undertaken to determine, under CFTR-selective recording conditions, the

* Corresponding authors. Fax: +44 (0) 117 331 2288.
E-mail addresses: jules.hancox@bristol.ac.uk (J.C. Hancox), a.james@bristol.ac.uk (A.F. James).

0006-291X © 2013 Elsevier Inc. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.bbrc.2013.01.087

response of β-adrenoceptor activated cardiac $I_{Cl.PKA}$ to Ni^{2+}. The results obtained demonstrate a marked, concentration-dependent inhibitory modulation by Ni^{2+} of β1-adrenoceptor mediated $I_{Cl.PKA}$.

2. Methods

Right ventricular cardiomyocytes were isolated from the hearts of Langendorff-perfused male New Zealand White rabbits as described previously [22]. All procedures were approved by the Ethics Committee of University of Bristol and conformed to the UK Animals (Scientific Procedures) Act, 1986. Prior to use, myocytes were stored at 4 °C in Kraft–Brühe (KB) solution [22,23].

2.1. Electrophysiological recording and data acquisition

Whole-cell patch-clamp recordings were made at 37 °C. The data acquisition and recording methods used here have been reported previously [20,24]. Cells were superfused with normal

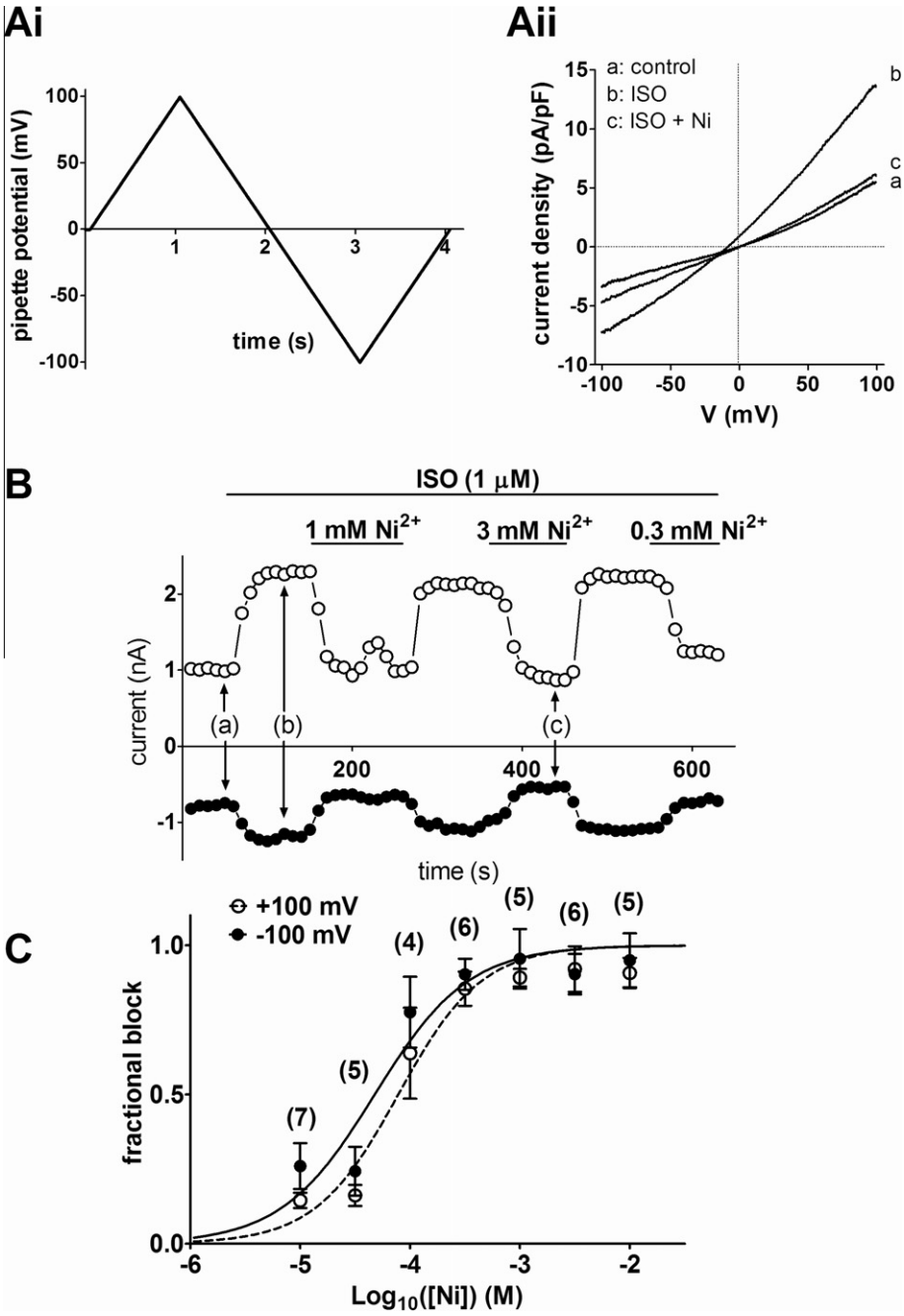

Fig. 1. The effect of extracellular Ni^{2+} on isoprenaline-activated $I_{Cl.PKA}$. (A) Panel (Ai) shows the voltage-ramp protocol (holding potential = 0 mV, frequency of application 1/10 s) used for recording Cl^- currents. Panel (Aii) shows representative currents, plotted against voltage, obtained during the descending phase of the ramp saw-tooth. Letters indicate traces obtained from the time-points indicated in panel (B). (B) Representative time course of an experiment with currents sampled at +100 mV (open circles) and −100 mV (filled circles) during saw-tooth voltage-ramps; the solid bars at the top indicate application of 1 μM isoprenaline (ISO) and Ni^{2+} at the concentrations indicated. (C) Concentration–response relationship of the effect of Ni^{2+} on $I_{Cl.PKA}$. Concentration–responses are shown at +100 mV (open circles) and −100 mV (filled circles). The '*n*' numbers at each respective concentration are shown in parentheses. Solid and dashed lines represent fits to the data with Eq. (2) at −100 mV and +100 mV respectively. The fitted logIC_{50} (M) at +100 and −100 mV were respectively −4.107 ± 0.075 and −4.322 ± 0.101; the n_H values for the fits were 1.145 ± 0.187 at +100 mV and 1.019 ± 0.214 at −100 mV.

Tyrode's solution containing (in mM): 140 NaCl, 5 HEPES, 10 D-glucose, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 1 $BaCl_2$, pH 7.45 with NaOH. CFTR-mediated $I_{Cl.PKA}$ was recorded as reported previously [20] using a Ca^{2+}, K^+-free external solution containing 1 mM $CdCl_2$ and was activated using 1 μM isoprenaline (ISO) [20,25].

Atenolol (Sigma–Aldrich) was made up as a 10 mM stock solution in distilled deionised (Milli-Q) water and was used at a final concentration of 1 μM in external solutions. ICI 118,551 (Tocris, Bristol, UK) was made up as a 4 mM stock solution in deionised water and was used at a final concentration of 100 nM.

2.2. Data analysis and presentation

Data were analyzed using Igor Pro (WaveMetrics, Inc., USA), Clampfit from the pClamp 10.0 software suite (Molecular Devices), Excel 2007 and GraphPad Prism 5 software. Data are presented as mean ± standard error of the mean (SEM), '*n*' values refer to numbers of cells for recordings (typically ⩾ two hearts). Statistical comparisons were made using a Student's paired *t* test and one- or two-way repeated measures (RM) ANOVA. $P < 0.05$ was considered to be statistically significant.

The fractional block of $I_{Cl.PKA}$ by Ni^{2+} ions was calculated from the fraction of β-adrenoceptor-activated current remaining in the presence of Ni^{2+} compared to that activated prior to Ni^{2+} addition, as follows Eq. (1):

$$\text{fractional block} = 1 - (I_{Ni} - I_{Control})/(I_{Iso} - I_{Control}) \quad (1)$$

where the $I_{Control}$, I_{Iso}, and I_{Ni} represent currents in the presence of control, isoprenaline and Ni^{2+} with isoprenaline, respectively.

The half-maximal inhibitory concentration (IC_{50}) of Ni^{2+} was calculated by plotting the mean ± SEM fractional block of $I_{Cl.PKA}$ against the Ni^{2+} concentration and fitting the data with a logistic equation:

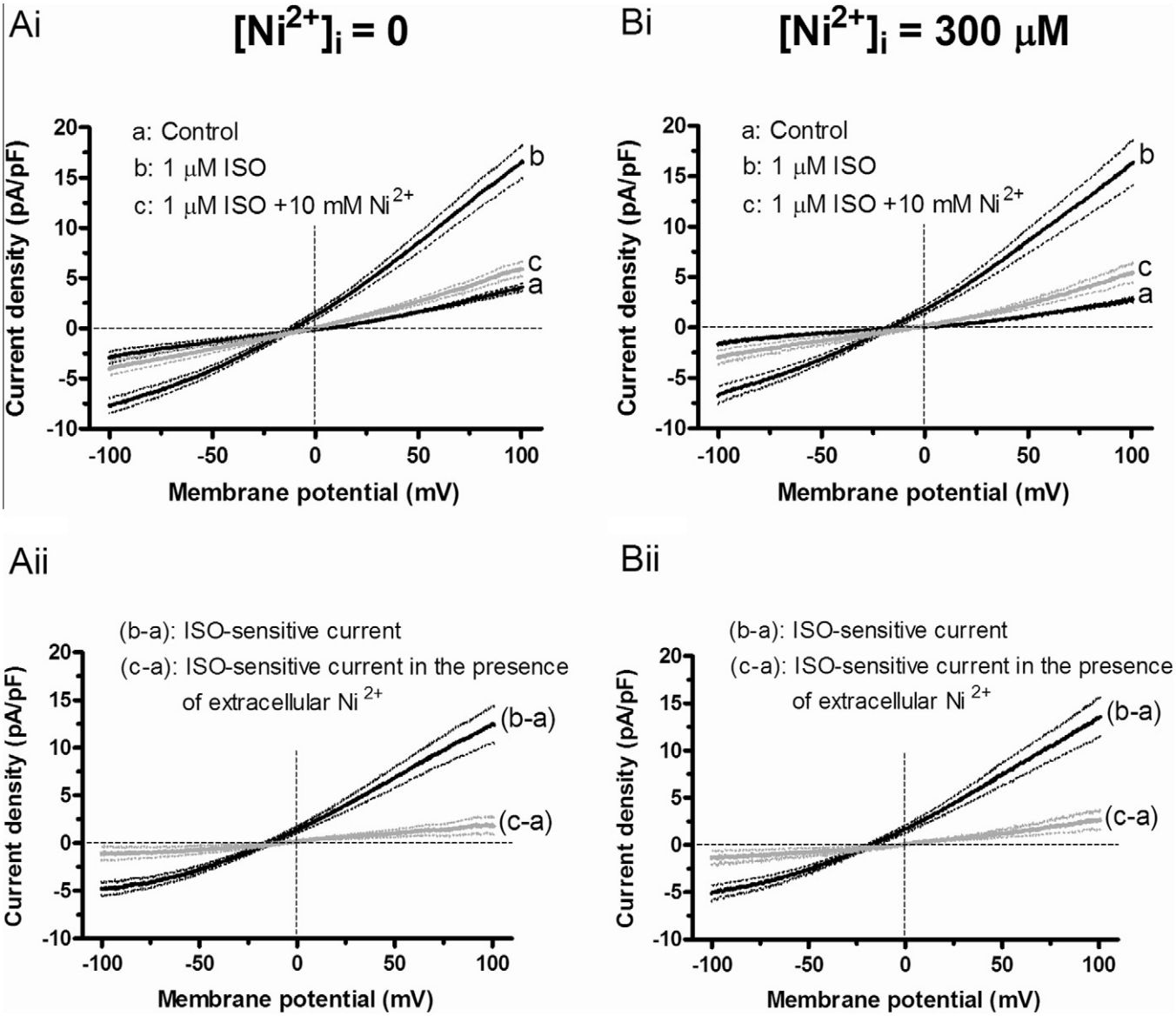

Fig. 2. Intracellular Ni^{2+} does not inhibit ISO-activated $I_{Cl.PKA}$. (Ai) Mean current–voltage relations (mean ± SEM; solid lines shows the mean values and the surrounding dotted lines show SEMs) recorded using pipette solution without intracellular Ni^{2+}. Currents were recorded in control extracellular solution (a), in the presence of 1 μM isoprenaline (ISO) (b) and in the presence of 1 μM ISO plus 10 mM Ni^{2+} (c) ($n = 6$). (Aii) Mean ISO-activated $I_{Cl.PKA}$ calculated from the data shown in panel (Ai). (Bi) Mean current–voltage relations (mean ± SEM; solid lines show the mean values and the surrounding dotted lines shows SEMs) recorded using pipette solution containing intracellular Ni^{2+} (300 μM). Currents were recorded in control extracellular solution (a), in the presence of 1 μM ISO (b) and in the presence of 1 μM ISO plus 10 mM Ni^{2+} (c) ($n = 6$). (Bii) Mean ISO-activated $I_{Cl.PKA}$ calculated from the data shown in panel (Bi).

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10(\text{Log IC}_{50} - X)^{\text{HillSlope}}} \quad (2)$$

where Y, Top and Bottom represent the response, maximal and minimum response to the drug respectively; X represents the logarithm of [Ni^{2+}] (μM).

3. Results and Discussion

Fig. 1(Ai) shows the 'saw-tooth' voltage command protocol used to record CFTR-mediated $I_{Cl.PKA}$[20,25]. It was applied continuously (at a frequency of 0.1 Hz) first in control solution, then in the presence of 1 μM isoprenaline and following subsequent Ni^{2+} ion application in the maintained presence of isoprenaline. Current between +100 mV and −100 mV was measured during the descending ramp phase of the voltage command. Fig. 1(Aii) shows representative currents during this ramp phase, from an individual experiment, plotted against voltage under the three conditions. Both inward and outward current components were increased markedly by isoprenaline, with an accompanying negative shift in zero-current potential, as anticipated for $I_{Cl.PKA}$ activation. In the presence of Ni^{2+} (3 mM), current was restored toward control values, indicative of substantial inhibition of the isoprenaline-activated current component. Fig. 1(B) shows a time-course plot (same experiment as Fig. 1A) of currents at +100 and −100 mV, illustrating (i) the rapid onset and reversal of Ni^{2+} effects and (ii) the fact that Ni^{2+} inhibition of isoprenaline-activated $I_{Cl.PKA}$ exhibited concentration-dependence. Fig. 1C shows concentration–response relations for inhibition by Ni^{2+} of isoprenaline-activated $I_{Cl.PKA}$. The IC_{50} values derived from the plots in Fig. 1(C) were 78.1 μM at +100 mV (95% confidence intervals of 55.0–111.0 μM) and 47.6 μM at −100 mV (95% confidence intervals of 29.7–76.3 μM); thus $I_{Cl.PKA}$ inhibition by Ni^{2+} was not strongly voltage dependent.

The rapid onset and reversal of Ni^{2+} effects evident in Fig. 1(B) indicates that Ni^{2+} was able to reach its site of action rapidly on application. One possible explanation for this rapidity of action is that Ni^{2+} ions may interact directly with an extracellular target to inhibit $I_{Cl.PKA}$. If this is the case, then *internally* applied Ni^{2+} ions should be ineffective at inhibiting $I_{Cl.PKA}$. This possibility was investigated by including Ni^{2+} in intracellular pipette dialysate. The Ni^{2+} concentration used (300 μM) was selected as it was sufficient to inhibit $I_{Cl.PKA}$ by >80% when applied externally (Fig. 1C). The mean current–voltage (I–V) plots in Fig. 2(Ai) and (Bi) show that there was little difference in control currents, those in isoprenaline or those in isoprenaline + externally applied Ni^{2+} between cells dialysed with Ni^{2+}-free and Ni^{2+}-containing pipette solutions. Fig. 2(Aii) and (Bii) show close similarities between isoprenaline-sensitive currents in cells dialysed with Ni^{2+}-free and Ni^{2+}-containing solutions and also in the remaining isoprenaline-sensitive current in the presence of externally applied Ni^{2+}. Thus, without Ni^{2+} in the pipette solution, the isoprenaline-sensitive current at +100 mV was 12.46 ± 1.90 pA/pF (n = 6), and decreased to 1.82 ± 0.91 pA/pF with 10 mM external Ni^{2+}. When 300 μM Ni^{2+} was included in the pipette solution, the isoprenaline-sensitive current at +100 mV was 13.53 ± 2.04 pA/pF (n = 6; P > 0.05, compared with pipette solution without Ni^{2+}), and decreased to 2.63 ± 0.99 pA/pF with 10 mM external Ni^{2+} (P > 0.05, compared with pipette solution without Ni^{2+}). These data demonstrate an external site of Ni^{2+} action to inhibit $I_{Cl.PKA}$. A direct effect of external Ni^{2+} on the CFTR channel mediating $I_{Cl.PKA}$ is precluded from previous observations that when $I_{Cl.PKA}$ is activated by forskolin (to activate adenylate cyclase downstream of the β-adrenoceptor), externally applied Ni^{2+} does not inhibit the current [19,20]. Thus,

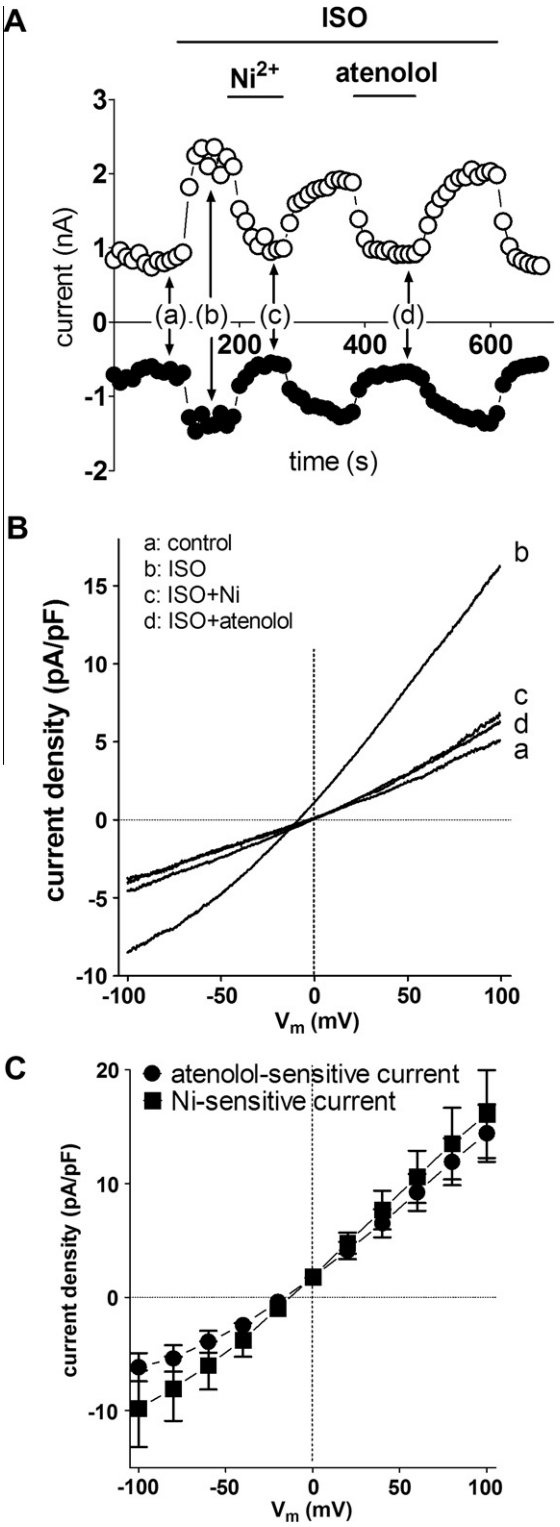

Fig. 3. Involvement of β-1 adrenoceptors in Ni^{2+}-sensitive $I_{Cl.PKA}$. (A) Representative time course of an experiment with currents sampled at +100 mV (open circles) and −100 mV (filled circles) during saw-tooth voltage-ramps; the solid bars at the top indicate application of 1 μM isoprenaline (ISO), 10 mM Ni^{2+} and 1 μM atenolol. (B) Representative current–voltage relations obtained during the descending phase of the ramp saw-tooth. Letters indicates traces obtained as indicated in panel (A). (C) Mean current–voltage relations for the Ni^{2+}-sensitive (n = 5) and atenolol-sensitive (n = 5) difference currents in the presence of 1 μM ISO. Bars indicate ±SEM. There was no statistically significant difference between the mean Ni^{2+}-sensitive and atenolol-sensitive current–voltage relations.

Ni^{2+} is most likely to exert its inhibitory action by direct interaction with β-adrenoceptors.

In order to determine whether β1 or β2 adrenoceptors are the likely targets of Ni^{2+}, isoprenaline was applied in the presence of β1 or β2 adrenoceptor inhibitors. Fig. 3 shows the results of experiments with the β1-adrenoceptor inhibitor atenolol. Fig. 3(A) and (B) show that 1 μM atenolol application produced a rapid inhibition of $I_{Cl,PKA}$ that was similar to that produced by 10 mM Ni^{2+}, with current in the presence of each agent close to that in control solution. Fig. 3(C) shows that mean *I–V* relations for Ni^{2+}-sensitive and atenolol-sensitive currents (i.e. the isoprenaline-activated $I_{Cl.PKA}$ inhibited by atenolol and Ni^{2+}) were closely superimposed. These observations implicate β1 adrenoceptors in the activation of Ni^{2+} sensitive $I_{Cl.PKA}$. In further experiments, isoprenaline was applied subsequent to application of the β2 adrenoceptor antagonist, ICI 118,551. As shown in Fig. 4(A) and (B), ICI 118,551 (at a substantial concentration of 100 nM) did not inhibit the ability of isoprenaline to activate $I_{Cl.PKA}$, nor was the ability of Ni^{2+} to inhibit isoprenaline-activated current impaired. Fig. 4(C) compares fractional inhibition of isoprenaline-activated $I_{Cl.PKA}$ by 10 mM Ni^{2+} in samples of cells treated with ICI 118,551 to cells not exposed to this agent ('control' in Fig. 4C) at both a positive and negative voltage during the descending ramp of the saw-tooth command: there was no significant difference between ICI 118,551 and control. Considered together with the data in Fig. 3, these observations demonstrate that Ni^{2+}-sensitive isoprenaline-activated $I_{Cl.PKA}$ was mediated by β1 adrenoceptor activation.

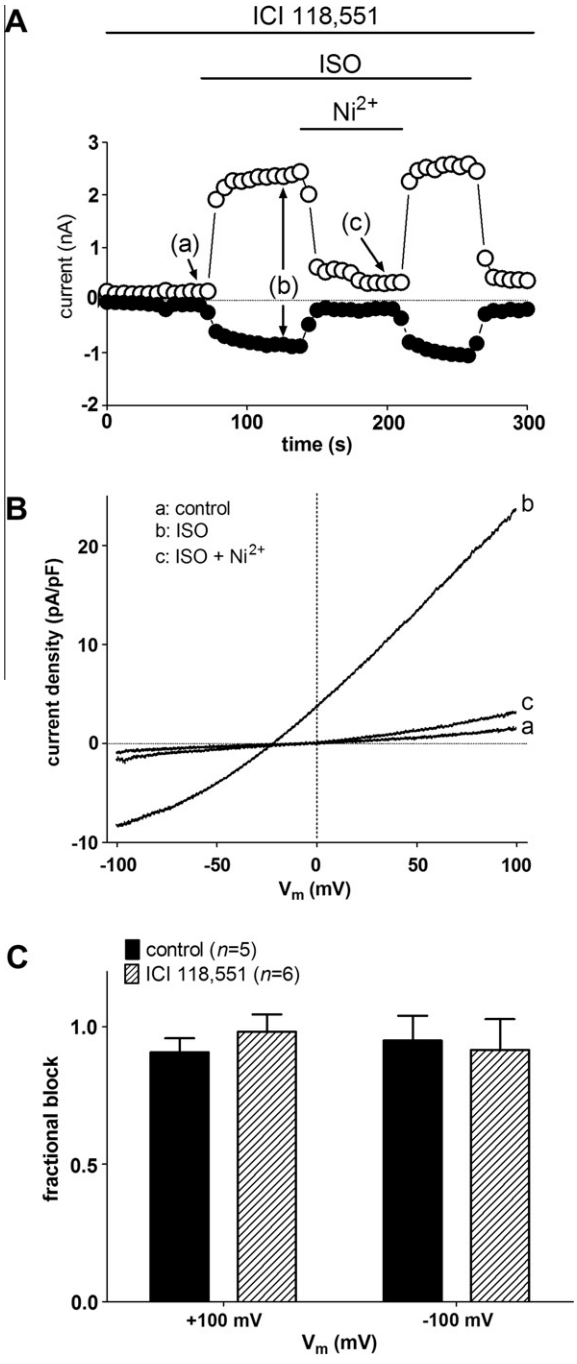

Fig. 4. β-2 adrenoceptors do not activate Ni^{2+}-sensitive $I_{Cl.PKA}$. (A) Representative time course of an experiment with currents sampled at +100 mV (open circles) and −100 mV (filled circles) during saw-tooth voltage-ramps; the solid bars at the top indicates application of 100 nM ICI 118,551, 1 μM isoprenaline (ISO) and 10 mM Ni^{2+}. (B) Representative current–voltage relations obtained during the descending phase of the ramp saw-tooth. Letters indicate traces obtained as indicated in panel (A). (C) Fractional block by 10 mM Ni^{2+} of ISO-activated $I_{Cl.PKA}$ at +100 mV and −100 mV in the absence (control) and presence of 100 nM ICI 118,551. ICI 118,551 had no statistically significant effect on fractional block at either voltage. Replicate numbers are given in parentheses.

The findings of the present study are inconsistent with a lack of Ni^{2+} modulation of $I_{Cl.PKA}$[21] and instead support and extend evidence for an inhibitory effect of Ni^{2+} on CFTR-encoded $I_{Cl.PKA}$, when activated via β-adrenergic stimulation [19,20]. This study reports for the first time the concentration-dependence of this effect and the β-adrenoceptor subtype responsible. Ni^{2+} has been reported to inhibit ventricular I_{NCX} with a K_D of ~290 μM in electrophysiological experiments with a cAMP-free pipette dialysate and of ~160 μM with raised (100 μM) cAMP [18], values higher than the IC_{50} values for inhibition of $I_{Cl.PKA}$ found in this study. The concentration-dependence of Ni^{2+} inhibition of $I_{Cl.PKA}$ observed here therefore indicates strong overlap between concentration-dependent inhibition of I_{NCX} and of β1 adrenoceptor activated $I_{Cl.PKA}$. Thus, an important consideration for the future study of β-adrenoceptor-mediated modulation of I_{NCX} is that this would best be undertaken under conditions in which recording solutions are chosen that either preclude CFTR activation, or in which alternative approaches (different NCX inhibitors to Ni^{2+} or ion replacement) are used to isolate I_{NCX}.

Ni^{2+} inhibits cardiac L-type Ca current ($I_{Ca,L}$) in electrophysiological experiments with a K_D of ~330–530 μM (the higher value with raised cAMP in the pipette dialysate) [26], and produces marked effects at submillimolar concentrations on the magnitude and kinetics of Kv1.5 channel current [27,28] and upon hERG channel activation kinetics [29]. These actions occur in the absence of β-adrenoceptor activation and, in contrast to the effect on β-adrenoceptor activated $I_{Cl.PKA}$ shown here, involve direct interactions between Ni^{2+} ions and the affected channels. However, extracellular divalent ions have also been reported to modify the neuromuscular junction acetylcholine receptor-channel complex [30] and can influence ligand binding to G-protein coupled receptors [31,32]. Zn^{2+} and Cu^{2+} ions have been reported to interact with α(1A) adrenoceptors with micromolar affinity and to act as allosteric modulators [33], whilst Mg^{2+} ions have been reported to decrease agonist affinity for platelet β-adrenoceptors [34]. Thus, the inhibitory effect of Ni^{2+} on $I_{Cl.PKA}$ reported here appears likely to result either from effects of the cation on isoprenaline binding to β1 adrenoceptors, or upon subsequent coupling between the receptor and adenylate cyclase. Future work to determine the underlying mechanism of this effect of extracellular Ni^{2+} is warranted. Perhaps most significantly, the findings of this study indicate that care is required in the use of Ni^{2+} in the study of cardiac conductances, particularly under conditions of sympathetic agonism, during which effects on receptor-activation of ionic

current may confound interpretation of direct effects of the cation on ion channels or transporters.

Acknowledgments

PPB and HWC were supported by the British Heart Foundation (FS/07/062; PG/11/24).

References

[1] S. Sorota, Insights into the structure, distribution and function of the cardiac chloride channels, Cardiovasc. Res. 42 (1999) 361–376.
[2] A.W. Mulvaney, C.I. Spencer, S. Culliford, J.J. Borg, S.G. Davies, R.Z. Kozlowski, Cardiac chloride channels: physiology, pharmacology and approaches for identifying novel modulators of activity, Drug Discov. Today 5 (2000) 492–505.
[3] C.I. Spencer, W. Uchida, R.Z. Kozlowski, A novel anionic conductance affects action potential duration in isolated rat ventricular myocytes, Br. J. Pharmacol. 129 (2000) 235–238.
[4] H. Li, H. Zhang, J.C. Hancox, R.Z. Kozlowski, An outwardly rectifying anionic background current in atrial myocytes from the human heart, Biochem. Biophys. Res. Commun. 359 (2007) 765–770.
[5] A. Bahinski, D.C. Gadsby, P. Greengard, A.C. Nairn, Chloride conductance regulated by protein kinase A in isolated guinea-pig ventricular myocytes, J. Physiol. 418 (1989) 32P.
[6] R.D. Harvey, J.R. Hume, Autonomic regulation of a chloride current in heart, Science 244 (1989) 983–985.
[7] G. Nagel, T.C. Hwang, K.L. Nastiuk, A.C. Nairn, D.C. Gadsby, The protein kinase A-regulated cardiac Cl-channel resembles the cystic fibrosis transmembrane conductance regulator, Nature 360 (1992) 81–84.
[8] P. Hart, J.D. Warth, P.C. Levesque, M.L. Collier, Y. Geary, B. Horowitz, J.R. Hume, Cystic fibrosis gene encodes a cAMP-dependent chloride channel in heart, Proc. Natl. Acad. Sci. USA 93 (1996) 6343–6348.
[9] A.F. James, T. Tominaga, Y. Okada, M. Tominaga, Distribution of cAMP-activated chloride current and CFTR mRNA in the guinea pig heart, Circ. Res. 79 (1996) 201–207.
[10] R.D. Harvey, C.D. Clark, J.R. Hume, Chloride current in mammalian cardiac myocytes. Novel mechanism for autonomic regulation of action potential duration and resting membrane potential, J. Gen. Physiol. 95 (1990) 1077–1102.
[11] M. Kuzumoto, A. Takeuchi, H. Nakai, C. Oka, A. Noma, S. Matsuoka, Simulation analysis of intracellular Na+ and Cl-homeostasis during beta 1-adrenergic stimulation of cardiac myocyte, Prog. Biophys. Mol. Biol. 96 (2008) 171–186.
[12] E. Carmeliet, K. Mubagwa, Antiarrhythmic drugs and cardiac ion channels: mechanisms of action, Prog. Biophys. Mol. Biol. 70 (1998) 1–72.
[13] J. Tamargo, R. Caballero, R. Gomez, C. Valenzuela, E. Delpon, Pharmacology of cardiac potassium channels, Cardiovasc. Res. 62 (2004) 9–33.
[14] K.D. Philipson, D.A. Nicoll, Sodium–calcium exchange: a molecular perspective, Annu. Rev. Physiol. 62 (2000) 111–133.
[15] Y.H. Zhang, J.C. Hancox, Regulation of cardiac Na^+–Ca^{2+} exchanger activity by protein kinase phosphorylation-still a paradox?, Cell Calcium 45 (2009) 1–10
[16] J. Kimura, S. Miyamae, A. Noma, Identification of sodium–calcium exchange current in single ventricular cells of guinea-pig, J. Physiol. 384 (1987) 199–222.
[17] T. Ehara, S. Matsuoka, A. Noma, Measurement of reversal potential of Na–Ca exchange current in single guinea-pig ventricular cells, J. Physiol. 410 (1989) 227–249.
[18] A.K. Hinde, L. Perchenet, I.A. Hobai, A.J. Levi, J.C. Hancox, Inhibition of Na/Ca exchange by external Ni in guinea-pig ventricular myocytes at 37°C, dialysed with cAMP free and cAMP containing solutions, Cell Calcium 25 (1999) 321–331.
[19] X. Lin, H. Jo, Y. Sakakibara, K. Tambara, B. Kim, M. Komeda, S. Matsuoka, Beta-adrenergic stimulation does not activate Na^+/Ca^{2+} exchange current in guinea pig, mouse, and rat ventricular myocytes, Am. J. Physiol. 290 (2006) C601–C608.
[20] P. Barman, S.C. Choisy, J.C. Hancox, A.F. James, Beta-adrenoceptor/PKA-stimulation, Na^+–Ca^{2+} exchange and PKA-activated Cl^- currents in rabbit cardiomyocytes: a conundrum, Cell Calcium 49 (2011) 233–239.
[21] K.S. Ginsburg, D.M. Bers, Isoproterenol does not enhance Ca-dependent Na/Ca exchange current in intact rabbit ventricular myocytes, J. Mol. Cell. Cardiol. 39 (2005) 972–981.
[22] J.C. Hancox, A.J. Levi, C.O. Lee, P. Heap, A method for isolating rabbit atrioventricular node myocytes which retain normal morphology and function, Am. J. Physiol. 265 (1993) H755–H766.
[23] G. Isenberg, U. Klockner, Calcium tolerant ventricular myocytes prepared by incubation in a "KB medium", Pflugers Arch. 395 (1982) 6–18.
[24] H. Cheng, G.L. Smith, C.H. Orchard, J.C. Hancox, Acidosis inhibits spontaneous activity and membrane currents in myocytes isolated from the rabbit atrioventricular node, J. Mol. Cell. Cardiol. 46 (2009) 75–85.
[25] P.P. Barman, S.C. Choisy, H.C. Gadeberg, J.C. Hancox, A.F. James, Cardiac ion channel current modulation by the CFTR inhibitor GlyH-101, Biochem. Biophys. Res. Commun. 408 (2011) 12–17.
[26] I.A. Hobai, J.C. Hancox, A.J. Levi, Inhibition by nickel of the L-type Ca channel in guinea pig ventricular myocytes and effect of internal cAMP, Am. J. Physiol. 279 (2000) H692–H701.
[27] D.C. Kwan, C. Eduljee, L. Lee, S. Zhang, D. Fedida, S.J. Kehl, The external K^+ concentration and mutations in the outer pore mouth affect the inhibition of Kv1.5 current by Ni^{2+}, Biophys. J. 86 (2004) 2238–2250.
[28] Y.M. Cheng, D. Fedida, S.J. Kehl, Kinetic analysis of the effects of H^+ or Ni^{2+} on Kv1.5 current shows that both ions enhance slow inactivation and induce resting inactivation, J. Physiol. 588 (2010) 3011–3030.
[29] W.K. Ho, I. Kim, C.O. Lee, J.B. Youm, S.H. Lee, Y.E. Earm, Blockade of HERG channels expressed in *Xenopus laevis* oocytes by external divalent cations, Biophys. J. 76 (1999) 1959–1971.
[30] K.L. Magleby, M.M. Weinstock, Nickel and calcium ions modify the characteristics of the acetylcholine receptor-channel complex at the frog neuromuscular junction, J. Physiol. 299 (1980) 203–218.
[31] P. Ernsberger, D.C. U'Prichard, Modulation of agonist and antagonist interactions at kidney α_1-adrenoceptors by nucleotides and metal ions, Eur. J. Pharmacol. 133 (1987) 165–176.
[32] E. Hermans, R.A. Challiss, Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors, Biochem. J. 359 (2001) 465–484.
[33] J. Ciolek, A. Maiga, E. Marcon, D. Servent, N. Gilles, Pharmacological characterization of zinc and copper interaction with the human alpha(1A)-adrenoceptor, Eur. J. Pharmacol. 655 (2011) 1–8.
[34] N. Cook, S.R. Nahorski, D.B. Barnett, (−)-[125]pindolol binding to the human platelet beta-receptor: characterization and agonist interactions, Eur. J. Pharmacol. 113 (1985) 247–254.