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

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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.

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

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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

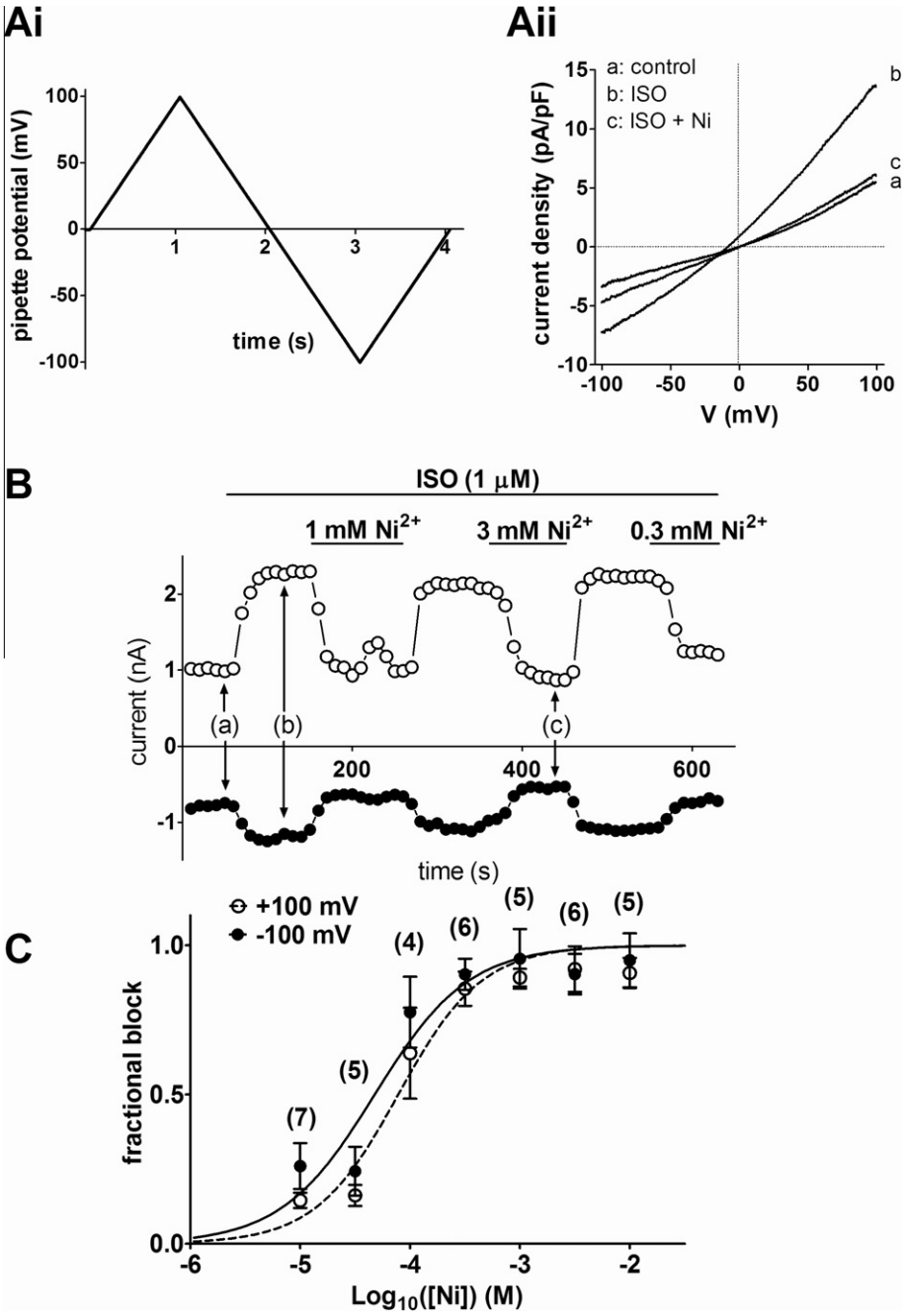

Fig. 1. The effect of extracellular Ni^{2+} on isoprenaline-activated $I_{Cl.PKA}$. (A) Panel (Ai) shows the voltage-ramp protocol (holding potential = 0 mV, frequency of application 1/10 s) used for recording Cl^- currents. Panel (Aii) shows representative currents, plotted against voltage, obtained 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.