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Enhanced inhibitory effect of acidosis on hERG potassium channels that incorporate the hERG1b isoform

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ABSTRACT

Extracellular acidosis occurs in the heart during myocardial ischemia and can lead to dangerous arrhythmias. Potassium channels encoded by *hERG* (*human ether-à-go-go-related gene*) mediate the cardiac rapid delayed rectifier K^+ current (I_{Kr}), and impaired hERG function can exacerbate arrhythmia risk. Nearly all electrophysiological investigations of hERG have centred on the hERG1a isoform, although native I_{Kr} channels may be comprised of hERG1a and hERG1b, which has a unique shorter N-terminus. This study has characterised for the first time the effects of extracellular acidosis (an extracellular pH decrease from 7.4 to 6.3) on hERG channels incorporating the hERG1b isoform. Acidosis inhibited hERG1b current amplitude to a significantly greater extent than that of hERG1a, with intermediate effects on coexpressed hERG1a/1b. I_{hERG} tail deactivation was accelerated by acidosis for both isoforms. hERG1a/1b activation was positively voltage-shifted by acidosis, and the fully-activated current–voltage relation was reduced in amplitude and right-shifted (by ~10 mV). Peak $I_{hERG1a/1b}$ during both ventricular and atrial action potentials was both suppressed and positively voltage-shifted by acidosis. Differential expression of hERG isoforms may contribute to regional differences in I_{Kr} in the heart. Therefore inhibitory effects of acidosis on I_{Kr} could also differ regionally, depending on the relative expression levels of hERG1a and 1b, thereby increasing dispersion of repolarization and arrhythmia risk.

1. Introduction

Ion channels responsible for the 'rapid' delayed rectifier potassium current (I_{Kr}) play an important role in electrical repolarization of the atria and ventricles of the heart [1–3]. The *human ether-à-go-go related gene* (*hERG*, alternative nomenclature *KCNH2*) is generally accepted to encode a protein responsible for I_{Kr} channels [1,2]. Loss-of-function *hERG* mutations are responsible for the LQT2 form of congenital long QT syndrome [2,4], whilst a gain-of-function mutation underlies variant 1 of the congenital short QT syndrome [5]. Inhibition of I_{Kr}/hERG by diverse pharmacological agents is strongly associated with drug-induced long QT syndrome [1–3].

Several hERG channel isoforms exist [6]. Whilst the majority of electrophysiological and pharmacological investigations of hERG in respect of the heart have focused on the "hERG1a" isoform, accumulating evidence suggests that native cardiac I_{Kr} channels are comprised of hERG1a together with an alternative transcript called "hERG1b" [7–12]. hERG1b possesses a truncated and unique N-terminus but is otherwise identical to hERG1a [6]. It is present in human ventricles and atria [9,13] and appears to be localised to the T tubules in ventricular myocytes [9]. Homomeric hERG1b is comparatively poorly expressed due to an N-terminal 'RXR' endoplasmic reticulum (ER) retention signal, but N-terminal interactions with hERG1a appear to facilitate hetero-oligomerization and trafficking to the surface membrane of heteromeric hERG1a/1b channels [10,11]. The N-terminus of hERG1a influences deactivation gating [14,15] and is able to interact with the channel's internal S4–S5 linker region to stabilise the open state during channel gating [14]. Consequently, ionic current (I_{hERG}) carried by heteromeric hERG1a/1b exhibits altered kinetics compared to that carried by hERG1a alone, with deactivation kinetics that may be closer to those of native I_{Kr} than those of hERG1a [12,13,16]. At present, there are few comparative data available on the pharmacological or physiological modulation of heteromeric hERG1a/1b, but recent reports suggest differences between hERG1a and hERG1a/1b in sensitivity to the selective I_{Kr} blocker E-4031 [12] and in regulation of rat ERG1a/1b compared to ERG1a by cGMP signalling [17]. It is possible that regional differences in the relative proportions of hERG1a and hERG1b could account for regional differences in I_{Kr} properties [16].

Extracellular acidosis occurs in the heart under a number of pathological conditions including myocardial ischemia and reperfusion [18]; it can alter the function of ion channels and predispose the heart to dangerous arrhythmias [19]. The effects of acidosis on

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hERG1a channels have been investigated, with decreases in current amplitude and accelerated deactivation both reported [20–28]. However, despite the emerging evidence for a physiological role for hERG1b in native cardiac I_{Kr}, to our knowledge no study has hitherto investigated the effects of acidosis on K^+ current mediated by hERG1b. Thus, it is not known whether or not channels incorporating this isoform respond similarly to acidosis as those mediated by hERG1a alone. We report here, for the first time, effects of acidosis on hERG channels incorporating the hERG1b isoform and demonstrate that hERG1b exhibits greater sensitivity to inhibition by extracellular acidosis than does the hERG1a isoform.

2. Materials and methods

2.1. Maintenance of hERG expressing CHO cells

hERG1b in pcDNA 3.1 was generously donated by Dr. Gail Robertson (University of Wisconsin). Chinese Hamster Ovary (CHO) cells were maintained in Kaighn's modification of Ham's F12 (F12-K) medium (GIBCO), supplemented with 10% foetal bovine serum (GIBCO) and 50 μg ml^{-1} gentamycin (GIBCO) and kept in a 5% CO_2 incubator (Hereaus) at 37 °C [27]. Cells were passaged and plated onto small sterilised glass coverslips in 40 mm petri dishes. After 24 h, cells were co-transfected with hERG and Green Fluorescent Protein at a ratio of 2:1 as described previously [29]. For hERG1a and 1b co-expression, hERG1a and 1b were co-transfected at a ratio of 1:1 [29]. Cells were incubated at 37 °C for at least 1 day prior to electrophysiological study [27,29].

2.2. Electrophysiological recording

Data acquisition and recording methods used were identical to those employed in recent studies from our laboratory [27,29]. Briefly, whole-cell voltage clamp recordings were made at 37 °C with an external solution containing (in mM): 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Glucose and 5 HEPES (titrated to pH 7.45 with NaOH). The pipette solution contained (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP and 10 HEPES (titrated to pH 7.2 with KOH). Patch pipette resistance ranged from 2–4 MΩ. Series resistance values typically lay between 3 and 7 MΩ and were compensated by ~60–80%. Acidic external solution was produced using normal HEPES-buffered Tyrode's solution titrated to pH 6.3 with HCl [27]. This pH, employed previously in our laboratory in the investigation of effects of acidosis on hERG1a [27], falls within the range of values reported in the myocardium during acute ischemia [30]. Acidic external solution was made up fresh on each experimental day. External superfusate was exchanged using an in-house built device capable of exchanging external solution in <1 s. Data digitization rates were 10 kHz during all protocols and an appropriate bandwidth of 2 kHz was set on the amplifier. The action potential (AP) waveforms used for 'AP clamp' experiments (Fig. 4) were identical to those described in [31].

2.3. Data analysis

Data were analysed using Clampfit 10.0 (Axon Instruments), Excel 2003, Origin 7 and Prism v3 (Graphpad Inc.) software. Data are presented as the mean ± standard error of the mean (SEM) and fits to the data employed standard exponential or Boltzmann functions. Statistical comparisons were made using a Student's *t*-test or one-way or two-way analysis of variance (ANOVA) followed by a Bonferroni post-test, as appropriate. *P* values of less than 0.05 were taken as being statistically significant.

3. Results and discussion

Fig. 1 shows the effect of reducing extracellular pH from 7.4 to 6.3 on I_{hERG} carried by hERG1a, hERG1b and hERG1a/1b channels, respectively. The voltage protocol shown in the lower traces in Fig. 1Ai–Aiii was used (*cf* [27,29]). At pH 7.4, each of hERG1a, hERG1b, and hERG1a/1b exhibited outward I_{hERG} on depolarization, followed by a deactivating 'tail' current on membrane potential repolarization to −40 mV. As anticipated [7,8,12,29], the rate of decline of the I_{hERG} tail was markedly slower for hERG1a than for either hERG1b or hERG1a/1b, whilst the tail current amplitude was greater in relation to the end-pulse current for hERG1a and hERG1a/1b than for hERG1b. In each case, reduction of external pH to 6.3 led to a rapid reduction of both pulse and tail current amplitude and also to a marked acceleration of tail current decline. Fig. 1B shows the mean level of inhibition of end-pulse I_{hERG} for each of the channels studied. End-pulse current inhibition of hERG1b was almost twice that of hERG1a and was also significantly greater than that of hERG1a/1b. The difference between extent of inhibition by acidosis for hERG1a and 1b was even greater for the I_{hERG} tail (Fig. 1C; with ~25% and ~64% tail current inhibition of hERG1a and 1b, respectively), with hERG1a/1b showing a response intermediate between the two. These data demonstrate that acidosis produces differential inhibition of I_{hERG} carried by hERG1a and 1b isoforms. The greater inhibitory effect of lowering extracellular pH on channels incorporating hERG1b seen here contrasts markedly with the reduced inhibitory potency of the selective hERG/I_{Kr} inhibitor E-4031 on hERG1a/1b compared to hERG1a [12].

The effect of acidosis on I_{hERG} tail deactivation rate was quantified by bi-exponential fitting of I_{hERG} tail current decline (Fig. 2). At pH 7.4 both fast and slow deactivation time-constants were greater for hERG1a than for hERG1b and hERG1a/1b, whilst hERG1a also exhibited a smaller proportion of fast deactivation than did hERG1b or hERG1a/1b, in which the faster process predominated (compare Fig. 2Aiii with Biii and Ciii). For each of the three expression conditions, pH 6.3 accelerated I_{hERG} deactivation, with both fast and slow time-constants of deactivation being markedly reduced. For hERG1a, the proportionate contribution of fast deactivation was also markedly increased at low pH (Fig. 2Aiii); this was not the case for either hERG1b (Fig. 2Biii) or 1a/1b (Fig. 2Ciii), for which fast deactivation already predominated at pH 7.4. These observations show for the first time that the shorter hERG1b isoform (819 amino-acids compared to 1159 for hERG 1a [6,12]), with its comparatively fast deactivation kinetics, responded to acidosis with a further acceleration in deactivation; they also implicate regions distal to the initial N-terminal segment of hERG1a channels in the modulatory effect of acidosis on I_{hERG} deactivation (*cf* [21]) .

Additional experiments elucidated further the effects of acidosis on I_{hERG} kinetics. These experiments focused on hERG1a/1b, because heteromeric hERG1a/1b (but not hERG1b alone) is proposed to contribute to native I_{Kr} [9–12]. The results of these experiments showed that differences between hERG1a and hERG1a/1b in the extent of current suppression by low pH could not be accounted for by a differential effect of acidosis on the voltage-dependence of I_{hERG} (examined via analysis of normalized tail current–voltage (I-V) relations [27] (Fig. 3Ai)). Thus, the half-maximal activation voltage ($V_{0.5}$) for I_{hERG} carried by hERG1a/1b was shifted rightwards by ~+6.6 mV, which is similar to that seen previously under identical conditions for hERG1a alone (~+5.6 mV [27]). The voltage-dependence of the inhibition of end pulse and tail currents produced by lowering pH from 7.4 to 6.3 was also determined (Fig. 3Aii). The two relations were similar at test potentials between −40 and ~ 0 mV, diverging at more positive voltages. As the region of maximum divergence included voltages at which