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Original article

Acidosis inhibits spontaneous activity and membrane currents in myocytes isolated from the rabbit atrioventricular node

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ABSTRACT

Recent evidence from intact hearts suggests that the function of cardiac nodal tissue may be particularly susceptible to acidosis. Little is currently known, however, about the effects of acidosis on the cellular electrophysiology of the atrioventricular node (AVN). This study was conducted, therefore, to determine the effect of acidosis on the spontaneous activity and membrane currents of myocytes isolated from the rabbit AVN, recorded at 35–37 °C using whole-cell patch-clamp. Reduction of extracellular pH (pH_e; from 7.4 to 6.8 or 6.3) produced pH-dependent slowing of spontaneous action potential rate and upstroke velocity, and reductions in maximum diastolic potential and action potential amplitude. Ionic current recordings under voltage-clamp indicated that acidosis (pH_e 6.3) decreased L-type Ca current ($I_{Ca,L}$), without significant changes in voltage-dependent activation or inactivation. Acidosis reduced the E-4031-sensitive, rapid delayed rectifier current (I_{Kr}) tail amplitude at −40 mV following command pulses to between −30 and +50 mV, and accelerated tail-current deactivation. In contrast, the time-dependent hyperpolarisation-activated current, I_f, was unaffected by acidosis. Background current insensitive to E-4031 and nifedipine was reduced by acidosis. Measurement of intracellular pH (pH_i) from undialysed cells using BCECF showed a reduction in mean pH_i from 7.24 to 6.45 (n=17) when pH_e was lowered from 7.4 to 6.3. We conclude that I_f is unlikely to be involved in the response of the AVN to acidosis, whilst inhibition of $I_{Ca,L}$ and I_{Kr} by acidosis are likely to play a significant role in effects on AVN cellular electrophysiology.

1. Introduction

The heart can be exposed to acidosis in a number of pathological conditions, such as myocardial ischaemia or systemic acidosis. In these situations, acidosis may be responsible for deleterious functional changes including decreased cardiac contractility and the development of arrhythmias associated with early after-depolarisations (EADs), delayed after-depolarisations (DADs), and the development of re-entrant circuits [1–4]. Whilst the effects of acidosis on ventricular cells and tissue have been studied extensively [1,4–7], effects on other regions of the heart are less well understood, although it is clear that such effects exist. For example, acidosis reduces the rate of action potential generation and contraction of isolated atria and Purkinje fibres [8,9] and experiments on isolated rat hearts suggest that the most marked effects of acidosis may be on pacemaker and conduction tissues: electrocardiogram measurements show that acidosis produces a substantial reduction in heart-rate and prolongation of the P-R interval, with little effect on the duration of the QRS complex [10]. These actions appear to be independent of possible alterations to neurotransmitter release from autonomic nerve terminals, as they persist in the presence of propranolol and atropine [10]. Data from canine and rabbit sinoatrial nodal (SAN) preparations also suggest a direct effect of pH on sinoatrial nodal electrophysiology [11,12]. Recent experiments employing an isolated right atrial and atrioventricular nodal (AVN) preparation from the rabbit heart have also provided evidence for a marked effect of acidosis on spontaneous rate and AVN conduction [13].

Since the AVN normally provides the only route by which cardiac excitation can pass from atria to ventricles and can function as a subsidiary pacemaker should the SAN fail [14,15], modulation of AVN activity can have profound physiological and pathophysiological consequences. It is therefore important that the effects of acidosis on AVN cells and tissues are well-understood. It is striking that, at present, there are no published studies on the effects of acidosis on AVN cellular electrophysiology, perhaps due to the technical challenges inherent in isolating and recording from cells from this cardiac region. Accordingly, the present study employed a well-established rabbit single-cell AVN preparation [16–19], in order to investigate effects of acidosis on AVN cellular electrophysiology. The study

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focused on establishing the effects of acidosis on spontaneous activity and ionic currents recorded from isolated AVN cells, using whole-cell patch-clamp recording.

2. Materials and methods

2.1. Cell isolation

Rabbit AVN cells were isolated as described previously [16,20] from the hearts of New Zealand White male rabbits (2–3 kg) killed humanely in accordance with UK Home Office legislation. The AVN region was identified by its relation to anatomical landmarks [21] and the entire AVN region within the Triangle of Koch was removed. Cells dispersed from this tissue were re-suspended and stored in refrigerated (4 °C) Kraftbruhe 'KB' solution [22] until use. In order to provide comparative data on cell size (measured as membrane capacitance), left atrial myocytes and right ventricular myocytes were isolated in parallel with AVN cells using the same isolation procedure.

2.2. Electrophysiological recording

Cells were transferred to an experimental chamber mounted on the stage of an inverted microscope (Eclipse TE2000-U, Nikon, Japan) and superfused with a standard control solution (see the Solutions and chemicals section, below). Whole-cell patch-clamp experiments were performed using an Axopatch-1D amplifier (Axon instruments, USA). Patch-pipettes (A-M Systems, USA) were pulled using a Narishige vertical electrode puller (Narishige PP-83, Japan), and were heat-polished to a final resistance of 2–3 MΩ (Narishige MF-83, Japan). Protocols were generated and data recorded on-line with pClamp 10.0 software (Axon instruments, USA) via an analogue-to-digital converter Digidata 1322 (Axon Instruments/Molecular Devices, USA). Spontaneous action potentials (APs) were recorded continuously with the gap-free acquisition mode by current clamping with a zero-current input. The AP digitization rate was 2 kHz. Membrane currents were recorded in whole cell voltage-clamp mode, with a digitization frequency of 10 kHz.

2.3. Solutions and chemicals

All chemicals were purchased from Sigma unless otherwise stated. All solutions were made with deionised Milli-Q water (Millipore Systems). The basic cell isolation and 'KB' solutions used for cell storage have been described previously [16,20]. To record spontaneous action potentials, the internal (pipette) solution was K^+-based containing (in mM): KCl 110, NaCl 10, HEPES 10, $MgCl_2$ 0.4, Glucose 5, K_2ATP 5, GTP–Tris 0.5, and pH 7.1 with KOH. Cells were bathed in control solution containing (mM): NaCl 93, KCl 5, $MgSO_4$ 1, Na_2HPO_4 1, $CaCl_2$ 1.9, Na-HEPES 20, Glucose 50, Na-acetate 20, and pH of 7.4—attained by addition of HCl. The composition of this solution was chosen to match that used in companion studies [13] on intact hearts/multicellular preparations. Acidic solutions (extracellular pH (pH_e) of 6.8 or 6.3) were made by titration with HCl. Throughout the text, pH values of pH 7.4, 6.8 and 6.3 refer to pH_e values unless otherwise indicated. Once the whole-cell patch-clamp recording mode had been obtained, external superfusate was provided by a rapid solution exchange (<1 s) device built in-house [23], and all experimental superfusion solutions were then applied from this device. The superfusate was maintained at 35–37 °C. For net whole-cell current recording, the external solutions were the same as those used for action potential recording.

For measurement of net ionic currents and selective recording of rapid delayed rectifier K^+ current (I_{Kr}), the pipette solution was similar to that used for action potential recording but with 5 mM BAPTA added to increase the buffering of intracellular Ca^{2+} and eliminate potential contamination of outward current tails with $[Ca^{2+}]_i$-activated inward tail currents [24]. Spontaneously active cells dialysed with this pipette solution ceased contracting once the whole-cell configuration was attained, consistent with effective buffering of bulk $[Ca^{2+}]_i$ by BAPTA from the pipette dialysate. For I_{Kr} measurement (Figs. 4 and 5), the external solution was the same as those used for net ionic current recording, but with the addition of 10 μM nifedipine to inhibit $I_{Ca,L}$. E-4031, an inhibitor of I_{Kr} [18,19], was added to external solution to give a final concentration of 10 μM.

In order to investigate the basis for alterations to $I_{Ca,L}$ with acidosis, the voltage-dependence of activation and inactivation (Fig. 3D) were assessed under $I_{Ca,L}$-selective conditions. For these experiments KCl was replaced by CsCl in the external and internal solutions and the pH of the pipette solution was adjusted to pH 7.1 with CsOH [25]. The small amount of K^+ in this solution (supplied from 5 mM K_2ATP) was insufficient to mediate significant K^+ currents, evidenced by an absence of outward tail currents on repolarization to −40 mV following depolarization to a positive voltage (not shown; see also [17,25,26]). 20 μM tetrodotoxin (TTX, Tocris, USA) was added to the external solutions to preclude potential contamination by Na current. For these experiments membrane potential was held at −80 mV and test commands were applied to voltages between −80 to +40 mV for 1 s. Each voltage-command was followed by a 3-ms step back to −80 mV, followed immediately by a 300-ms pulse to 0 mV. The frequency of application of the protocol was 0.2 Hz.

Nifedipine was dissolved in dimethylsulphoxide (DMSO) to give a 20 mM stock solution which was kept at room temperature in a light-tight container. TTX and E-4031 were made up as 20 and 10 mM stock solutions, respectively, in Milli-Q water, which were kept at −20 °C.

2.4. Measurement of intracellular pH in response to extracellular acidosis

The intracellular pH (pH_i) of AVN cells was measured using the pH-sensitive fluorescent indicator BCECF (2′,7′-bis-(2-carboxyethyl)-5,6-carboxyfluorescein). Cells were loaded with 10 μM BCECF AM (Invitrogen) at room temperature for 10 min [27]. The cells were then centrifuged, the supernatant removed, and the cells were resuspended in Tyrode's solution and kept at room temperature until use. The BCECF-loaded cells were then placed in an experimental chamber and imaged using confocal laser scanning microscopy (Pascal LSM, Zeiss, Germany). The dye was excited with 488 nm light and emitted fluorescence was collected at 505–530 nm. Line-scans across the width of cell were performed at 1 s intervals. Cells were superfused with external solutions of pH_e 7.4 (Control) or pH_e 6.3 (acidosis). Fluorescence intensities were converted to pH_i values using nigericin-containing calibration solutions of different pHs between pH 6.0 and pH 7.5. Nigericin calibration solutions contained (in mM): KCl 140, $MgCl_2$ 1.0, HEPES 10, PIPES 10 and nigericin 10 μM, and were adjusted to the desired pH with NaOH [28].

2.5. Data analysis and statistics

Action potential and current analysis was performed using Clampfit from the pClamp 10.0 software suite. Statistical analysis was performed using Microsoft Office Excel (Microsoft Corporation), Origin (OriginLab Corporation) and Prism (Graphpad Software, Inc.). $I_{Ca,L}$ activation and inactivation relations were fitted using Graphpad Prism. Graphs were drawn using Graphpad Prism. All data are expressed as mean±SEM. Statistical comparisons were made using two-sample paired, independent *t*-test or one-sample *t*-test, or ANOVA as appropriate. $P<0.05$ was regarded as statistically significant.

To quantify voltage-dependent activation of $I_{Ca,L}$ the following equations were used:

$$G = I/(V_m - E_{rev}) \quad (1)$$