An outwardly rectifying anionic background current in atrial myocytes from the human heart

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Abstract

This report describes a hitherto unreported anionic background current from human atrial cardiomyocytes. Under whole-cell patch-clamp with anion-selective conditions, an outwardly rectifying anion current (I ANION ) was observed, which was larger with iodide than nitrate, and with nitrate than chloride as charge carrier. In contrast with a previously identified background anionic current from small mammal cardiomyocytes, I ANION was not augmented by the pyrethroid tefluthrin (10 μM); neither was it inhibited by hyperosmolar external solution nor by DIDS (200 μM); thus I ANION was not due to basal activity of volume-sensitive anion channels. I ANION was partially inhibited by the Cl - /C0 channel blockers NPPB (50 μM) and Gly H-101 (30 μM). Incorporation of I ANION into a human atrial action potential (AP) simulation led to depression of the AP plateau, accompanied by alterations to plateau inward calcium current, and to AP shortening at 50% but not 90% of complete repolarization, demonstrating that I ANION can influence the human atrial AP profile.

Keywords: Action potential; Anion; Anionic; Atrium; Atrial myocyte; Background current; Cardiac; Computer modelling; Heart; Patch-clamp

The electrophysiological behaviour of cardiac myocytes from mammalian hearts is determined by the combined activity of a range of different cation and anion channel types. The reversal potential for chloride (Cl - ) ions in the heart (E Cl ) lies between ~−60 and −40 mV [1]. Negative to E Cl outward Cl - movement generates depolarizing ionic current, whilst positive to E Cl inward Cl - movement generates repolarizing ionic current. Therefore, the activation of Cl - channels can influence both the resting membrane potential and the duration of cardiac action potentials (APs) ([1–3] for reviews). Several different anion channel types have been identified that may contribute to cardiac physiology and pathophysiology [1–3]. Of the cardiac anion channel currents thus far identified, the three major types are: (i) a cystic fibrosis transmembrane conductance regulator (CFTR) current-activated through cAMP-dependent phosphorylation (I Cl,cAMP ; e.g. [4–6]); (ii) a stretch- or swelling-activated Cl - current (I Cl,Swell ; e.g. [7–9]) and (iii) a Ca 2+ -activated Cl - current (I Cl,Ca ; e.g. [10–12]).

Recently, an outwardly rectifying anionic background current (I AB ) has been identified in cardiac myocytes from two commonly studied model species (rat and guinea-pig) using whole-cell patch-clamp measurements [13,14]. I AB is distinct from previously identified Cl - currents as it has a distinct permeability sequence (NO 3 - > I - > Cl - ) and is insensitive to the stilbene diphosphonate Cl - channel inhibitor DIDS, to cell swelling and to intracellular Ca 2+ and cAMP [13,14]. I AB can also be differentiated from other major cardiac anion currents as it can be activated by the pyrethroid agent tefluthrin [14]. Anion substitution experiments have provided evidence that I AB can influence AP duration (APD) [13]. There is some disagreement as to
whether or not a basally active anionic current exists in human atrium [15,16] and there is no information as to whether humans exhibit an $I_{AB}$ with the characteristics of that seen in small mammal hearts. Therefore, the present study was undertaken to determine whether or not $I_{AB}$ exists in adult human cardiac myocytes. The resulting findings indicate the presence in human atrial myocytes of an outwardly rectifying anionic background current ($I_{ANION}$). Notably, the $I_{ANION}$ observed in this study has the potential to contribute to human atrial electrophysiology, but is distinct from both the $I_{AB}$ recorded previously from myocytes from small mammal hearts [13,14] and from outwardly rectifying stilbene diphosphonate-sensitive anionic currents recorded previously from human atrium [17].

Methods

Atrial myocyte isolation

The study was approved by the local Central and South Bristol Research Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki. Human right atrial appendages were obtained, with consent, from 32 patients (27 males, 5 females, average age 69.7 ± 1.7 years) undergoing coronary artery bypass surgery. Tissue samples were quickly immersed in cardioplegic solution (see Table 1; solution G, 100% O$_2$, ice cold). The samples were chopped into small chunks and washed with an EGTA-containing solution (see Table 1; solution H) gassed with 100% O$_2$ for 15 min at 37°C. The chunks were then incubated in the same solution from which EGTA was excluded and protease type XXIV (3 U/ml, Sigma) and collagenase type V (250 U/ml, Sigma) were added. The medium was continuously gassed with 100% O$_2$ at 37°C. After 15 min, the incubation medium was substituted for the same solution containing collagenase only. Myocytes were progressively released from the chunks into the supernatant and their yield monitored under a microscope. The suspension was washed in enzyme-free solution and the myocytes were stored at room temperature until use (within ~8 h of cell isolation).

Electrophysiology

Solutions used. Experimental solutions for the investigation of anionic current were similar to those used previously to study $I_{AB}$ [14]; the composition of all solutions used is given in Table 1. Osmolarity values given for each of the solutions listed in Table 1 were measured using a micro-osmometer employing a freezing-point method (Advanced Instruments, Norwood, MA, USA). Myocytes used in whole-cell voltage-clamp experiments were superfused (20–25°C) with a standard Hepes-buffered Tyrode’s solution (see Table 1; solution A) until the whole-cell recording configuration had been obtained. For isolation of background anion current, sodium-free Tyrode’s solutions were used (solutions B–E) in which Na was replaced by N-methyl-D-glucamine (NMDG), with one of several possible dominant anions: solution B, chloride; solution C, aspartate; solution D, iodide; solution E, nitrate. All drugs used were added to solution E from stock solutions made in dimethyl sulfoxide.

Table 1

Composition of solutions used in this study

| Chemical          | Solution (concentration, mM) | A$^1$ | B$^2$ | C$^2$ | D$^2$ | E$^2$ | F$^2$ | G$^2$ | H$^1$ | I$^2$ |
|-------------------|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| NaCl              |                              | 145   |      |      |       |       |       |       | 137   |       |
| KCl               |                              | 4     |      |      |       |       |       |       |       |       |
| MgCl$_2$         |                              | 1     | 2.5  | 2.5  | 2.5   | 2.5   | 2.5   | 2.5   | 2.5   | 0.5   |
| CaCl$_2$         |                              | 2     |      |      |       |       |       |       |       |       |
| Glucose          |                              | 10    | 10   | 10   | 10    | 10    | 10    | 10    | 10    | 10    |
| HEPES            |                              | 10    | 5    | 5    | 5     | 5     | 5     | 10    | 5     |       |
| NMDG-Cl          |                              |       | 135  |      |       |       |       |       |       |       |
| NMDG-Aspartate   |                              |       | 135  |      |       |       |       |       |       |       |
| NMDG-I           |                              |       |      | 135  |       |       |       |       |       |       |
| NMDG-NO$_3$      |                              |       |      |      | 135   |       |       |       |       |       |
| TEACl            |                              |       |      |      |       |       |       |       |       |       |
| BaCl$_2$         |                              |       |      |      |       |       |       |       |       |       |
| CdCl$_2$         |                              |       |      |      |       |       |       |       |       |       |
| Sucrose          |                              |       |      |      |       |       |       |       | 70    |       |
| Cs glutamate     |                              |       |      |      |       |       |       |       |       | 75    |
| CsCl             |                              |       |      |      |       |       |       |       |       | 20    |
| CsEGTA           |                              |       |      |      |       |       |       |       |       | 0.05  |
| MgATP (tris-salt)|                              |       |      |      |       |       |       |       |       | 10    |
| Tris-phosphocreatine      |                      |       |      |      |       |       |       |       | 5     |       |
| Tris-GTP         |                              |       |      |      |       |       |       |       |       | 0.1   |
| Pyruvic acid     |                              |       |      |      |       |       |       |       |       | 5     |
| $^t$Pipes        |                              |       |      |      |       |       |       |       |       | 30    |
| Adenosine        |                              |       |      |      |       |       |       |       | 5     |       |
| EGTA             |                              |       |      |      |       |       |       |       | 0.2   |       |
| KH$_2$PO$_4$     |                              |       |      |      |       |       |       |       | 50    | 5     |
| Mannitol         |                              |       |      |      |       |       |       |       | 100   |       |
| MgSO$_4$         |                              |       |      |      |       |       |       |       | 8     | 1     |
| Cl$^-$ concentration |                        | 155   | 150  | 15   | 15    | 15    | 15    | 0     | 137   | 21    |
| Osmolarity (mOsm)|                              | 308 ± 2.5 | 305 ± 1.2 | 306 ± 0.3 | 310 ± 3 | 305 ± 0.2 | 365 ± 1.5 | 383 ± 0.3 | 282 ± 0.6 | 308 ± 1.2 |

Osmolarities for each solution are measured values (see Methods).

Note. pH 7.4 with $^1$NaOH, $^3$CsOH, $^4$KOH; pH 7.1 with CsCO$_3$. Minus sign indicates absence.
(DMSO) with an exception of N-(2-naphthalenyl)-(3,5-dibromo-2,4-dihydroxyphenyl)methylene)glycine hydrazide (Gly H-101), which was solved in distilled water. The hyperosmotic external solution (solution F) was prepared by adding 70 mM sucrose to solution E. A Cs-based pipette solution (solution I) was used for all experiments. Solution I was sodium-free to prevent contamination of chloride currents by the sodium–calcium exchanger current.

Drugs. Disothiocyanostilbene-2,2′-disulfonic acid (DIDS, final concentration 200 μM), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, final concentration 50 μM) and tefluthrin (TEF, final concentration 10 μM) were purchased from Sigma Chemical Co. (Poole, UK). N-(2-naphthalenyl)-(3,5-dibromo-2,4-dihydroxyphenyl)methylene)glycine hydrazide (Gly H-101, final concentrations of 10 and 30 μM) was purchased from Merck (Frankfurt, Germany). All the drug-containing solutions were protected from light throughout.

Electrophysiological recording

In electrophysiological experiments, junction potential changes were minimized by immersing the reference Ag/AgCl electrode in a 3 M KCl solution with a continuous agar bridge (4% agar in 3 M KCl). Borosilicate glass pipettes (Harvard Apparatus, UK) were pulled using a vertical two-step Narishige PP-830 microelectrode puller (Narishige, Japan) and had a tip resistance of 5–7 MΩ when filled with the pipette solution. During anion substitution experiments, background anion current was elicited from voltage-clamped myocytes (superfused with solutions B, C, D and E in the whole-cell configuration) by depolarizing ramps from −90 to +70 mV from a holding potential of −50 mV (ramp rate of 0.32 V s⁻¹; sweep duration 1.03 s). A holding potential of −50 mV was used to inactivate the Na⁺-current and T-type Ca²⁺-current.

Recordings were made using an Axopatch 200A amplifier, and data were recorded on computer using pClamp v. 9.0 software (Axon Instruments, Forster City, CA). Data were analyzed using the Clampfit program of pClamp v. 9.0.

Mean values of averaged original signals over five command pulses were used for statistical analysis. Hyperpolarizing voltage steps of −20 mV and 5 ms duration were applied at 20 Hz to record the capacitance transients required for direct integration and the calculation of cell capacitance. The statistical significance between control and the drug periods or NO₃⁻ and other anions were determined by the Paired Student’s t-test using either Microsoft Excel or GraphPad Prism v. 4.0. The statistical significance between the normal and hyperosmotic NO₃⁻ solutions was calculated with two-way ANOVA test using GraphPad Prism v. 4.0. Statistical significance was considered to refer to the 95% level of confidence (p < 0.05).

**Human atrial action potential simulations**

The Courtemanche et al. human atrial action potential (AP) model [18] was modified to incorporate a formulation for I_{ANION} based on the experimental data obtained with NO₃⁻ and Cl⁻ in Figs. 1 and 2. Readers are referred to [18] for the general equations required to set up the model. The following equation was used to simulate anionic background current (I_{ANION})

\[ I_{ANION} = \frac{(V - E_{ANION})}{1 - e^{-V/V_s}} \] (1)

where \( E_{ANION} \) represents the current reversal potential and \( g_{ANION} \) is the conductance of \( I_{ANION} \). By fitting Eq. (1) to experimental data shown in Fig. 1B and scaled to the mean data shown in Fig. 2A, we obtained \( g_{ANION} = 0.37 \, pS/pF, E_{ANION} = -45.64 \, mV, c = 0.87, d = 8.4 \times 10^{-4} \, mV^{-1} \) for the NO₃⁻-sensitive \( I_{ANION} \), and \( g_{ANION} = 0.19 \, pS/pF, c = 0.94, d = 2.5 \times 10^{-4} \, mV^{-1} \) for the Cl⁻-sensitive \( I_{ANION} \).

**Results and discussion**

The voltage protocol used for these experiments was similar to that used previously to study I_{AB} present in cardiomyocytes from small mammal hearts [14] and is shown as an inset to Fig. 1A. From a holding potential of −50 mV, ascending voltage ramps were applied between −90 to +70 mV. This protocol was applied to cells superfused first with aspartate (Asp)-containing solution (solution C) and then with different superfusates containing more permeant anions. Fig. 1A shows an example of the net current traces obtained from a cell superfused serially with solutions containing Asp⁻, Cl⁻, NO₃⁻ and I⁻. Both inward and, particularly, outward current components were greater with Cl⁻, NO₃⁻ and I⁻ than with Asp⁻ in the external superfusate. Fig. 1B shows current traces for the same cell, obtained by subtracting the current in Asp⁻ from that with each of the more permeant anions. With each of Cl⁻, NO₃⁻ and I⁻, the Asp⁻-sensitive difference current showed marked outward rectification. Fig. 1C compares the mean outward current amplitude at +60 mV (normalized to membrane capacitance) for the three anions. Compared to NO₃⁻, the observed current...
was significantly greater with I \(^{-}\) and smaller with Cl\(^{-}\) as charge carrier. These observations support the presence in human atrial cells of a basally active, anionic current (I\(_{\text{ANION}}\)): however, the relative current amplitudes with the three permeant anions differ from those observed previously with for the I\(_{\text{AB}}\) observed in myocytes from guinea-pig and rat hearts, where I\(_{\text{AB}}\) was largest with NO\(_3\)\(^{-}\) (permeability sequence NO\(_3\)\(^{-}\) \(>\) I \(^{-}\) \(>\) Cl\(^{-}\); [13,14]).

Fig. 2A shows the mean I\(_{\text{ANION}}\)-voltage relation for 19 atrial cells, with NO\(_3\)\(^{-}\) as the major external anion (with I\(_{\text{ANION}}\) measured as the NO\(_3\)\(^{-}\) – Asp\(^{-}\) difference current). The mean current–voltage relation for the resulting current showed clear outward rectification, with an observed reversal potential (E\(_{\text{rev}}\)) for I\(_{\text{ANION}}\) in these experiments of \(-45.7 \pm 2.2\) mV (obtained by pooling E\(_{\text{rev}}\) values from individual experiments). Previous studies provide evidence that human atrial cells exhibit I\(_{\text{Cl, Swell}}\) (e.g. [15,17,19–21]).

Therefore, in order to determine whether or not I\(_{\text{ANION}}\) could be attributed to basal activity of channels mediating I\(_{\text{Cl, Swell}}\), Asp\(^{-}\) to NO\(_3\)\(^{-}\) substitutions were also made using hyperosmolar external solution [14]. The mean data from eight such experiments are shown in Fig. 2B. There was no statistically significant difference between the plotted densities of I\(_{\text{ANION}}\) from the I–V relation obtained in hyperosmolar solution and that shown in Fig. 2A, suggesting that I\(_{\text{ANION}}\) is distinct from I\(_{\text{Cl, Swell}}\).

In order to characterize further I\(_{\text{ANION}}\) from human atrial myocytes, the sensitivity of the current to a range of pharmacological interventions was tested. Fig. 2C summarises the effects of the various interventions (expressed as % changes in NO\(_3\)\(^{-}\)-sensitive current at \(+60\) mV). The stilbene disulphosphate DIDS failed to inhibit I\(_{\text{ANION}}\) at a concentration (200 \(\mu\)M) that would be anticipated to inhibit I\(_{\text{Cl, Swell}}\) [19,21]. On the other hand, tefluthrin (10 \(\mu\)M), which we have previously reported to activate the I\(_{\text{AB}}\) seen in myocytes from guinea-pig hearts [14], failed to alter significantly the amplitude of I\(_{\text{ANION}}\) from human atrial myocytes. Together with the relative I\(_{\text{ANION}}\) amplitudes in I\(^{-}\), Cl\(^{-}\) and NO\(_3\)\(^{-}\), the lack of effect of tefluthrin indicates that I\(_{\text{ANION}}\) is distinct from the previously reported rat/guinea-pig I\(_{\text{AB}}\) [13,14]. Moreover, the lack of significant inhibition of the current by DIDS or hyperosmolar solution makes the I\(_{\text{ANION}}\) observed in the present study distinct from I\(_{\text{Cl, Swell}}\) [1] and from an osmolarity- and stilbene-sensitive outwardly rectifying chloride current recently reported by Demion and colleagues [17]. NPPB (50 \(\mu\)M) produced a partial, statistically significant (p < 0.05) inhibition of I\(_{\text{ANION}}\). The glycine hydrazide Cl\(^{-}\) channel inhibitor Gly H-101 failed to produce a significant blockade of I\(_{\text{ANION}}\) at 10 \(\mu\)M (~7-fold greater than the reported IC\(_{50}\) for CFTR channel inhibition at \(+60\) mV [22]); but produced partial attenuation of the current at 30 \(\mu\)M (~20-fold the reported IC\(_{50}\) for CFTR [22]). Evidence for the presence of CFTR (I\(_{\text{Ca,cAMP}}\)) in human atrial cells is mixed [1,15,19–21,23], with a number of studies failing to observe the current in response to \(\beta\)-adrenergic stimulation, forskolin or cAMP (e.g. [15,19–21]). Previous work has failed to find evidence for I\(_{\text{Cl, Ca}}\) in human atrial myocytes [24] and, moreover, the presence of EGTA in the pipette dialysate (Table 1, solution I) and external [Ca\(^{2+}\)] replacement in our experiments would have inhibited any [Ca\(^{2+}\)]-activated conductances on membrane depolarization. Therefore, the I\(_{\text{ANION}}\) seen here appears to differ not only from guinea-pig and rat I\(_{\text{AB}}\) [13,14] but also from the three major reported cardiac anion conductances in: (i) being basally active and (ii) its overall pharmacological profile and sensitivity to anion substitution.

In order to gain insight into the physiological role of I\(_{\text{ANION}}\), the current was incorporated into human atrial AP simulations as outlined in the ‘Methods’. Fig. 3A shows the simulated APs (at an AP frequency of 1 Hz) from the Courtemanche et al. model [18] both without (Control) and with inclusion of I\(_{\text{ANION}}\), whilst Fig. 3B shows the corresponding current profiles during the time-course of the
In summary, the extent to which the incorporation/omission of $I_{\text{ANION}}$ influences the susceptibility of human atrial cells and tissue to arrhythmia and to pursue the underlying identity and regulation of this novel background conductance.

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