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Supplementary Methods

**Rat left atrial myocyte isolation**

All animal procedures were approved by the ethics committee of the University of Bristol and performed in accordance with UK legislation (Animals (Scientific Procedures) Act, 1986). Left atrial myocytes were isolated from adult male Wistar rats (200-320 g) as described previously. In brief, anaesthetised animals (sodium pentobarbital 60-100 mg/kg i.p.) were subject to thoracotomy and the heart rapidly excised into Tyrode’s solution at room temperature (~22 °C). The heart was retrogradely perfused via the aorta with Tyrode’s solution containing collagenase (Worthington Class 1, Lorne Laboratories, UK) at 37 °C using a modified Langendorff apparatus. The left atrium was then removed, finely chopped and triturated in Kraftbrühe solution (KB). Dissociated cells were stored in KB at 4 °C and used within 10 hours of isolation.

**Whole-cell recording**

Currents were filtered with a corner frequency of 1 kHz and recorded to the hard drive of a PC via an EPC-9 amplifier using Pulse software (version 8.11, HEKA GmbH, Germany) at a sampling frequency of 5 kHz. The junction potential on immersing the tip of the pipette in the bath solution was compensated electronically and no further compensation was applied. On forming the whole-cell configuration, capacitance transients were compensated electronically and currents were normalised to whole-cell capacitance ($C_m = 57.5\pm1.43$ pF, $n=137$) as a measure of cell size (surface area). The series resistance ($R_s = 3.1\pm0.08$ MΩ, $n=137$) was not compensated and no corrections were made for voltage-drop error. Voltage-gated inward and outward currents were investigated using a square-shaped pulse protocol: from a holding potential of -80 mV, 500 ms pulses were applied to potentials from -40 mV to +50 mV increasing in 10 mV increments. Pulses were applied every 5 s and a 50 ms pre-pulse was applied to inactivate the Na$^+$ current. The steady-state outward and inward rectifier currents were recorded using a ramp protocol: from a holding potential of -80 mV, a step to +20 mV for 100 ms was followed by a ramp to -120 mV over 500 ms. Ramps were applied at
3 s intervals. The background inward-rectifier K⁺ current was obtained by subtraction of the background current obtained during superfusion with a K⁺-free solution ([K⁺]₀ =0) from the current obtained in the presence of external K⁺ ([K⁺]₀=4 mM) (Supplementary Figure 1). The concentration-dependence of noradrenaline action on I<sub>CaL</sub> and I<sub>Kss</sub> was examined using a modified ramp protocol as described above but incorporating a 50 ms prepulse to -40 mV to inactivate Na⁺ currents. I<sub>Kss</sub> was measured as the steady-state outward current at the end of the 100 ms step to +20 mV. I<sub>CaL</sub> was measured as the difference between I<sub>Kss</sub> and the peak inward current activated on stepping to +20 mV. For the power spectral analysis, recordings were made in the presence of 10 µM nifedipine to block I<sub>CaL</sub>. For the current-clamp recording of action potentials (APs), pipettes were filled with a pipette solution comprising 10 mM NaCl, 110 mM KCl, 0.4 mM MgCl₂, 5 mM D-glucose, 10 mM HEPES, 5 mM K-ATP and 0.5 mM Tris-GTP, pH 7.3 (KOH). APs were elicited by 2 ms depolarising current pulses at a frequency of 1 Hz.

**Data analysis**

Current and voltage recordings were analysed using IgorPro (vs3.16B, Wavemetrics Inc., USA). Mean current-voltage relations for the L-type Ca<sup>2+</sup> current (I<sub>CaL</sub>) were fitted with a modified Boltzmann equation, as follows:

**Equation 1:**

\[
I_{CaL}(V_m) = \frac{G_{max}(V_m-V_{rev})}{(1+\exp(-\frac{V_{half}-V_m}{k}))}
\]

where \(V_m\) was the membrane potential, \(G_{max}\) represented the maximum conductance of the membrane, \(V_{rev}\) was the reversal potential of I<sub>CaL</sub>, \(V_{half}\) represented the voltage of half-maximal activation and \(k\) was a slope factor.
The voltage-dependent activation curve for the noradrenaline-sensitive outward current was created by calculating the conductance \( G \), assuming perfect \( K^+ \)-selectivity, of the noradrenaline-sensitive component of the outward current activated by square-shaped pulses and normalising to the maximal conductance at positive potentials \( G_{\text{max}} \). The voltage-dependent activation curve was then fitted with the following equation:

\[
\frac{G}{G_{\text{max}}} = \frac{1}{1 + \exp \left( \frac{V_{\text{half}} - V_m}{k} \right)}
\]

where \( G, G_{\text{max}}, V_{\text{half}}, V_m \) and \( k \) have the definitions described above.

**Concentration-dependence of noradrenaline action**

The percentage increase in \( I_{\text{CaL}} \) by noradrenaline was calculated as follows:

\[
\text{Equation 3: } \% \text{ increase} = 100 \times \left( \frac{I_{\text{noradrenaline}} - I_{\text{control}}}{I_{\text{control}}} \right).
\]

On the other hand, the percentage inhibition of \( I_{Kss} \) by noradrenaline was calculated using the following equation:

\[
\text{Equation 4: } \% \text{ inhibition} = 100 \times \left( \frac{I_{\text{control}} - I_{\text{noradrenaline}}}{I_{\text{control}}} \right).
\]

In both equations, \( I_{\text{control}} \) and \( I_{\text{noradrenaline}} \) represent, respectively, the control currents and the currents in the presence of noradrenaline. Percentage changes in currents were plotted against the logarithm (base 10) of the corresponding noradrenaline concentration in molar \( (X) \) and the relations fitted by the following logistic equation:
Equation 5: \[
\%\text{response} = \frac{\text{bottom} + \text{top} - \text{bottom}}{(1 + 10^{(\log EC_{50} - X) \times \text{HillSlope})}},
\]

where \text{bottom} and \text{top} represent the responses at, respectively, minimally and maximally effective concentrations, \(\log EC_{50}\) represents the logarithm (base 10) of the half-maximally effective concentration and \text{HillSlope} represents the Hill coefficient representing the slope of the concentration-response relationship. Note that the \(\log EC_{50}\) and \(EC_{50}\) for the inhibition of \(I_{Kss}\) by noradrenaline are reported in the manuscript as \(\log IC_{50}\) and \(IC_{50}\).

**Power spectral analysis**

Analysis of the power spectrum was performed using IgorPro (vs3.16B, Wavemetrics Inc., USA). The variance of the noradrenaline-sensitive steady-state outward current was calculated from the integral of spectral density function, as described previously \cite{4, 5}. Each 500 ms current trace was analyzed as a block of 2450 data points, giving a range of 2.04 Hz – 2.5 kHz (Nyquist frequency). The spectrum in the presence of noradrenaline was assumed to represent background noise and subtracted from the spectrum in control solution to obtain the spectrum of the noradrenaline-sensitive current. Frequencies of greater than 400 Hz were disregarded due to the filtering effect of the whole-cell recording mode \((f_{c, RC} = \frac{1}{2\pi R_g C_m})\). The noradrenaline-sensitive spectrum was fitted with a double Lorentzian function:

Equation 6: \[
S(f) = \frac{S(0)_1}{1+(\frac{f}{f_{c1}})^2} + \frac{S(0)_2}{1+(\frac{f}{f_{c2}})^2},
\]

where \(S(0)_1\) and \(S(0)_2\) (with units of \(A^2 s\)) and \(f_{c1}\) and \(f_{c2}\) (Hz) are the low frequency asymptotes and corner frequencies for the low and high frequency Lorentzian, respectively. The current variance ascribable for each Lorentzian was calculated from the integral of this function:
Assuming that the noradrenaline-sensitive current represented a single population of \(K^+\)-selective channels gating between a single closed state and open state with an open probability \(\leq 0.1\), the single channel conductance was calculated as:

\[
\text{Equation 8: } \gamma = \frac{\sigma^2}{\Delta (V_m - V_{rev})},
\]

where \(\Delta I\) is the noradrenaline-sensitive macroscopic current, \(V_m\) is the membrane potential (i.e. +50 mV) and \(V_{rev}\) is the reversal potential for a \(K^+\)-selective channel (i.e. \(E_K = -91\) mV).

**Supplementary Results & Discussion**

Although hyperpolarising-shifts in the voltage-dependent activation of cardiac \(I_{Ca\text{L}}\) on \(\beta\)-adrenoceptor-stimulation are widely reported (e.g. references cited in \(^6\)), the apparent shift observed in this study is likely largely due to voltage-drop error across the uncompensated series resistance (Fig. 1Bi): For example, an increase in current of \(~0.95\) nA (approximate mean noradrenaline-induced increase in \(I_{Ca\text{L}}\) at 0 mV) across an \(R_s\) of \(~3.1\) M\(\Omega\) would result in \(~3\) mV shift in voltage. This conclusion is consistent with reports that shifts in voltage-dependent activation of L-type \(Ca^{2+}\) channel currents are not reproduced in studies of the phosphorylation-dependent regulation of recombinant channels in expression systems \(^7,^8\). Comparison of the slope factor, \(k\), of \(17.11\) mV for the noradrenaline-sensitive outward current with that for the strongly
voltage-dependent $I_{CaL}$ ($k=5.5 \text{ mV}$) illustrates the shallow-nature of voltage-dependence of the steady-state outward current.

The effect of noradrenaline (1 µM) on the background current was investigated using a modified ramp protocol (Supplementary Figure 1). Step depolarisation to +20 mV produced a large, fast and poorly resolved inward current that rapidly inactivated to a noisy outward current at the end of the 100 ms step (Supplementary Fig. 1B). The noisy outward current was strongly reminiscent of the steady-state outward current observed during square-shaped depolarising pulses (Fig. 1A). During the subsequent descending voltage ramp to -120 mV, currents showed inward rectification, the rectification being particularly marked at negative potentials. Removal of external K$^+$ (K$^+e$) resulted in a marked reduction in the outward current at +20 mV, a reduction in the noise of the outward current and a loss of the inward rectification so that the inward current at -120 mV was also markedly reduced. The K$^+e$-dependent background current was calculated by subtraction of the current in the absence of external K$^+$ from that in control Tyrode’s solution ([K$^+e$]=4 mM) $^3$. The K$^+e$-dependent difference current showed marked inward rectification negative to the calculated K$^+$ equilibrium potential ($E_K=-90.9 \text{ mV}$), consistent with the contribution of K$_{ir}$2.x channels to this current (Supplementary Fig. 1C). In addition, at potentials of -20 mV and positive, the K$^+e$-dependent current also showed outward rectification. Superfusion of the cells with 1 µM noradrenaline had no effect on the inward rectifier current but caused a marked inhibition of the outwardly rectifying current (Supplementary Fig. 1D). For example, noradrenaline inhibited the K$^+e$-dependent current at +20 mV by 89.2±11.6 % (P=0.0003) but had no significant effect on the current at -120 mV (1.9±2.6 %, P=0.2531, n=6).

Thus, the noradrenaline-sensitive difference current represents an outwardly rectifying current evident at potentials of -40 mV and positive (Supplementary Fig. 1E).

To examine whether noradrenaline elicited similar responses in other species, whole-cell current recordings were made from mouse isolated atrial myocytes (Supplementary Figure 2). The whole-cell currents of mouse atrial myocytes were very similar to those of rat atrial cells, with a peak inward current activated by depolarisation to voltages between -30 mV and +40 mV that was maximal at 0 mV.
(Supplementary Fig. 2A) and a time-independent steady-state outward current (\(I_{KSS}\)) that was activated at voltages positive to -40 mV (Supplementary Fig. 2A & Supplementary Fig. 2B). Similar to the findings in rat isolated atrial myocytes, superfusion of the cells with 1 \(\mu\)M noradrenaline increased the transient inward current (\(I_{CaL}\)) over the entire voltage range (Supplementary Fig. 2C) and reduced \(I_{KSS}\) at positive potentials (Supplementary Fig. 2B). The inward rectifier (\(I_{K1}\)) during ramp pulses was unaffected by noradrenaline although, similar to rat atrial cells, the steady-state current at +20 mV was reduced by 32.9\%±3.2\% (P<0.001, \(n=5/1\), Supplementary Fig. 2D).

The ionic basis of the outward currents was investigated in rat atrial myocytes using \(\mathrm{K}^+\)-free internal and external solutions. Under \(\mathrm{K}^+\)-free conditions, the outward current was markedly attenuated and the noise very much reduced (Supplementary Fig. 3A) and the current-voltage relations right-shifted to reverse at 0 mV (Supplementary Fig. 3B), demonstrating the contribution of \(\mathrm{K}^+\) to both the inward rectifier currents at negative potentials and the steady-state outward currents positive to -20 mV (cf. Fig. 1Bii). Most significantly, 1 \(\mu\)M noradrenaline had no effect on the steady-state outward current in the absence of \(\mathrm{K}^+\) (Supplementary Fig. 3B).

Fluoxetine and mibefradil have been reported to block TREK-1 channels\(^9\)-\(^12\). Their effects on the background currents were therefore investigated using the ramp protocol (Fig. 1C and Supplementary Fig. 1). It was striking that both agents markedly reduced the currents over the full voltage range (Supplementary Fig. 4A & Supplementary Fig. 4C). Subtraction of the current in the presence of drug from the corresponding control value generated the drug-sensitive difference currents for fluoxetine (Supplementary Fig. 4B) and mibefradil (Supplementary Fig. 4D). The fluoxetine-sensitive currents reversed at -81.4±2.4 mV (\(n=7/2\)) while the mibefradil-sensitive currents reversed at -91.9±0.8 mV (\(n=6/2\)). Since these values are close to \(E_K\), these data demonstrate that the predominant effect of these drugs was to inhibit \(\mathrm{K}^+\)-selective currents. It was notable that the noradrenaline-sensitive current was completely abolished in the presence of the TREK-1 channel blockers (cf. Supplementary Fig. 4B & Supplementary Fig. 4D with Supplementary Fig. 1E). While fluoxetine and mibefradil have previously been shown to block...
cardiac voltage-gated K\(^+\) currents, to the best of our knowledge, this is the first report demonstrating an effect of either of these drugs on cardiac \(I_{K1}\) \(^{13-16}\). Indeed, fluoxetine has been reported not to inhibit \(I_{K1}\) in ventricular cells \(^{14,16}\). Presumably, atrial-ventricular differences in the molecular composition of \(I_{K1}\) contribute to regional differences in sensitivity to fluoxetine \(^{17}\).
Supplementary Figure Legends

**Supplementary Figure 1.** Recording of background K\(^+\) currents. A Ramp-voltage protocol. B Representative ramp-current traces in control solution and in the absence of extracellular K\(^+\). C Mean current density-voltage relations from 6 cells/3 animals in control (filled circles), in the absence of extracellular K\(^+\) (filled squares) and K\(^+\)-sensitive difference currents (open squares). D Mean K\(^+\)-sensitive background current-voltage relations from 6 cells/3 animals in control (filled circles) and 1 µM noradrenaline (open circles). *, P<0.05; ***, P<0.001; two-way RM ANOVA with Bonferroni post hoc test. Data correspond to those shown in Figure 1C. E Mean noradrenaline-sensitive difference current calculated from the data shown in Figure 1C and Supplementary Fig. 1D.

**Supplementary Figure 2.** Effects of noradrenaline in mouse atrial myocytes. A Example current traces obtained using square-shaped depolarising pulses to 0 mV and +50 mV in the absence and presence of 1 µM noradrenaline. B Mean \(I_{\text{Kss}}\)-voltage relations in control (filled squares) and in the presence of 1 µM noradrenaline (n=5/1). C Mean \(I_{\text{CaL}}\)-voltage relations in control (filled circles) and in the presence of 1 µM noradrenaline (n=5/1). D Mean background current-voltage relations obtained using the ramp protocol in control (filled circles) and in the presence of 1 µM noradrenaline (n=5/1). For B, C & D, *, P<0.05; **, P<0.01; ***, P<0.001; two-way RM ANOVA with Bonferroni post hoc test.

**Supplementary Figure 3.** Effects of noradrenaline in K\(^+\)-free solutions. A Example current traces in the absence of noradrenaline using square-shaped depolarising pulses to +50 mV obtained in K\(^+\)-containing (control) and K\(^+\)-free intracellular and extracellular solutions. B Mean steady-state current-voltage relations using K\(^+\)-free intracellular and extracellular solutions in control (filled squares) and in the presence of 1 µM noradrenaline (open squares) (n=7/3). *, P<0.05; two-way RM ANOVA with Bonferroni post hoc test.

**Supplementary Figure 4.** Effects of the TREK-1 blockers, fluoxetine and mibefradil on background currents. A Mean background current density-voltage relations from 7 cells/2 animals in control solution (filled
circles), in the presence of fluoxetine (100 μM, filled squares) and in the presence of fluoxetine and noradrenaline (1 μM, open squares). **, P<0.01; ***, P<0.001; two-way RM ANOVA with Bonferroni post hoc test; fluoxetine vs control. **, P<0.01; ***, P<0.001; two-way RM ANOVA with Bonferroni post hoc test; fluoxetine vs control. 

**Supplementary Figure 5.** Effects of the protein kinase A-inhibitor, H-89, on current modulation by noradrenaline. 

A Representative $I_{CaL}$ traces at 0 mV. B Mean $I_{CaL}$ density-voltage relations from 8 cells/3 animals in control (filled circles), following treatment with H-89 (10 μM, filled squares) and in the presence of noradrenaline following H-89 treatment (open circles). Solid lines represent fits to equation 1. C Representative outward current traces at +50 mV. D Mean $I_{Kss}$ density-voltage relations from 6 cells/3 animals in control (filled circles), following H-89 treatment (filled squares) and in the presence of noradrenaline following H-89 treatment (open circles). *, P<0.05; ***, P<0.001; two-way RM ANOVA with Bonferroni post hoc test.

**Supplementary Figure 6.** Correlation between percentage increase in APD$_{30}$ and percentage inhibition of $I_{Kss}$ in response to 1 μM noradrenaline in the presence of $\beta_2$-adrenoceptor antagonism. Data are those...
presented in Figure 4A and Figure 6D. Left-most symbol represents data obtained in the presence of 1 μM propranolol, central symbol is data obtained in the presence of 10 μM atenolol plus 100 nM ICI-118,551 and the right-most symbol represents the data obtained in 10 μM atenolol alone. The Pearson’s correlation coefficient was 0.9891 (P<0.05). Solid line represents a linear regression of APD$_{30}$ prolongation on $I_{KsS}$ inhibition.
References
