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Inhibition of a TREK-like K^+ channel current by noradrenaline requires both β_1- and β_2-adrenoceptors in rat atrial myocytes

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Aims Noradrenaline plays an important role in the modulation of atrial electrophysiology. However, the identity of the modulated channels, their mechanisms of modulation, and their role in the action potential remain unclear. This study aimed to investigate the noradrenergic modulation of an atrial steady-state outward current (I_{Kss}).

Methods and results Rat atrial myocyte whole-cell currents were recorded at 36°C. Noradrenaline potently inhibited I_{Kss} ($IC_{50} = 0.90$ nM, 42.1 ± 4.3% at 1 μM, $n = 7$) and potentiated the L-type Ca^{2+} current (I_{CaL}, $EC_{50} = 136$ nM, 205 ± 40% at 1 μM, $n = 6$). Noradrenaline-sensitive I_{Kss} was weakly voltage-dependent, time-independent, and potentiated by the arachidonic acid analogue, 5,8,11,14-eicosatetraynoic acid (EYTA; 10 μM), or by osmotically induced membrane stretch. Noise analysis revealed a unitary conductance of 8.4 ± 0.42 pS ($n = 8$). The biophysical/pharmacological properties of I_{Kss} indicate a TREK-like K^+ channel. The effect of noradrenaline on I_{Kss} was abolished by combined β_1-/β_2-adrenoceptor antagonism (1 μM propranolol or 10 μM β_1-selective atenolol and 100 nM β_2-selective ICI-118,551 in combination), but not by β_1- or β_2-antagonist alone. The action of noradrenaline could be mimicked by β_2-agonists (zinterol and fenoterol) in the presence of β_1-antagonist. The action of noradrenaline on I_{Kss}, but not on I_{CaL}, was abolished by pertussis toxin (PTX) treatment. The action of noradrenaline on I_{CaL} was mediated by β_1-adrenoceptors via a PTX-insensitive pathway. Noradrenaline prolonged APD_{30} by 52 ± 19% ($n = 5$; $P < 0.05$), and this effect was abolished by combined β_1-/β_2-antagonism, but not by atenolol alone.

Conclusion Noradrenaline inhibits a rat atrial TREK-like K^+ channel current via a PTX-sensitive mechanism involving co-operativity of β_1-/β_2-adrenoceptors that contributes to atrial APD prolongation.

Keywords Background K^+ current • Beta-adrenoceptor • K_{2P} channel • Steady-state outward current • Osmotic stretch • TREK-1 • Arachidonic acid

1. Introduction

Cardiac sympathetic activity plays an important role in electrophysiological responses of the atria and in the genesis of atrial tachyarrhythmias.[1,2] Noradrenaline is the principal neurotransmitter released from sympathetic postganglionic fibres and acts via α_1-, β_1-, and β_2-adrenoceptors through multiple signalling pathways to modulate atrial ion channel and transporter function and thereby electrophysiological activity.[1–3] For example, the action of β-adrenoceptor stimulation on Ca^{2+} entry via L-type Ca^{2+} channel current (I_{CaL}) and sarcoplasmic reticulum function underlies the positive inotropic response to sympathetic activity and, under pathological conditions in the predisposed heart, may contribute to triggered activity through after-depolarizations and abnormal automaticity.[4,5] On the other hand, the effects of noradrenaline on K^+ channel currents are likely to contribute changes in membrane excitability and repolarization.[1,2] The inwardly rectifying background K^+ current (I_{K1}) and the steady-state outward K^+ current (I_{Kss}) have been reported to be inhibited by α_1- and β-adrenoceptor agonists[6–14] and may be important in the noradrenergic control of excitability, action potential (AP) profile, and refractoriness, and may thereby contribute to the arrhythmic substrate.[2,15,16]

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However, the channels contributing to the effects of noradrenergic activation on atrial I_{K1} and I_{Kss} and the receptor/signalling pathways remain unresolved. Moreover, since many of the previous studies on I_{K1} and I_{Kss} have been conducted using receptor-selective combinations of agonists and antagonists, the net effect of the physiological agonist, noradrenaline, is often unclear.[6–14] I_{K1} is largely carried by inward rectifier K^+ ($K_{ir}2.x$) channels, although the two-pore domain K^+ (K_{2P}) channel, TWIK-1 ($K_{2P}1.1$), may also contribute.[16,17] The inward rectifier channels, $K_{ir}3.1$ and $K_{ir}3.4$, form a heteromultimeric G-protein-gated channel that underlies the current activated by acetylcholine (I_{KACh}) via a pertussis toxin (PTX)-sensitive mechanism in atrial myocytes.[16] A number of K^+ channels have been proposed to be involved in I_{Kss}, although the precise molecular composition of the underlying channels remains unclear. The voltage- and time-dependent kinetics of I_{Kss} are distinct from those of the delayed rectifier currents, I_{Kr} and I_{Ks}.[18] The lack of detectable inactivation, together with relative insensitivity to 4-aminopyridine (4-AP), distinguishes I_{Kss} from the ultra-rapidly activating delayed rectifier current (I_{Kur}).[15,19] The two-pore domain K^+ (K_{2P}) channel, TREK-1 ($K_{2P}2.1$), is a weakly voltage-dependent arachidonic acid- and mechano-sensitive channel expressed in the heart that is suggested to contribute to I_{Kss} and is modulated by isoprenaline in atrial myocytes.[17,20–24] TASK-1 ($K_{2P}3.1$) is an acid-sensitive K_{2P} channel proposed to contribute to cardiac background currents and suggested to be under G-protein-coupled receptor control.[25–30] In addition, the six transmembrane-domain voltage-gated K^+ channel, SLICK (slo2.1 or $K_{Ca}4.2$), is a Ca^{2+}-independent homologue of the BK channel (slo1.1 or $K_{Ca}1.1$) that is expressed in the heart and has been suggested to contribute to α_{1A}-adrenoceptor-sensitive I_{Kss} in ventricular myocytes.[14,31]

Much of the work characterizing the properties of I_{Kss} and its modulation by adrenoceptors has been conducted in rat cardiac myocytes.[6,12,14,15,20–22,26–28,30] Therefore, the objectives of this study were to investigate the channels and the receptor pathways involved in the effect of noradrenaline on whole-cell I_{Kss} in atrial myocytes from the rat heart and to examine the contribution of I_{Kss} to changes in the atrial AP.

2. Methods

Further details are available in Supplementary material online.

2.1 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.[32]

2.2 Whole-cell recording

Whole-cell currents and potentials were recorded using the whole-cell patch-clamp technique. Myocytes were superfused with Tyrode's solution comprising the following (in mM): 140 NaCl, 4 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 D-glucose, and 5 HEPES, pH 7.4 at 36°C. For whole-cell, voltage-clamp current recording, the internal solution comprised the following (in mM): 10 NaCl, 110 KCl, 0.4 $MgCl_2$, 5 D-glucose, 10 HEPES, 5 BAPTA, 5 K-ATP, and 0.5 Tris–GTP, pH 7.3 (KOH). Inward rectifier K^+ currents were isolated using a voltage-ramp protocol and subtraction of the background current obtained in K^+-free external solution (see Supplementary material online, *Figure S1*). For K^+-free recordings, the external and internal solutions were created by equimolar replacement of KCl with CsCl. Iso-osmotic solution consisted of (in mM) 90 NaCl, 4 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 D-glucose, 5 HEPES, and 100 Mannitol, pH 7.4 at 36°C (osmolality = 305 mOsm/kg H_2O). Hypo-osmotic solution was produced by the omission of mannitol (osmolality = 202 mOsm/kg H_2O). Recordings for power spectral analysis were made in the presence of 10 μM nifedipine. For current-clamp AP recording, the internal solution comprised the following (in mM): 10 NaCl, 110 KCl, 0.4 $MgCl_2$, 5 D-glucose, 10 HEPES, 5 K-ATP, and 0.5 Tris–GTP, pH 7.3 (KOH). APs were elicited by 2 ms depolarizing current pulses at 1 Hz stimulation frequency.

2.3 PTX treatment of atrial myocytes

Atrial myocytes were incubated with PTX (1.5 μg/mL) at 37°C for 3 h. Treatment with PTX under these conditions was sufficient to abolish the activation of I_{KACh} by 1 μM ACh.

2.4 Drugs and reagents

All reagents were purchased from Sigma-Aldrich (Poole, UK), except PTX (Merck Chemicals Ltd, Nottingham, UK). Noradrenaline, fenoterol, atenolol, propranolol, ICI-118,551, zinc, fluoxetine, and mibefradil were dissolved in deionized water (dH_2O) as 10 mM stock solutions and dissolved to the final concentration in the extracellular solution on the day of experiment. Prazosin was dissolved in near-absolute ethanol to make a 1 mM stock solution and dissolved to the final concentration in the extracellular solution on the day of experiment. Chromanol 293B, zinterol, and nifedipine were dissolved in dimethyl sulfoxide to make 10 mM stock solution of each drug and stored at −20°C. 4-AP was dissolved in dH_2O to make 10 mM stock solution and the pH adjusted to 7.4 at 36°C with HCl. To avoid oxidation, 5,8,11,14-eicosatetraynoic acid (EYTA—a non-metabolizable arachidonic acid analogue) was dissolved in chloroform under a nitrogen atmosphere, and all chloroform evaporated before storage at −20°C. Stock solutions were diluted to the final concentration in the extracellular solution on the day of experiment. 1 μM of the neurotransmitter was used as an effective concentration previously reported to inhibit I_{K1} and I_{Kss}.[6,7] The concentrations of prazosin (5 μM), atenolol (10 μM), ICI-118,551, and propranolol (1 μM) used were chosen to achieve maximal effective blockade of, respectively, α_1-, β_1-, β_2-, and combined β_1-/β_2-adrenoceptors.[7,33–35] Zinterol (1 μM) and fenoterol (10 μM) were used in the presence of 10 μM atenolol to achieve selective β_2-adrenoceptor activation.[34,35] Protein kinase A (PKA) was inhibited using 10 μM H-89, a concentration that has been shown to inhibit phosphorylation of the cardiac α_{1c} L-type Ca^{2+} channel subunit.[36]

2.5 Data analysis

Current and voltage recordings were analysed using IgorPro (vs3.16B, Wavemetrics, Inc., USA). The voltage-dependence of the L-type Ca^{2+} current (I_{CaL}) and the noradrenaline-sensitive steady-state outward current were fitted with modified Boltzmann equations. The variance of the noradrenaline-sensitive, steady-state outward current was calculated from the integral of the spectral density function, as described previously.[14]

2.6 Statistical analysis

Data are presented as the mean ± SEM. Sample sizes are provided in the figure legends (numbers of cells/numbers of animals). Statistical analyses were performed using Prism (vs5.01, GraphPad Software, Inc., USA). Current–voltage relations were analysed by two-way repeated-measures (RM) analysis of variance (ANOVA) with the Bonferroni *post hoc* test. All other results were analysed by one-way ANOVA with Bonferroni's multiple comparisons test. A *P*-value of ≤ 0.05 was considered significant.

3. Results

3.1 Whole-cell currents modulated by noradrenaline

Two distinct current components were activated by square-shaped depolarizing pulses (500 ms) to potentials of −20 mV and positive:

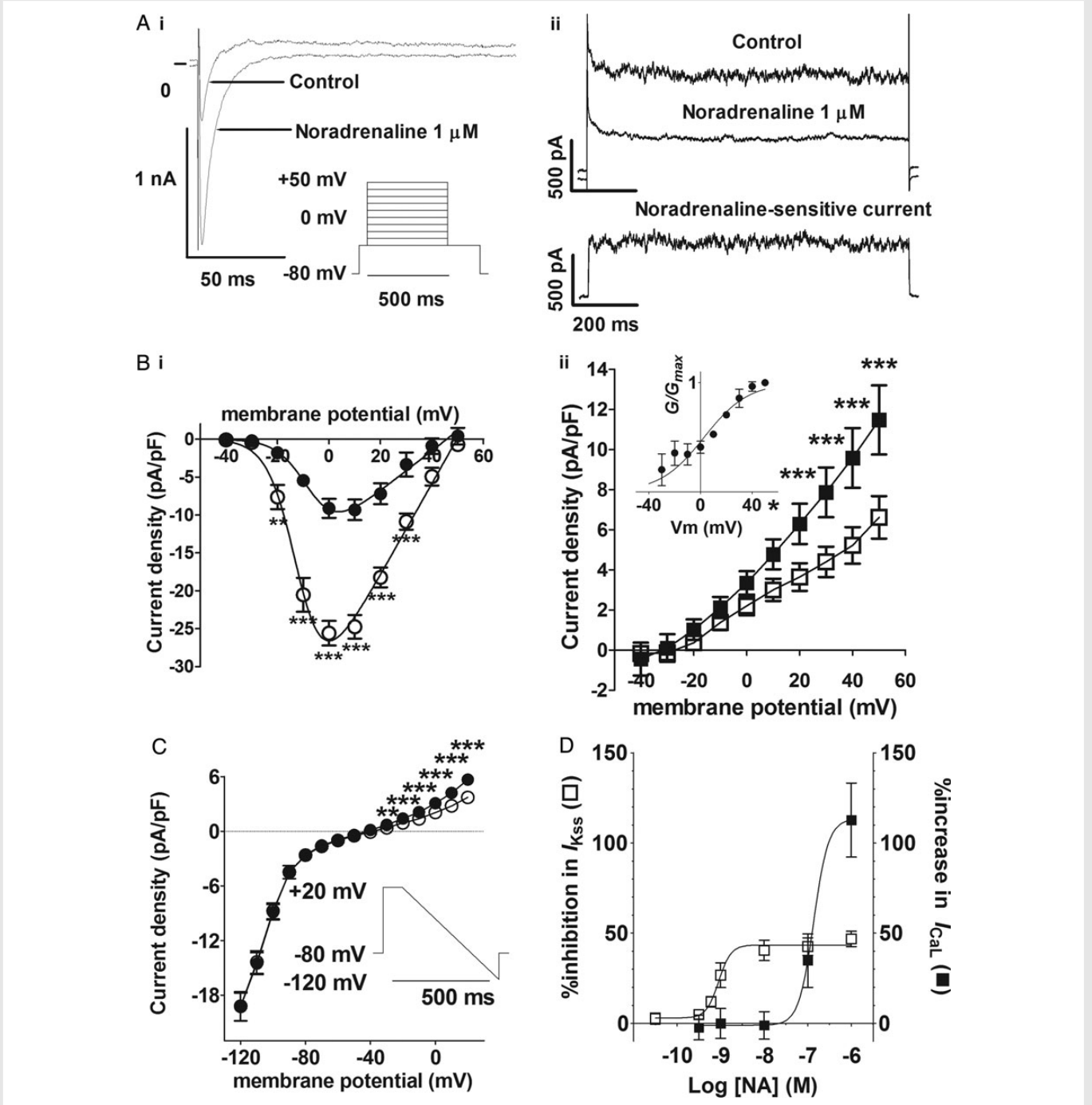

Figure 1 Effects of noradrenaline on L-type Ca^{2+} (I_{CaL}) and steady-state currents. (*A*) Example traces in control solution and in the presence of noradrenaline (1 μM) at 0 mV (i) and at +50 mV (ii). Inset in (i) shows the voltage protocol. Lower panel in (ii) shows the noradrenaline-sensitive current at +50 mV calculated as $I_{control} - I_{noradrenaline}$ (scale bars, the same for upper and lower panels). (*B*) Mean current density–voltage relations from seven cells (five animals) for the peak inward current (i, circles) and the steady-state current (ii, squares). Control—filled symbols; 1 μM noradrenaline—open symbols. Solid lines in (i) represent fits to a modified Boltzmann equation. G_{max}, V_{rev}, V_{half}, and k were, respectively, 0.265 kS F^{-1}, +45.8 mV, −6.9 mV, and +5.8 mV in control and 0.603 kS F^{-1}, +49.7 mV, −11.9 mV, and +5.5 mV in noradrenaline. Inset in (ii) shows the voltage-dependence of activation for the 1 μM noradrenaline-sensitive difference current; solid line represents fit to a modified Boltzmann equation. (*C*) Effect of 1 μM noradrenaline on mean background current–voltage relations ($n = 6/2$). *$P < 0.05$; ***$P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test. Inset shows a voltage-ramp protocol. (*D*) Concentration-dependent action of noradrenaline on the percentage inhibition of I_{Kss} (open squares) and percentage increase in I_{CaL} (filled squares) at +20 mV. Data from 4 to 16 cells/1–8 animals at each concentration. Solid lines represent a fit to a logistic equation.

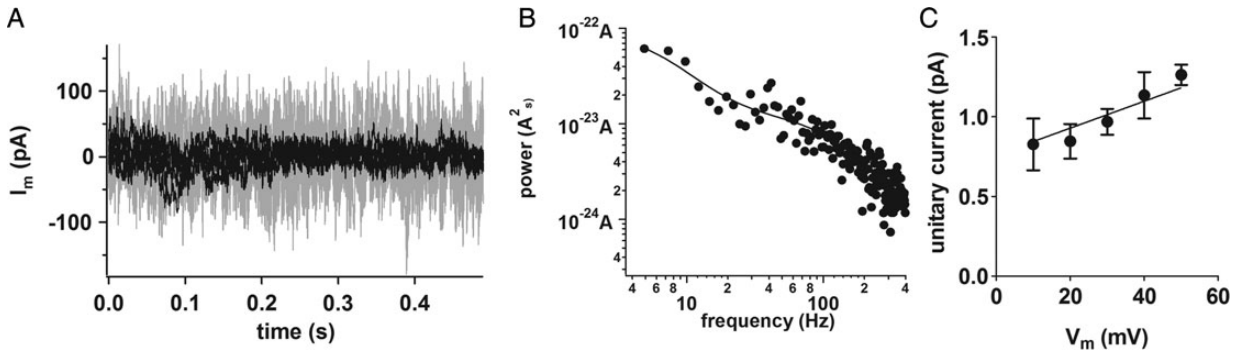

Figure 2 Power spectral analysis of the noradrenaline-sensitive I_{Kss}. (*A*) Five consecutive DC-subtracted traces recorded at +50 mV in control (grey traces) and in noradrenaline-containing (black traces) solution. (*B*) A representative power spectrum for the noradrenaline-sensitive current; data correspond to that shown in (*A*). Solid line represents a fit to a double Lorentzian equation; $S(0)_1 = 8.15$ pA^2 s^{-1}, $f_{c1} = 6.3$ Hz, $S(0)_2 = 1.15$ pA^2 s^{-1}, $f_{c2} = 135.6$ Hz. (*C*) Mean unitary current–voltage relations for the noradrenaline-sensitive current ($n = 8/6$). Solid line was fitted by linear regression assuming zero current at E_K (−90.9 mV).

(i) an inward current that rapidly reached a peak and subsequently inactivated to a steady-state outward current level by the end of the pulse, representing the L-type Ca^{2+} current (I_{CaL}) (*Figure 1*Ai) and (ii) a steady-state outward current that showed little inactivation at the end of the pulse (*Figure 1*Aii). I_{CaL} showed voltage-dependent activation with $V_{half} \approx -7$ mV ($k \approx 6$ mV) and a maximal inward current density at 0 mV under control conditions (*Figure 1*Bi). Superfusion of the cells with 1 μM noradrenaline produced a marked increase in I_{CaL} across the voltage range, and this was associated with a ∼2.3-fold increase in G_{max} and a ∼5 mV negative shift in V_{half}. In addition, 1 μM noradrenaline also inhibited the steady-state outward current at positive potentials, the current at +50 mV being reduced by 42.1 ± 4.3% ($n = 7/5$) (*Figure 1*Bii). Note the time-independent nature of the noradrenaline-sensitive outward current at +50 mV (lower panel, *Figure 1*Aii). The noradrenaline-sensitive outward current component was weakly voltage-dependent ($V_{half} = 2.71 \pm 3.22$ mV, $k = 17.11 \pm 3.04$ mV), being activated from potentials positive to −40 mV (inset, *Figure 1*Bii). The inwardly rectifying current obtained at negative potentials using a modified ramp protocol was abolished in the absence of external K^+ (see Supplementary material online, *Figure S1*), demonstrating the involvement of $K_{ir}2.x$ channels.[37] While 1 μM noradrenaline inhibited the steady-state outward currents evident at potentials positive to −40 mV (*Figure 1*Bii), the currents negative to −40 mV were unaffected (*Figure 1C*). Thus, under the conditions of this study, noradrenaline inhibited a time-independent, outwardly rectifying weakly voltage-dependent current, whereas I_{K1} was unaffected (see Supplementary material online, *Figure S1E*). Noradrenaline (1 μM) had similar effects on whole-cell currents in isolated mouse atrial myocytes (see Supplementary material online, *Figure S2*). The effects of noradrenaline on the steady-state outward current were abolished in the absence of internal and external K^+, demonstrating the K^+-selective nature of the noradrenaline-sensitive I_{Kss} (see Supplementary material online, *Figure S3B*). In rat atrial cells, I_{Kss} was inhibited concentration-dependently by noradrenaline with $\log(IC_{50}) = -9.048 \pm 0.0979$ M ($EC_{50} = 0.895$ nM), whereas I_{CaL} was potentiated with a $\log(EC_{50}) = -6.687 \pm 2.882$ M ($EC_{50} = 136$ nM) (*Figure 1D*). Thus, noradrenaline was ∼150-fold more potent in the inhibition of I_{Kss} than in the increase of I_{CaL}.

3.2 Power spectral analysis of noradrenaline-sensitive I_{Kss}

It was notable in the recordings of steady-state outward currents in the presence of K^+ that the level of noise in I_{Kss} was reduced by noradrenaline (*Figure 1*Aii). This is demonstrated in *Figure 2A*, in which 5 DC-component-subtracted currents at +50 mV in the presence of noradrenaline (black traces) are superimposed on 5 DC-subtracted control currents (grey). This reduction in noise reflects the action of noradrenaline on the gating of the underlying K^+-selective channels. Because the open-channel conductance is a function of the current variance, the power spectra provide a measure of the unitary conductance.[38] The power spectra could be described by a double Lorentzian (*Figure 2B*), in which the corner frequencies (mean values at +50 mV in parentheses), f_{c1} (4.1 ± 1.6 Hz) and f_{c2} (148.5 ± 25.3 Hz), are functions of channel gating and the low-frequency asymptotes; $S(0)_1$ (572 ± 251 pA^2 s^{-1}) and $S(0)_2$ (4.70 ± 1.04 pA^2 s^{-1}) are functions of the power of the noise arising from channel gating ($n = 8/6$ cells). The open-channel conductance calculated by a linear regression of the unitary current–voltage relations was 8.44 ± 0.42 pS (*Figure 2C*, $n = 8/6$).

3.3 Sensitivity of I_{Kss} to K^+ channel modulators

The outward currents at positive potentials were reduced by the blocker of voltage-gated K^+ channels, 4-AP (3 mM),[39] although noradrenaline in the continued presence of 4-AP inhibited the current further so that the response to noradrenaline at +20 mV was reduced to ∼36% of control (*Figure 3A* and *B*). On the other hand, neither the KCNQ1/I_{Ks} delayed rectifier channel blocker, chromanol 293B (10 μM),[40] nor the TASK-1/TASK-3 blocker, Zn^{2+} (1 mM),[41,42] had any significant effect on the response to noradrenaline, although the control steady-state outward current was reduced by 28.9 ± 4.1% ($n = 5/2$) in the presence of the divalent cation (*Figure 3B*). These data are consistent with the proposition that TASK-1/3 contributes to an outward current in rat atrial myocytes that is not modulated by noradrenaline. On the other hand, the background currents at both positive and negative potentials were markedly inhibited by the TREK-1 blockers, fluoxetine and mibefradil (see Supplementary material online,

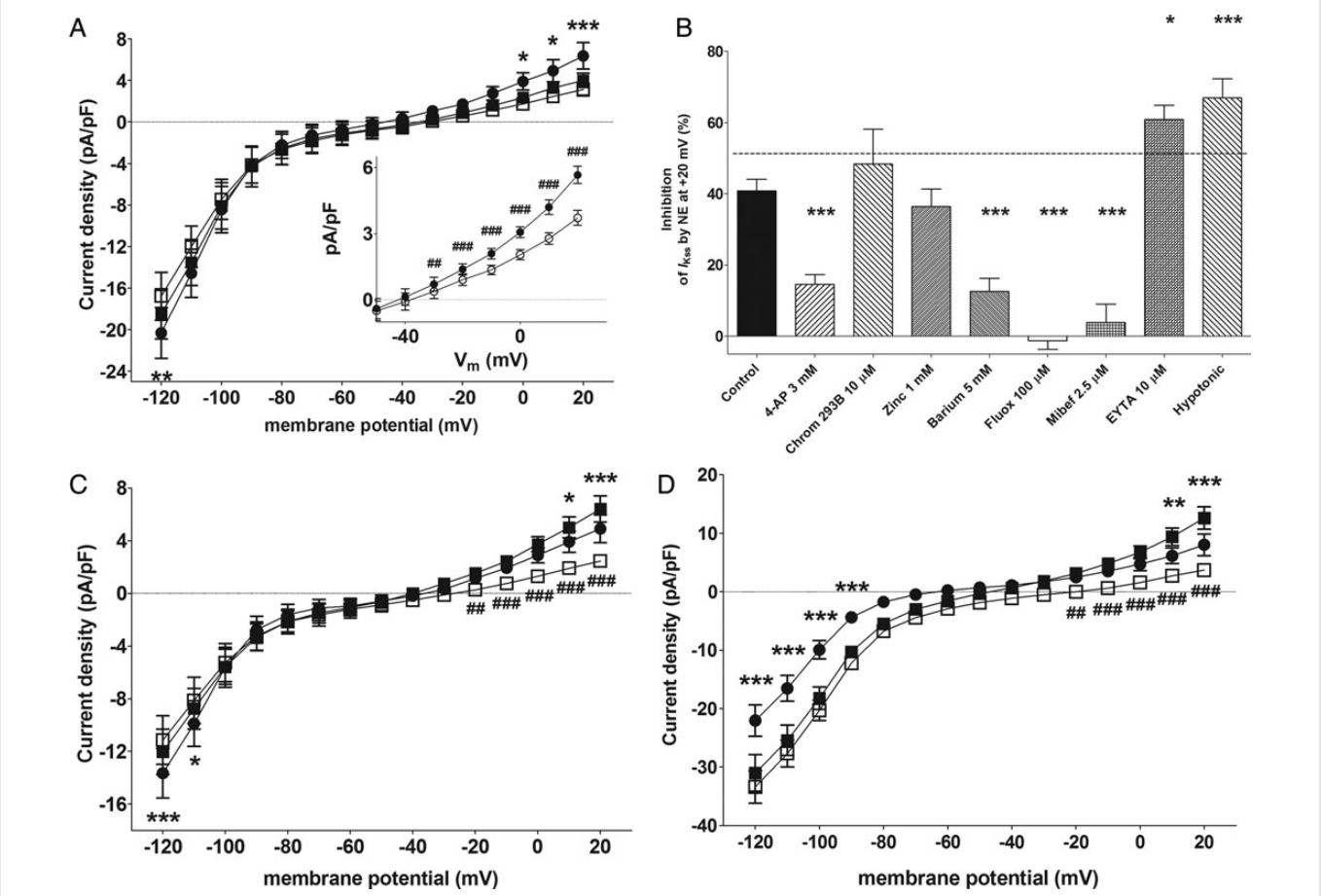

Figure 3 Effect of K^+ channel modulators on the noradrenaline-sensitive current. (*A*) Mean background current density–voltage relations from six cells/two animals in control solution (filled circles), in the presence of 4-AP (3 mM, filled squares) and in the presence of 4-AP + noradrenaline (1 μM, open squares). Inset reproduces the effect of noradrenaline under control conditions shown in *Figure 1D* over the voltage range, −50 to +20 mV. 4-AP vs. control: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; inset noradrenaline vs. control: $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test. (*B*) Effect of K^+ channel modulators on the mean percentage inhibition by noradrenaline of I_{Kss} at +20 mV. Control ($n = 8/6$), 4-AP ($n = 6/2$), chromanol 293 B (Chrom 293B; $n = 5/1$), Zn^{2+} ($n = 5/2$), Ba^{2+} ($n = 7/1$), fluoxetine (fluox; $n = 7/2$), mibefradil (mibef; $n = 6/2$), EYTA ($n = 5/3$), and hypotonic solution ($n = 7/2$). $^{**}P < 0.01$; $^{***}P < 0.001$; one-way ANOVA with the Bonferroni multiple comparisons test vs. control. (*C*) Mean background current density–voltage relations in control solution (filled circles) and in the presence of EYTA (1 μM, filled squares) and in the presence of EYTA + noradrenaline (1 μM, open squares, $n = 5/3$). $^*P < 0.05$; $^{***}P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test, EYTA vs. control. $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test, noradrenaline + EYTA vs. EYTA. (*D*) Mean background current density–voltage relations in control solution (filled circles), in the presence of hypotonic solution (filled squares) and in the presence of hypotonic solution + noradrenaline (1 μM, open squares, $n = 7/2$). $^{**}P < 0.01$; $^{***}P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test, hypotonic vs. control. $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; two-way RM ANOVA with the Bonferroni *post hoc* test, noradrenaline + hypotonic vs. hypotonic.

Figure S4).[43–46] The fluoxetine- and mibefradil-sensitive difference currents reversed close to the K^+ equilibrium potential (E_K), demonstrating their K^+-selectivity (see Supplementary material online, *Figure S4B* and *D*). Most strikingly, the effect of noradrenaline on the steady-state outward current was abolished in the presence of the TREK-1 channel blockers (*Figure 3B*). TREK-1 channel currents are reported to be activated by arachidonic acid and by stretch.[20–22,24,47] The effect of the non-metabolizable analogue of arachidonic acid, EYTA (10 μM), on the noradrenaline-sensitive current was investigated (*Figure 3C*). Superfusion of the cells with EYTA increased the steady-state outward current at +20 mV by 38.6 ± 16.2% ($P < 0.05$; $n = 5/3$; *Figure 3C*). Addition of noradrenaline in the continued presence of EYTA resulted in a reduction of the steady-state outward current by 60.9 ± 3.9% ($P < 0.001$; $n = 5/3$; *Figure 3C*), representing an augmentation of the response to noradrenaline of ∼50% (*Figure 3B*). Similarly, hyposmotic stretch also increased I_{Kss} at +20 mV (69.7 ± 17.0%, $n = 7/2$, $P < 0.05$), and noradrenaline inhibited the hyposmotically potentiated current (67.0 ± 12.6%; $P < 0.001$), representing ∼63% augmentation of the response to noradrenaline (*Figure 3B* and *D*).

3.4 Adrenoceptor subtypes in the noradrenaline response

The inhibitory effect of noradrenaline on I_{Kss} at concentrations of 1–100 nM was strongly reduced by either the β_1-adrenoceptor blocker, atenolol (10 μM), or β_2-selective ICI-118,551 (100 nM; *Figure 4A*). However, neither antagonist was effective against the response to 1 μM noradrenaline when applied alone (*Figure 4A*). On the

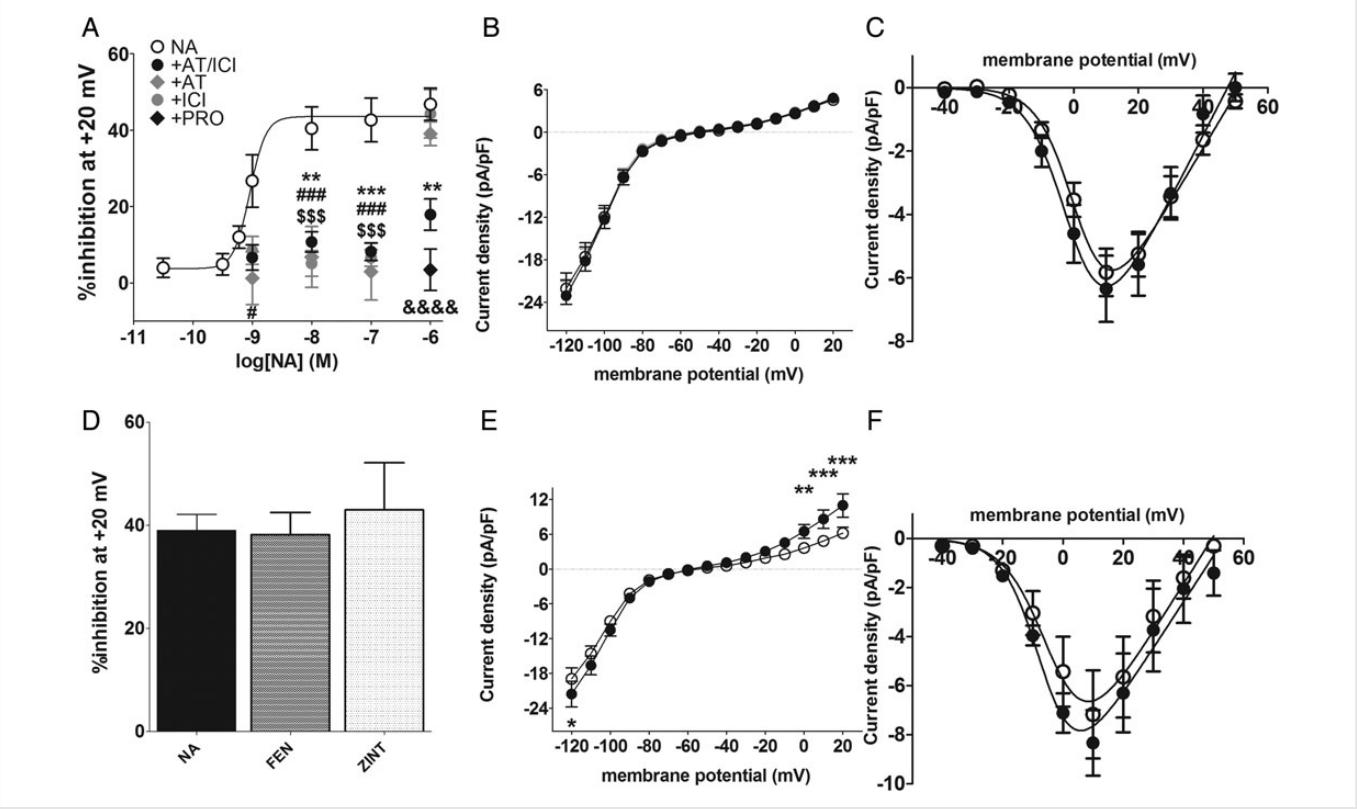

Figure 4 Effect of adrenoceptor antagonists on the effects of noradrenaline. (*A*) Effect of adrenoceptor antagonists on the concentration-dependence of mean percentage I_{Kss} inhibition by noradrenaline at +20 mV. Control (open circles, $n = 8/6$), atenolol (grey-filled diamonds, $n = 11/4$), ICI-118,551 (grey-filled circles, $n = 10/4$), atenolol plus ICI-118,551 (black-filled circles, $n = 5/2$), and propranolol (black-filled diamonds, $n = 8/4$). Solid line represents a fit to a logistic equation. Control data as shown in *Figure 1D*. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$; two-way ANOVA with the Bonferroni *post hoc* test, atenolol vs. control. $^{\$\$\$}P < 0.001$, two-way ANOVA with the Bonferroni *post hoc* test, ICI-118,551 vs. control. $^{**}P < 0.01$ and $^{***}P < 0.001$; two-way ANOVA with the Bonferroni *post hoc* test, atenolol/ICI-118,551 vs. control. $^{\&\&\&\&}P < 0.0001$, Student's paired *t*-test, propranolol vs. control. (*B*) Mean background current–voltage relations in control (grey-filled circles), in the presence of 1 μM propranolol (black-filled circles) and in the presence of propranolol + noradrenaline (open circles, $n = 8/4$). Note that the IV curves are almost completely superimposed. (*C*) Mean I_{CaL} density–voltage relations in the presence of propranolol (filled circles) and in the presence of propranolol + noradrenaline (open circles, $n = 9/4$). I_{CaL}–V relation in the presence of propranolol alone was not significantly different from control (*Figure 1Bi*, $P = 0.1388$, two-way ANOVA with RM for voltage). (*D*) Mean percentage inhibition of I_{Kss} at +20 mV by β_2-agonists, noradrenaline (1 μM, $n = 11/4$), fenoterol (10 μM, $n = 8/3$), and zinterol (1 μM, $n = 6/2$) in the presence of 10 μM atenolol. (*E*) Effect of 1 μM zinterol on mean background current density–voltage relations in the presence of 10 μM atenolol ($n = 6/2$). Filled circles—control; open circles—1 μM zinterol. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; two-way ANOVA with the Bonferroni *post hoc* test, zinterol vs. control at each voltage. (*F*) Effect of 1 μM zinterol on I_{CaL} density–voltage relations in the presence of 10 μM atenolol ($n = 5/2$). Solid lines in (*C* and *F*) represent fits to modified Boltzmann equations.

other hand, the effect of 1 μM noradrenaline was markedly inhibited when the β_1- and β_2-antagonists, atenolol (10 μM) and ICI-118,551 (100 nM), were applied in combination (*Figure 4A*). The apparent rightward-shift in the concentration- dependence of I_{Kss} inhibition by noradrenaline produced by either atenolol or ICI-118,551 when applied alone, taken together with the cumulative effect against 1 μM noradrenaline when the antagonists were applied in combination, demonstrates the involvement of both β_1- and β_2-adrenoceptor subtypes in response to noradrenaline. Consistent with this, the effect of 1 μM noradrenaline on I_{Kss} was abolished by the non-selective β_1-/β_2-adrenoceptor antagonist, propranolol (1 μM; *Figure 4A* and *B*). The effect of 1 μM noradrenaline on the steady-state outward current at +20 mV (46.8 ± 2.56%, $n = 13$) was unaffected by the α-adrenoceptor antagonist, prazosin (5 μM), either applied alone (46.6 ± 4.06%, $n = 5$) or in combination with 10 μM atenolol (42.3 ± 5.11%, $n = 5$). I_{Kss} was inhibited by β_2-adrenoceptor-selective agonism with either zinterol (1 μM) or fenoterol (10 μM) in the presence of 10 μM atenolol, in a manner similar to noradrenaline, consistent with a role for β_2-adrenoceptors in the inhibition of I_{Kss} (*Figure 4D* and *E*). The augmentation of I_{CaL} by 1 μM noradrenaline was also completely inhibited in the presence of propranolol (*Figure 4C*). The increase of I_{CaL} by 1 μM noradrenaline (201 ± 54.6% at +10 mV, $n = 6$, *Figure 1Bi*) was also completely abolished in the presence of the β_1-adrenoceptor antagonist, 10 μM atenolol (−3.1 ± 2.69%, $n = 5$). On the other hand, zinterol had no effect on I_{CaL} (*Figure 4F*), indicating that the effect of noradrenaline on I_{CaL} was mediated predominantly by β_1-adrenoceptors.

3.5 Cell signalling pathways

The β_2-adrenoceptor couples with both G_s- and G_i-mediated signalling pathways.[3] G_s-protein activates the adenylyl cyclase/PKA cascade involving cyclic AMP.[3] The inhibitory effect of noradrenaline on the steady-state outward current could still be observed following

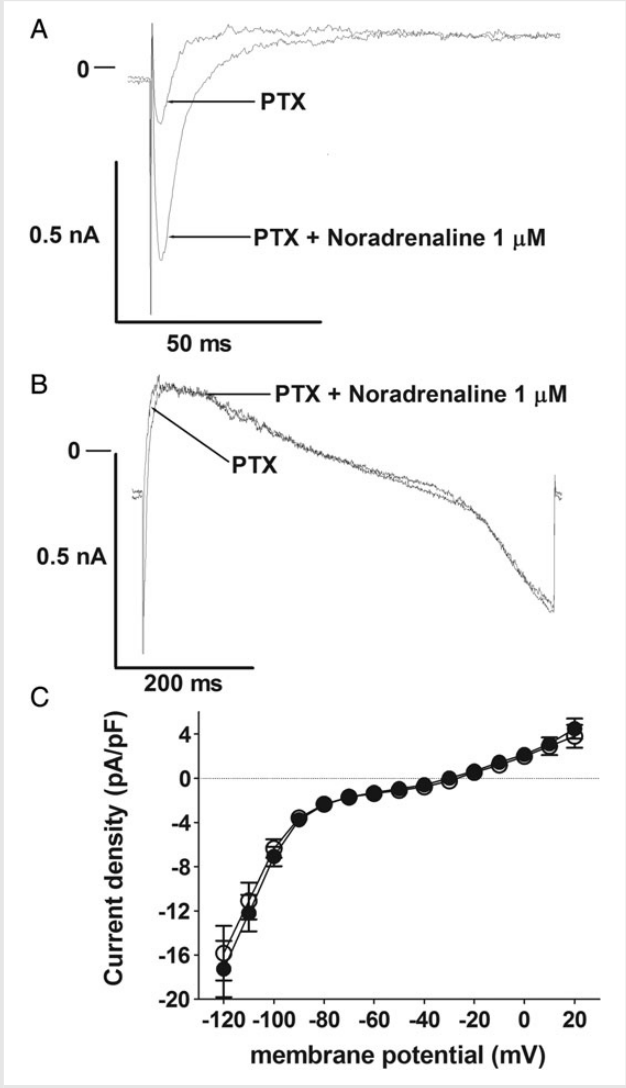

Figure 5 Effect of PTX on the action of noradrenaline. (*A*) An example of I_{CaL} traces in control and 1 μM noradrenaline at 0 mV after pretreatment with PTX. The effect of noradrenaline is comparable with that seen in PTX-untreated cells (*Figure 1*Ai). (*B*) An example of ramp current traces in control and noradrenaline following pretreatment with PTX. (*C*) Mean current density–voltage relations in control (filled circles) and noradrenaline (open circles) in PTX pretreated cells ($n = 5/2$). In contrast to PTX-untreated cells (*Figure 1C*), the effect of noradrenaline on currents at positive potentials is lost.

treatment of the cells with the PKA inhibitor, H-89 (10 μM), at a concentration sufficient to ablate completely the augmentation of I_{CaL} (see Supplementary material online, *Figure S5*), indicating that PKA was not essential to the action of noradrenaline on I_{Kss}. The role of G_i-proteins in the inhibitory effect of noradrenaline on the steady-state outward current was investigated by pretreatment of the cells in PTX (*Figure 5*). While the effect of noradrenaline on I_{CaL} remained intact in PTX-treated cells (*Figure 5A*), the steady-state outward current inhibition was completely abolished (*Figure 5B* and *C*). Taken together, these results show that the inhibitory effect of noradrenaline on the steady-state outward current was mediated via a PTX-sensitive mechanism that did not require PKA. On the other hand, β_2-adrenoceptors did not contribute to the potentiation of I_{CaL} by noradrenaline, which likely involved β_1-adrenoceptors acting via PKA.

3.6 AP prolongation by noradrenaline

The effects of noradrenaline (1 μM) on the AP were investigated (*Figure 6*). Noradrenaline caused a delay in the early and mid-phases of repolarization, consistent with changes in I_{CaL} and I_{Kss} (*Figure 6A*). The effect of noradrenaline in the presence of the β_1-antagonist, atenolol, and the non-selective β_1-/β_2-blocker, propranolol, is shown in *Figure 6B* and *C*, respectively. The effects of noradrenaline on mean AP duration at 30% repolarization (APD_{30}) in the absence of and the presence of the β-blockers are shown in *Figure 6D*. Noradrenaline caused ~65% prolongation of APD_{30}. Although β_1-antagonism produced a partial reduction in APD_{30} prolongation, the effect of noradrenaline was completely abolished with combined β_1-/β_2-blockade using either 100 nM ICI-118,551 in combination with 10 μM atenolol or 1 μM propranolol (*Figure 6D*).

4. Discussion

This study demonstrates for the first time the potent inhibition of a steady-state outward K^+ current in atrial myocytes by nanomolar concentrations of noradrenaline ($IC_{50} = 0.90$ nM). In contrast, noradrenaline was ~150-fold less potent in potentiating I_{CaL} ($EC_{50} = 136$ nM). Both β_1- and β_2-adrenoceptors were co-operatively involved in the inhibition of I_{Kss}, which was via a PTX-sensitive pathway that did not require PKA. On the other hand, the potentiation of I_{CaL} by noradrenaline was mediated via β_1-adrenoceptors through PKA. The noradrenaline-sensitive current had pharmacological and biophysical properties consistent with the involvement of a TREK-like channel. Inhibition of I_{Kss} contributed to the noradrenaline-induced prolongation of the AP in atrial myocytes.

4.1 Properties of noradrenaline-sensitive I_{Kss}

The effectively instantaneous activation of the noradrenaline-sensitive steady-state outward current on depolarization positive to −40 mV and the lack of time-dependent inactivation over the course of the pulse are representative of adrenoceptor-modulated I_{Kss} in rat ventricular myocytes reported previously and rule out contribution of the transient outward current, I_{to}.[6,12,14] Moreover, the observation that 36% of the noradrenaline-sensitive current remained in the presence of 3 mM 4-AP indicates that I_{Kss} was distinct from the comparatively 4-AP-sensitive I_{Kur}/K_v1.5 channel currents that would be almost completely inhibited by the concentration of this voltage-gated K^+ channel blocker.[15,19]

Although control I_{Kss} was inhibited by Zn^{2+}, consistent with the contribution of TASK-1 channels to the whole-cell currents, the response to noradrenaline was unaffected, demonstrating that this K_{2P} channel did not contribute to the noradrenaline-sensitive current. On the other hand, the weak voltage-dependence, the potentiation by the arachidonic acid analogue, EYTA, and by osmotic stretch and the inhibition by fluoxetine, mibefradil, and Ba^{2+} are all properties that noradrenaline-sensitive I_{Kss} shares with TREK-1 channels.[20–22,43–47] The effects of noradrenaline on the power spectrum demonstrate unequivocally the involvement of an ion channel with flickery kinetics in I_{Kss}. The calculated unitary conductance (8.4 pS) is close to the 14 pS originally proposed for TREK-1.[48] However, unitary current recordings of TREK-1-like

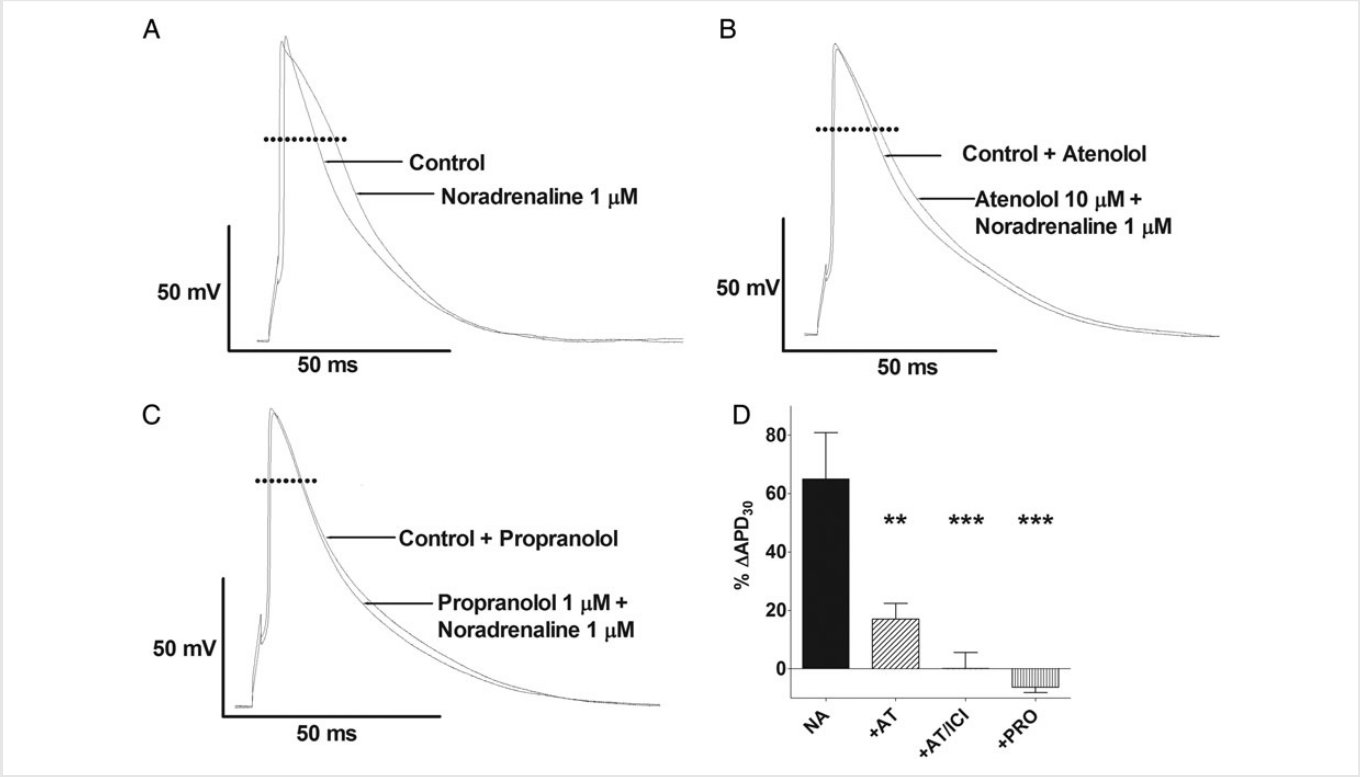

Figure 6 Effects of noradrenaline on APs. (*A*) Representative APs in control and in 1 μM noradrenaline. (*B*) Representative APs in the presence of 10 μM atenolol and 1 μM noradrenaline in the continued presence of atenolol. (*C*) Representative APs in the presence of 1 μM propranolol and 1 μM noradrenaline in the continued presence of propranolol. (*D*) Mean noradrenaline-induced percentage APD_{30}-change (%ΔAPD_{30}) in the absence of antagonist (NA, $n = 5/4$), in the presence of 10 μM atenolol (+AT, $n = 8/4$), in the presence of 100 nM ICI-118,551 plus 10 μM atenolol (+AT/ICI, $n = 6/2$), and in the presence of 1 μM propranolol (+PRO, $n = 5/2$). $^{**}P < 0.01$; $^{***}P < 0.001$; one-way ANOVA with the Bonferroni *post hoc* test.

channel currents in rat cardiomyocytes have suggested a conductance of ~41 pS.[21,22] The basis for the differences in reported conductance are unclear, but a number of conductance modes for the TREK-1 channel have been reported, and it has been suggested that the conductance mode depends on interaction with cytosolic regulatory proteins.[49] It is conceivable, therefore, that the noradrenaline-sensitive channel represents a lower conductance mode of TREK-1 than was evident previously in cell-attached and excised-patch recordings.[21,22] It is notable that I_{Kss} was also reduced by the removal of external K^+ in a manner reminiscent of K_{ir} channels. Nevertheless, I_{Kss} was distinct from I_{K1}, because noradrenaline had no effect on the inward rectifier currents. Interestingly, a tendency to pore collapse and non-conductive states of the channel in external K^+-free conditions similar to $K_{ir}2.1$ has recently been suggested for TREK-1.[50] Thus, our data are consistent with the involvement of a TREK-1-like channel in the noradrenaline-sensitive I_{Kss} of rat atrial myocytes.

4.2 Inhibition of I_{Kss} is mediated via a PTX-sensitive mechanism involving both β_1- and β_2-adrenoceptors

The effect of receptor subtype-selective concentrations of atenolol (10 μM) and ICI-118,551 (100 nM)[33–35] on the concentration response of I_{Kss} inhibition by noradrenaline demonstrated the involvement of both β_1- and β_2-adrenoceptors. Either atenolol or ICI-118,551 was effective at inhibiting responses to 1–100 nM noradrenaline, indicating co-operativity between β_1- and β_2-adrenoceptor pathways. It is notable that noradrenaline was effective at inhibiting I_{Kss} at concentrations far below the reported dissociation constant at either β_1- (K_d~ 1–4 μM) or β_2-adrenoceptors (K_d~ 4–26 μM).[51–53] Interestingly, it has been reported that β_1-/β_2-adrenoceptor subunits can form heterodimeric receptors in cardiomyocytes that have an increased agonist sensitivity relative to the respective homodimeric receptors.[54] Moreover, the action of noradrenaline in inhibiting I_{Kss} with greater potency than potentiation of I_{CaL} is consistent with a previous report of the effects of the β-agonist, isoprenaline, on I_{Kss} and I_{CaL} in rat ventricular myocytes.[12]

The abolition of I_{Kss} responses following pretreatment with PTX demonstrated the involvement of a G_i-protein, consistent with reports of coupling of β_1-, as well as β_2- adrenoceptors, to G_i-protein pathways in cardiomyocytes.[55,56] The involvement of β-adrenoceptors in the inhibition of TREK-1 channel-like currents in rat atrial myocytes has been suggested previously.[21] However, the inhibitory response to noradrenaline was not abolished by treatment with H-89, indicating that PKA was not required for the regulation of I_{Kss}. Although the effect of noradrenaline on I_{Kss} was reduced in the presence of H-89, this was likely due to non-specific actions of the PKA inhibitor.[57] The requirement of both β_1- and β_2-adrenoceptors for I_{Kss} inhibition by noradrenaline and the PTX sensitivity of this response suggest that the receptors and G_i-proteins modulating TREK-like channel activity are co-localized, consistent with the ability of β_1-/β_2-adrenoceptor subtypes to form heterodimers.[54]

The potentiation of I_{CaL} by noradrenaline was mediated predominantly via a PTX-insensitive, H-89-sensitive β_1-adrenoceptor pathway, consistent with an essential role for PKA in the regulation of cardiac L-type Ca^{2+} channels.[56,58] I_{CaL} was not potentiated by zinterol, indicating that, in contrast to ventricular cells, β_2-adrenoceptors were not involved in the regulation of L-type Ca^{2+} channels in rat atrial myocytes.[56,59] Consistent with this view, the potentiation of I_{CaL} by 1 μM noradrenaline was completely abolished in the presence of β_1-receptor-selective atenolol.

4.3 Noradrenergic inhibition of I_{Kss} contributes to AP prolongation

Noradrenaline caused prolongation of the AP at relatively depolarized potentials, but had little effect closer to the resting membrane potential. The prolongation of APD_{30} by noradrenaline was consistent with the actions of noradrenaline on I_{CaL} and I_{Kss}. Under the conditions of this study, it was possible to discriminate between the actions of noradrenaline on I_{CaL} and I_{Kss} through differences in their sensitivity to β-adrenoceptor-selective antagonists. For example, in the presence of atenolol alone, noradrenergic potentiation of I_{CaL} would be blocked while the inhibition of I_{Kss} would remain intact. On the other hand, in the presence of combined β_1-/β_2-antagonism, the effect of noradrenaline on I_{Kss} would also be reduced. Thus, the correlation between the degree of noradrenaline-induced APD_{30} prolongation and the degree of noradrenergic inhibition of I_{Kss} in the presence of β_1-/β_2-non-selective propranolol, β_1-selective atenolol, and β_2-selective ICI-118,551 in combination and atenolol alone (see Supplementary material online, *Figure S6*) illustrated the contribution of I_{Kss} to the noradrenergic prolongation of the AP. AP prolongation through I_{Kss} inhibition is likely to affect sarcolemmal Ca^{2+} fluxes via L-type Ca^{2+} channels and the Na^+/Ca^{2+} exchanger and thereby to contribute to the effects of noradrenaline on atrial contractility and arrhythmogenesis.

4.4 Possible physiological significance of noradrenaline-sensitive I_{Kss}

It has not been possible, to date, to measure directly the concentration of noradrenaline achieved at the neuroeffector junction of cardiac postganglionic fibres during sympathetic activity, but it is generally estimated to be >100 nM.[60] The EC_{50} for noradrenergic potentiation of I_{CaL} corresponds well with this value, consistent with the potentiation via β_1-adrenoceptors of atrial Ca^{2+} entry during sympathetic activity.[2,4] The 'basal' concentration of noradrenaline at the cardiac neuroeffector junction is also unknown, but it is likely to be similar to circulating concentrations of noradrenaline, which are reportedly very low (1–2 nM).[60] Since the IC_{50} for inhibition of I_{Kss} by noradrenaline was ~0.90 nM, it seems likely that this current would be inhibited, contributing to AP prolongation at relatively modest levels of sympathetic activity. On the other hand, this TREK-like channel current is activated by stretch and may make a greater contribution to AP repolarization under physiological mechanical loads *in vivo*.[22] The inhibition by noradrenaline may provide a mechanism by which AP configuration is optimized to mechanical load and degree of autonomic activation.

4.5 Limitations

This study provides good evidence of a role for a TREK-like I_{Kss} in repolarization of rat atrial myocytes and demonstrates the existence of a similar noradrenaline-sensitive current in mouse atrial cells. Expression of TREK-1 has recently been demonstrated in the human heart.[61] However, it is currently unclear to what extent I_{Kss} contributes to the repolarization of the human atrium. Moreover, it is not clear whether these TREK-like currents are atrial selective. Further work is therefore merited to establish the contribution of I_{Kss} and K_{2P} channels to human cardiac electrophysiology.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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