Properties of a Time-Dependent Potassium Current in Pig Atrium: Evidence for a Role of Kᵥ1.5 in Repolarization

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ABSTRACT

Cardiac electrical activity is modulated by potassium currents. Pigs have been used for antiarrhythmic drug testing, but only sparse data exist regarding porcine atrial ionic electrophysiology. Here, we used electrophysiological, molecular, and pharmacological tools to characterize a prominent porcine outward K⁺ current (Iₖ,PCO) in atrial cardiomyocytes isolated from adult pigs. Iₖ,PCO activated rapidly (time to peak at +60 mV; 2.1 ± 0.2 ms), inactivated slowly (τᵢ = 45 ± 10; τᵢ = 215 ± 28 ms), and showed very slow recovery (τᵢ = 1.54 ± 0.73 s; τᵢ = 7.91 ± 1.78 s; n = 9; 36°C). Activation and inactivation were voltage-dependent, and current properties were consistent with predominant K⁺ conductance. Neurotoxins (heteropodatoxin, hongatoxin, and blood depressing substance) that block K₄.4, K₁,1.1, -1.2, -1.3, and -3.4 in a highly selective manner as well as H₂O₂ and tetraethylammonium, did not affect the current. Drugs with K₁.5-blocking properties (flecainide, perhexiline, and the novel atrial-selective antiarrhythmic 2’-[(4-methoxyphenyl)-acetylamo]-methyl-biphenyl-2-carboxylic acid (AVE0118) inhibited Iₖ,PCO (IC₅₀ of 132 ± 47, 17 ± 10, and 1.25 ± 0.62 μM, respectively). 4-Aminopyridine suppressed the current and accelerated its decay, reducing charge carriage with an IC₅₀ of 39 ± 15 μM. Porcine-specific Kᵥ channel subunit sequences were cloned to permit real-time quantitative reverse transcription-polymerase chain reaction on RNA extracted from isolated cardiomyocytes, which showed much greater abundance of Kᵥ1.5 mRNA compared with Kᵥ1.4, Kᵥ4.2, and Kᵥ4.3. Action potential recordings showed that Iₖ,PCO inhibition with 0.1 mM 4-AP delayed repolarization (e.g., action potential duration at −50 mV increased from 45 ± 9 to 69 ± 5 ms at 3 Hz; P < 0.05). In conclusion, porcine atrium displays a current that is involved in repolarization, inactivates more slowly than classic transient outward current, is associated with strong Kᵥ1.5 expression, and shows a pharmacological profile typical of Kᵥ1.5-dependent currents.

Atrial fibrillation (AF) is a very common arrhythmia contributing to morbidity and mortality of affected patients (Wolf et al., 1998). Medical treatment remains a mainstay of therapy (Nattel and Opie, 2006), although the use of antiarrhythmic drugs is limited by potentially deleterious side effects (Hohnloser and Singh, 1995). Porcine models have been used to test Kᵥ1.5-targeting atrial-selective drugs that are designed to circumvent this proarrhythmic risk inherent to treatment with conventional antiarrhythmic drugs (Wirth et al., 2003). However, to date there has been no clear demonstration of Kᵥ1.5-related currents in porcine atrium.

Potassium currents [in particular transient outward currents (Iₒ) and Iₖur] are among the targets of novel atrial-selective drugs developed for treating AF (Knobloch et al., 2004). Kinetic differences of activation, inactivation and recovery from inactivation discriminate between various potassium currents and are related to properties of the underlying ion channel subunits. The properties of Iₒ have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004). The properties of Iₖur have been characterized extensively in many species (Patel and Campbell, 2004).
Fast-inactivating \( I_{\text{to}} \) is carried by Kv4.3 subunits in dog and human, whereas Kv1.4 subunits contribute to \( I_{\text{to,f}} \) in rabbit atria (Wang et al., 1999; Patel and Campbell, 2005). Rabbit \( I_{\text{to}} \) is characterized by particularly slow recovery from inactivation, which is related to the participation of Kv1.4 subunits (Wang et al., 1999). In human atrium, \( I_{\text{Kur}} \) is carried by Kv1.5 subunits, a member of the delayed rectifier current family, and has been shown to inactivate slowly over a period of seconds (Feng et al., 1998; Nattel et al., 1999). At room temperature, \( I_{\text{Kur}} \) may show a non-inactivating phenotype (Li et al., 2004) although recording conditions importantly modulate inactivation kinetics of this current (Snyders et al., 1993). Likewise, \( \beta \)-subunits may modify inactivation properties of Kv1.5 currents (Ubele et al., 1996).

Porcine atrial cellular electrophysiology has been studied to a limited extent and is poorly understood, although this species is commonly used in experimental studies (Jansen et al., 1998). A recent investigation demonstrated the presence of a \( \text{Ca}^{2+} \)-dependent chloride current \( (\text{ICl,Ca}) \) and \( I_{\text{Kur}} \) in porcine atria (Li et al., 2004). In preliminary studies, we noted a robust time-dependent current in porcine atrium that activates rapidly (like \( I_{\text{Kur}} \)) and inactivates more slowly than classic \( I_{\text{to}} \) and somewhat more rapidly than previously reported \( I_{\text{Kur}} \). This study aimed to characterize this porcine outward current (which we abbreviate \( I_{\text{Kur,PO}} \)) with respect to electrophysiological properties, expression of potential corresponding underlying subunit transcripts, pharmacological responses, and functional role. In particular, we were interested in studying the pharmacological profile of the current with respect to known selective blockers of potential underlying \( \text{K}^+ \) channel subunits, with a view to determining whether \( I_{\text{Kur,PO}} \) can potentially account for previous reports of anti-AF actions of Kv1.5 blockers in pig hearts.

**Materials and Methods**

**Animal and Tissue Handling.** Male castrated pigs of the German landrace \((n = 62; 19 \pm 0.5 \text{ kg})\) were anesthetized with intravenous application of 30 mg/kg pentobarbital. During deep anesthesia, hearts were excised via left thoracotomy, resulting in humane euthanasia. Hearts were immediately immersed in oxygenated Tyrode’s solution. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication 85-13, revised 1996) and were performed by technicians specifically trained and experienced in animal care.

For isolation of single cardiomyocytes, the proximal circumflex coronary artery was cannulated and the atrial preparation was perfused with oxygenated Tyrode’s solution on a Langendorff apparatus. The perfusion solution was then switched to \( \text{Ca}^{2+} \)-free Tyrode’s solution until all contraction ceased \((-10 \text{ min})\), and 100 U/ml collageenate \((\text{type II}; \text{Worthington Biochemicals, Freehold, NJ})\)-containing \( \text{Ca}^{2+} \)-free Tyrode’s solution was used for cell isolation as reported previously (Gogelein et al., 2004). After isolation, cells were stored in a high-\( [\text{K}^+] \) storage solution at room temperature and studied within 12 h. Only healthy-looking cells with clear cross-striations and sharp edges were used for electrophysiological measurements. For real-time RT-PCR measurements, aliquots of isolated atrial cardiomyocytes were used, whereas the remainder of the cell isolation was used for electrophysiological experiments on the same days.

**Solutions and Drugs.** The high-\( [\text{K}^+] \)-containing cell storage solution contained 120 mM KCl, 10 mM KH2PO4, 10 mM dextrose, 40 mM mannitol, 70 mM L-glutamic acid, 10 mM \( \beta\)-OH-butyric acid, 20 mM taurine, 10 mM EGTA, and 0.1% bovine serum albumin \((\text{pH} 7.3; \text{KOH})\). Tyrode (extracellular) solution contained 136 mM NaCl, 5.4 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 0.33 mM Na2HPO4, 5 mM HEPES, and 10 mM dextrose, \((\text{pH} 7.35 \text{ at } 36^\circ \text{C}; \text{NaOH})\). CidCl2 \((200 \mu\text{M}; \text{Sigma-Aldrich, St. Louis, MO})\) and 1 \( \mu\text{M} \) HMR 1556 \((\text{Sanofi-Aventis, Frankfurt, Germany}; \text{Gerlach et al., 2001})\) were added to suppress L-type calcium current and \( I_{\text{Na}} \). \( I_{\text{Na}} \) contamination was avoided by using a 50-ms prepulse to \(-50 \text{ mV}\) or by substitution of equimolar Tris-HCl for external NaCl. The internal solution for current recording contained 110 mM K+-aspartate, 20 mM KCl, 1 mM MgCl2, 5 mM MgATP, 0.1 mM Li-GTP, 10 mM HEPES, 5 mM Na-phosphocreatine, and 5 mM EGTA \((\text{pH} 7.3; \text{KOH})\). For action potential \((\text{AP})\) recording, EGTA was omitted. Fresh solutions were prepared daily. Drugs were from Sigma-Aldrich unless otherwise indicated, and toxins were from Alomone Labs (Jerusalem, Israel). Stock solutions were initially prepared and used throughout the study; for individual experiments, cells were incubated until steady-state current inhibition or washout was reached. Toxin-containing solutions were prepared fresh on each day of experimentation.

**Data Acquisition and Analysis.** Currents were recorded in voltage-clamp mode with whole-cell patch-clamp at 36 ± 0.5°C (Gogelein et al., 2004). Data sampling was performed at 1 kHz, and filtering was at 25 Hz. Borehole glass electrodes had tip resistances between 1.5 and 3.0 MΩ when filled with internal solution. Mean \( \pm \text{S.E.M.} \) compensated series-resistance was 5.6 ± 0.1 MΩ. Cell capacitance averaged 47.6 ± 1.9 pF \((n = 141)\). To control for cell size variability, currents were expressed as densities \((\text{pA/pF})\). Junction potentials between bath and pipette solution averaged 3.6 ± 0.5 mV. APs were recorded in current-clamp mode and elicited with 2-ms twice threshold depolarizations.

Nonlinear algorithms were used for curve-fitting. \( t \) tests were used for two-group statistical comparisons. \( P < 0.05 \) indicated statistical significance. Data are expressed as mean \( \pm \text{S.E.M.} \).

**Cloning of Porcine \( K_\text{C} \), Channel Subunits.** To define primers for real-time RT-PCR, partial DNA sequences for pig Kv4.3 \((\text{KCND3})\), Kv4.2 \((\text{KCND2})\), Kv1.4 \((\text{KCNA4})\), and KChIP2 \((\text{KCNP2})\) were identified. The complete sequence for porcine Kv1.5 \((\text{KCNA5})\) has been reported \((\text{NM001006593})\) (Gogelein et al., 2004). PCR was first performed on pig brain \((\text{for KCND3, KCND2, and KCNA4})\) or heart \((\text{for KCNP2})\) cDNA with degenerate primers based on sequences from other species. Pig-specific sequences were then used to design primers (Table 1) to amplify 660 bp \((\text{KCND3})\), 532 bp \((\text{KCND2})\), 630 bp \((\text{KCNA4})\), and 591 bp \((\text{KCNP2})\) cDNA fragments. The fragments were cloned into pCRIIblunt or pCR2.1Topo vectors and sequenced \([\text{GenBank accession nos. DQ285632 (KCND3), DQ285631 (KCND2), DQ285633 (KCNA4), and DQ285634 (KCNP2)}]\).

**Quantitative Real-Time RT-PCR.** Isolated cardiomyocytes were homogenized \((\text{Mixermill 300; QIAGEN, Valencia, CA})\), and total RNA was extracted \((\text{QIAGEN})\). DNase digestion was performed with 10 \( \mu\text{g}\) of RNA, 5 \( \mu\text{L}\) of 10X DNase buffer \((\text{Ambion, Austin, TX})\), 1 \( \mu\text{L}\) of RNase inhibitor \((\text{Applera, Norwalk, CT})\), and 1 \( \mu\text{L}\) of DNase \((2 \text{ U}\,\text{mL}^{-1}; \text{Ambion})\) in 50 \( \mu\text{L}\). cDNA synthesis was performed from 2 \( \mu\text{g}\) of RNA \((\text{reverse transcriptase kit; Applera})\). Samples were incubated at 25°C for 10 min and 42°C for 60 min. The reaction was stopped by heating to 95°C for 5 min.

The RT product was then used as a template for subsequent PCR with gene-specific primers (Table 1). Real-time PCR was performed using an ABI Prism 7900 \((\text{Applera})\) and the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles at 95°C for 15 s, and 1 min at 60°C. Multiplex PCR used 0.125 \( \mu\text{L}\) of target probe \((50 \mu\text{M})\), 0.45 \( \mu\text{L}\) of target forward primer \((50 \mu\text{M})\), 0.45 \( \mu\text{L}\) of target reverse primer \((50 \mu\text{M})\), 12.5 \( \mu\text{L}\) of TaqMan \(2X\) PCR master mix \((\text{Applera})\), 1.25 \( \mu\text{L}\) of \(20X\) target primers and probes \((\text{PreDeveloped TaqMan assay reagents; 18S RNA control; Applera})\), and 10 \( \mu\text{L}\) of cDNA sample \((1:20 \text{ diluted with water})\) or used undiluted. RNA abundance was expressed as \( \Delta\text{Ct} \), subunit expression normalized to that of the internal control \((18S)\) \((\text{Bustin, 2005})\).
### Results

**Voltage and Time Dependence.** One-second depolarizing pulses from a holding potential of ~80 mV to potentials between ~60 and +60 mV (0.1 Hz; Fig. 1A) elicited rapidly activating outward currents showing time-dependent inactivation. Based on this observation and further evidence detailed below, we termed this current $I_{K,PO}$ for porcine outward potassium current. $I_{K,PO}$ amplitude was quantified as the difference between peak and end-pulse steady-state current unless stated otherwise. Threshold to current activation was positive to ~20 mV, and myocytes had a mean ± S.E.M. $I_{K,PO}$-density of 11.6 ± 1.6 pA/pF upon depolarization to +60 mV ($n = 20$; Fig. 1B).

Inactivation voltage dependence was examined with 1000-ms prepulses followed by 750-ms test pulses to +60 mV ($n = 10$ cells; Fig. 1C). Current amplitudes were normalized to current at ~100 mV and plotted against the voltage of the conditioning pulse. Activation voltage dependence was determined from the $I_{K,PO}$-voltage relationship, corrected for driving force according to the equation $\alpha_V = I_v / (I_{\text{max}}(V - E_{\text{rev}}))$, where $\alpha_V$ and $I_v$ are the activation variable and $I_{K,PO}$ amplitude at voltage $V$, $I_{\text{max}}$ is $I_{K,PO}$ amplitude at +60 mV, and reversal potential ($E_{\text{rev}}$) was ~69.2 ± 2.3 mV (obtained from deactivating tail currents recorded at potentials between ~100 and ~60 mV after brief, ~2- to 5-ms depolarizations to +60 mV). $E_{\text{rev}}$ was corrected for liquid junction potentials. Voltages for half-maximal activation and inactivation (Boltzman fits), and corresponding slope factors were 16.8 ± 3.8 mV (slope of 15.4 ± 1.7) and ~28.2 ± 2.9 mV (slope of ~6.1 ± 1.5).

Current activation-speed assessed as time to peak was voltage dependent and became faster at more positive potentials (e.g., ~13.5 ± 1.8 ms at 0 mV, 2.1 ± 0.24 ms at +60 mV; Fig. 1E). Inactivation kinetics were best fitted by biexponential functions, and resulting time constants ($\tau$) were slow (e.g., at +60 mV $\tau_1$ averaged 45 ± 10 and $\tau_\infty$ was 215 ± 28 ms; $n = 10$; Fig. 1E).

Recovery from inactivation was assessed with a paired pulse protocol with depolarizations (P1 and P2) to +60 mV at increasing P1-P2 intervals (Fig. 1F) and a holding potential of ~80 mV. Current during P2 was normalized to current during P1 and showed biexponential recovery with time constants of 1.54 ± 0.73 s ($\tau_1$) and 7.91 ± 1.78 s ($\tau_\infty$; $n = 9$; Fig. 1G).

To study frequency dependence, cells were repetitively depolarized from ~80 to +60 mV (410-ms pulses) in Tris-Cl-containing, Na$^+$-free external solution. Currents during the 10th pulse were normalized to current during the first pulse, showing a frequency-dependent decline ($n = 15$; Fig. 1H). Similar experiments were performed in the presence of extracellular NaCl with 50-ms prepulses to ~50 mV to inactivate $I_{Na}$. These recordings showed slightly greater frequency dependence, but they were qualitatively comparable with those obtained with Tris-Cl ($n = 7$; Fig. 1H). In Tris-Cl, $I_{K,PO}$ elicited with the 10th pulse were ~46 ± 3% (0.5 Hz) of P1, declining to ~13 ± 3% (2 Hz), compared with ~38 ± 3% (0.5 Hz) and ~7 ± 1% (2 Hz) in NaCl-containing solution ($P = 0.07$ and 0.05, respectively). The effect of equimolar Na$^+$ replacement by Tris and prepulse protocols to inactivate $I_{Na}$ in Na$^+$-containing solution on peak to steady-state current was subtle. In nine cells, $I_{K,PO}$ at +60 mV without prepulses averaged ~8.3 ± 1.4 and 9.5 ± 1.4 pA/pF ($P = N.S.$) in the presence and absence of extracellular Na$^+$, respectively. In Na$^+$-containing Tyrode’s solution, currents recorded at +60 mV with 50-ms prepulses to ~50 mV, averaged 8.4 ± 1.5 compared with 9.3 ± 1.4 pA/pF without prepulses ($P = N.S.$).

**Effects of Cl$^-$ and K$^+$ Substitution on $I_{K,PO}$.** To assess the potential charge carrier of $I_{K,PO}$, we investigated the effects of Cl$^-$ and K$^+$ substitution on the current under study. The reversal potential of the current averaged ~69.2 ± 2.3 mV ($n = 3$ cells). After correction for liquid-junction potentials this value was ~72 mV, compatible with a predominately K$^+$-carried conductance.

$I_{K,PO}$ was also recorded in individual cells before and after replacement of external chloride by equimolar cyclamate to assess potential contributions of a previously described Ca$^{2+}$-dependent Cl$^-$ current (Li et al., 2004). This intervention did not alter $I_{K,PO}$ (Fig. 2, A and B). Mean ± S.E.M. $I_{K,PO}$ density was similar over a range of voltages, averaging 7.1 ± 1.8 pA/pF at +60 mV in NaCl and 7.0 ± 1.9 pA/pF with Na-cyclamate ($n = 5$; $P = N.S.$; Fig. 2C). The effects of intracellular K$^+$ replacement by Cs$^+$ were then determined. Currents were recorded from 10 cells with regular internal solution and from nine other cells isolated from the same pigs on the same days with equimolar substitution of CsCl for internal KCl. Intracellular K$^+$ removal abolished $I_{K,PO}$; currents at +60 mV averaged 12.1 ± 0.9 pA/pF (KCl) versus

### Table 1

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Forw., forward primer; Rev., reverse primer.
0.2 ± 0.05 pA/pF (CsCl; P < 0.001; Fig. 2F). Together, these results strongly suggest that $I_{K,PO}$ is primarily a K$^+$ current.

**Pharmacological Characterization.** After having characterized the current as a K$^+$-dependent current with slow inactivation and recovery from inactivation, we set out to obtain the pharmacological profile of $I_{K,PO}$. The K$^+$ channel blocker 4-aminopyridine (4-AP) was applied at concentrations between 0.1 μM and 100 mM. The left panel of Fig. 3A illustrates 4-AP effects on $I_{K,PO}$. Reversible suppression was seen, with an IC$_{50}$ on peak to steady-state current of 0.81 ± 0.16 mM (n = 7; Fig. 3A, right, closed circles). Washout returned current amplitude to 81 ± 5% of control. 4-AP at lower concentrations significantly accelerated current inactivation: inactivation $\tau_i$ and $\tau_f$ averaged was 135 ± 70 and 35 ± 14 ms, respectively, after application of 100 μM 4-AP, compared with 315 ± 51 and 115 ± 7 ms, respectively, under control conditions (P < 0.05 for each), a behavior suggestive of open channel block (Fedida, 1997). To consider the reduction of charge carried by $I_{K,PO}$, we determined the 4-AP IC$_{50}$ based on the area under the current-time curve (Dukes et al., 1990; Gogelein et al., 2004). Integration of the area between the remaining current at maximal 4-AP concentration and the transient outward currents for determination of fractional block yielded an IC$_{50}$ of 39 ± 15 μM (Fig. 3A, closed circles; P < 0.01).

Use-dependent 4-AP-unblocking is characteristic of K$_v$4.2 and K$_v$4.3 currents (Campbell et al., 1993; Tseng et al., 1996).

Fig. 1. Biophysical current characterization. A, $I_{K,PO}$ recorded with 1000-ms depolarizations from a holding potential of −80 mV to potentials between 0 and +60 mV (protocol in inset, 0.1 Hz). B, mean ± S.E.M. $I_{K,PO}$-voltage relationship (n = 20). C, voltage dependence of steady-state inactivation with 1000-ms prepulses to various potentials and 750-ms test pulses to +60 mV. D, mean ± S.E.M. data for voltage dependence of $I_{K,PO}$ activation and inactivation (n = 10 each). E, mean ± S.E.M. time to peak $I_{K,PO}$ and inactivation time constants (n = 10 each). F, example of $I_{K,PO}$ recovery from inactivation. G, mean ± S.E.M. current during the second pulse ($I_{P2}$) normalized to current during the first pulse ($I_{P1}$), as a function of P1-P2 interval (protocol in inset) with biexponential fit to mean ± S.E.M. data (n = 9). H, $I_{K,PO}$ during the 10th pulse ($I_{P10}$) to +60 mV normalized to that during first pulse ($I_{P1}$) plotted over different frequencies with and without extracellular sodium. Act., activation; inact., inactivation; $\tau_i$, fast time constant; $\tau_s$, slow time constant; TP, test potential.
After current stabilization, 2 mM 4-AP was added to the bath, and cells were repetitively depolarized for 10 pulses (1 Hz; n = 4). There was no significant difference between currents elicited with the first compared with the last pulse (19 ± 7% versus 12 ± 8% of first-pulse current; P = N.S.) incompatible with use-dependent 4-AP unblocking.

We next determined the effect of flecainide (a moderately potent blocker of K,1 channels) on I_{KPO}. Flecainide was applied at 100 nM to 1 mM (Fig. 3B, left) and suppressed I_{KPO} with an IC_{50} of 132 ± 47 μM (n = 10; Fig. 3B, right). The effect was reversible upon washout (83 ± 12% of control). The inactivation of K,1.4 is substantially slowed by oxidative stress as imposed by H_{2}O_{2} (Dixon et al., 1996). We applied H_{2}O_{2} at an external concentration of 0.01% (Fig. 3C) and found no effect of H_{2}O_{2} on I_{KPO} inactivation (e.g., at +60 mV; τ_{a} = 292 ± 19 ms before versus 299 ± 15 ms after H_{2}O_{2}; P = N.S.). Likewise, current amplitude remained unaffected and averaged 8.4 ± 4.5 pA/pf (at +60 mV) before versus 8.2 ± 4.5 pA/pf after H_{2}O_{2} arguing against a significant role for K,1.4. Tetraethylammonium (TEA; 10 mM) did not significantly affect peak to steady-state I_{KPO}, e.g., under control conditions, current density at +60 mV averaged 13.6 ± 1.6 compared with 15.0 ± 1.7 pA/pF with TEA and 14.9 ± 2.1 pA/pF after 15 min washout (Fig. 3D; n = 9; P = N.S.).

**Effects of Specific Neurotoxins.** The slow inactivation of I_{KPO} made an important contribution of K,4 subunits unlikely. To assess further any possible K,4 contribution, heteropodatoxin (a potent blocker of native I_{Ko1} and heterologously expressed K,4 channels; Sanguinetti et al., 1997) was applied at 100 and 500 nM. Heteropodatoxin had no effect on I_{KPO} [e.g., at +60 mV, mean ± S.E.M. control current density was 14.2 ± 5.3 versus 16.2 ± 6.7 (100 nM) and 15.9 ± 6.6 pA/pF (500 nM) (n = 7; P = N.S.)]. Hongatoxin blocks heterologously expressed K,1.1, -1.2, and -1.3 channels with IC_{50} values of 31, 170, and 86 pM, respectively (Koschak et al., 1998). No change in I_{KPO} was seen with 0.1 nM (e.g., at +60 mV, mean ± S.E.M. I_{KPO} was 32.9 ± 5.6 before versus 32.5 ± 5.8 pA/pF after hongatoxin, respectively; n = 5; P = N.S.). Blood depressing substance is an Anemonia sulcata toxin that blocks K,3.4 transient outward currents (Diochot et al., 1998). I_{KPO} density at +60 mV in six cells averaged 26.4 ± 4.8 before versus 26.7 ± 5.0 pA/pF after application of 100 nM blood depressing substance (P = N.S.).

After the pharmacological exclusion of a significant contribution of K,4, K,1.1, -1.2, -1.3, and -3.4 subunits, we assessed the response to K,1.5 blockers. We first studied the effect of perhexiline (an antianginal drug that inhibits heterologously expressed K,1.5 with an IC_{50} of 1.5 μM) (Rampe et al., 1995). Figure 4A depicts representative currents recorded under control conditions and in the presence of perhexiline. I_{KPO} was clearly suppressed (IC_{50} of 17 ± 10 μM; n = 9; Fig. 4B). The atrial-selective compound AVE0118 suppresses K,1.5 current in heterologous systems with an IC_{50} of 1.1 ± 0.2 μM (Gogelein et al., 2004). AVE0118 inhibited I_{KPO} with an IC_{50} of 1.25 ± 0.62 μM (Fig. 4, C and D). Consistent with open channel block, τ_{a} accelerated from 76 ± 18 ms (control) to 17 ± 2 ms with 1 μM AVE0118 (n = 8; P < 0.05), whereas τ_{a} remained unaltered (τ_{a} = 332 ± 26 versus 327 ± 53 ms for control and AVE0118, respectively; P = N.S.).

**Effect of I_{KPO} Inhibition on Atrial Action Potentials.** To investigate the potential physiological role of I_{KPO} in porcine atrial repolarization, we recorded effects on APs (Fig. 5A). The addition of 0.1 mM 4-aminoypyridine prolonged terminal AP repolarization (Fig. 5B).

**Quantitative Real-Time RT-PCR.** Results of quantitative real-time RT-PCR on RNA extracted from isolated cardiomyocytes of animals that were used for patch-clamp experiments demonstrated predominant expression of K,1.5 subunit mRNA (Fig. 6, A and B). K,1.5 mRNA expression was ∼15-fold that of K,4.3 and KChIP2 (which were similar) and ∼153-fold that of K,1.4 (n = 6; P < 0.001 for each). K,4.2 mRNA was barely detectable.

**Discussion**

**Major Findings.** This study provides evidence for the presence of a time-dependent K^{+} conductance in pig atrium with physiological properties and pharmacological responses compatible with the participation of K,1.5 α-subunits, and a role in porcine atrial repolarization. These findings identify I_{KPO} as the likely target of K,1.5 blockers in previous in vivo studies of novel antiarrhythmic compounds and suggest that pigs may represent a model for the study of atrial-selective antiarrhythmic drugs that act by inhibiting K,1.5-based currents.
Previous Studies on Porcine Electrophysiology. Pigs have been used for a variety of experimental studies of cardiac arrhythmias (Janse et al., 1998; Wirth et al., 2003), but information about the cardiac cellular electrophysiology of the pig is limited. Porcine sinoatrial cells exhibit $I_{\text{Ks}}$ (Ono et al., 2000) and ventricular myocytes exhibit an $I_{\text{Cl,Ca}}$ that contributes to repolarization (Li et al., 2003). Another study by the latter investigators documented the presence of $I_{\text{Cl,Ca}}$, $I_{\text{Kur}}$, $I_{\text{Kr}}$, and $I_{\text{Ks}}$ in pig atrial myocytes (Li et al., 2004). The $I_{\text{Kur}}$ (which the authors called $I_{\text{Kur,p}}$) reported in the latter study was relatively small and apparent primarily at slow frequencies (0.05 Hz) at room temperature. $I_{\text{Kur,p}}$ showed weak inward rectification, use dependence, 4-AP sensitivity ($IC_{50}$ of 72 ± 4 μM), and TEA resistance. This work differs from ours in the use of low EGTA concentration in the pipette and recording at room temperature.

Our study adds to previous results in showing the presence of a substantial time-dependent outward $K^+$ current that is sensitive to blockers of $K_v1.5$, but not other possible underlying subunits, and that contributes to porcine atrial repolarization. Several lines of additional evidence presented here (including biophysical properties as well as mRNA expression) are consistent with a potential role for underlying $K_v1.5$ $K^+$ channel subunits.

Relation of Biophysical Properties to Other Transient Outward Currents. The major candidate $K^+$ channel subunits generating rapidly activating and inactivating (so called “fast”) $I_{\text{to}}$ phenotypes are $K_v4.2$ and $K_v4.3$ (Nerbonne,
Thus, the predominant subunit underlying native \(I_{\text{to}}\) in human ventricular cells is Kv4.3, which generates a current that inactivates rapidly as a single exponential process (\(\tau_{\text{inact.}} = 7.9 \pm 0.3\) ms; 35°C) (Nabauer et al., 1996). Canine \(I_{\text{to},f}\) is similarly carried by Kv4.3 subunits (Dixon et al., 1996). Rat ventricular \(I_{\text{to}}\) (predominantly Kv4.2) also inactivates rapidly (\(\tau_{\text{inact.}} = 48 \pm 7\) ms; 22°C) (Himmel et al., 1999). In contrast, a functionally distinct \(I_{\text{to}}\) phenotype (\(I_{\text{to,slow}}\)) has been identified in many mammalian species, which inactivates with a double exponential process (\(\tau_{\text{slow}}\) in the order of hundreds of milliseconds) and is carried by Kv1.4 subunits (Patel and Campbell, 2005). This \(I_{\text{to}}\) phenotype is distinguished from Kv4-based currents by its slow recovery from inactivation, with time constants on the order of seconds (Xu et al., 1999; Wickenden et al., 1999). For example, ferret \(I_{\text{to,slow}}\) recovers with \(\tau = 3.0 \pm 0.45\) s (at 22°C) and mouse \(I_{\text{to,slow}}\) recovers with similar time constants (Brahmajoithi et al., 1999; Xu et al., 1999).

Relation of Biophysical Properties to \(I_{\text{Kur}}\) and \(I_{\text{K,slow}}\). Mouse ventricular myocytes express a current termed \(I_{\text{K,slow}}\) with kinetic properties consistent with Kv1.5 α-subunits (Zhou et al., 1998). In other species, Kv1.5 underlies the atrially expressed ultrarapid delayed rectifier current (\(I_{\text{Kur}}\)), which is often described as noninactivating. Although Kv1.5 current has generally been described as a delayed rectifier, it can show substantial time-dependent inactivation, with complete inactivation for depolarizations of sufficient duration (Feng et al., 1998; Lin et al., 2001; Snyders et al., 1993). The inactivation kinetics that we found for \(I_{\text{K,PO}}\) were faster than those published for hKv1.5 in heterologous systems (e.g., \(\tau_{\text{inact.}} = 250\) ms; \(\tau_{\text{slow}} = 1500\) ms; Lin et al., 2001), although the latter studies were performed at room temperature, which in itself substantially slows inactivation (Snyders et al., 1993). It is also possible that \(I_{\text{K,PO}}\) involves a contribution of β-subunits, which are known to interact with Kv1.5 and accelerate its inactivation (Uebele et al., 1998). A full study of the molecular biology of \(I_{\text{K,PO}}\) would be very interesting, but it is beyond the scope of the present article.

**Pharmacological Profile of \(I_{\text{K,PO}}\).** Kv1.5-based currents are sensitive to 4-AP. For example, mouse ventricular \(I_{\text{K,slow}}\) is inhibited by 4-AP with an IC\(_{50}\) of \(32 \pm 5\) μM (Zhou et al., 1998). Significant interspecies differences in 4-AP sensitivity of \(I_{\text{to}}\) currents attributed to Kv1.5 exist, with IC\(_{50}\) values ranging up to 600 μM in rat atrium (Zhou et al., 1998). However, despite differences in affinity, all Kv1.5-carried currents are 4-AP sensitive, as was \(I_{\text{K,PO}}\) in this study. Relevant \(I_{\text{K,PO}}\) charge carriage inhibition (based on assessment
of area under the current-time curve accounting for open channel block) occurred with an IC$\text{_{50}}$ of 39 ± 15 μM. IC$\text{_{50}}$ for heterologously expressed hK$\text{v}_{1.5}$ peak currents ranges between 50 and 290 μM (Grissmer et al., 1994; Bouchard and Fedida, 1995), consistent with the results for $I_{K,PO}$ in the present study. Although 4-AP is nonspecific in that it blocks both $I_{to,f}$ and $I_{to,s}$, the underlying mechanisms are distinct. Block of $I_{to,s}$ occurs predominantly in the open state (Campbell et al., 1993). In contrast, 4-AP block of $I_{to,f}$ occurs through closed state binding and displays use-dependent unblocking and reverse use dependence (Patel and Campbell, 2005). No use-dependent unblocking was observed in the present study, and block was consistent with open-state dependence. Both Kv$\text{1.4}$ and Kv$\text{1.5}$ currents show predominant open-state 4-AP block, but Kv$\text{1.4}$ is insensitive to flecanide (Akar et al., 2004) and sensitive to H$\text{2}O_\text{2}$, inconsistent with the response of $I_{K,PO}$. A contribution of other Kv$\text{1}$ subunits to $I_{K,PO}$ was excluded by the absence of any effect of hongatoxin application. In contrast, $I_{K,PO}$ was sensitive to perhexiline, AVE0118, flecainide, and 4-AP at concentrations fully compatible with Kv$\text{1.5}$ inhibition (Rampe et al., 1995; Zhou et al., 1998; Gogelein et al., 2004). A relevant contribution of Kv$\text{3.1}$ subunits that have been shown to underlie $I_{Kur}$ in dogs is excluded, because this current is exquisitely sensitive to TEA (IC$\text{_{50}}$ of 0.3 mM).

**Limitations of This Study.** Variability in cell isolation can affect the results obtained. To minimize errors introduced by this process, we studied APs and mRNA levels from cells in pigs that were used for current recordings on the same day. Furthermore, native cells express a variety of ionic currents, and their electrophysiological isolation requires selective protocols and pharmacological agents with imperfect specificity. One-second pulses were chosen to allow for almost complete inactivation of the current. In some instances, incomplete inactivation might have caused biophysical inaccuracy, but longer pulses were poorly tolerated, and the results had minimal effect on the analyses. Another limitation of this study is the lack of protein expression data. We tried to
obtain Western blots from porcine atrial protein preparations, but we were unable to obtain specific bands with commercially available antibodies, none of which have been raised against porcine-specific epitopes.

References


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