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Cloning and expression of a rat cardiac delayed rectifier potassium channel

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ABSTRACT **We have cloned a cDNA (designated RAK) coding for a delayed-rectifier K current (I_{RAK}) from adult rat heart atrium and expressed it in *Xenopus* oocytes. RAK differs from the cloned rat brain K current, BK2 [McKinnon, D. (1989) *J. Biol. Chem.* 264, 8230–8236], by one amino acid at residue 411. RAK expressed in oocytes compares closely to the intrinsic adult rat atrial delayed-rectifier current measured by using whole-cell recording of single isolated cells. Northern blot analysis confirmed the presence of the channel in adult rat atrium, and to a lesser extent, in rat ventricle. I_{RAK} activates with time constants ranging from 58 ms at −20 mV to 6 ms at +60 mV and does not show significant inactivation over 800 ms. It is blocked by 4-aminopyridine > barium >> tetraethylammonium chloride, which is similar to the relative potencies of these blockers on the native delayed rectifier current. We conclude that the main delayed rectifier K current in adult rat atria is virtually identical to a neuronal delayed rectifier, BK2.**

The delayed rectifier K current in heart cells regulates the duration of the plateau of the cardiac action potential by countering the depolarizing, inward calcium current. Delayed rectifier K currents characteristically are activated upon depolarization from rest, display a sigmoidal or delayed onset, and have a nonlinear, or rectifying, current–voltage relation. Several types of delayed-rectifier K conductances have been identified in cardiac cells based on measured single-channel conductances (for review, see ref. 1). Heart rate and contractility are regulated by second messenger modification of delayed-rectifier K conductances, and species differences in the shape of the plateau must be influenced by the type and level of channel expression.

Cloning and expression of K channels makes it possible to describe the cardiac K currents at a new level of detail. At least two cardiac plateau currents have already been described. One, a very slow onset delayed rectifier in neonatal rat (I_{sK}) identical to the K channel found in rat kidney and diethylstilbestrol-primed uterus (2, 3), is dissimilar to any previously expressed K current. The I_{sK} protein is a mere 130 amino acids in length with one putative transmembrane-spanning segment. When expressed, it displayed extremely slow activation, failing to reach steady state over 20 s (activation was fit with three time constants: 0.36 s, 1.5 s, and 20 s), and was insensitive to 4-aminopyridine (4-AP) and tetraethylammonium chloride (TEA) (3). A second cardiac K channel (RHK1; ref. 4) homologous to the rat cortical delayed-rectifier current, RCK4 (5), displayed fast activation and inactivation, similar to the transient outward current described in rat ventricular myocytes (6). Here we describe the cloned and expressed rat atrial delayed-rectifier current and compare it to the native rat atrial K current measured in whole-cell recordings from dissociated myocytes. I_{RAK} is distinct from previously described expressed cardiac currents.

METHODS

Cloning of K Channels. Cellular atrial, ventricular, and forebrain RNA was isolated from adult rat tissues as described (7), and polyadenylylated mRNA was purified by oligo(dT) chromatography (8). To obtain cDNA clones encoding putative K channels, double-stranded cDNA was synthesized from atrial or forebrain mRNA by using an oligo(dT)$_{12-18}$ primer as described (9); synthetic *Eco*RI adaptors were added; and cDNAs of greater than 1.0 kilobase (kb) were ligated with *Eco*RI-digested λgt10 and packaged *in vitro* to yield individual cDNA libraries of 2×10^6 clones. Sequence information from a mouse homologue of the *Drosophila* Shaker gene product was used to design an oligonucleotide probe encoding amino acids 72–98 of the mouse sequence (10). This probe was used to screen the rat forebrain cDNA library under low-stringency conditions as described (11). This screening identified a cDNA clone encoding a rat homologue of the mouse K channel. The coding sequence of the rat K channel clone was uniformly labeled with ^{32}P and used to screen the rat atrial cDNA library under low-stringency conditions. This screening identified ≈20 candidate clones; clone RAK displayed the strongest hybridization signal and was subjected to nucleotide sequence analysis as described (12).¶ The proposed translation initiation codon is preceded by an in-frame stop codon and is similar to the consensus sequence for eukaryotic initiation codons (13).

Synthesis of RNA for Injection Studies. For expression of the putative atrial K channel in *Xenopus laevis* oocytes, the plasmid pSP64T (kindly provided by D. Melton, Harvard University, Cambridge, MA) was used. The coding sequence of RAK was inserted into this plasmid downstream of an SP6 promoter and flanked by 5′ and 3′ noncoding sequences and a poly(A) tract derived from a *Xenopus* β-globin mRNA (14). The plasmid was linearized by digestion with *Eco*RI, and capped transcripts were synthesized *in vitro* with SP6 RNA polymerase as described by the supplier (Promega). SP6 transcripts were resuspended in deionized H_2O at a final concentration of 1–2 μg/μl.

Northern Blots. Polyadenylylated mRNA (8 μg per lane) was fractionated on a 1% agarose/formaldehyde gel (8), transferred to nitrocellulose, and hybridized under high-stringency conditions with a ^{32}P-labeled restriction fragment encoding the entire RAK sequence as described (11). Hybridization was in 50% formamide/5× standard saline citrate

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Abbreviations: 4-AP, 4-aminopyridine; TEA, tetraethylammonium chloride; *I*, current.

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¶The nucleotide sequence of cDNA clone RAK has been deposited in the GenBank data base (accession no. M74449).

(SSC)/50 mM sodium phosphate (pH 6.8)/0.1% sodium pyrophosphate/5× Denhardt's solution/sonicated salmon sperm DNA (50 μg/ml)/0.1% SDS/10% dextran sulfate at 42°C. Washes were carried out in 0.2× SSC/0.1% SDS at 55°C.

Due to the low abundance of mRNA detected by the RAK probe and the large size of the transcript, it has been difficult to obtain a clear hybridization signal with shorter (oligonucleotide) probes encoding untranslated regions of the RAK sequence. We attribute this to the poor transfer of long RNAs, relatively low specific activity of the oligonucleotide probes, and low abundance of the transcript. However, we have conducted rapid amplification of cDNA 3′ ends from atrial mRNA and have sequenced more than 12 independent cDNA clones; all these clones correspond to the RAK sequence, rather than any closely related clone. The sizes of the transcripts were determined by comparison with the positions of 28S and 18S rRNA as indicated. The filter was rehybridized with a ^{32}P-labeled oligonucleotide probe derived from a portion of a mouse α-actin coding sequence; this confirmed that equivalent amounts of mRNA were present in each lane (data not shown).

Electrophysiology. Adult female *Xenopus laevis* toads were obtained from Nasco (Fort Atkinson, WI) and Carolina Biological Supply. Ovarian lobes were removed from anesthetized animals under sterile conditions. The incisions were sutured, and the toads were allowed to recover in isolation before being returned to the aquaria. Stage V and VI oocytes were mechanically defolliculated with fine forceps and kept in L-15 medium (diluted 50% with sterile H_2O, buffered with 15 mM Hepes, supplemented with 0.25 unit of nystatin per ml and 0.2 g of gentamicin per ml at pH 7.6) at 19°C and injected with mRNA within 24 hr of surgical removal. Transcripts (50–100 ng per oocyte) were injected in a 50-nl bolus, and the oocytes were stored for 2 days in supplemented L-15 media.

Two-electrode voltage clamp (Turbo TEC 01C; NPI Instruments, Tamm, F.R.G.) was used to measure the K currents from *Xenopus* oocytes. Recordings were made in bathing solution containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM Hepes (pH 7.5). Current and potential measuring electrodes were pulled to resistances of 0.5 and 2 MΩ, respectively, when filled with 2 M KCl. Records were stored and analyzed on an Indec 11/73 system (Indec, Sunnyvale, CA). Current records were filtered with a tunable Bessel filter set to 300 Hz. All experiments were carried out at 22°C.

Whole-cell recordings (15) of dissociated adult rat (described in ref. 6) atrial myocytes were made using an Axopatch-1B (Axon Instruments, Burlingame, CA) patch clamp recorder with series resistance compensation. Whole-cell capacitance averaged 53 ± 9 pF (mean ± SEM; n = 6). Current signals were filtered at 3–5 kHz, digitized on an AST 286 equipped with a Tecmar Labmaster interface and PCLAMP software (Axon Instruments), and stored on floppy disks. Recording pipettes contained 135 mM KCl, 10 mM EGTA, 10 mM Hepes, 5 mM glucose, 3 mM Mg·ATP, and 0.5 mM Tris·GTP (pH 7.2). The bath solution contained 136 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, 5 mM $CoCl_2$, and 0.02 mM tetrodotoxin. Experiments were carried out at 22°C.

RESULTS

An adult rat atrial cDNA library was screened under low-stringency conditions with a probe encoding a rat forebrain K channel; this brain K channel is homologous to the mouse brain K channel (10) and identical to one previously described by other investigators as RCK1 (16). One atrial cDNA clone obtained by this screen, designated RAK, containing a 2.2-kb insert, was chosen for further characterization. Nucleotide sequence analysis of the RAK cDNA identified a single long open reading frame encoding a predicted protein of 499 amino acids with a relative molecular weight of 56,766.

Analysis of the RAK protein sequence revealed several characteristics that strongly suggested that this protein was a member of the K channel family. Most significantly, the RAK protein contains six putative transmembrane segments, including an S4 voltage sensor domain, which are highly conserved among the delayed rectifier K channels characterized in mouse brain (10), rat brain (4, 16) and the A-type K channels encoded by the Shaker locus of *Drosophila* (17, 18). The predicted RAK protein is most similar to a putative rat brain K channel termed BK2 (19). The deduced RAK sequence differs from BK2 only at residue 411, where a phenylalanine residue (RAK) replaces a serine residue (BK2). The nucleotide sequences of RAK and BK2 differ in only 3 out of 1500 base pairs within this region; two nucleotide differences are silent (nucleotides 396 and 420), whereas the third results in the serine to phenylalanine substitution at residue 411 of the RAK sequence.

Northern blot analysis confirmed the presence of the RAK transcript in adult rat atrial RNA (Fig. 1). One intense band of 8.5 kb was observed, indicating that the RAK transcript contains large untranslated regions. Similar observations have been made in Northern analysis of other K channels (10, 19, 20). A band at 8.6 kb was also detected in adult rat ventricular RNA, but at significantly lower levels. Two fainter bands were detected at 4.2 and 2.8 kb in atrium and again at reduced levels in ventricle. These may represent degraded forms of the transcripts or cross-hybridization with another transcript.

RAK Encodes Delayed-Rectifier K Channels. Messenger RNA specific for RAK was synthesized by *in vitro* transcription of the cloned cDNA and injected into *Xenopus* oocytes. Two days after injection, large outward currents could be measured in 90% of the oocytes tested. Fig. 2*A* shows the measured currents, and Fig. 2*C* shows the current–voltage relation elicited upon depolarization from a holding potential of −70 mV. Currents began to activate above −40 mV and showed some degree of saturation above +40 mV. The average peak current at +40 mV was 37.1 + 8.9 μA (mean ± SEM; n = 11). The trace of Fig. 2*A* was selected from an oocyte expressly injected with a lower amount of mRNA in order to limit the net K current and prevent changes of the potassium gradient. Large K currents resulted in shifts in the potassium equilibrium potential (E_K) during long pulses and gave rise to artifactual slow inactivation. Fig. 2 *B* and *D* shows analogous currents measured from single adult rat atrial cells in whole-cell clamp. The average peak current was 1.99 ± 13 nA at +40 mV (mean ± SEM, n = 56).

I_{RAK} currents (*Xenopus*) showed rapid activation and little to no inactivation within 800 ms. The current onset was best

FIG. 1. Northern blot analysis of RNA from adult rat atrium and ventricle. Eight micrograms of poly(A) mRNA was loaded per lane and fractionated on a 1% agarose/formaldehyde gel and transferred to nitrocellulose. The blot was hybridized with a ^{32}P-labeled probe made by random priming of a restriction fragment containing the entire coding sequence of RAK. The autoradiogram was exposed for 24 hr at −70°C with an intensifying screen. Rehybridization of the filter with a probe to mouse α-actin confirmed that equivalent amounts of RNA were present in each lane (results not shown). Results are representative of two independent RNA samples from each tissue. Lane 1, adult rat atrial RNA; lane 2, adult rat ventricular RNA.

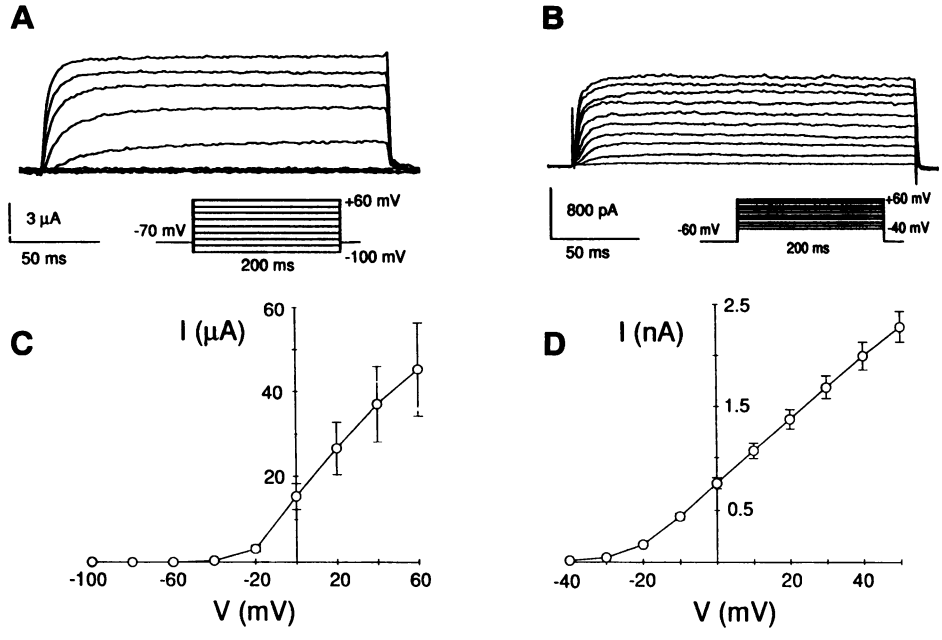

FIG. 2. Ionic currents measured in *Xenopus* oocytes expressing RAK (*A*) and single dissociated adult rat atrial cells (*B*). Two-electrode voltage clamp of RAK-expressing oocytes revealed a fast onset, noninactivating delayed-rectifier K current similar to the predominant K current in rat atrium. Voltage clamp protocols are shown from holding potentials of −70 and −60 mV, respectively. The average peak current–voltage relations are shown for *Xenopus* (mean ± SEM; $n = 12$) (*C*) and rat atrium ($n = 56$) (*D*). In each case, peak current is plotted against membrane voltage.

fit by a rising exponential of the form $I_{RAK} = I_K [1 - e^{(-t/\tau)}]$, where I_K was the steady-state current and τ was the time constant of the exponential. The time constant ranged from 57.8 ± 8.6 ms (mean ± SEM, $n = 11$) at −20 mV to 6.4 ± 0.4 ms (mean ± SEM, $n = 8$) at +60 mV (Fig. 3*A*). In contrast, the activation in single rat atrial cells was fit best by the same function raised to the fourth power, giving time constants of activation ranging from 5 ms at −10 mV to 2 ms at +40 mV (data not shown; ref. 21). The two activation models do not necessarily indicate important differences in gating of the expressed and native channels. For comparison to other channels, the half activation time was 5.8 ± 0.8 ms ($n = 5$) at +40 mV for RAK in the oocyte and 3.1 ± 0.26 ms ($n = 12$) in rat atrial cells.

I_{RAK} is K selective; reversal potentials were measured in oocytes expressing RAK by varying the external K concentration from 2 to 20 mM and 50 mM. From a holding potential of −70 mV, the voltage was stepped to +40 mV to fully activate the K current, followed by a return to variable voltages between +40 and −140 mV. Tail current reversals were then plotted as a function of external K concentration (Fig. 3*B*). The resulting relations were fit to yield a slope of 47 mV per decade, close to the expected slope of 58 mV per decade at 22°C for a perfectly selective K electrode. I_{RAK} was

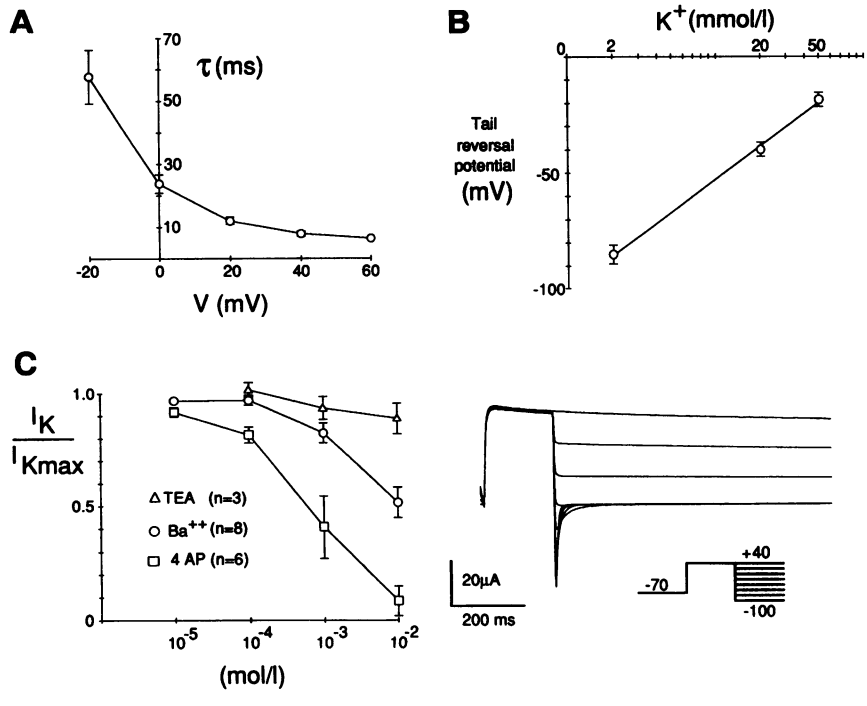

FIG. 3. Properties of I_{RAK} expressed in *Xenopus* oocytes. (*A*) The time constant (τ) of I_{RAK} onset was determined by stepping from a holding potential of −70 mV to depolarized membrane potentials, and the current onsets were fitted to a single exponential. The time constant (τ) was then plotted as a function of membrane voltage (mean ± SEM; $n = 8$–10). (*B*) K selectivity of I_{RAK}. Tail reversal potentials were measured by stepping the voltage from −70 mV to +40 mV for 200 ms to fully activate I_{RAK} before returning to the potentials shown in *Inset* in *Lower*. Current tail reversal potentials were measured at three external potassium concentrations (mean ± SEM; $n = 8$) and plotted as a function of $[K]_o$ (*Upper*). The current record (*Lower*) shows the reversal potential measured in 50 mM extracellular K. (*C*) I_{RAK} is inhibited by K channel blockers. Oocytes expressing RAK were held at −70 mV before jumping to +40 mV to fully activate I_{RAK}. Relative reduction in peak current is shown for three K channel blockers as a function of concentration.

blocked by the K channel blockers, 4-AP and barium, but was relatively insensitive to TEA (Fig. 3*C*). The peak current was blocked 50% by 600 μM 4-AP and 10 mM barium. The relative insensitivity to TEA is common to other K channels closely homologous to RAK. NGK1, the K channel cloned from NG108-15 neuroblastoma–glioma cells and that has a sequence identical to BK2, was not blocked 50% by 10 mM TEA (22). The delayed-rectifier current in whole-cell clamped rat atrial cells was similarly insensitive to TEA and was not blocked 50% by concentrations of TEA up to 50 mM.

DISCUSSION

We have shown that the RAK message is present in rat atrium (and at lower levels in rat ventricle) and, when expressed in the *Xenopus* oocyte expression system, produces a current that is practically identical to the native rat atrial delayed rectifier current. In rat atrium and when expressed in oocytes, the delayed-rectifier current displays fast activation time constants (4 and 24 ms at 0 mV) compared to the cardiac delayed rectifier in other commonly studied cardiac preparations (at 0 mV) such as chicken ventricle (2.5 s; ref. 23), calf Purkinje (>100 ms; ref. 24), and bullfrog atrium (3 s; ref. 25). In both native and expressed forms, the current displays little inactivation. Similarly, the activation voltage range for both is between −40 and −30 mV. Although many K currents have been expressed in oocytes from various tissues, there have been few data to date comparing the native and recombinant K currents. Significantly, the endogenous rat atrial delayed rectifier current and oocyte-expressed RAK current display very similar electrophysiological properties. The slight alteration in kinetics may arise from a number of differences between expression in oocytes and the rat cardiac cell. For example, the number and regulation of subunits comprising a K channel is unknown and may vary in the assembly process from frog oocyte to rat myocyte. However, given the small discrepancies between the expressed and native currents, it seems more likely that subtle differences in the lipid environment or state of phosphorylation of the channel protein can account for these changes.

Cardiac delayed rectifiers from frog atrium, chicken ventricle, and guinea pig atrium have been shown to be modified by receptor-gated mechanisms, such as the β-adrenergic-mediated increase in I_K (26) responsible in part for shortening of the cardiac action potential. In fact, I_K has been shown to be modified by both protein kinase A and protein kinase C in guinea pig ventricle (27, 28). The sequence of RAK has one poorly conserved putative phosphorylation site based on the consensus sequence for protein kinase A substrates (29) at residues 446–450 and two consensus sites for phosphorylation by protein kinase C (30) at residues 320–322 and 324–326.

Note Added in Proof. While this paper was under review, Roberds and Tamkun (31) described the sequences of several voltage-gated K channels from rat heart.

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