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Functional characterization of RK5, a voltage-gated K^+ channel cloned from the rat cardiovascular system

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A voltage-sensitive K^+ channel previously cloned from rat heart designated RK5 (rat Kv4.2) (Roberds and Tamkun, 1991, Proc. Natl. Acad. Sci. USA 88, 1798–1802) was functionally characterized in the *Xenopus* oocyte expression system. RK5 is a homolog of the *Drosophila Shal* K^+ channel, activates with a rise time of 2.8 ms, has a midpoint for activation of −1 mV and rapidly inactivates with time constants of 15 and 60 ms. RK5 is sensitive to 4-AP, IC_{50}= 5 mM, and is insensitive to TEA and dendrotoxins. The voltage dependence and kinetics of the RK5 induced currents suggest this channel contributes to the I_{to} current in heart.

Electrophysiology; *Shal*; Transient current; Dendrotoxin; *Xenopus* oocytes

1. INTRODUCTION

Numerous K^+ currents have been identified in the heart which exhibit distinct electrophysiological properties [1]. However the diversity and low density of cardiac K^+ channels has made it difficult to successfully study a specific type of K^+ channel in cardiac cells. The expression of cloned K^+ channels in systems amenable to voltage clamp has allowed the detailed characterization of K^+ channel isoforms present in brain [2–4]. Recently, Roberds and Tamkun [5] cloned five K^+ channels, RK1 through RK5, from a rat cardiac cDNA library. We have chosen in this paper to functionally characterize RK5 channels in the *Xenopus* oocyte expression system. RK5 is a mammalian K^+ channel demonstrating extensive sequence identity (70%) to the *Drosophila Shal* K^+ channel [6]. A K^+ channel recently cloned from mouse brain (m*shal*) [7] shows 72% amino acid identity to RK5. The identification of two different mammalian *Shal*-like channels suggests that a large mammalian *Shal*-like channel family may exist.

2. MATERIALS AND METHODS

2.1. *Materials*

β, γ and δ dendrotoxins were purified from *Dendroaspis angusticepis* venom (Sigma) and Toxin I and Toxin K from *Dendroaspis polylepis* (Sigma) using the procedure of Benishin et al. [8].

Abbreviations: 4-AP, 4-aminopyridine; TEA, tetraethylammonium

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2.2. *Expression in Xenopus oocytes*

The polymerase chain reaction (PCR) was used to amplify nucleotides −90 to +1495 of RK5 [5] and the resulting fragment subcloned into pOEV which was used for both germinal vesicle injections and as a template for cRNA preparation. To guard against potential polymerase induced sequence error two separate reactions were run, with the products being individually subcloned and used for cRNA synthesis. Both templates produced identical currents. cRNA synthesis with T7 polymerase and polyadenylation was according to standard protocols [9]. cRNA (10–20 ng) was injected directly into the cytoplasm of the vegetal pole of *Xenopus laevis* oocytes or cDNA-containing pOEV was injected into the germinal vesicle of the oocytes. The oocytes were stored at 18°C in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvic acid, pH 7.5) supplemented with 0.5 mM theophylline and 50 μg/ml gentamicin.

2.3. *Electrophysiological recordings*

Electrophysiological experiments were performed 24–72 h following either the cytoplasmic or germinal microinjections of the cRNA or pOEV construct, respectively. Macroscopic currents were recorded using the two microelectrode voltage clamp (Dagan 8500 Preamp-Clamp) in a continuously perfused bath of ND96 at 20–22°C. The microelectrodes were filled with 3 M KCl and had resistances of approximately 1 M. Each current trace was low pass filtered at 30 kHz, digitized with 12-bit precision and sampled at a rate of 2400 Hz. All current records had linear leak and capacitive transients subtracted using the P/4 procedure. Voltage pulse protocols and data acquisition were controlled by a pCLAMP program (Axon Instruments, Inc. Burlingame, CA) through a TL-1 DMA interface and a Labmaster DMA board (Scientific Solutions).

3. RESULTS AND DISCUSSION

Injection of *Xenopus* oocytes with RK5 cRNA produced voltage-dependent, outward K^+ current activating near −40 mV when examined under voltage clamp (Fig. 1A). Germinal vesicle injection of RK5 cDNA resulted in the expression of essentially identical K^+ currents (data not shown). The time required to go

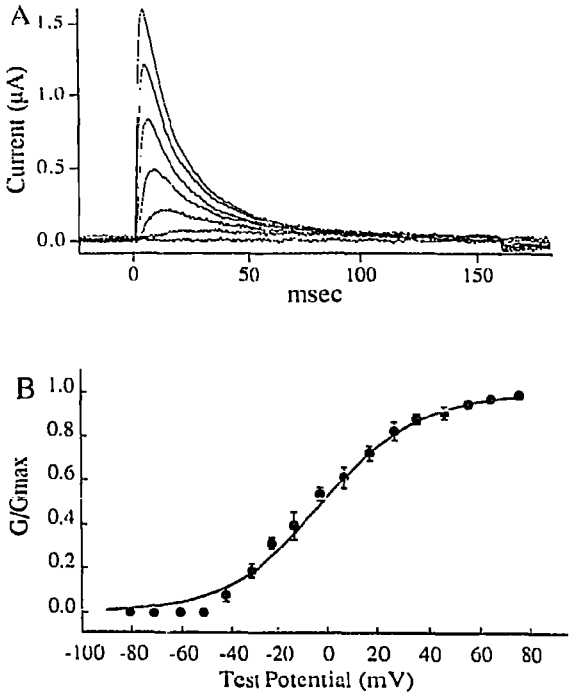

Fig. 1. Currents in *Xenopus* oocytes injected with mRNA derived from RK5 cDNA. In (A) a voltage-dependent, transient current was evoked in response to 160 ms test pulses from a holding potential of −90 mV to test voltages ranging from −60 mV to +60 mV in 20 mV increments. A small non-inactivating current seen in uninjected oocytes has been subtracted. (B) A plot of normalized conductance (G/G_{max}) versus test potential for RK5 is shown. The conductance (G) was calculated from peak current values, assuming a V_{rev} of −100 mV. The line is a result of a least-squares fit of a Boltzmann function ($G/G_{max}=1/(1+\exp((V_{½}-V)/K'))$) to the data. The error bars are ± SEM, n= 5.

from 10 to 90% of the peak current was measured for voltage-step pulses to 20 mV from a holding potential of −90 mV and showed that RK5 activated rapidly with a rise time of 2.8 ± 0.1 ms. The conductance–voltage relationship for RK5 channels is shown in Fig. 1B. The conductance was fit by a Boltzmann function from which the voltage generating half-maximal conductance ($V_{½}$= −1.0 ± 0.9 mV) and the slope factor (K'=19.6 ± 0.9 mV/e-foid increase in conductance) was determined. The slope of the voltage dependence of conductance curve for RK5 and other *Shal*-like channels is much less than for *Shaker* homologs. This may be due to a phenylalanine in position 303 in the S4 region of RK5 rather than a leucine seen in the corresponding position in all other families of voltage gated ion channels. Substitution of valine for this leucine in *Shaker* results in a large decrease in the slope and a shift in the midpoint of the conductance–voltage curve to a more positive voltage [10].

The ion selectivity of RK5 channels was determined by measuring the reversal potential of tail currents in various extracellular potassium concentrations using a modified ND96 in which NaCl was replaced by KCl. The reversal potential is increased by 51.7 mV for a tenfold increase in extracellular K^+ concentration. Comparison of this value to the theoretical value of 58 mV predicted by the Nernst equation for a perfectly K^+ selective semipermeable membrane demonstrates that this channel is much more permeable to K^+ than Na^+ or Cl^-.

RK5 shows rapid voltage dependent activation and is highly selective for potassium ions, but inactivates much more rapidly than mammalian *Shaker*-like K^+ channels. RK5 currents were 90% inactivated within 70 ms following a depolarization to 20 mV. The time course for inactivation of RK5 currents is well fit by the sum of two exponentials, with inactivation time constants of 15 ± 2 ms and 61 ± 6 ms and fractional amplitudes of 0.68 and 0.32 respectively at 20 mV (n=4). The inactivation time constants for RK5 are only weakly voltage dependent ranging from 27 ± 4 ms and 96 ± 15 ms at −20 mV to 12 ± 2 ms and 43 ± 6 ms at 60 mV with the fractional amplitudes unchanged (n=4). The steady-state inactivation properties of RK5 were analyzed by measuring the current elicited by a depolarization to 50 mV following a one second prepulse varying between −90 mV and −30 mV. The fraction of the peak current remaining after the prepulse is plotted in Fig. 2 as a function of the prepulse potential. The data were fit by a Boltzmann function to give a steady-state inactivation curve with a $V_{½}$ of −55.0 ± 3.5 mV and a K' of −6.8 ± 0.8 mV/e-fold change in conductance. Steady-state inactivation is nearly complete at voltages below the threshold of activation.

A profile of the pharmacological sensitivity of RK5 channels to a variety of K^+ channel blockers was determined. The concentration dependence of the block was determined by measuring the peak K^+ current following a voltage step to 20 mV from a holding potential of −90 mV before and after application of a drug and fractional

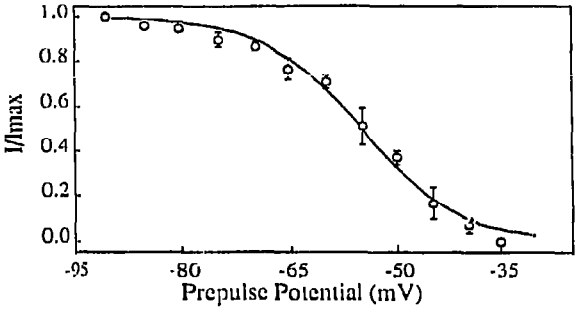

Fig. 2. RK5 steady-state inactivation. The effect of prepulse depolarization on the inactivation of RK5 currents is shown. A small residual current similar to that seen in uninjected oocytes, which is constant following prepulses more positive than −35 mV is subtracted. The ratio (I/I_{max}) of the outward current following a prepulse (I) and in the absence of a prepulse (I_{max}) is plotted as a function of prepulse voltage. The line is a result of a non-linear least-squares fit of a Boltzmann function. The error bars are ± SEM, n=5.

current calculated. RK5 channels were sensitive to 4-AP (IC_{50} of 5.0 mM) and insensitive to concentrations of up to 100 mM TEA. RK5 channels were also completely insensitive to 300 nM concentrations of all the dendrotoxins examined (*Dendroaspis polylepis* Toxins I and K and γ, β and δ dendrotoxins) and to relatively high concentrations of lidocaine (1 mM) and procainamide (5 mM). The lack of sensitivity to external TEA of RK5 is in agreement with predictions based on its amino acid sequence. MacKinnon and Yellen [11] have shown that block of *Shaker* channel mutants by external TEA involves an uncharged amino acid in position 449 the side chain of which is capable of hydrogen bonding and to a lesser extent a negatively charged amino acid in position 431. RK5 has valine (377) and alanine (359) at the corresponding positions and therefore should be insensitive to external TEA.

The cardiac current that RK5 most closely resembles is the transient outward current first described by Josephson et al. [12]. RK5 and I_{to} currents activate following depolarization to −40 mV and above and share a shallow voltage dependence of activation. Both channels open rapidly with peak currents occurring within 10 ms of depolarization to 0 mV and above. The steady-state inactivation curve of RK5 is slightly shifted in the positive direction ($V_{\frac{1}{2}}$=−55 mV) compared to that of I_{to} ($V_{\frac{1}{2}}$=−62 mV) and the time course of inactivation of both currents is similar. The greatest difference between RK5 and I_{to} lies in their sensitivity to block by external 4-AP. RK5 is less sensitive to 4-AP (IC_{50}=5 mM) than I_{to} which is completely blocked by 2–4 mM 4-AP [12,13]. 4-AP sensitivity of A-current channels expressed from size-fractionated brain mRNA in *Xenopus* oocytes increases when the channels are co-expressed with a 2–4 kb mRNA fraction that expresses no K^+ channel activity on its own [14]. Therefore the difference in 4-AP sensitivity between RK5 and I_{to} could be due to the lack of a small subunit or a post-translational modification that occurs in vivo but not in the oocyte expression system in the absence of the smaller RNA.

Baldwin et al. [15] have recently reported the cloning and expression of a longer version of RK5 from rat brain (rat *shal*1) that has an additional 139 amino acids on the carboxyl terminal end and differs in sequence from the last 14 amino acids of RK5. These amino acid differences are due to an additional two nucleotides in the rat *shal*1 sequence which shift the reading frame. A second RK5 clone from our lab contains these two nucleotides. The RK5 described in this communication may represent an allelic difference or possibly a cloning artifact. However, since the RK5 currents are similar to rat *shal*1, the possibility that RK5 represents an allelic variation must be explored further. The major functional difference between the channels is that rat *shal*1 has a very slow component to its inactivation time course not seen with RK5. The addition of 139 amino acids to the C-terminal of RK5 as seen in rat *shal*1 apparently interferes with the inactivation process.

RK5 codes for a *Shal*-like potassium selective channel that is expressed in both cardiac and neuronal tissue [5]. This channel opens and inactivates rapidly in response to depolarizing stimuli and may contribute to I_{to} in the heart. All three members of the *Shal* family of K^+ channels that have been cloned and expressed to date show rapid voltage dependent activation and inactivate rapidly suggesting other members of the family will behave similarly and form a class of transient K^+ channels.

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REFERENCES

[1] Rudy, B. (1988) Neuroscience 25, 729–749.
[2] Timpe, L., Schwarz, T., Tempel, B., Papazian, D., Jan Y. and Jan, L. (1988) Nature 331, 143–145.
[3] Pongs, O. (1989) Pflügers Arch. 414, S71–75.
[4] Christie, M., Adelman, J., Douglass J. and North, R. (1989) Science 244, 221–224.
[5] Roberds, S. and Tamkun, M. (1991) Proc. Natl. Acad. Sci. USA 88, 1798–1802.
[6] Wei, A., Covarrubias, M., Butler, A., Baker, K., Pak M. and Salkoff, L. (1990) Science, 248, 599–603.
[7] Pak, M., Baker, K., Covarrubias, M., Butler, A., Ratcliffe A. and Salkoff, L. (1991) Proc. Natl. Acad. Sci. USA 88, 4386–4390.
[8] Benishin, C., Sorensen, R., Brown, W., Krueger B. and Blaustein, M. (1988) Mol. Pharmacol. 34, 152–159.
[9] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K., eds. (1989) Current Protocols in Molecular Biology, Wiley, New York.
[10] McCormack, K., Tanouye, M., Iverson, L., Lin, J., Ramaswami, M., McCormack, T., Campanelli, J., Mathew, M. and Rudy, B. (1991) Proc. Natl. Acad. Sci. USA. 88, 2931–2935.
[11] MacKinnon, R. and Yellen, G. (1990) Science. 250, 276–279.
[12] Josephson, I., Sanchez-Chapula, J. and Brown, A. (1984) Circ. Res. 54, 157–162.
[13] Dukes, I., Cleemann, L. and Morad, M. (1990) J. Pharmacol. Exper, Ther. 254, 560–569.
[14] Rudy, B., Hoger, J.H., Lester, H.A. and Davidson, N. (1988) Neuron 1, 649–658.
[15] Baldwin, T.J., Tsaur, M., Lopez, G., Jan, Y.N. and Jan, L.Y. (1991) Neuron 7, 471–483.