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Molecular cloning and expression of a bovine endothelial inward rectifier potassium channel

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Abstract A 5.1 kb cDNA encoding an inward rectifier K^+ channel (BIK) was isolated from a bovine aortic endothelial cell library. The cDNA codes for a 427-amino-acid protein with two putative transmembrane regions. Sequence analysis reveals that BIK is a member of the Kir2.1 family of inward rectifier K^+ channels. Expression in *Xenopus* oocytes showed that BIK is a K^+-specific strong inward rectifier channel that is sensitive to extracellular Ba^{2+}, Cs^+, and a variety of anti-arrhythmic agents. Northern analysis revealed that endothelial cells express a 5.5 kb BIK mRNA that is sensitive to shear stress.

Key words: Endothelial cell; K^+ channel; Inward rectifier; cDNA cloning; Shear stress; Gene expression

1. Introduction

The endothelial lining of blood vessels is exposed to a wide range of hemodynamic shear-stress environments [1,2]. Arterial endothelial cells sense mechanical shear stress and transduce it into a variety of biophysical, biochemical, and gene regulatory responses [3–6]. The initial mechanotransduction mechanism(s) has not been identified, however. A logical starting point to look for the primary mechanotransduction mechanism is with the fastest responses. One of the most rapid responses of endothelial cells to shear stress is the opening of an inwardly rectifying K^+ channel with simultaneous hyperpolarization of the endothelial cell membrane [7,8]. A previous study using potential sensitive dyes demonstrated a flow-induced hyperpolarization of endothelial cells [9]. Furthermore, studies of unidirectional Rb^+ efflux by Alevriadou et al. [10] confirm shear stress-dependent membrane permeability to K^+.

A variety of ionic conductances are observed in electrophysiological studies of arterial endothelial cells [11,12]. The major voltage-gated current observed in endothelial cells (I_{K1}) is K^+ specific [8,11,13–16]. I_{K1} has the properties of a K^+ inward rectifier: it is blocked by both extracellular Ba^{2+} and Cs^+; it is strongly inwardly rectifying with a reversal potential near the potassium equilibrium potential; and its amplitude is a function of extracellular potassium concentration [11,12,17]. Depolarization of endothelial cells relative to the membrane potential (V_m) elicits small outward currents, while hyperpolarizing voltage steps give rise to large inward currents [11,12,18]. The assumed role of the endothelial K^+ inward rectifier is to set the endothelial cell V_m [15,18,19]. In bovine aortic endothelial cells (BAEC) the V_m is reported to be approximately −64 mV, which is near the −70 mV physiological potassium equilibrium potential [20,21]. This indicates that, in BAECs, V_m is primarily determined by K^+. The addition of Ba^{2+} and Cs^+ at inhibitory concentrations for inward rectifiers (100 μM and 2 mM, respectively) causes depolarization of BAECs [8,20]. Thus changes in the inwardly rectifying current can modify the V_m of endothelial cells. This change in V_m modulates the electrochemical gradient for Ca^{2+}, which determines the magnitude of Ca^{+2} influx from the extracellular space [22,23]. Intracellular Ca^{2+} is an important endothelial signaling molecule that mediates a wide variety of intracellular events [24].

In this paper we report the cDNA cloning and functional expression of a bovine aortic endothelial cell K^+ inward rectifier channel, BIK. The primary structure and electrophysiological properties of BIK are presented. Inhibition of the BIK current by a variety of cations and pharmacological agents is examined. Finally, we demonstrate that the expression BAEC BIK mRNA significantly decreases in response to fluid flow. Preliminary results of this study were presented in abstract format [25].

2. Materials and methods

2.1. Endothelial cell isolation and growth

Clonal endothelial cells were mechanically isolated and cultured [26] using a modification of the techniques described by Gajdusek [27]. The identity of the cell lines was verified by morphology, diacetylated LDL uptake [28] and immunofluorescent labeling of factor VIII expression [29]. All cells were used before the 15th passage. A parallel plate flow chamber [26] was used to subject the endothelial cells to a well-defined laminar shear stress of 30 dyn/cm^2 for 24 h.

2.2. cDNA cloning and sequencing

Polymerase chain reaction (PCR) was used to obtain a DNA probe for hybrid screening of a bovine aortic cDNA library. Two degenerate primers were synthesized according to the amino acid sequences conserved between the mouse Kir2.1 [30] and the rat Kir2.2 [31]. The sequences of the sense and antisense oligonucleotide primers were 5′-ATYGTNGGNTGYATHATHGA-3′ and 5′-YTCRTTYTCRTA-RCARAANSYRTTNGC-3′, respectively, corresponding to amino

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Abbreviations: I_{K1}, arterial endothelial cell K^+ inward rectifier current; V_m, membrane potential; Kir, inward rectifier potassium channel; cDNA, complementary deoxyribonucleic acid; PCR, polymerase chain reaction; M-MLV, Moloney murine leukemia virus; cRNA, complementary ribonucleic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BAEC, bovine aortic endothelial cell; mRNA, messenger ribonucleic acid; compound II, (1-(4-methanesulphonamidophenoxy)-3-(*N*-methyl 3,4-dichlorophenylethylamino)-2-propanol); TEA, tetraethyl ammonium; I_{BK}, BIK-induced current; K_i, dissociation constant

The nucleotide sequence data reported in this paper has been submitted to GenBank with Accession Number U95369.

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acid residues 166–171 and 371–379 of the mouse Kir2.1 [30]. The cDNA was synthesized using M-MLV reverse transcriptase (Gibco), random hexamers (Gibco), and BAEC total RNA. PCR was carried out by Taq DNA polymerase (Promega) with 100 ng of cDNA and 2 mM Mg^{2+} for 35 cycles under the following conditions: 95°C, 1 min; 50°C, 1.5 min; 72°C, 2 min. The amplified product was subcloned into the PCR II vector (Invitrogen) and sequenced using an Applied Biosystems 373A DNA Sequencer with DyeDeoxy terminators (ABI). Sequence analysis of the 640 bp fragment (PCGene software package) revealed that it was 88% homologous to the corresponding region of the mouse Kir2.1 [30]. This 640 bp fragment (Ba10) was used to screen a bovine aortic endothelial cell cDNA library constructed in the phage λZAPII (Stratagene). Screening was done on 1.5×10^6 plaque forming units using the ECL direct nucleic acid labeling and detection system (Amersham) according to the manufacturer's protocols. In vivo excision and rescue of pBluescript SK(−) from the positive λZAPII clones were performed according to the manufacturer's instructions. We selected one clone with a 5.0 kb insert for further characterization. Both strands of the clone were sequenced as described above.

2.3. Northern blot analysis

Total RNA was isolated from clonal bovine aortic endothelial cells using the acid guanidinium/phenol method [32]. RNA (10 μg) was electrophoresed on a 1% agarose gel containing formaldehyde and transferred by pressure blotting using the PosiBlot Pressure Blotter (Stratagene) onto a BrightStar Plus positively charged nylon membrane (Ambion). BrightStar BIOTINscript (Ambion) was used to make biotin labeled RNA probes for hybridization. The Ba10 clone and the mouse GAPDH Control Vector (Ambion) were used as templates for probe synthesis. The filter was first hybridized with the Ba10 probe for 16 h at 65°C and washed at 65°C according to the manufacturer's protocols from NorthernMax (Ambion). Detection of the biotinylated RNA probe was done using BrightStar BioDetect (Ambion). Autoradiography was performed on Kodak Biomax MR film for 5 min at room temperature. Rehybridizations were done subsequently with the GAPDH probe following the protocol described above. The films with the GAPDH signal were exposed for 1 min. The intensity of each hybridization band was determined by optical densitometry (Personal Densitometer SI and ImageQuant, Molecular Dynamics).

2.4. Functional expression of BIK

The pBluescript SK(−) plasmid containing the BIK clone was linearized with *Eco*RV and capped run-off cRNA was synthesized in vitro with T3 RNA polymerase. The transcribed RNA was injected into defolliculated *Xenopus* oocytes (27 ng RNA/oocyte) [33] followed by incubation for 48 h. Channel expression was measured by two microelectrode voltage clamping with a cutoff frequency of 2 kHz (8-pole Bessel filter). Electrodes (1–3 MΩ) filled with 3 M KCl were used. The pClamp software was used for data acquisition and analysis. Bath solution (ND96) contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.3 mM niflumic acid and 5 mM HEPES (pH 7.5). Solutions with various concentrations of K^+ were made by substituting equimolar concentrations of K^+ for Na^+ in ND96.

2.5. Pharmacological agents

Quinidine [34], disopyramide [35] and niflumic acid were purchased from Sigma. Sotalol [36] and compound II [37] were purchased from Tocris Cookson.

3. Results

3.1. Primary structure of BIK

Fifteen positive cDNA clones were isolated from a bovine aortic endothelial cell cDNA library. The nucleotide sequence of the largest clone (BIK) contains one long open reading frame encoding a protein of 427-amino-acid residues with a calculated M_r of 48 236 (Fig. 1). Sequence analysis of the other 14 positive clones established that they were shorter cDNA clones identical to portions of BIK. The nucleotide sequence of the coding region is 84% identical to the mouse Kir2.1 [30] with seven amino-acid differences. Sequence comparison of both the 5′ and 3′ non-coding regions of BIK to the corresponding regions in the mouse Kir2.1 [30] reveals overall low homology although stretches of high homology exist. Hydrophobicity analysis of the BIK amino-acid sequence shows the presence of two transmembrane segments (M1 and M2), and the pore-forming region H5 which is characteristic of inwardly rectifying K^+ channels [38,39]. The BIK sequence has putative phosphorylation sites for protein kinase C (residues 3, 6, 357 and 383), protein kinase A (residue 425), and tyrosine kinase (residues 242 and 366).

3.2. Northern blot analysis of BIK mRNA in shear-stressed BAECs

In order to determine if BIK mRNA is altered by shear stress, BAECs were subjected to flow prior to RNA isolation. Northern blot analysis was used to compare BIK mRNA from clonal BAECs exposed to shear stress to BIK mRNA from non-sheared control cells. BIK is expressed in BAECs as a single mRNA with estimated size of 5.5 kb (Fig. 2A). Fig. 2B demonstrates that BIK mRNA is significantly reduced in BAECs exposed to 30 dyn/cm^2 shear stress for 24 h as compared with RNA from BAECs not exposed to shear stress. GAPDH served as the internal standard to normalize the BIK signal because the expression of GAPDH in BAECs is not affected by changes in shear stress [40]. The GAPDH signal indicates equal RNA was loaded in each lane.

3.3. Electrophysiological properties of BIK

The electrophysiological properties of BIK were examined using a *Xenopus* oocyte expression system. When expressed in oocytes, BIK produced a current (I_{BK}) that was activated at hyperpolarized potentials (Fig. 3A). I_{BK} was rapidly activating and showed slight inactivation over the time course of the voltage pulses (Fig. 3A). I_{BK} increased in a nonlinear fashion as a function of external $[K^+]$ (Fig. 3A,B). No significant inward current was observed in uninjected or water-injected oocytes under similar conditions (data not shown). The voltage at which I_{BK} rectified shifted to lower potentials at lower external $[K^+]$ (Fig. 3B). The BIK current in oocytes was highly potassium selective; the potential at which the current reverses changed 55.8 mV per decade change in external $[K^+]$ (Fig. 3C).

External Ba^{2+} (Fig. 4A) and Cs^+ (Fig. 4B) both exhibited a concentration- and voltage-dependent block of I_{BK} which is typical for inward rectifier currents [41,42]. Both Ba^{2+} and Cs^+ demonstrated a greater block of the steady-state current at more negative potentials (Fig. 4A,B). We examined the voltage dependence of the Cs^+ and Ba^{2+} block by first determining the K_i (concentration producing 50% block) for each at a variety of voltages. This is done by plotting [steady-state current level without blocker]/[steady-state current level with blocker] versus the external blocker concentration. The K_i is the inverse slope of the linear fit of the data. Fig. 4C shows this graph for various $[Cs^+]$. The K_i for Ba^{2+} was similarly calculated to be 89 ± 7 μM at −120 mV ($n=5$). The Woodhull model of ionic blockage of channels [43] was used to quantify the voltage dependence of the Cs^+ block. The logarithms of the K_i values for Cs^+ were plotted against the membrane potential (Fig. 4D). A 10-fold change in K_i corresponds to a change in membrane potential of 37 mV. This implies that the fractional distance of the Cs^+ binding site in the membrane electric field is 1.6, which is similar to the Cs^+ block observed

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5'...GCGCCAGCAACAGGACCTGTTCTCTGGATGTCAGCTGAGTTACTAAGGTGACTCTGCATGTCAAGAGACAGACCTTGGTAGCCAGAACCTCAAGGCCCCTGGAGACCCCATCCC -120
TCCTTCTTTTTTTGTGCGTGTGGGTCCTCACTGAACATTCAAACCTGTTTCTCCAAAGGGTTTTGCAAAAACTGAGACTGTTTCCCAAAGCAGAAGCGCTGGCGTCCACAGCAGAAGCG -1

                ϕ       ϕ
Met Gly Ser Val Arg Thr Asn Arg Tyr Ser Ile Val Ser Ser Glu Glu Asp Gly Met Lys Leu Ala Thr Leu Ala Val Ala Asn Gly Phe 30
ATG GGC AGC GTG CGC ACC AAC CGC TAC AGC ATC GTC TCT TCA GAG GAG GAC GGC ATG AAG CTG GCC ACC CTG GCG GTG GCC AAC GGA TTC 90

Gly Asn Gly Lys Ser Lys Val His Thr Arg Gln Gln Cys Arg Ser Arg Phe Val Lys Lys Asp Gly His Cys Asn Val Gln Phe Ile Asn 60
GGG AAT GGC AAG AGC AAA GTC CAC ACC CGC CAG CAG TGC AGG AGC CGC TTC GTG AAG AAG GAC GGA CAC TGC AAC GTG CAG TTC ATC AAC 180

Val Gly Glu Lys Gly Gln Arg Tyr Leu Ala Asp Ile Phe Thr Thr Cys Val Asp Ile Arg Trp Arg Trp Met Leu Val Ile Phe Cys Leu 90
GTG GGC GAG AAG GGC CAG CGG TAC CTG GCG GAC ATC TTC ACC ACG TGC GTG GAC ATC CGC TGG CGG TGG ATG CTG GTC ATC TTC TGC CTG 270

                        M1
Ala Phe Val Leu Ser Trp Leu Phe Phe Gly Cys Val Phe Trp Leu Ile Ala Leu Leu His Gly Asp Leu Asp Ala Ser Lys Glu Ser Lys 120
GCT TTC GTG CTC TCC TGG CTC TTC TTC GGC TGT GTG TTT TGG TTG ATC GCG CTG CTC CAC GGG GAC CTG GAT GCG TCC AAG GAG AGC AAA 360

                                                        H5
Ala Cys Val Ser Glu Val Asn Ser Phe Thr Ala Ala Phe Leu Phe Ser Ile Glu Thr Gln Thr Thr Ile Gly Tyr Gly Phe Arg Cys Val 150
GCC TGC GTG TCC GAG GTC AAC AGC TTC ACG GCT GCC TTC CTT TTC TCC ATC GAG ACG CAG ACC ACC ATC GGC TAC GGC TTC CGC TGC GTC 450

                                M2
Thr Asp Glu Cys Pro Val Ala Val Phe Met Val Val Phe Gln Ser Ile Val Gly Cys Ile Ile Asp Ala Phe Ile Ile Gly Ala Val Met 180
ACG GAC GAG TGC CCT GTG GCC GTC TTC ATG GTG GTC TTC CAG TCC ATC GTG GGC TGC ATC ATC GAC GCC TTC ATC ATC GGT GCG GTA ATG 540

Ala Lys MET Ala Lys Pro Lys Lys Arg Asn Glu Thr Leu Val Phe Ser His Asn Ala Val Ile Ala Met Arg Asp Gly Lys Leu Cys Leu 210
GCC AAG ATG GCC AAG CCC AAA AAG AGA AAC GAG ACG CTG GTC TTC AGC CAC AAC GCC GTG ATC GCC ATG AGG GAC GGC AAG CTC TGC CTC 630

Met Trp Arg Val Gly Asn Leu Arg Lys Ser His Leu Val Glu Ala His Val Arg Ala Gln Leu Leu Lys Ser Arg Ile Thr Ser Glu Gly 240
ATG TGG CGG GTG GGC AAC CTC CGG AAG AGC CAC TTG GTG GAG GCG CAC GTG CGC GCG CAG CTC CTC AAG TCC AGA ATC ACC TCC GAG GGG 720

    ϕ
Glu Tyr Ile Pro Leu Asp Gln Ile Asp Ile Asn Val Gly Phe Asp Ser Gly Ile Asp Arg Ile Phe Leu Val Ser Pro Ile Thr Ile Val 270
GAG TAC ATC CCC CTG GAT CAG ATA GAC ATC AAC GTG GGC TTC GAC AGC GGC ATC GAC CGC ATA TTT CTG GTG TCT CCC ATC ACC ATC GTC 810

His Glu Ile Asp Glu Asp Ser Pro Leu Tyr Asp Leu Ser Lys Gln Asp Ile Asp Asn Ala Asp Phe Glu Ile Val Val Ile Leu Glu Gly 300
CAC GAG ATC GAT GAG GAC AGT CCT CTG TAC GAT CTG AGC AAG CAG GAC ATC GAC AAC GCA GAC TTT GAG ATC GTG GTC ATC CTC GAG GGT 900

Met Val Glu Ala Thr Ala Met Thr Thr Gln Cys Arg Ser Ser Tyr Leu Ala Asn Glu Ile Leu Trp Gly His Arg Tyr Glu Pro Val Leu 330
ATG GTG GAG GCC ACG GCC ATG ACC ACG CAG TGC CGG AGC TCG TAC CTG GCC AAC GAG ATC CTC TGG GGT CAC CGC TAC GAG CCG GTG CTC 990

                                                        ϕ
Phe Glu Glu Lys His Tyr Tyr Lys Val Asp Tyr Ser Arg Phe His Lys Thr Tyr Glu Val Pro Asn Thr Pro Leu Cys Ser Ala Arg Asp 360
TTC GAG GAG AAA CAC TAC TAC AAA GTA GAC TAC TCC AGG TTC CAC AAG ACG TAC GAA GTC CCC AAC ACG CCC CTG TGC AGC GCT AGG GAC 1080

        ϕ                                               ϕ
Leu Ala Glu Lys Lys Tyr Ile Leu Ser Asn Ala Asn Ser Phe Cys Tyr Glu Asn Glu Val Ala Leu Thr Ser Lys Glu Glu Asp Asp Ser 390
TTA GCG GAG AAG AAA TAC ATC CTG TCG AAC GCT AAC TCG TTT TGC TAC GAA AAT GAG GTC GCC CTC ACG AGC AAA GAG GAA GAC GAC AGT 1170

Glu Asn Gly Val Pro Glu Ser Thr Ser Thr Asp Thr Pro Pro Asp Ile Asp Leu His Asn Gln Ala Ser Val Pro Leu Glu Pro Arg Pro 420
GAG AAC GGG GTC CCC GAG AGC ACA AGC ACG GAC ACG CCC CCG GAC ATA GAC CTG CAC AAC CAG GCC AGT GTA CCT CTA GAG CCC AGG CCG 1260

      ϕ
Leu Arg Arg Glu Ser Glu Ile  -                                                                                        427
TTA CGA CGG GAG TCG GAG ATA TGA CTGAGTCCTCCTGGGGAGTGCTTCCTGTGAAACACGGTCTGTTGGTCAAAGGCCCAAAACAGTTACGCACACGACGGTACCAGGGGC 1371
CTGAGTCCTCCTGGGGAGTGCTTCCTCTGAAACACGGTCTGTTGGTCAAAGGCCCAAAACAGTTACGCACACGACGGTACCAGGGGCAGGTGGTTCGAGGCAAGTGACCACAAGGGACT 1490
GGGGCTAGCGCGGTGGTTTTAGAGAAAGACTGTAAGCGCGAAGACGA...3'
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Fig. 1. Nucleotide and deduced amino acid sequences of BIK. Nucleotides numbered from the initiating ATG and amino acid residues numbered from the initiating MET. Boxed regions are the putative transmembrane regions (M1 and M2) and the pore forming region (H5). ϕ indicates potential phosphorylation sites for protein kinase C (S3, T6, S357, T383), protein kinase A (S425) and tyrosine kinase (Y242, Y366). Residues in bold (N172 and E224) are required for the strong rectification of the mouse Kir2.1 [54–56]. Only part of the 5′ and 3′ untranslated nucleotide sequences are shown.

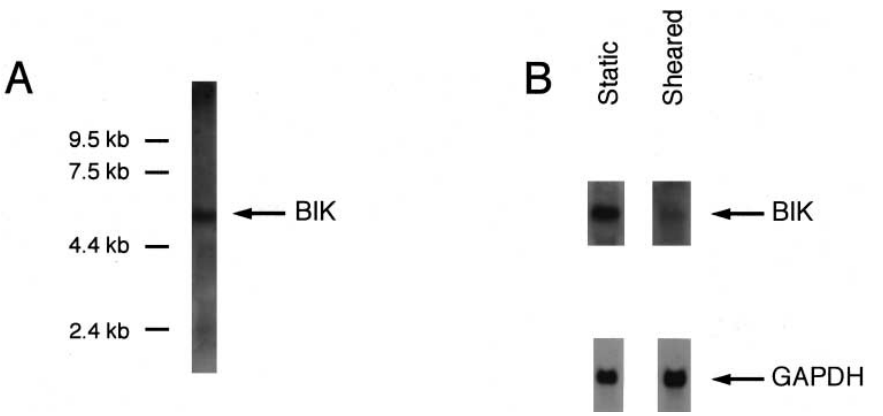

Fig. 2. Northern blot analysis of BIK mRNA. A: Expression of BIK mRNA (~5.5 kb) in clonal bovine aortic endothelial cells. B: Comparison of BIK mRNA expression in sheared versus static controls in BAECs. GAPDH mRNA (~1.4 kb) expression also shown. RNA size markers (BRL) are shown on the left of (A).

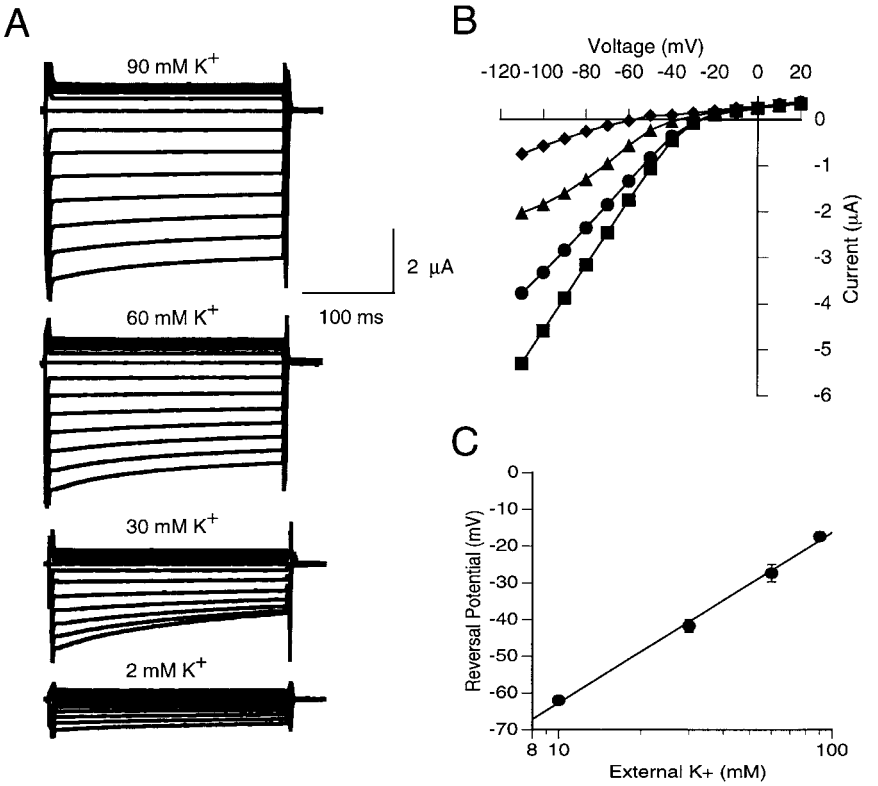

Fig. 3. Inwardly rectifying currents from BIK expressed in *Xenopus* oocytes. A: Currents were recorded from an BIK cRNA injected oocyte that was clamped at −40 mV and subjected to 260 ms test potentials ranging from −110 mV to +20 mV in 10 mV increments. B: Current–voltage relationships obtained from same oocyte as (A). Current amplitudes at 100 ms after initiation of pulses are plotted against the membrane potential. Concentration of external K^+: ■, 90 mM; ●, 60 mM, ▲, 30 mM; ♦, 2 mM. C: Semilogarithmic plot of extracellular K^+ concentration versus reversal potential. Data shows mean ± SE for three oocytes. The straight line is a best fit of the data.

in the Kir2.1 channel cloned from mouse [30]. This result is indicative of a multi-ion block of permeant (K^+) and blocking (Cs^+) ions in a multi-ion pore [44]. TEA was a much less potent blocker of I_{BK} then either Ba^{2+} or Cs^+ with a K_i of 39 ± 3 mM at −110 mV ($n = 5$).

The sensitivity of I_{BK} to four antiarrhythmic agents was examined by application of the drugs to oocytes previously injected with BIK specific cRNA. Quinidine and compound II were the most potent inhibitors of the BIK mediated currents (Fig. 5A,D). The K_i values for quinidine and compound II were determined to be 110 ± 30 μM and 39 ± 14 μM at −130 mV respectively ($n = 3$–5). These K_i values did not demonstrate any voltage dependence. Disopyramide and sotalol both showed only small effects on I_{BK} (Fig. 5B,C).

4. Discussion

In this report we describe the cDNA cloning and functional expression of a bovine aortic endothelial cell K^+ inward rectifier channel, BIK. We present the entire primary structure and characterize the electrophysiological properties of the clone. Inhibition of the BIK current by a variety of cations and pharmacological agents are reported. Finally, we demonstrate that the expression of BIK messenger RNA in BAECs is significantly reduced in response to fluid flow.

The BIK induced current (I_{BK}) shares electrophysiological properties with the endothelial cell current (I_{K1}). I_{BK} and I_{K1} have similar strongly inwardly rectifying whole cell current–voltage relationships, they both show rapid activation kinetics, and they are both blocked by extracellular Ba^{2+} and Cs^+ at similar concentrations [8,11,12,18]. Both currents respond to increases in extracellular potassium with an increase in their respective current reversal potentials and an increase in their slope conductances [12]. Because of these similarities we propose that BIK is the predominant inward rectifier described in endothelial cells.

Regulation of a K^+ current in BAECs could have significant impact on a variety of cell signal transduction systems. BIK mRNA was found to be greatly decreased in BAECs exposed to 30 dyn/cm^2 shear stress for 24 h. Therefore the cells regulate ion channel mRNA as a response to the shear-stress environment. This result is significant because it is the first report of the shear-stress regulation of a primary signaling molecule in endothelial cells. The regulation of several genes in BAECs has been shown to be sensitive to the level of shear stress induced by blood flow. These include genes encoding basic fibroblast growth factor [45], nitric oxide synthase [46,47], transforming growth factor β1 [48] and endothelin I [49]. The regulation of BIK may have affects on a wide range of other shear-related events including the regulation of the genes mentioned above.

Antiarrhythmic agents are in widespread clinical use for the management of cardiac arrhythmias [50]. These drugs inhibit cardiac ion channels. BIK has structural and functional similarity to cardiac ion channels. Two different drugs, quinidine, a class Ia agent, and compound II, a class III agent, were each found to significantly inhibit I_{BK} at physiologically relevant concentrations [34,37]. Interestingly, sotalol, a close analog of

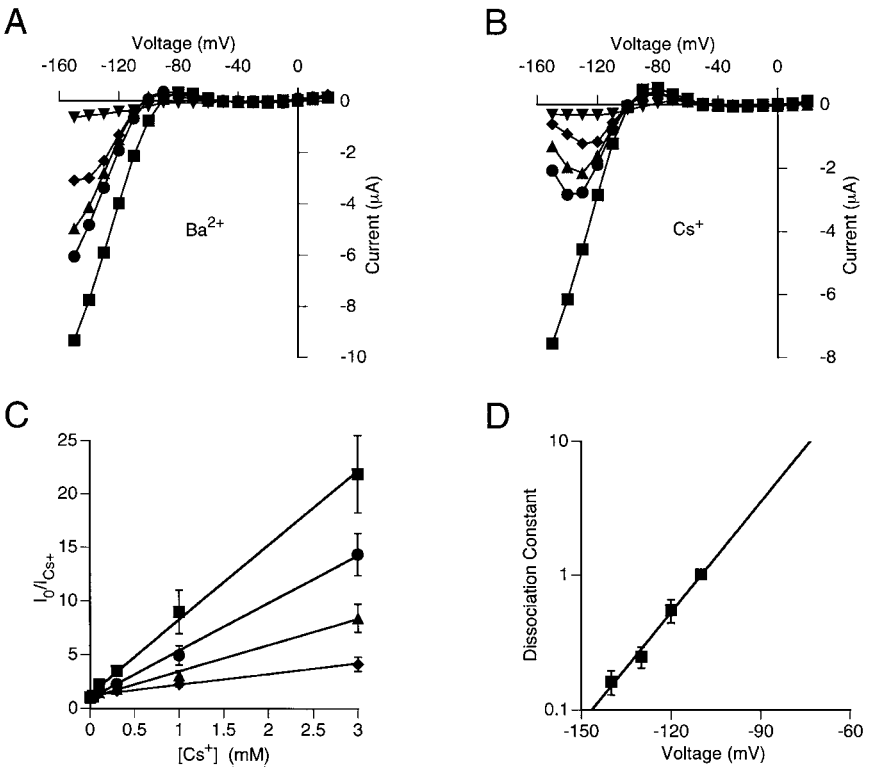

Fig. 4. Analysis of I_{BK} block by Ba^{2+} and Cs^+. A: Current–voltage relationships for representative oocyte in various concentrations of Ba^{2+}: ●, 30 μM; ▲, 100 μM; ♦, 300 μM; ▼, 1 mM. B: Current–voltage relationships for representative oocyte in various concentrations of Cs^+: ●, 100 μM; ▲, 300 μM; ♦, 1 mM; ▼, 3 mM. In both (A) and (B) the ■ symbol represents no drug. Cell subjected to 260 ms voltage steps in 10 mV increments between −150 mV and +20 mV from a holding potential of −40 mV in ND96. Current amplitudes measured 100 ms after initiation of test pulses. C: Plot of the ratios of steady-state current levels in the absence and presence of Cs^+ as a function of $[Cs^+]_o$ at the indicated membrane potentials: ■, −140 mV; ●, −130 mV; ▲, −120 mV; ♦, −110 mV. Least squares fit derived from $I_0/I_{Cs+} = 1+[Cs^+]/(1+K_i)$ [57]. D: Logarithms of K_i values for Cs^+ block plotted as a function of voltage. Data in (C) and (D) shows the mean ± SE for five oocytes.

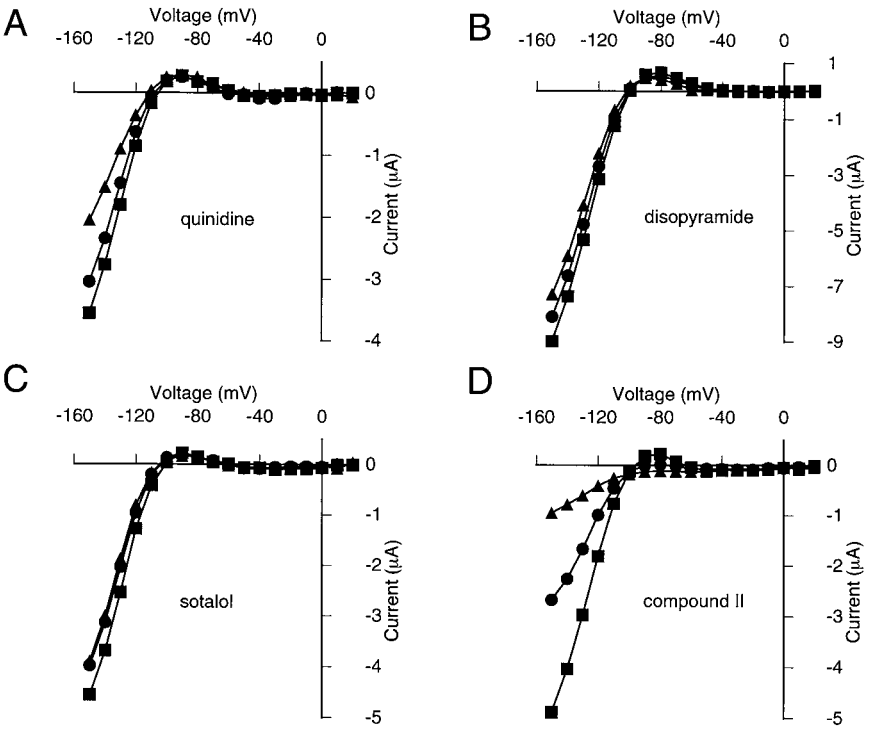

Fig. 5. Analysis of I_{BK} inhibition by four antiarrhythmic agents. I/V plots obtained from representative oocytes as in Fig. 4. The ■ symbol represents the control (no agent) in each plot. Drugs and concentrations: (A) quinidine: ●, 10 μM; ▲, 100 μM; (B) disopyramide: ●, 180 μM; ▲, 720 μM; (C) sotalol: ●, 250 μM; ▲, 1 mM; (D) compound II ●, 10 μM; ▲, 50 μM.

compound II [51], displayed very little inhibition of I_{BK}. This is the first report of the inhibition of an endothelial cell ion channel with these agents. These results indicate that both quinidine and compound II may have significant effects on vascular endothelial cells.

The BIK current may be the primary determinant of resting potential in endothelial cells. Modulation of BIK current by shear stress or other factors would have a direct effect on the membrane potential. Since the influx of extracellular calcium is dependent on V_m, an understanding of resting potential maintenance is crucial to understanding calcium homeostasis within endothelial cells. Moreover, it has been postulated that endothelial cells are electrically coupled not only to each other but also to vascular smooth muscle cells [52,53]. Thus the maintenance of a hyperpolarized membrane potential may be of importance to both endothelial cell function and vascular smooth muscle cell relaxation.

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