Differential Expression of Voltage-Gated K⁺ Channel Genes in Arteries From Spontaneously Hypertensive and Wistar-Kyoto Rats

Robert H. Cox, Kimberly Folander, Richard Swanson

Abstract—Voltage-gated K⁺ currents play an important role in determining membrane potential, intracellular Ca²⁺, and contraction in arterial smooth muscle. In this study, the expression of genes encoding voltage-gated K⁺ channels of the Kv1.X family was compared in arteries from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Expression of Kv1.X in thoracic aorta, mesenteric arteries, tail artery, and heart was determined, both qualitatively and quantitatively, by reverse transcription–polymerase chain reaction. Our results demonstrate distinct but overlapping patterns of expression in vascular tissues. In general, Kv1.2 and Kv1.5 were most highly represented, and the levels of Kv1.2 were significantly larger in all tissues from SHR. Levels of Kv1.5 in arteries did not differ significantly between strains but were greater in SHR heart. Moderate levels of Kv1.3 and Kvβ1.1 expression were also found in all tissues and were larger in SHR. Kv1.1 expression was not different between the 2 strains, and no significant expression of Kv1.4 (except in heart and aorta), Kv1.6, or Kvβ2.1 was observed in either strain. Kv1.2 and Kv1.5 transcripts represent ~1 to 2 parts/10⁶ of total mesenteric arterial RNA with ~2- to 5-fold lower levels in aorta and tail artery. Whole-cell voltage-gated K⁺ channel currents, recorded from mesenteric arterial myocytes, were larger in SHR than WKY (eg, at 0 mV: 7.3±0.8 versus 10.9±1.2 pA/pF). The voltage dependence of activation was more negative in SHR (V₅₀: −20±4 mV versus −32±3 mV) but that of availability was not different. These results indicate that Kv1.X genes are differentially expressed between WKY and SHR (especially Kv1.2 and Kvβ1.1). These differences in gene expression are associated with a greater voltage-gated K⁺ channel current density in SHR and shifted voltage-dependent activation compared with WKY. These differences may be a compensatory mechanism related to the membrane potential depolarization in SHR or some manifestation thereof. (Hypertension. 2001;37:1315-1322.)

Key Words: potassium channels ■ gene expression ■ hypertension, genetic ■ arteries ■ Shaker potassium channels

In vascular smooth muscle (VSM) cells, force development and vascular resistance are closely coupled to membrane potential,¹,² which, in turn, is primarily determined by potassium conductance.³,⁴ Thus, blockade of K⁺ channels by tetraethylammonium, 4-aminopyridine, or charybdotoxin depolarizes Vₘ and results in tonic contraction of VSM.⁵-⁷ Alternatively, some vasodilators and antihypertensive compounds, such as NO and β-adrenergic agonists, exert their actions in part by increasing K⁺ conductance.⁴,⁸,⁹ Consistent with a central role in the normal regulation of smooth muscle contraction, K⁺ conductance is altered in some pathological states such as hypertension¹⁰,¹¹ and atherosclerosis,¹² contributing to the changes in VSM function that occur in these disorders.

At least 4 different K⁺ channel classes have been reported to contribute to K⁺ conductance in smooth muscle. These include voltage-dependent, Ca²⁺-activated, ATP-dependent, and inwardly rectifying K⁺ channels.³ The relative roles of these different classes vary in accordance to vascular location, animal species, and pathological state.³,⁴ Ca²⁺-activated K⁺ channels (KᵥCa) may provide a negative feedback mechanism in the regulation of membrane potential by opening in response to intracellular Ca²⁺ elevations.¹³ ATP-dependent K⁺ channels (KᵥATP) have been suggested to play a role in the metabolic regulation of smooth muscle contraction¹⁴ as well as in mediating vascular relaxation induced by a number of vasodilator drugs.⁴ Inwardly rectifying K⁺ channels contribute to the regulation of resting membrane potential in some tissues as well as to vascular relaxation to increases in extracellular [K⁺].⁴,¹⁵

Voltage-gated K⁺ channels (Kᵥ) play a major role in the regulation of K⁺ conductance in many different types of smooth muscle.¹⁶,¹⁷ A large number of functionally distinct Kᵥ α-subunits are known to exist, many of which are the unique products of individual members of a large superfamily of genes.¹⁸ The differential expression of these genes in various smooth muscles and the coassembly of the α-subunits they encode (along with distinct, modulatory β-subunits) may
contribute to the large functional diversity that has been noted for native K_C currents in different myocytes.18–24

The purpose of this study was to identify the Kv1.X subunits that underlie the K_C current in arterial myocytes and to determine whether the relative abundance of their transcripts is different in the hypertensive spontaneously hypertensive rat (SHR) model. We demonstrate here that Kv1.2 and Kv1.5 are the dominant Shaker family transcripts expressed in arterial myocytes and that there is a significantly greater expression of Kv1.2 in SHR arterial smooth muscle cells compared with Wistar-Kyoto rats (WKY). These and other differences in expression of this gene family correlate with the large functional diversity that has been noted for native K_C currents in different myocytes.18–24

**Methods**

**RNA Isolation**

Twelve- to 15-week-old WKY or SHR were killed by CO_2 narcosis, and tissue samples, including the thoracic aorta from the arch to the diaphragm, a 10-cm length of tail artery, the arch to the diaphragm, a 10-cm length of tail artery, the mesenteric arterial network, and the heart, were rapidly removed and placed in iced isolation buffer. The isolation buffer had the following composition (in mmol/L): 140 NaCl, 5 KCl, 1 MgCl_2, 10 HEPES, and 10 glucose with pH 7.4 (NaOH) and an osmolality of 298±2 mOsm/L. Tissues from groups of 6 animals were rapidly cleaned of extraneous material, pooled, and frozen in liquid nitrogen. Total RNA was isolated by homogenization in guanidinium isothiocyanate and centrifugation through a CsCl step gradient as previously described.25 RNA was ethanol precipitated and dissolved in diethylpyrocarbonate-treated water at a concentration of ~1 µg/µL, and a 5-µg sample of each was qualitatively evaluated by agarose gel electrophoresis. Rat brain poly A⁺ mRNA was purchased from Clontech (Palo Alto, Calif.) for use as a standard.

**Reverse Transcription–Polymerase Chain Reaction**

First-strand cDNA was synthesized in a 20-µL reaction that contained 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 0.5 mmol/L each dNTP, 10 mmol/L diithiothreitol, 10 U SuperScript II reverse transcriptase (GibcoBRL), 2 U RNasin, 2 µg of total RNA, and either 7.5 ng/µL random hexamer primers or 100 mmol/L of a gene-specific primer (Table 1). A unique sequence (5’-TCTAGAGGTTCAAGCTT-3’, called “Tag” below) was added to the 5’ end of each reverse gene-specific primer to enable specific amplification from cDNA in subsequent quantitative polymerase chain reaction (PCR) reactions. First-strand syntheses were performed at 42°C for 50 minutes. PCR from the randomly primed first-strand cDNAs was performed with gene-specific forward and reverse primers (Table 1). Amplifications from the specifically primed first-strand cDNAs used a gene-specific forward primer and the Tag sequence as the reverse primer. The Tag was used in place of a gene-specific reverse primer to restrict amplification to the cDNA templates into which it had been incorporated and, thereby, to minimize amplification from potential trace amounts of contaminating genomic DNA. Control experiments empirically demonstrated the lack of amplification of Kv genes from genomic DNA using the Tag sequence as the reverse primer. The 50-µL PCR reaction contained 2 µL of the first-strand reaction, 0.2 µmol/L each primer, 40 mmol/L Tricine-KOH, 15 mmol/L KOAc, 3.5 mmol/L Mg(OAc)_2, 0.2 µmol/L each dNTP, 3.75 µg/mL BSA, and 1 µL of Advantage cDNA polymerase (Clontech). PCR (20 or 25 cycles) was performed using a Perkin Elmer Model 480 thermocycler with the following parameters: melting at 94°C for 30 seconds, annealing at 62°C for 1 minute, and extension at 68°C for 3 minutes. These PCR parameters were selected on the basis of the results of preliminary experiments designed to ensure that data were obtained from the exponential phase of amplification. Control templates included 500 ng of rat brain poly A⁺ mRNA to control for cDNA synthesis (ie, as a reverse transcription [RT] control) and 500 ng of rat genomic DNA or water as positive and negative PCR controls, respectively.

**Quantitative PCR**

Kv channel transcripts contained in total RNA isolated from tissues were quantitated by inclusion of competitive, internal standard RNAs (IS-RNAs) in the cDNA synthesis reactions. Templates for the synthesis of the IS-RNAs were generated by mutagenesis of the Kv1.X cDNAs to create a 100-bp tandem repeat within the region to be amplified in the PCR. The 100-bp insertion fragments were

<table>
<thead>
<tr>
<th>Primer Sequences</th>
<th>Name</th>
<th>Location</th>
<th>Sense/Antisense</th>
<th>Size, nt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.1F</td>
<td>1310–1330</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1Tag</td>
<td>1310–1330</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1F</td>
<td>1684–1704</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1Tag</td>
<td>1684–1704</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1F</td>
<td>2143–2163</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1Tag</td>
<td>2143–2163</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1F</td>
<td>1921–1944</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.1Tag</td>
<td>1921–1944</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.2F</td>
<td>1863–1884</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.2Tag</td>
<td>1863–1884</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.3F</td>
<td>1684–1704</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.3Tag</td>
<td>1684–1704</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.4F</td>
<td>2281–2301</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.4Tag</td>
<td>2281–2301</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.5F</td>
<td>2267–2287</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.5Tag</td>
<td>2267–2287</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.6F</td>
<td>2666–2686</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.6Tag</td>
<td>2666–2686</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.7F</td>
<td>1799–1819</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.7Tag</td>
<td>1799–1819</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.8F</td>
<td>2145–2165</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.8Tag</td>
<td>2145–2165</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.9F</td>
<td>181–200</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.9Tag</td>
<td>181–200</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.10F</td>
<td>520–540</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.10Tag</td>
<td>520–540</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.11F</td>
<td>400–420</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.11Tag</td>
<td>400–420</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.12F</td>
<td>681–701</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Kv1.12Tag</td>
<td>681–701</td>
<td>ATA TAT GAA TTT CAC TTT GTC CTT CAT GTT AGT CCT</td>
<td>375</td>
<td></td>
</tr>
</tbody>
</table>

The GenBank submissions from which primer sequences were obtained are as follows: Kv1.1, M26161; Kv1.2, X16003; Kv1.3, M31744; Kv1.4, X16002; Kv1.5, M27158; Kv1.6, X17621; Kv1.7, X70662; and Kv1.8, X76724.
synthesized by PCR (using the wild-type cDNAs as the templates) and ligated into the cDNAs at unique restriction sites located within the regions to be amplified. Mutated cDNAs were cloned by standard techniques and sequenced on both strands to verify the insertion. IS-RNAs were transcribed in vitro from the mutated channel cDNAs as described, quantitated by A 260, and then serially diluted to yield stock solutions that ranged in concentration from 0.1 pg/µL to 1 ng/µL.

To quantitatively determine the amount of a Kv channel transcript in total RNA, a series of RT-PCR amplifications was performed, each containing (in the first-strand cDNA synthesis reaction) 1 µg of the total tissue RNA plus 1 µL of 1 of the serial dilutions of the competing IS-RNA. First-strand synthesis and PCR amplification were performed as described above. Preliminary experiments were performed to determine if the presence of this additional 100-bp insert altered the efficiency of PCR amplification by determining product accumulation during successive PCR cycles by Southern blot analysis. We found that PCR efficiency (~0.85, ie, product accumulated by 1.7-fold/cycle of PCR) was not different between wild-type RNA and IS-RNA for either Kv1.2 or Kv1.5. Furthermore, because the regions of the transcripts that were amplified were relatively short (360 to 480 bp), the product amplified from the IS-RNA that contained the additional 100 bp could easily be distinguished from the wild-type product by 2% agarose gel electrophoresis. Over the appropriate concentration range, increasing amounts of the IS-RNA competitively inhibited amplification of the wild-type RNA (Figure 3).

Analysis

PCR products were fractionated by agarose gel electrophoresis and analyzed by Southern blotting as described previously. Blots were probed with 32P-labeled oligonucleotides (Table 1), which recognized specific sequences within the regions amplified by the PCR but outside of the tandem-repeat region present in the IS-RNAs. After washes to high stringency (0.1% SSC and 0.1% SDS at 65°C), hybridization was recorded using either conventional x-ray film (Kodak X-AR) or a Molecular Dynamics PhosphorImager. Quantitation was performed by densitometric analysis of the digital images. For the initial RT-PCR reactions (from randomly primed first-strand

Figure 1. Kv1.X gene expression. Representative Southern blots from WKY (A) and SHR (B) showing regional differences in expression Kv1.X genes between heart (H), aorta (A), mesenteric arteries (M), and tail arteries (T). Transcripts encoding Kv1.1, Kv1.2, Kv1.3, Kv1.5, and Kvβ1.1 were found in all arterial samples with Kv1.2 and Kv1.5 being the dominant species relative to their expression levels in brain poly A+ RNA (B*). Lanes show cDNA synthesis performed in the presence (+) and absence (−) of reverse transcriptase. PCR controls were performed using rat genomic DNA (G) or water (W) as the template. Note that PCR products are expected from the genomic DNA template for all reactions except Kvβ2.1, because the primer pairs used in that reaction span an intron.

Figure 2. Normalized expression of Kv1.X genes in vascular tissues of SHR and WKY. Digital images of Southern blots were analyzed by quantitative dosimetry, and values for each tissue were divided by the value obtained from rat brain in each experiment before averaging. Higher levels of expression of Kv1.2, Kv1.3, and Kvβ1.1 were found in SHR, but there were no statistically significant differences in Kv1.1, Kv1.4, or Kv1.5 between the groups. No significant amounts of mRNA for Kv1.6 or Kvβ2.1 were found in these tissues. Columns represent mean values, and bars represent ±1 SEM (n=10 for both WKY and SHR). Asterisks represent significant differences (P<0.05) between SHR and WKY. Note the differences in scale for each of the panels.
PCR Products

[Image: M 100+ 100- 100 10 1 0.1 X+ X- Brain]

Southern Blot

[Image: ]

PhosphoImager

[Image: ]

Regression Analysis

[Image: ]

Figure 3. Quantitative PCR methods. Summary of the methods used to quantitate Kv transcripts in total RNA using an IS-RNA. PCR products were size fractionated on 2% agarose gels then transferred to nylon membranes. The membranes were hybridized to 32P-labeled gene-specific oligonucleotide probes, washed to high stringency, and recorded on either x-ray film or digitally with a PhosphorImager. Images were quantitated by volume integration, and the results were used to determine the amount of IS-RNA that produced equimolar levels of the 2 products by least-squares regression analysis. The numbers in labeling above the gel indicate the pg of IS-RNA; + and −, cDNA synthesis performed in the presence and absence of reverse transcriptase; X, unknown tissue RNA; and M, marker lane. The lanes marked 100+, 100−, X+, and X− contain only IS-RNA or tissue RNA. The other 4 lanes contain a mixture of tissue and IS-RNA.

Figure 4. Quantitative analysis of Kv1.2 and Kv1.5 gene expression. Expression levels are represented as pg of Kv1.2 and Kv1.5 mRNA/µg total RNA in each tissue. Values for Kv1.2 were significantly greater in all SHR arteries and heart compared with WKY, but differences in Kv1.5 were not significantly different between strains (except for heart). Columns represent average results, and vertical bars represent ± SEM (n=10 for both WKY and SHR). Asterisks represent significant differences (P<0.05) between SHR and WKY.

Electrophysiology

Myocytes were freshly dispersed from mesenteric artery as previously described.28 Membrane currents were recorded using the whole-cell patch-clamp configuration29 at room temperature (~23°C). Series resistance and capacitance compensation were adjusted maximally, and currents were recorded using a patch-clamp amplifier with a 100 Mohm/ohm head stage (Dagan, model 8900). Experimental protocols were controlled using a computer (Dell 466/L) and PCLAMP software (version 5.5.1, Axon Instruments). Current signals were converted from analog to digital form at a sampling rate of 10 kHz using a Labmaster A/D board (Axon Instruments) and stored in the computer for analysis. Experimental current records were analyzed using PCLAMP software.

The external solution for patch-clamp studies was the same as the isolation solution with the addition of 0.2 mmol/L Ca2+ and 1.8 mmol/L Mg2+. The pipette solution had the following composition (mmol/L): 120 KCl, 5 NaCl, 5 MgATP, 10 HEPES, and 10 BAPTA at pH 7.2 (titrated with KOH) and had an osmolality of 306±3 mOsm/L.

Chemicals

Collagenase was purchased from Worthington Biochemical (CLS3), and elastase was purchased from ICN Pharmaceuticals (porcine pancreas, Cleveland, Ohio). All other chemicals were obtained from Sigma Chemical Co.

Results

Kv1.X Genes Are Differentially Expressed in Vascular Tissues

The expression of genes encoding Kv1.X α- and β-subunits was studied in several different arterial tissues by RT-PCR. Southern blot analysis of products amplified from total RNAs isolated from the aorta, mesenteric artery, and tail artery demonstrated that these genes are differentially expressed in these vascular tissues (Figure 1). The products amplified from the same sequences in total heart RNA and commercial
preparations of rat genomic DNA and rat brain poly A⁺ RNA are also included for comparison as external standards. Multiple Kv1.X transcripts were detected in each tissue and, qualitatively, the levels of Kv1.2 and Kv1.5 appeared highest relative to either the levels in rat brain mRNA or the level of the product that could be amplified from rat genomic DNA. Significant but smaller relative amounts of Kv1.1, Kv1.3, and Kvβ1.1 were present in all arteries, and Kv1.3 and Kv1.4 were also present in heart and in aorta. Neither Kv1.6 nor Kvβ2.1 was found in any of these tissues. Expression levels of the various Kv1.X genes in aorta and tail artery were similar whereas expression in mesenteric arteries was generally larger than the other 2 sites in both animal groups. Kv gene expression was, therefore, nonuniform among the different vessels, and the data demonstrate distinct but overlapping patterns of transcription of the various genes in these tissues.

A semiquantitative comparison of Kv subunit expression in tissues from SHR and WKY to that of rat brain showed selective differences between the 2 animal groups that were generally similar for all 4 tissues. Normalized values for the expression of Kv1.2, Kv1.3 (except heart and tail artery), and Kvβ1.1 (except heart) were larger in tissues from SHR compared with WKY. No differences in values of normalized expression of Kv1.1, Kv1.4, or Kv1.5 (except heart) were found between the 2 groups (Figure 2).

Quantitative Analysis of Transcription

With the determination that Kv1.2 and Kv1.5 were the dominant Shaker transcripts expressed in arterial tissues, a competitive RT-PCR approach was used to quantitatively compare their expressions (Figure 3). We found significant strain and tissue differences in the levels of expression of the Kv1.2 gene (Figure 4). The amount of Kv1.2 mRNA was 60% to 110% larger in all 4 tissue sources isolated from SHR compared with WKY. In both strains, the mesenteric artery expressed the highest levels of Kv1.2 whereas the tail artery, aorta, and heart contained smaller but similar amounts.

In the case of Kv1.5, there were significant tissue but not strain differences in the levels of expression of the Kv1.5 gene (Figure 4). Only in heart were there significant differences in Kv1.5 expression between WKY and SHR. The level of expression of the Kv1.5 gene showed significant differences between tissues with the following rank order for both WKY and SHR: mesenteric artery > tail artery > aorta > heart. Overall, levels of Kv1.2 and Kv1.5 gene expression were similar in mesenteric and tail artery, but Kv1.2 gene expression was larger in aorta and heart.

Electrophysiological Differences in Kv Current Recorded From Mesenteric Myocytes of SHR and WKY

To study the relationship between gene expression and Kv current density, measurements of whole-cell K⁺ current (I_K) were made in mesenteric arterial myocytes under conditions in which other K⁺ currents were blocked. To inhibit KCa and KATP currents, 100 nmol/L iberiotoxin plus 10 μmol/L glybenclamide were added to the perfusion solution and 10 mmol/L BAPTA plus 5 mmol/L MgATP were included in the pipette solution. Values of whole-cell I_K recorded under these conditions are summarized in Figure 5. Kᵢ current density recorded from a holding potential of −80 mV was significantly larger in SHR over the entire voltage range studied.

The voltage dependence of Kᵢ current activation and availability were also determined in mesenteric myocytes. Activation curves determined from tail currents were significantly shifted to more hyperpolarized potentials in SHR compared with WKY (Figure 6). These data were fit with a Boltzmann equation, and the differences in the voltage at half activation (V0.5) were statistically significant (WKY = −20 ± 4 mV [n = 21], SHR = −32 ± 3 mV [n = 18]; P < 0.01). Values for the slope constant (k) did not, however, differ (WKY = 12.2 ± 2.5 mV [n = 21], SHR = 11.3 ± 1.8 mV [n = 18]). Data from the availability curves were also fit with a Boltzmann equation but no significant differences were found in either V0.5 or k between the 2 groups.

Discussion

In this study we document, for the first time, differences in the expression of Kv1.X genes among systemic arteries of normotensive and hypertensive animals. A distinct but overlapping pattern of gene expression was observed in all cardiovascular tissues investigated. Multiple Kv1.X transcripts were detected in each of the tissues studied, but the level of expression of some of the transcripts differed between the arterial sites, resulting in a distinct, tissue-specific pattern of expression for this related family of Kv
Overall, the Kv1.2 and Kv1.5 genes were most highly expressed when normalized to expression levels in brain. Their relative abundance among the arterial sites varied in the following order: mesenteric artery > tail artery > aorta. Furthermore, selective differences between the normotensive WKY and hypertensive SHR strains were observed. Transcripts encoding Kvβ1.1 were present in SHR but not in WKY arteries, and levels of Kv1.2 and Kv1.3 mRNAs were greater in SHR compared with WKY animals at all arterial sites studied. No significant differences between the strains were found in Kv1.5, Kv1.1, or Kv1.4 expression whereas expression of Kv1.6 or Kvβ2.1 was not detected in any of these arteries from either strain. Kv1.5 expression was significantly higher in SHR heart.

These studies were performed in both qualitative and quantitative ways. In the first series of experiments, we sought to determine which Kv1.X transcripts were expressed in various systemic arteries and which of those were dominant by normalizing their expression to that observed in rat brain. For comparison of RT-PCR products from WKY and SHR, we wanted to use an invariant external source of RNA for normalization. In this way, we could allow for variations in the efficiency of synthesis and amplification through the sequence of RT-PCR reactions. Fulfilling the external requirement precluded the use of RNA from either WKY or SHR. Any quality source of genomic DNA-free RNA would have sufficed for this purpose. We selected 1 previously used in this laboratory and characterized it extensively to ensure that all members of the Kv1.X family were represented and that it was genomic DNA free.

To address absolute levels of expression of the dominant genes, a quantitative, competitive PCR method was used. In these studies, IS-RNAs, which differed from the wild-type RNAs only by the presence of a small tandem-sequence repeat, were included with the total tissue RNA in the RT-PCR reactions. The inclusion of these extremely similar internal standards allowed for amplification of the 2 RNA species in the same reaction, by the same primers, and with equivalent efficiency and provided a competitive substrate for amplification of the wild-type RNAs. Such experiments allowed quantitation of the levels of expression of the Kv genes and demonstrated that the 2 dominant transcripts in mesenteric artery, Kv1.2, and Kv1.5 were present at levels of ∼1 to 2 parts/10⁵ in total RNA in that tissue and at ∼2- to 5-fold lower levels in tail artery and aorta.

We were not able to extent the quantitative comparisons to Kv1.3 and Kvβ1.1 because the amount of total RNA isolated in each preparation amounted to ∼20 to 30 μg for tail and mesenteric arteries from both groups of animals. Because the first-strand synthesis reaction required the use of gene-specific primers for each combination of RNAs, we had to limit the scope of these comparisons to Kv1.2 and Kv1.5, which were the more abundant transcripts present.

We have focused on the expression of members of the Shaker Kv1 family in this study. In preliminary experiments, we also screened arterial RNA for the expression of members of the Kv2, Kv3, and Kv4 families. Primers designed from regions of homology among all members of each of these Kv families were used in RT-PCR reactions. While transcripts from all 3 of these families were found in rat brain, none were found in these arteries from both WKY and SHR.

The significance of the finding of multiple Kv1.X transcripts in arteries is not clear. In tissues such as the brain and heart, in which cells fulfill different functional roles, the presence of multiple transcripts would not be surprising. While arteries do have cell types other than smooth muscle cells, neither endothelial cells nor fibroblasts have been reported to express Kv channel transcripts. Either all smooth
muscle cells express multiple Kv1.X transcripts or there are populations of cells with different expression patterns. This remains to be determined.

We have assumed that the whole-cell current recorded under the conditions of these experiments is Kᵥ current. In preliminary studies, we found no inward rectifier K’ current in mesenteric myocytes. Iᵥ is not effected by 500 µmol/L Ba²⁺, and no inward current can be activated by hyperpolarizing voltage steps to −140 mV (data not shown). Kᵥa and KᵥATP channels were inhibited by the experimental conditions. Furthermore, in cells dialyzed with symmetrical [Cl⁻]Cl⁻ currents are small and show nearly linear current-voltage relations.⁷,²⁸ There are no Na⁺ currents in this tissue, and nonselective (NS)-cation currents are small and linear with voltage.²⁸ Both Cl⁻ and NS-cation currents appear as “linear leak” currents, which were subtracted from whole-cell current records, and never accounted for more than 5% of the current at +60 mV. Also, because of the symmetrical ionic conditions for their respective charge carriers, Ic1 and Ic2 would be zero at 0 mV at which whole-cell (ie, Kᵥ) currents were significantly larger in SHR.

Several members of the Shaker family, including Kv1.1, Kv1.2, and Kv1.5, have previously been reported to be expressed in smooth muscle from vascular,¹⁹,²³,³² gastrointestinal,²¹,²²,³³ and airway sources.³⁰ Yuan et al¹⁰ have reported decreased expression of the Kv1.5 gene (but not Kvβ1.1) in pulmonary arteries of human subjects with primary pulmonary hypertension. There are, however, no data in the literature comparing levels of expression of Kv1.X genes on a quantitative basis in arterial smooth muscle or differences associated with systemic hypertension. We report here, for the first time, a greater level of expression of some (Kv1.2, Kv1.3, and Kvβ1.1) but not all (Kv1.1, Kv1.4, and Kv1.5) members of this family of genes in arteries of SHR. Furthermore, we report a correlation of this expression with differences in Kᵥ current density and shifted activation gating. On the basis of the assumption that these differences in RNA levels accurately reflect differences in protein levels, it is plausible that the differences in expression of these genes in SHR tissues reported herein are responsible for the differences in Kᵥ currents. The larger Kᵥ current density could be the result of the larger Kv1.2 expression. It is likely that the differences in voltage dependence of activation are the result of the differences in Kv1.X expression. However, the exact manner in which the reported differences contribute is difficult to assess at this time. Factors, such as differences in subunit composition of the tetrameric Kᵥ channels or the association pattern with Kvβ subunits, may also be involved in these differences. Additional work will be required to test the assumption that the differences in RNA levels are reflected at the protein level and to elucidate the molecular mechanism underlying differences in current density in this hypertensive model.

The finding of a larger gene expression of some Kv1.X transcripts and larger Kᵥ currents in SHR compared with WKY was surprising in view of previous studies.⁷,¹¹,³⁴ Previous studies using native myocytes reported smaller Kᵥ currents in interlobar arteries from SHR and DOCA-salt hypertensives,¹¹ in SHR aorta,⁷ and in SHR mesenteric artery myocytes recorded with perforated patch methods.³⁴ In this study, however, we found larger Kᵥ current density in SHR myocytes when recorded under conditions designed to eliminate other K’ current components and in the presence of low Ca²⁺. There is a major methodological difference between the present study and previous ones, ie, the level of intracellular Ca²⁺ buffering.

Gelband and Hume have demonstrated that increases in intracellular Ca²⁺ can inhibit Kᵥ current in arterial myocytes.²⁸ We recently reported that influx through L-type Ca²⁺ channels can also inhibit Kᵥ currents in mesenteric arterial myocytes.²⁸ It is plausible that the smaller Kᵥ currents recorded from SHR myocytes in the absence of strong intracellular Ca²⁺ buffering in previous studies is the result of a greater inhibitory effect of intracellular Ca²⁺ on Kᵥ channels. This could be the result of a larger Ca²⁺ influx in SHR, a larger near-membrane [Ca²⁺]² in SHR myocytes,²⁶ and/or a greater sensitivity of Kᵥ channels to Ca²⁺ in SHR, perhaps as a result of the differential expression of Kv genes (this study).

This mechanism (Ca²⁺ inhibition of Kᵥ current) can explain a number of previously reported observations in hypertensive arterial smooth muscle (ASM). The larger Ca²⁺ current in SHR³⁷ would cause a larger inhibition of Kᵥ current, resulting in membrane depolarization.³⁵ The membrane depolarization produces a further increase in Ca²⁺ influx³⁸ and a secondary increase in Kᵩ current.¹³ This process continues until the increase in Kᵩ current compensates for the decrease in Kᵥ current and their sum balances the inward current. These changes result in an increased contribution of L-type Ca²⁺ channels to agonist-mediated responses in hypertensive ASM.¹,²,³⁹ This in turn increases the effectiveness of L-type Ca²⁺ blockers in decreasing vascular tone and blood pressure in hypertensives.⁴⁰ Kᵥ channels are an important determinant of resting membrane potential in ASM.⁴,¹⁶,¹⁷ The increased Kᵥ gene expression in SHR may be a compensatory response to normalize membrane potential and Ca²⁺ influx. The mechanisms responsible for the differential expression of Kv1.X genes in SHR arteries remain to be determined. From previous studies, it is clear that a number of the phenotypic characteristics of hypertensive ASM could be involved. For example, it has recently been demonstrated that membrane depolarization–induced increases in L-type Ca²⁺ influx activates gene expression in ASM.⁴¹ Indeed, it is well known that Ca²⁺ signaling in a variety of cell types is a versatile regulator of differential gene expression.³² Furthermore, membrane depolarization alone can alter Kᵥ gene expression independent of changes in intracellular Ca²⁺.³³ The involvement of these factors and others such as hormonal modulation⁴⁴ as well as the signaling pathways involved remain to be determined.

Acknowledgment

This study was supported by research grant HL-28476 from the United States Public Health Service.

References


34. Cox RH, Lozinskaya IM, Dietz JN. Differences in K⁺ current components in mesenteric artery myocytes from WKY and SHR. *Am J Hypertens*. In press.


Differential Expression of Voltage-Gated K⁺ Channel Genes in Arteries From Spontaneously Hypertensive and Wistar-Kyoto Rats
Robert H. Cox, Kimberly Folander and Richard Swanson

_Hypertension_. 2001;37:1315-1322
doi: 10.1161/01.HYP.37.5.1315

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/37/5/1315

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/