Diverse Expression of Delayed Rectifier K⁺ Channel Subtype Transcripts in Several Types of Smooth Muscles of the Rat

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Abstract

Diverse expression of voltage dependent K⁺ (Kv) channels was examined in smooth muscles (SMs); carotid artery (CA), mesenteric artery (MA), urinary bladder (UB), and vas deferens (VD) of the rat, using RT-PCR based analyses. Among eight Kv channel subtypes examined (Kv 1.1, Kv 1.2, Kv 1.5, Kv 1.6, Kv 2.1, Kv 2.2, Kv 3.1, and Kv 3.2), expression of three delayed rectifier Kv (K_D) channel (Kv 1.2, Kv 1.5, and Kv 2.1) transcripts was observed in these SMs. To determine precisely the expression levels of the transcripts encoding K_D subtypes, those of three K_D subtypes (Kv 1.2, Kv 1.5, and Kv 2.1) were determined by competitive PCR. In vascular SM tissues, CA and MA, Kv 1.2 and Kv 1.5 transcripts were expressed at relatively high levels, whereas in visceral SM tissues, UB and VD, Kv 2.1 transcripts were expressed at the relatively high levels. These results suggest that the diverse expression of K_D subtypes is, at least in part, responsible for differences in electrical excitability and also for the variation of the electrophysiological and pharmacological phenotypes as tonic and phasic SMs.

Key word: delayed rectifier K⁺ channel, Kv 1.2, Kv 1.5, Kv 2.1, mesenteric artery, carotid artery, urinary bladder, vas deferens, competitive PCR

Introduction

Differences in membrane excitability between vascular SMs, which are electrically quiescent and have tonic features in muscular contraction, and visceral SMs, which show clear excitation-contraction coupling and have phasic features of contraction, are one of the major issues to understand the diversity of SM functions. The difference in membrane excitability in SMs may mainly depend upon the density of voltage-dependent Ca²⁺ channels but also upon,

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at least in part, the types and density of K^+ channels. Several K^+ currents are observed in both types of SMs and belong to roughly two categories; ligand (Ca^{2+} , ATP, and pH) gated K^+ currents and voltage gated ones. The functions of voltage gated K^+ (K_v) channels are associated with the regulation of the resting membrane potential (Yuan, 1995; Archer *et al.*, 1998) and the control of action potential shape and frequency in SMs (Carl, 1995; Imaizumi *et al.*, 1996; Jagger *et al.*, 1998; Koh *et al.*, 1999).

Kinetically distinct two types of voltage gated K^+ currents, a fast-inactivating A-type and a slowly or not inactivating delayed rectifier-type were identified in SMs (Kuriyama *et al.*, 1995 & 1998). The delayed rectifier K^+ currents (I_{KD}) together with Ca^{2+} activated K^+ currents play major roles in the electrical activities in both types of SMs. I_{KD} in colonic SMs has been suggested to be resolved into at least three components based on characteristic voltage dependencies, kinetics, and pharmacology (Carl, 1995). Similar pharmacological separation of I_{KD} into two or more components has been shown also in tonic SMs (Muraki *et al.*, 1990; Volk *et al.*, 1991).

A large number of mammalian cDNAs that encode K_v channels have been cloned. K_v channels are homo- or hetero-tetramers formed by the assembly of α subunits and four major subfamilies have been identified: K_v 1, K_v 2, K_v 3, and K_v 4 (Chandy & Gutman, 1995). Recently, new families of the electrically silent γ subunits, K_v 5, K_v 6, K_v 8, and K_v 9, which associate with K_v 2 subfamily and modulate the biophysical properties, have been cloned (Zhao *et al.*, 1994; Post *et al.*, 1996; Salinas *et al.*, 1997; Patel *et al.*, 1997). In canine gastrointestinal SMs, three molecular components of I_{KD} have been identified; K_v 1.2, K_v 1.5, and K_v 2.2 (Hart *et al.*, 1993; Overturf *et al.*, 1994; Schmalz *et al.*, 1998; Epperson *et al.*, 1999). In rat pulmonary artery, both K_v 2.1 and K_v 1.5 contribute to the initiation of hypoxic pulmonary vasoconstriction and K_v 9.3 also contributes to I_{KD} activation at resting potential by associating with K_v 2.1 (Archer *et al.*, 1998; Patel *et al.*, 1997).

Thus, some molecular approaches gain insights into the complexity and the diversity of K_D subtypes in SMs. However, little is known about the difference in expression patterns of K_D channel components between vascular and visceral SMs. In this study, the expression patterns of three K_D subtype transcripts (K_v 1.2, K_v 1.5, and K_v 2.1) were examined in tonic vascular SMs (carotid artery and mesenteric artery) and phasic visceral SMs (urinary bladder and vas deferens) using competitive PCR. Molecular identification and diversity of K_D channel subtypes are fundamental to our understanding of the differences in membrane excitability and the regulation of tone in SMs.

Methods

RNA extraction, reverse transcription, and non-quantitative PCR

RNA extraction and reverse transcription (RT) from carotid artery (CA), mesenteric artery (MA), urinary bladder (UB), and vas deferens (VD) of the 9–10 week-old male Wistar rat were performed as reported previously (Ohya *et al.*, 1997). Scraping with spatula denuded endothelium and epithelium. Specific primers for the 8 known subtypes of K_D channel α subunits (K_v 1.1: Baumann *et al.*, 1988; K_v 1.2: Stuhmer *et al.*, 1989; K_v 1.5: Swanson *et al.*,

1990; Kv 1.6: Grupe *et al.*, 1990; Kv 2.1: Frech *et al.*, 1989; Kv 2.2: Hwang *et al.*, 1992; Kv 3.1 & Kv 3.2: Luneau *et al.*, 1991a & 1991b), and glyceralhyde-3-phosphate-dehydrogenase (GAPDH) of the rat were designed. Primer sequences, along the positions of the termini of each primer corresponding to numbering of the original published sequence, are indicated as follows:

- Kv 1.1 (+) 5'-CTACCCAAGGCAGGCGGACCA-3'
 (−) 5'-GCGCTGGTACTCCTTCTCGGGT-3'
 (69 to 453; 385 bp, GenBank accession number; X12589)
- Kv1.2 (+) 5'-GATGAGCGAGATTCCCAGTT-3'
 (−) 5'-CTGTTGTTTACTCCCCCTG-3'
 (1051 to 1400; 350 bp, X16003)
- Kv 1.5 (+) 5'-TTCGCAGAGGCAGACAATCAC-3'
 (−) 5'-AGCTACCCCTTCTGATACTGTC-3'
 (1327 to 1708; 382 bp, M37145)
- Kv 1.6 (+) 5'-TGGAAGCAAGGGGAGTGG-3'
 (−) 5'-CTGACCACTGCCACCACTCACA-3'
 (615 to 993; 379 bp, X17621)
- Kv 2.1 (+) 5'-GAAGCGCCAGGAGAAAGCCATCAAGC-3'
 (−) 5'-TTGGTGTTGAGGATGGGCTGGGACTG-3'
 (1257 to 1631; 375 bp, X16476)
- Kv 2.2 (+) 5'-GCCAAGAACAGCCAGAGAGG-3'
 (−) 5'-AAGCCCCAGCATCGAGGTTCA-3'
 (1679 to 2047; 369 bp, M77482)
- Kv 3.1 (+) 5'-GACGTATCGCCAGCACCGC-3'
 (−) 5'-GGGTCCTCGAACAGTGCCC-3'
 (322 to 549; 228 bp, M68880)
- Kv 3.2 (+) 5'-CTCAACTACTACCGCACG-3'
 (−) 5'-TGCCCAGTCTCTTGCCC-3'
 (343 to 574; 232 bp, M59211)
- GAPDH (+) 5'-GCCAAAAGGGTCATCATCTCCG-3'
 (−) 5'-ACATTGGGGGTAGGAACACGGA-3'
 (341 to 713; 373 bp, X02231).

PCR amplification for 35 cycles was carried out at a final volume of 10 μ l. One cycle of the thermal cycler program used for PCR amplification included a 0.5 min denaturation step at 94°C, a 0.5 min annealing step at 55°C, and a 0.5 min primer extension step at 72°C. Amplified products were separated on 1.5% agarose gels in Tris acetate/EDTA buffer, visualized with 1 mg/ml ethidium bromide, and documented on FAS 1000 (TOYOBO).

Quantitative comparison of K_v subtype transcripts by competitive PCR

cDNAs obtained from four tissues (CA, MA, UB, and VD) were subjected to quantitative PCR using the PCR MIMIC Construction kit (Clontech). PCR MIMICs were constructed for Kv 1.2, Kv 1.5, Kv 2.1, Kv 9.3, and GAPDH and serially diluted according to Clontech protocol.

The following sequences (MIMIC) were added to 5' site of each gene specific primer. The sequences for MIMIC and Kv 9.3-specific primers (Patel *et al.*, 1997) are indicated as follows :

MIMIC (+) 5'-CGCAAGTGAAATCTCCTCCG-3'

(-) 5'-ATTTGATTCTGGACCATGGC-3' (8 to 513, 506 bp)

Kv 9.3 (+): 5'-TCTTCCTCTCTGTGGGTATCTCC-3'

(-): 5'-GACTGGTGATGAAGGCGTGTACT-3'

(986 to 1363 ; 378 bp, AF029056)

Competitive PCR was carried out by titration of target cDNA with known amounts of PCR MIMIC constructs (10 or 2-fold serial dilution) using specific primers for Kv 1.2, Kv 1.5, Kv 2.1, and GAPDH as described above. The thermal cycle program used for PCR amplification : 5 sec denaturation step at 94°C, 10 sec annealing step at 60°C, 30 sec primer extension step at 72°C for 36 cycles in GeneAmp 2400 (Perkin Elmer) with AmpliTaq-Gold DNA polymerase (Perkin Elmer). Amplified products were separated by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. Resultant gels were imaged by a FluorImager 595 (Molecular Dynamics) and the digitized signals were quantified with ImageQuant software (Molecular Dynamics). The log of the band intensity ratio between the target cDNA and MIMIC was then plotted against the log of MIMIC molecules added to the PCR reaction. The data points excluding the values that fell out the linear portion of the curve were fitted with a line drawn by a linear regression analysis. The amount of target cDNA was calculated by determining the x-intercept for the point on the line where the ratio of the target to MIMIC equals one. Experiments were performed from six different animals with duplicate or triplicate and the concentration of the target cDNA was normalized to the expression level of GAPDH transcripts. To identify the specificity of each amplified product, it was separated on 1.0% agarose gels, ligated into *Sma* I site of pBluescript II SK(+) (Stratagene), and sequenced by the dideoxy sequencing methods with a DSQ-1000L sequencer (Shimadzu).

Statistics

Data are expressed as means $\pm$ SEM. Statistical significance between two and multi groups was examined by Student's unpaired *t* test and Scheffe's test after one-way ANOVA, respectively. A value of $p < 0.01$ was considered to be significant.

Results

Identification of delayed rectifier K^+ (K_D) channel subtypes

Based on the known sequences, the expression levels of eight K_D channel subtype : Kv 1.1, Kv 1.2, Kv 1.5, Kv 1.6, Kv 2.1, Kv 2.2, Kv 3.1, and Kv 3.2, were analyzed by non-quantitative RT-PCR in rat smooth muscles (SMs) (carotid artery, CA ; mesenteric artery, MA ; urinary bladder, UB ; vas deferens, VD). Amplified products were separated on 1.5% agarose gels and visualized by ethidium bromide staining. On the basis of the predicted sizes of the amplified products, significant expression of Kv 1.2, Kv 1.5, and Kv 2.1 transcripts was detected in all tissues examined and other subtypes were expressed at low levels or not detected (Fig. 1). Amplified products of GAPDH were observed in all tissues at almost identical concentrations

Fig. 1. Ethidium bromide stained gels showing the expression of eight delayed rectifier K⁺ channel subtypes: Kv 1.1, Kv 1.2, Kv 1.5, Kv 1.6, Kv 2.1, Kv 2.2, Kv 3.1, and Kv 3.2 in smooth muscles. Carotid artery, CA; mesenteric artery, MA; urinary bladder, UB; vas deferens, VD. As a positive control, amplified products of all subtypes were obtained from brain (Br).

(not shown). As a positive control, the same experiment was carried out using rat brain and all products were obtained (Fig. 1, top panel; Br). As a negative control to identify genomic contamination, RNA from each sample was used as a template in the PCR. Also, to show that PCR reactants were free from contamination, two other control reactions were performed; one contained only primers and no template DNA, and the other contained only template DNA and no primer. PCR products were not obtained in these negative control experiments (not shown). These results suggest that molecular candidates encoding K⁺ channel subtypes responsible for components of endogenous I_{KD} in these SMs are Kv 1.2, Kv 1.5, and Kv 2.1 but not Kv 1.1, Kv 1.6, Kv 2.2, Kv 3.1, and Kv 3.2.

Diverse expression of K_D subtype transcripts

To determine precisely the expression levels of transcripts encoding three K_D subtypes (Kv 1.2, Kv 1.5, and Kv 2.1) in vascular and visceral SMs, a quantitative method based on competitive PCR (cPCR) was performed. First, a constant amount of target cDNA was titrated against ten-fold serial dilution of competitor (not shown). Based on the results, a fine-tuned two-fold serial dilution for the quantitative PCR was prepared. The point where the equal amount of product was formed was calculated as shown in *Methods*.

Representative gels used for digital analyses were shown in Figure 2A (Kv 1.2) and Figure 3A (Kv 1.5), respectively. The digitized signals of the specific amplification for Kv 1.2 and Kv

Fig. 2. Expression levels of Kv 1.2 transcripts in CA, MA, UB, and VD determined by competitive PCR. A: Gel electrophoresis of competitor (comp.) and target PCR products (target) for Kv 1.2 in CA, MA, UB, and VD. Competitive PCR for 36 cycles was carried out as described in Methods. Amplified products were resolved on 1.5% agarose gel and visualized by ethidium bromide for Kv 1.2 (350 bp; target), Kv 1.2 MIMIC (546 bp; competitor). Each PCR reaction solution included 2-fold serial dilutions (left to right) of competitor. In lane 1 (*) of each panel, the amount of competitor in a PCR reaction solution is 5×10^{-1} attomole in CA and MA and 10^{-1} attomole in UB and VD, respectively. B: After correcting for size differences between the target cDNA and the competitor, the concentration of target transcript was calculated and expressed as % of GAPDH transcript concentration. Columns and bars indicate mean $\pm$ SE, respectively. Experiments were performed using six separate animals ($n=6$) with duplicate or triplicate for each ($p < 0.01$, UB vs. CA, UB vs. MA, VD vs. CA, VD vs. MA).

1.5 were compared with those of the competitors for them, respectively (see *Methods*). Similarly, amplified products of GAPDH were identified in all tissues examined by cPCR. GAPDH transcripts were expressed at an essentially constant concentration of approximately 2 attomole/ μ g of total RNA; 2.08 ± 0.18 , 2.20 ± 0.09 , 2.58 ± 0.12 , and 2.43 ± 0.08 attomole/ μ g in CA, MA, UB, and VD, respectively ($n=6$ for each; $p > 0.05$ between four groups). After correcting for the size differences between the target cDNA and the competitor, the concentration of the target cDNA was calculated and expressed as % of GAPDH transcript. As a consequence, it was found that the expression pattern of Kv 1.2 and Kv 1.5 in vascular SMs was

Fig. 3. Expression levels of Kv1.5 transcripts in CA, MA, UB, and VD determined by competitive PCR. A: Gel electrophoresis of competitor (comp.) and target PCR products (target) for Kv 1.5 in CA, MA, UB, and VD. Competitive PCR for 36 cycles was carried out as described in Methods. Amplified products were resolved on 1.5% agarose gel and visualized by ethidium bromide for Kv 1.5 (382 bp; target), Kv 1.5 MIMIC (549 bp; competitor). Each PCR reaction solution included 2-fold serial dilutions (left to right) of competitor. In lane 1 (*) of each panel, the amount of competitor in a PCR reaction solution is 5×10^{-1} attomole (CA, MA, and VD) and 5×10^{-2} attomole (UB), respectively. B: After correcting for size differences between the target cDNA and the competitor, the concentration of target transcript was calculated and expressed as % of GAPDH transcript concentration. Columns and bars indicate mean $\pm$ SE, respectively. Experiments were performed using six separate animals ($n=6$) with duplicate or triplicate for each ($p < 0.01$, UB vs. CA, UB vs. MA, VD vs. CA, VD vs. MA).

strikingly different from those in visceral SMs. Both subtype transcripts were highly expressed in vascular SMs (CA and MA), as compared with those in visceral SMs (UB and VD) (Fig. 2A, 3A). The expression levels of Kv 1.2 transcripts versus that of GAPDH were 2.59 ± 0.25 , 3.09 ± 0.54 , 0.15 ± 0.02 , and $0.25 \pm 0.02\%$ and those of Kv 1.5 transcripts were 4.52 ± 0.46 , 4.08 ± 0.24 , 0.15 ± 0.03 , and $1.52 \pm 0.11\%$ in CA, MA, UB, and VD, respectively ($n=6$ for each, $p < 0.01$, UB vs. CA, UB vs. MA, VD vs. CA, VD vs. MA in both Fig. 2B and 3B).

In addition, the digitized signals of the specific amplification for Kv 2.1 were compared with those of the competitors for Kv 2.1. A representative gel used in digital analysis was shown

Fig. 4. Expression levels of Kv 2.1 transcripts in CA, MA, UB, and VD determined by competitive PCR. A: Gel electrophoresis of competitor (comp.) and target PCR products (target) for Kv 2.1 in CA, MA, UB, and VD. Competitive PCR for 36 cycles was carried out as described in Methods. Amplified products were resolved on 1.5% agarose gel and visualized by ethidium bromide for Kv 2.1 (375 bp; target), Kv 2.1 MIMIC (558 bp; competitor). Each PCR reaction solution included 2-fold serial dilutions (left to right) of competitor. In lane 1 (*) of each panel, the amount of competitor in a PCR reaction solution is 5×10^{-1} attomole (CA) and 1 attomole (MA, UB, and VD), respectively. B: After correcting for size differences between the target cDNA and the competitor, the concentration of target transcript was calculated and expressed as % of GAPDH transcript concentration. Columns and bars indicate mean $\pm$ SE, respectively. Experiments were performed using six separate animals ($n=6$) with duplicate or triplicate for each ($p < 0.01$, UB vs. CA, MA, and VD, respectively).

in Figure 4A. Expression of Kv 2.1 transcripts was relatively abundant in all tissues examined, especially in UB (Fig. 4B). The expression levels of Kv 2.1 transcripts were 4.11 ± 0.46 , 2.27 ± 0.31 , 7.15 ± 0.36 , and $2.88 \pm 0.18\%$ of GAPDH in CA, MA, UB, and VD, respectively ($n=6$ for each, $p < 0.01$, UB vs. CA, MA, and VD, respectively). On the other hand, the expression levels of Kv 2.2 transcripts, which expressed at relatively low levels in non-quantitative RT-PCR analysis, were under 0.6% of GAPDH in all tissues examined (not shown).

Data from Fig. 2B, Fig. 3B and Fig. 4B were summarized and the expression levels of Kv 1.2, Kv 1.5, and Kv 2.1 transcripts were illustrated as percentages versus the total of them in

Fig. 5. Comparison of expression levels of K_v subtype transcripts in four types of smooth muscles. A: carotid artery (CA); B: mesenteric artery (MA); C: urinary bladder (UB); D: vas deferens (VD). The expression levels of Kv 1.2, Kv 1.5, and Kv 2.1 transcripts were expressed as percentages versus the total of them in each smooth muscle.

each tissue (Fig. 5). In CA and MA, Kv 1.2, Kv 1.5, and Kv 2.1 transcripts were expressed at relatively high levels. The mRNA levels of Kv 1.2, Kv 1.5, and Kv 2.1 transcripts were 23, 40, and 37% in CA and 33, 43, and 24% in MA, respectively. On the other hand, in UB and VD, Kv 2.1 transcript was the major one among three. The mRNA levels of Kv 1.2, Kv 1.5, and Kv 2.1 were 2, 2, and 96% in UB and 5, 33, and 62% in VD, respectively.

In conventional RT-PCR experiments, among the electrically silent γ subunits in mammals which per se do not form K⁺ channels but associated with Kv2 subfamily, the significant expression of Kv 9.3 transcripts was detected in all tissues examined and other subtypes (Kv 5.1 and Kv 6.1) were expressed at very low levels (not shown). Subsequently, in cPCR experiments, Kv 9.3 transcript was extensively expressed in MA but also moderately observed in CA, UB, and VD; the expression levels were 2.54 ± 0.26 , 6.78 ± 0.88 , 1.30 ± 0.07 , and $2.28 \pm 0.44\%$ of GAPDH in CA, MA, UB, and VD, respectively ($n=6$ for each; $p < 0.01$ MA vs. CA, UB, and VD, respectively) (Fig. 6).

Discussion

The present study clearly shows a large difference in the expression patterns of Kv channel subtype transcripts between tonic vascular SMs (CA and MA) and phasic visceral SMs (UB and VD).

Fig. 6. Expression levels of Kv 9.3 transcripts in CA, MA, UB, and VD determined by competitive PCR. A: Gel electrophoresis of competitor (comp.) and target PCR products (target) for Kv 9.3 in CA, MA, UB, and VD. Competitive PCR for 36 cycles was carried out as described in Methods. Amplified products were resolved on 1.5% agarose gel and visualized by ethidium bromide for Kv 9.3 (378 bp; target), Kv 9.3 MIMIC (543 bp; competitor). Each PCR reaction solution included 2-fold serial dilutions (left to right) of competitor. In lane 1 (*) of each panel, the amount of competitor in a PCR reaction solution is 5×10^{-1} attomole. B: After correcting for size differences between the target cDNA and the competitor, the concentration of target transcript was calculated and expressed as % of GAPDH transcript concentration. Columns and bars indicate mean $\pm$ SE, respectively. Experiments were performed using six separate animals ($n=6$) with duplicate or triplicate for each ($p < 0.01$, MA vs. CA, UB, and VD, respectively).

Most of previous studies dealing with I_{K_D} in SMs focused on isolating and characterizing K_D channels/currents in native SM cells using electrophysiological techniques coupled with pharmacological manipulations (Kuriyama *et al.*, 1998). The present study extended the study to identify molecular distributions of K_D subtypes using competitive PCR (cPCR). cPCR method is sensitive and capable of detecting and quantifying transcript levels less than attomolar concentration. It is therefore considered as the most rigorous method for quantitation of mRNA levels (Wang *et al.*, 1989; Jung *et al.*, 1998). The data from the quantitative cPCR analyses of Kv 1 and Kv 2 support the following conclusion. The vascular SMs (CA and

MA) are rich sources of Kv 1 and Kv 2 transcripts (Kv 1.2, Kv 1.5, and Kv 2.1), whereas the visceral SMs (UB and VD) are rich sources of Kv 2 transcripts (Kv 2.1). Especially, in UB, Kv 2.1 was expressed almost homogeneously, suggesting that only Kv 2.1 could be responsible for I_{KD}.

Although there are very few systematical and comparative studies of I_{KD} in rat SMs, the pharmacological separation of I_{KD} components in SMs has been performed in the other species. I_{KD}s in electrically quiescent SMs such as rabbit pulmonary artery (Okabe *et al.*, 1987), canine trachea (Muraki *et al.*, 1990), rabbit coronary artery (Volk *et al.*, 1991) are highly sensitive to 4-AP, whereas those in SMs with relatively high excitability; rabbit ileum (Ohya *et al.*, 1986), guinea pig gastric antrum (Noack *et al.*, 1992) are less sensitive. In SMs with relatively high excitability, large conductance Ca²⁺ activated K⁺ channels mainly contribute to K⁺ conductance, whereas, in electrically quiescent SMs such as coronary artery and trachea, 4-AP sensitive K⁺ channels contribute to K⁺ conductance to a larger extent. Moreover, three components of I_{KD} were classified in canine colon (Carl, 1995): (1) a rapidly activating, 4-AP sensitive and TEA insensitive current, I_{KD(f)}; (2) a more slowly activating 4-AP insensitive and TEA sensitive current, I_{KD(s)}; and (3) a low inactivation threshold current, I_{KD(n)}, which is also less sensitive to TEA than I_{KD(s)} and activates more rapidly than I_{KD(s)}. Based on molecular pharmacological studies, followings have been shown; Kv 1 subfamily is sensitive to 4-AP but resistant to TEA, whereas Kv 2 subfamily is insensitive to 4-AP but relatively sensitive to TEA and the currents mediated by Kv 2 are activated more slowly than Kv 1 (Hart *et al.*, 1993; Grissmer *et al.*, 1994; Ikeda & Korn, 1995; Schmalz *et al.*, 1998; Epperson *et al.*, 1999). Therefore, Kv 1.2 and Kv 1.5 may contribute to I_{KD(f)}, whereas Kv 2.1 may contribute to I_{KD(s)} in rat SMs. Taken together, I_{KD} in the vascular SMs could be composed of both I_{KD(f)} and I_{KD(s)}, whereas that in the visceral SMs could be mainly composed of I_{KD(s)}. The characteristics of endogenous I_{KD} components reported so far, however, have varied widely and appear to be more complicated.

Alternatively spliced variants of K_D channel subtypes may possibly be the cause of the complexity of endogenous I_{KD} in SMs. Currently, the several splice variants of Kv 1 and Kv 4 have been isolated in mammalian (Luneau *et al.*, 1991a; Kim *et al.*, 1997; Ohya *et al.*, 1997). In the present study, the partial and/or complete sequences of Kv 1.1, Kv 1.2, Kv 1.4, Kv 1.5, Kv 1.6, Kv 2.1, and Kv 2.2 were determined in MA and/or VD. No alternatively spliced variants for all subtypes are, however, found in all tissues examined (unpublished data). A further source of increased complexity in pharmacological and biophysical properties is the potential for different K⁺ channel subunits to form heteromultimers that have properties distinct from those of the parent homomultimers (Ruppersberg *et al.*, 1990; Russell *et al.*, 1994). In the present study, two members of Kv 1 subfamily (Kv 1.2 and Kv 1.5) are expressed in the vascular SMs. Whether heteromultimers are formed *in vivo* or not, is remained to be determined.

The contribution of regulatory subunits to I_{KD}s should also be considered as the cause of endogenous I_{KD} complexity. Kv 1 family β subunits (Kv β 1) have been reported as the modulator to fasten or induce the inactivation of the currents via Kv 1 α subunit. The combination of α and β subunits of Kv 1 results in A-type like K⁺ currents and is apparently resolved from I_{KD}s in SMs. Preliminary study showed that Kv β 1.1 and Kv β 1.3 transcripts

were not highly expressed in the SMs examined (Ohya, unpublished observation). Recent evidence indicates the existence of regulatory subunits that interact with members of the Kv 2: Kv 5-Kv 9 (Zhao *et al.*, 1994; Post *et al.*, 1996; Salinas *et al.*, 1997; Patel *et al.*, 1997). Patel *et al.* have shown that the activity of Kv 2.1/Kv 9.3 heteromultimer may play an important role in pulmonary artery (PA) vasoconstriction and in the development of PA hypertension. Of interest is that, in not only vascular SMs (CA and MA) but also visceral SMs (UB and VD), Kv 9.3 transcripts were substantially expressed (Fig. 6). Therefore, in both types of SMs, Kv 9.3 may contribute to I_{KD} activation at resting potential by associating with Kv 2.1. Moreover, several proteins including multiple PDZ domains, which can sort the ion channel proteins into subcellular compartments and induce channel clustering, have been cloned (Kim *et al.*, 1996; Topinka & Brecht, 1998). The increasing number of modulatory components for functional K_D subtypes may provide amazingly novel capacities of functional diversity. Further work will be required to distinguish between these possibilities.

In conclusion, the determination of the expression patterns of Kv channel subtypes responsible for I_{KD} in CA, MA, UB and VD is of great value in elucidating the physiological roles of specific K^+ channels, and giving insights into the functional heterogeneity of the membrane excitability of SMs. The present study gives further evidence supporting that high expression of Kv 1.2 and Kv 1.5 encoding rapidly activating I_{KD} components susceptible to 4-AP may be essential for keeping electrical quiescence in CA and MA as in other elastic arteries or tonic SMs (Hara *et al.*, 1980; Doi *et al.*, 2000). In addition, Kv 2.1 may be responsible for the major component of I_{KD} in visceral/phasic SMs.

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