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Analysis of Voltage-Gated Potassium Channel $\beta 1$ Subunits in the Porcine Neonatal Ductus Arteriosus

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ABSTRACT: The voltage-gated potassium channels (Kv) are partially responsible for the contraction/relaxation of blood vessels in response to changes in the P_{O_2} level. The present study determined the expression of Kv $\beta 1$ and four oxygen-sensitive Kv α subunits (Kv1.2, Kv1.5, Kv2.1, and Kv9.3) in the ductus arteriosus (DA), the aorta (Ao), and the pulmonary artery (PA) in porcine neonates immediately after birth. We cloned three Kv $\beta 1$ transcript variants (Kv $\beta 1.2$, Kv $\beta 1.3$, and Kv $\beta 1.4$), Kv1.2, Kv1.5, and Kv9.3 from piglets. Three Kv $\beta 1$ transcripts, Kv1.2, Kv1.5, and Kv9.3, encode predicted proteins of 401, 408, 202, 499, 600, and 491 residues. These Kv showed a high degree of sequence conservation with the corresponding Kv in human. Northern and quantitative real-time PCR (qr-PCR) analyses showed that Kv $\beta 1.2$ expression was high in the DA and Ao but low in the PA. Kv1.5 expression was high in the Ao and PA but low in the DA. Expression of Kv $\beta 1.3$, Kv $\beta 1.4$, Kv1.2, Kv2.1, and Kv9.3 was low in these blood vessels. The inactivation property of Kv $\beta 1.2$ against Kv1.5 was confirmed using *Xenopus laevis* oocytes. Our findings suggest that the molecular basis for the differential electrophysiological characteristics including opposing response to oxygen in the DA and the PA are partially due to diversity in expression of Kv1.5 and Kv $\beta 1.2$ subunits. The high expression of Kv $\beta 1.2$ and relatively low expression of Kv1.5 in the DA might be partially responsible for the ductal closure after birth. (*Pediatr Res* 59: 167–174, 2006)

The DA plays a pivotal role in fetal development by diverting the passage of blood from the PA into the Ao (1). At birth, functional closure of the DA is initiated by an increase in P_{O_2} (2–4). Previously, we reported that oxygen increases the intracellular calcium concentration and causes contraction of the DA (5). In that report, closure of the

ATP-sensitive potassium channel was suggested to be involved in the DA contraction due to depolarization of the membrane potential, which, in turn, increases calcium influx via the voltage-dependent calcium channel. However, mechanisms other than the ATP-sensitive potassium channel may also be involved in the oxygen-induced contraction and these remain to be studied.

Although the mechanism has not been elucidated, Kv channels in SMC may also be involved in oxygen-induced vascular constriction (6). The oxygen sensitivity of Kv has been studied extensively, and Kv1.2, Kv1.5, Kv2.1, Kv3.1b, Kv4.2, Kv4.3, and Kv9.3 are major candidates for the oxygen-sensitive potassium channel (7–13). Among them, it was reported that SMC in the DA expresses Kv1.5 and Kv2.1 (14).

Functional Kv are composed of a tetramer of Kv α subunits (15), which co-assemble with Kv β subunits (16–21). The Kv β subunits have variants known as Kv $\beta 1.1$, Kv $\beta 1.2$, and Kv $\beta 1.3$ (22,23), Kv $\beta 2.1$ and Kv $\beta 2.2$ (23), Kv $\beta 3$ (24), and Kv $\beta 4.1$ (25), which show distinct modulatory effects on the Kv α subunits (16,18). Kv $\beta 1.2$ is a potent blocker for Kv1.1, Kv1.2, Kv1.4, and Kv1.5 (20) and confers the ability to respond to hypoxia to Kv4.2 (12).

The interaction of the Kv $\beta 1$ subunits with α subunits is consistent with the $\alpha_4\beta_n$ model, where n equals 0, 1, 2, 3, or 4, depending upon the relative concentration of α and β subunits. The $\alpha_4\beta_n$ stoichiometry allows for gradual changes in Kv $\beta 1$ -mediated inactivation (19).

Thus, oxygen-sensitive Kv α subunits are possibly regulated by Kv β subunits based on the relative expression levels. Therefore, we hypothesized that diversity in the expression of oxygen-sensitive Kv α or Kv β subunits in the DA as well as in the contiguous Ao and PA might be responsible for various responses to change in P_{O_2} at birth.

Abbreviations: Ao, aorta; b-PA, third and fourth branched pulmonary artery; DA, ductus arteriosus; EC, endothelial cells; Kv, voltage-gated potassium channel; PA, pulmonary artery; SkM, skeletal muscle; SMC, smooth muscle cells

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METHODS

Animals and tissue samples. We chose the pig model for our studies to obtain enough starting blood vessel specimens. Various tissues were taken from newborn piglets of the Landrace/Large White/Duroc composite killed with chloroform immediately after birth. The adventitia and the endothelial layer of the DA, Ao, and PA were removed in saline. All tissues were quickly frozen and stored at -135°C . Treatment of animals conformed to the guiding principles of the American Physiologic Society. The experiment was approved by the Ethical Committee of Animal Experiments of Tokyo Women's Medical University.

RNA isolation. Total RNA of DA, Ao, and PA were isolated from 14, 2, or 3 pooled segments, respectively. We followed the RNA extraction methods described by Chomczynski and Sacchi (26). To reduce the level of contamination with proteoglycans and polysaccharides, RNA extraction solution (27) was used to precipitate the RNA.

Cloning. A strategy combining a homology-based PCR and rapid amplification of cDNA ends (RACE) was used to identify Kv β 1, Kv1.2, and Kv1.5 cDNA. Total RNA extracted from the DA, brain and SkM were reverse-transcribed into cDNA using PowerScript Reverse Transcriptase (BD Biosciences Clontech, Palo Alto, CA). Based on the conserved regions of the human, mouse, and rat Kv β cDNA, we designed primers to amplify partial Kv β cDNA and to carry out RACE (Table 1). RACE was performed using a SMARTRACE cDNA amplification kit (BD Biosciences Clontech). Obtained cDNA were cloned into pT7Blue T-Vector (Novagen, Darmstadt, Germany), and sequenced with a BigDye terminator sequencing kit (Applied Biosystems, Foster City, CA) and a 3100 genetic analyzer (Applied Biosystems). Sequences were analyzed with SeqMan II (DNASTAR, Madison, WI) and GENETYX-MAC (Software Development, Tokyo, Japan).

A porcine lambda cDNA library, constructed from cultured smooth muscle thoracic aorta (Stratagene, La Jolla, CA), was screened using a probe prepared from rat pCIneo-Kv9.3 labeled with horseradish peroxidase using an enhanced chemiluminescence (ECL) direct nucleic-acid labeling system (Amersham Pharmacia Biotech UK, Ltd., Little Chalfont, Buckinghamshire, UK). Positive phages were located with ECL detection reagents (Amersham Pharmacia Biotech UK, Ltd.).

Porcine genomic DNA was extracted from the kidney with a DNeasy Tissue kit (QIAGEN, Hilden, Germany), and used as a template to obtain the PCR products containing the 5'-end region of the Kv β 1.4 cDNA, using a DNA Walking SpeedUp Premix kit (Seegene, Seoul, Korea) with three target-specific primers.

Northern blot analysis. Northern blot analysis was performed as described previously with modification (28). Isolated total RNA (20 $\mu\text{g}/\text{lane}$) was electrophoresed on denaturing 1.0% agarose-formaldehyde gels and transferred to nitrocellulose membranes (NitroPure, GE Osmonics, Trevose, PA). cDNA probes were generated using either restriction enzyme digestion or PCR followed by gel-purification. A Kv β 1 common probe (1050 bp), corresponding to almost all the outermost half of the 3'-untranslated region (UTR), was prepared by digestion of the Kv β 1 3'-RACE clone with *Hind*III (in the 3'-UTR) and *Sal*I (in the multiple cloning site). A Kv β 1.2-specific probe (170 bp), corresponding to nucleotides 67–236 of the porcine Kv β 1.2, and a Kv β 1.3-specific probe (1094 bp), corresponding to nucleotides –875 to 219 of the porcine Kv β 1.3, were generated using PCR. Kv1.2, Kv1.5, and Kv9.3 probes (710, 804, and 1476 bp), corresponding to nucleotides 1352–2061 of the porcine Kv1.2, nucleotides 1737–2541 of the porcine Kv1.5, and nucleotides 1–1476 of the porcine Kv9.3, were generated using PCR. A glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe (682 bp), corresponding to nucleotides 549–1230 of the porcine GAPDH (GenBank accession number AF017079), was also generated using PCR. The cDNA probes were labeled using a Nick translation kit (Roche Diagnostics, Mannheim, Germany) with [α - ^{32}P]dCTP. Hybridization was performed overnight at 65°C . The blot was washed with $1\times$ SSC and 0.1% SDS and visualized with an image analyzer BAS 2000 (Fuji Photo Film, Tokyo, Japan).

qr-PCR. DNase I digestion of total RNA was carried out on an RNeasy Mini Kit (QIAGEN) extraction column using an RNase-Free DNase Set (QIAGEN). The total RNA was reverse-transcribed into cDNA with random hexamers and MultiScribe Reverse Transcriptase (Applied Biosystems). As a control for genomic DNA contamination, all cDNA synthesis reactions were set up with additional samples lacking reverse transcriptase.

We designed all primers and probes used for qr-PCR using Primer Express software (Applied Biosystems) (Table 2). The 25- μL qr-PCR reaction included $1\times$ Premix Ex TaqTM (TAKARA BIO, Shiga, Japan), 900 nM of the respective primers, 250 nM of the respective probe, $1\times$ ROX reference dye, and template cDNA corresponding to 5 ng total RNA. 18S ribosomal RNA (Applied Biosystems) was used as an internal control. For standard curve production, the plasmids harboring the corresponding Kv, EC-specific marker, SMC-specific marker, and ribosomal 18S cDNA fragments were linearized by appropriate restriction enzyme digestion, purified, quantified, and diluted serially in water containing yeast tRNA (50 ng/ μL). The second passage of primary culture of porcine aortic EC (Cell Applications, San Diego, CA) and the first passage of primary culture of porcine aortic SMC (Cell Applications) were used as positive controls to estimate potential

Table 1. Sequence of primers used in the clonings

Gene	Primer sequence	
cDNA cloning		
<i>Kcna2</i>	5'-RACE GSP-1	CACTTGCAAATACTGGGCCTGCTCCTC
(Kv1.2)	5'-RACE GSP-2	TGACAGGGACCGGTAAGGCAATTGGTTA
	5'-RACE GSP-3	AAAAAGTACTCGTTCGGAGGGGGTCAA
	5'-RACE GSP-4	AGATGTTGATAAACCACCTCTCGCAGCA
	3'-RACE GSP	CTGTCTATTTTCGAGAGGCCGATGAGC
<i>Kcna5</i>	5'-RACE GSP-1	TCCGGGATGCTGGAGAAGTGGGTCTCT
(Kv1.5)	5'-RACE GSP-2	GGCCTCGGCGAAGTAGACGGCGCTGGAGA
	5'-RACE GSP-3	CCACCAGCTCGGTGCCAGGGGTGATGAA
	5'-RACE GSP-4	TTGGGTCCATCTCCGAGCGCAGCCGTCG
	5'-RACE GSP-5	CCCGCCTCCCTCCATCCTTGACGATC
	3'-RACE GSP-1	AAGGGGCTGCAGATCTGGGCAAGAC
	3'-RACE GSP-2	GGCCTCCATGCGGGAGCTGGGCCTGCTC
<i>Kcnab1</i>	Conserved region-F	TGCTTGGNCTNGGNAACNTGGGTSAC
(Kv β 1)	Conserved region-R	ACNCCCTCRTNCKSAGRCACCA
	3'-RACE GSP-1	CGGCGTGCTGAAAGTTCCAGGGCTTCA
	5'-RACE GSP-1	TGCTCCCCAGAATCACTTCAGCCTTCC
<i>Kcnab1</i> , member 2		
(Kv β 1.2)		
<i>Kcnab1</i> , member 3	5'-RACE GSP-1	GCTCTCATAAGCAATTGTCATCAGCCGT
(Kv β 1.3)		
<i>Kcnab1</i> , member 4	5'-RACE GSP-1	TCCTTCTTGCTGTAGGGCTTGTTGGG
(Kv β 1.4)	5'-RACE GSP-2	CCTGAATGGCACCAGGTTTTCAATG
Genomic cloning		
<i>Kcnab1</i> , member 4	TSP-1	CTCCAACCTTTCTTCTTGATG
(Kv β 1.4)	TSP-2	GATGCTCCCCAGAATCACTT
	TSP-3	CCCCAGAATCACTTCAGC

GSP, gene-specific primer; TSP, target-specific primer.

Table 2. Sequences of primers and probes used in the real-time PCR experiment

Gene		Primer or probe sequence	Amplicon (bp)	Accession no.
<i>Kcna2</i> (Kv1.2)	Probe	CCCAGCATCCCGGATGCCTTC	69	DQ005441
	Forward	ATGAGCGCGAGTCCCAGTT		
	Reverse	CAGTTGTCATGGAGACGACTGC		
<i>Kcna5</i> (Kv1.5)	Probe	TGCGCTTCTTCGCCCTGTCCCA	76	DQ005442
	Forward	CACGTGTGTCGTCTGGTTCAC		
	Reverse	CCGGGAAAACCTCAGCCTTG		
<i>Kcnb1</i> (Kv2.1)	Probe	TCGTCCTCTCCACCATTGCCCTG	73	NM_214218
	Forward	TCCTCGCCATAATTTCCATCA		
	Reverse	AGCTCAGGCAGCGTGTGA		
<i>Kcns3</i> (Kv9.3)	Probe	CCCGGCACTCTGTGGGCCTTC	68	DQ005443
	Forward	ATGAGGATTTTCCGAATTCTCAAG		
	Reverse	AGTGTGGCCCCCAACGA		
<i>Kcnab1</i> , member 2 (Kv β 1.2)	Probe	CCAAGCCTCCGCCTCAGGCA	66	AY866528
	Forward	AAATGTAGACGGCACCTAGCAGTA		
	Reverse	GGGCCTAACAGGCCAACAG		
<i>Kcnab1</i> , member 3 (Kv β 1.3)	Probe	ATGTGGTGAACGCAGCCCGGG	84	AY866527
	Forward	TGAGGACCGACTTCTGAGTAAGC		
	Reverse	ATAGCGACCGTGCAGGAATT		
<i>Kcnab1</i> , member 4 (Kv β 1.4)	Probe	CAAGGAAGGGAATCTA-MGB*	78	AY866526
	Forward	TCGGGACATGTAGGCACTGA		
	Reverse	TGTCCTGGAAATGCAAGAGTCA		
<i>Icam-2</i> (Intercellular adhesion molecule-2)	Probe	CCACCACCACGGGCCACTGG	72	NM_001001631
	Forward	cagcttgcatgagcagaagtg		
	Reverse	GACCACAACGCTAAGGAACCA		
<i>Sm22a</i> (Smooth muscle protein 22-alpha)	Probe	tgcccagagaacccacctcca	72	AF053629
	Forward	TCCTGACGGCTCCAAACC		
	Reverse	acctgtccatttgctgaag		

* Minor Groove Binder.

contribution of EC. Real-time PCR of standards and internal controls was performed each time on the same 96-well plate with the samples being quantified.

To quantify target mRNA expression, cDNA copy numbers were calculated based on the results of the standard curve of the same run. The correlation coefficients were always above 0.98. Each sample was analyzed in triplicate, and arithmetic means were calculated. The cDNA copy numbers were then normalized using the calculated 18S cDNA copy number of the same sample.

cRNA synthesis, injection, and electrophysiological measurement in *Xenopus* oocytes. Capped cRNA were synthesized *in vitro* from the linearized plasmids harboring the coding sequences of Kv1.5 or Kv β 1.2 using T7 RNA polymerase (Promega, Madison, WI). Defolliculated *Xenopus laevis* oocytes were prepared, as described previously (29), and injected with 50 nL of cRNA. A single oocyte was impaled with two standard glass microelectrodes (1–2.5-megohm resistance) filled with 3 M KCl and maintained under a voltage clamp using a Gene Clamp 500B amplifier (Axon Instruments, Burlingame, CA). Stimulation of the preparation, data acquisition, and analysis were performed using pClamp software (Clampex 8.2 and Clampfit 8.2, Axon Instruments). All experiments were performed at room temperature in ND96 solutions containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES at pH 7.4.

RESULTS

Cloning. The structures of the three porcine Kv β 1 subunit nucleotide sequences were aligned with the human Kv β 1 subunit (Fig. 1A). Predicted amino acid sequences of porcine Kv β 1 variants are presented in Figure 1B. Two porcine Kv β 1 subunits cloned from DA and brain encoded the predicted proteins of 408 and 401 amino acids that share a high degree of sequence conservation with Kv β 1.2 (97.8%) and Kv β 1.3 (99.5%) in human, respectively. Over the C-terminal 328 amino-acids, they were identical and 99.4% identical to human Kv β 1. Kv β 1.2 and Kv β 1.3 differed in length, 80 and 73 amino-acids, respectively, and in the sequence of their N-

terminal regions. The N-terminal regions of porcine Kv β 1.2 and Kv β 1.3 shared an identity of 91.3% and 100% to the corresponding region of human Kv β 1.2 and Kv β 1.3.

The third porcine Kv β 1 variant cloned from SkM contained 116 distinct nucleotides at the 5'-end and 823 nucleotides at the opposite end of the conserved C-terminal cDNA, but lacked 164 nucleotides near to the 5'-end of C-terminal conserved cDNA and the entire cDNA encoding the variable N-terminal regions. No translational start codon was present in the 116 distinct nucleotides. Taking the first in-frame ATG as the start codon and the stop codon as being similar to other Kv β 1 subunits, an open reading frame of 609 bp encoding a protein of 202 amino-acids, that would be completely identical with the latter half of the C-terminal region of other Kv β 1 subunits, was predicted. The 116 distinct nucleotides at the 5'-end of this porcine Kv β 1 subunit were not similar to the N-terminal cDNA sequence of human Kv β 1.1. This previously unrecognized Kv β 1 subunit was designated Kv β 1.4. Genomic sequencing of the 5'-end region of Kv β 1.4 revealed that this transcript retained a highly homologous sequence with the 3'-end region of human Kv β 1 intron 4 (Fig. 2). (GenBank accession numbers: Kv β 1.2 AY866528; Kv β 1.3, AY866527; and Kv β 1.4, AY866526)

Porcine Kv1.2 (Fig. 3A), Kv1.5 (Fig. 3B), and Kv9.3 (Fig. 3C) subunits cloned from the brain, DA, and Ao encoded the predicted proteins of 499, 600, and 491 amino-acids that share a high degree of sequence conservation with the homologous proteins, Kv1.2 (99.6%), Kv1.5 (86.5%), and Kv9.3 (96.3%) in human, respectively (GenBank accession

Figure 1. Schematic presentation of nucleotide sequences (A) and predicted amino acid sequences (B) of porcine and human Kv β 1 transcripts. (A) Code regions are shown in thick lines and UTR regions in thinner lines. The closed triangle indicates a critical stop codon on a Kv β 1.4 nucleotide sequence. Due to this stop codon, the Kv β 1.4 transcript has a smaller code region than the other Kv β 1 transcripts. p: porcine, h: human (B) Kv β 1 amino acid sequences were deduced from the cloned Kv β 1.2, Kv β 1.3, and Kv β 1.4 nucleotide sequences, and aligned with amino acid sequences of human Kv β 1 transcript variants. Amino acid residues identical to the porcine Kv β 1.2 are shaded.

Figure 2. Comparison of Kv β 1.4 partial cDNA, genomic DNA, and human genomic Kv β 1 DNA. The 116 nucleotides at the 5'-end of the Kv β 1.4 cDNA and the near 3'-end of human intron 4 DNA sequences were aligned. The dashed box indicates a stop codon at the 5'-end of Kv β 1.4 cDNA. Open boxes denote nucleotides that are not conserved between porcine and human Kv β 1.

Figure 4. Northern blot analysis of Kv β 1 subunits using (A) Kv β 1 common, (B) Kv β 1.2-, and (C) Kv β 1.3-specific probes. (A) The 2.8 kb band was detected in the SkM. The 3.2 kb band was detected exclusively in the DA, Ao, UA, and UV, weakly in the b-PA and faintly in the PA and brain. The 3.8 kb band was detected mainly in the brain. Multiple transcripts of approximately 15 kb were detected in many of the samples. (B) The Kv β 1.2-specific probe detected the 3.2 kb transcript in the DA, Ao, b-PA, UA, and UV. (C) The Kv β 1.3-specific probe detected the 3.8 kb transcript in the brain. Approximately 15 kb transcripts were detected in the DA, Ao, b-PA, UA, and UV. To document the presence of intact RNA in each lane, the same membrane was rehybridized with a porcine GAPDH probe (middle). Equal loading was demonstrated by ethidium bromide staining of the 28S ribosomal RNA (bottom). UA, umbilical artery; UV, umbilical vein; LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium; IS, interventricular septum.

numbers: Kv1.2, DQ005441; Kv1.5, DQ005442; and kv9.3, DQ005443).

Northern blot analysis. The Kv β 1 common probe hybridized to multiple bands (Fig. 4A). The 3.2 kb transcript was expressed highly in the DA and Ao, and faintly in the PA. The 3.8 kb transcript was expressed very weakly in the DA and Ao. Expression of the 3.2 kb transcript in the DA and Ao was confirmed by a Kv β 1.2-specific probe (Fig. 4B). The Kv β 1.3-specific probe was not hybridized to any of the DA, Ao, and PA as the 3.8 kb band. However, approximately 15 kb transcripts were detected in the DA and Ao (Fig. 4C). Although

Figure 5. Northern blot analysis using (A) Kv1.2, (B) Kv1.5, and (C) Kv9.3 probes. (A) The Kv1.2 probe was hybridized to the approximately 10 kb transcripts in the brain, b-PA, and lung. (B) The Kv1.5 probe detected the 2.8 kb transcript abundantly in the Ao, PA, b-PA, LA, RA, and IS, but weakly in the DA, UA, UV, lung, LV, RV, SkM, and brain, and the 10 kb transcript in the b-PA and brain. (C) The Kv9.3 probe detected the 2.8 kb transcript in the b-PA, UA, and UV and weakly in the lung and brain. The same membrane was rehybridized with a porcine GAPDH probe (middle). Ethidium bromide staining of 28S ribosomal RNA is shown below. Abbreviations are as in Figure 4.

we tried to detect Kv β 1.4 using the Kv β 1.4-specific probe, we could not obtain any signal on the Northern blot (data not shown).

Expression of Kv1.2 was not detected in the DA, Ao, or PA (Fig. 5A). Expression of the 2.8 kb transcripts was high in the Ao and PA, but low in the DA using the Kv1.5 probe (Fig. 5B). Kv9.3 was not detected in the DA, Ao, or PA (Fig. 5C).

qr-PCR. Expression of the Kv β 1.2 transcript in the DA and Ao was high, and low in the PA (Fig. 6A). Kv β 1.3 was detected very faintly in the DA and Ao. Expression of Kv β 1.4 was not detected in the DA, Ao, or PA. Expression of Kv β 1.2 dominated the other Kv β 1s among the blood vessels examined.

Kv1.2 was not detected in the DA, Ao, or PA (Fig. 6B). The expression of Kv1.5 in the Ao and PA was high, but relatively low in the DA. Kv2.1 was detected faintly in the Ao and PA. Expression of Kv9.3 was low in the DA, Ao, and PA. Expression of Kv β 1.2 in the DA was higher than that of Kv1.5, and in the Ao and PA it was lower than that of Kv1.5 (Fig. 6C).

To estimate the potential contribution of EC in the DA, Ao, and PA samples, we used intracellular adhesion molecule 2 (ICAM-2) as EC-specific and SM22 α as SMC-specific

Figure 3. Predicted amino acid sequences of Kv1.2, Kv1.5, and Kv9.3. Porcine Kv1.2 (A), Kv1.5 (B), Kv9.3 (C) amino acid sequences were deduced from the cloned nucleotide sequences, and aligned with amino acid sequences of corresponding Kv1.2, Kv1.5, and Kv9.3 from human, mouse, rat, respectively. Amino acid residues identical to the corresponding porcine Kv are shaded. p, porcine; h, human; m, mouse; r, rat.

Figure 6. qPCR analysis of the expression of Kv β 1, Kv1.2, Kv1.5, Kv2.1, and Kv9.3 transcripts. (A) Expression of the Kv β 1.2 transcript was high in the DA, Ao, b-PA, UA, and UV and relatively low in the PA. Expression of Kv β 1.3 was low in the brain and very low in the DA, Ao, b-PA, UA, and UV. Expression of Kv β 1.4 was low in the SkM. Expression of Kv β 1.2 dominated the other Kv β 1 in the DA, Ao, b-PA, UA, and UV. (B) Expression of Kv1.2 was low in the brain and very low in the b-PA and lung. Expression of Kv1.5 was high in the Ao, PA, b-PA, LA, RA, and IS, and relatively low in the DA, UA, UV, Lung, LV, RV, SkM, brain, and kidney. Expression of Kv2.1 was low in the brain, and very low in the Ao, PA, b-PA, and SkM. Expression of Kv9.3 was high in the b-PA, UA, UV, lung, and brain, and low in the DA, Ao, LA, RA, SkM, liver, and kidney. (C) Comparison of the expression of Kv β 1.2 and Kv1.5 mRNA. Expression of Kv β 1.2 in the DA, UA, and UV was higher than that of Kv1.5. The DA, Ao, and PA express high level of SM22 α (D), but low level of ICAM-2 (E). Porcine aortic EC and SMC were used as positive controls. All mRNA levels were expressed as the copy number of the corresponding standard plasmid relative to that of the 18S standard plasmid.

marker. Expression of SM22 α was high in the DA, Ao, and PA (Fig. 6D), but that of ICAM-2 was low (Fig. 6E).

Electrophysiology. A current activated by depolarization from a holding potential of -60 mV was evoked in the oocytes injected with cRNA encoding Kv1.5 (Fig. 7A) and Kv1.5/Kv β 1.2 (Fig. 7B). The outward currents evoked by a range of depolarizations from a holding potential of -60 mV to test potentials ranged from -40 to $+60$ mV. A

Figure 7. K $^{+}$ currents elicited from oocytes injected with porcine Kv1.5 cRNA alone (A) or Kv1.5 and Kv β 1.2 cRNA (B). K $^{+}$ currents elicited from oocytes by steps from a holding potential of -60 mV to a range of test potentials from -30 to $+60$ mV in 10-mV increments. Measurable current was seen with voltage steps to potentials positive of -30 mV, which increased in a voltage-dependent manner. The current-voltage relationship for five oocytes is shown in (C).

small current was detected at a test potential of -40 mV, and this increased in a voltage-dependent manner with increasing depolarization. The I-V relationships of Kv1.5 and Kv1.5/Kv β 1.2 are shown in Figure 7C. When Kv β 1.2 was co-expressed with Kv1.5, the amplitudes of the currents were decreased significantly.

DISCUSSION

In the present study, we demonstrated the first quantitative difference in expression of three porcine Kv β 1 variants, Kv1.2, Kv1.5, Kv2.1, and Kv9.3 mRNA in various oxygen-sensitive blood vessels at birth. We used the adventitia and endothelial denuded DA, Ao, and PA as sample specimens. These expressed a high level of the SMC-specific marker but a low level of the EC-specific marker (Fig. 6, D and E). Although some fibroblasts and EC may remain in the tissue, it is most likely that the specimens consist of the vascular SMC.

We found that expression of Kv β 1.2, Kv1.5, and Kv9.3 were relatively high and characteristic in each blood vessel. Expression of Kv β 1.2 in the DA was higher than that of

Kv1.5. In contrast, expression of Kv β 1.2 in the Ao, PA, and b-PA was lower than that of Kv1.5. We confirmed the inactivation property of Kv β 1.2 against Kv1.5 using *Xenopus laevis* oocytes.

From these findings, Kv β 1.2 in the DA might inactivate the Kv1.5 current effectively because of the higher expression of Kv β 1.2. This inactivation might lead to SMC depolarization, opening of calcium channels, an increase in intracellular calcium, and vasoconstriction. In contrast, Kv β 1.2 in the PA might inactivate the Kv1.5 current poorly, because of the much lower expression of Kv β 1.2. The molecular basis for the differential electrophysiological characteristics in the PASMC and the DASMC might be due to a blood vessel-specific difference in expression of Kv1.5 and Kv β 1.2.

Kv β belongs to the aldose-reductase superfamily of NADPH, suggesting that it is an intracellular redox-sensing device for Kv channels (30). Kv β 1.2 might play the role of sensing the cellular redox state and directly modifying the properties of the Kv1.5 subunit, depending upon the redox state, leading to the regulation of vascular constriction. The DA is known to constrict with an increase in oxygen at birth but the PA does not. The opposing response to oxygen might result from the difference in the relative expression levels of Kv1.5 and Kv β 1.2.

The b-PA showed relatively high expression of Kv9.3 and a faint but detectable amount of Kv1.2 and Kv2.1 in addition to Kv1.5, suggesting acquisition of oxygen-sensitive properties under hypoxia and a physiologic distinction between the main PA and the b-PA.

Although expression of Kv β 1.2 in heart has been described in adult specimens (31,32), we did not detect Kv β 1.2 in the porcine neonate heart. The diverse tissue distribution of Kv β 1.2 may result from either differences in the developmental stage or species specificity.

The highly conserved sequences of Kv β 1.2 and Kv β 1.3 cDNA corresponding to human cDNA indicate that Kv β 1.2 and Kv β 1.3 might be generated through alternative splicing similar to human Kv β 1.2 and Kv β 1.3. The porcine Kv β 1 gene might have a different splicing point compared with the corresponding human Kv β 1 gene (Fig. 2). A high sequence similarity between the 5'-end of Kv β 1.4 cDNA and the near 3'-end of the human intron 4 DNA was observed. Because the Kv β 1.4 subunit lost its DNA sequences corresponding to human exons 1–4 by this splicing, the putative start codon on exon 1 in ordinary Kv β 1 genes was missing. Therefore, Kv β 1.4 has the putative first initiating start codon on putative exon 8, leading to a shorter predicted amino acid sequence.

Kv β 1.2 and Kv β 1.3 subunits both contain a “ β ball” in their NH₂-terminal end with structural similarities to the “ α ball” that works as an inactivating domain in A-type Kv channels. Therefore, Kv β 1.2 and Kv β 1.3 are expected to confer certain inactivation properties to the Kv α subunits. In contrast, Kv β 1.4 does not contain a “ β ball,” suggesting that it is not able to inactivate the Kv α subunits. Mouse Kv β 4.1 also lacks a “ β ball” and enhances expression of Kv2.2 (25). Therefore, Kv β 1.4 may have a role similar to that of Kv β 4.1.

In this study, we demonstrated the quantitative difference in expression of Kv β 1 variants, Kv1.2, Kv1.5, Kv2.1, and Kv9.3 mRNA in various oxygen-sensitive blood vessels at birth. Our results suggest that the molecular basis for the differential electrophysiological characteristics including opposing response to oxygen in the DA and the PA are partially due to diversity in expression of Kv1.5 and Kv β 1.2 subunits. The high expression of Kv β 1.2 and relatively low expression of Kv1.5 in the DA might be partially responsible for the ductal closure after birth. The potential involvement of Kv β 1.2 in vascular constriction at birth as an intracellular redox-sensing device for Kv channels is one of the most interesting questions to be addressed in the future. Protein levels of the Kv α and β subunits as well as developmental changes in the expression of these proteins should be determined in a future study.

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