J. Membrane Biol. 196, 61–69 (2003)
DOI: 10.1007/s00232-003-0625-z

The Journal of
Membrane
Biology

Differential Expression of Kir6.1 and SUR2B mRNAs in the Vasculature of Various Tissues in Rats

L. Li, J. Wu, C. Jiang
Department of Biology, Georgia State University, 24 Peachtree Center Avenue, Atlanta, GA 30302-4010, USA

Received: 11 June 2003 Revised: 4 September 2003

Abstract. ATP-sensitive K^+ channels (K_{ATP}) couple the intermediary metabolism to cellular excitability and play an important role in the cardio-protective effect of ischemic preconditioning and the activity-dependent autoregulation of cerebral circulation. Although previous studies using PCR and Northern blot suggest that the vascular isoform may consist of Kir6.1 and SUR2B, their expression and precise distribution in various vasculatures remain unknown. To illustrate their vascular expression, we performed this study using in situ hybridization histochemistry. Antisense riboprobes were synthesized by in vitro transcription and labeled with digoxigenin. Distributions of these mRNAs in the various blood vessels were revealed under a bright-field microscope. The expression of Kir6.1 and SUR2B mRNAs was observed in small and intermediate arteries as well as arterioles in several tissues, including basilar, vertebral, mesenteric, coronary and renal arteries. The transcripts were found in arterial smooth muscles. Also, we observed Kir6.1/SUR2B expression in capillary beds. The Kir6.1 and SUR2B expression pattern showed clear overlap, suggesting that they may form heteromeric K_{ATP} channels in these tissues. The Kir6.1 and SUR2B stains were detected in aorta and renal tubular cells although their expression level was extremely low. In contrast, the Kir6.1 and SUR2B mRNAs were not seen in vena cava, other small veins, myocardium and skeletal muscles. With their strong expression in small arteries and capillaries, it is very likely that the Kir6.1 and SUR2B form the vascular isoform of K_{ATP} channels in these vasculatures.

Key words: K_{ATP} Channels — Vascular smooth muscle — In situ hybridization

Correspondence to: C. Jiang; email: cjiang@gsu.edu

Introduction

ATP-sensitive K^+ (K_{ATP}) channels couple the intermediary metabolism to cellular excitability, and play an important role in autoregulation of vascular tones (Ashcroft, 1988; Samaha et al., 1992). These K_{ATP} channels are composed of four Kir6.x subunits and four sulfonylurea receptors (SURs), distributed widely in various cells and tissues such as pancreatic β-cells, myocardium, neurons, skeletal muscles, and smooth myocytes. In these tissues they function in insulin secretion, maintenance of membrane potential, and the regulation of cellular excitability and vascular tones (Noma, 1983; Quayle et al., 1997; Ashcroft & Gribble, 1998). The K_{ATP} channels are inhibited by intracellular ATP and sulfonylureas, which act on the Kir6.x and SURx subunits, respectively (Gribble & Ashcroft, 2000; Loussouarn et al., 2001; Song & Ashcroft, 2001).

The vascular K_{ATP} channels are regulated by multiple intra- and extracellular signal molecules, including ATP, nucleotide diphosphates (NDP), protons, nitric oxide, phospholipids, protein kinases, etc. (Larsson et al., 1993; Baukrowitz et al., 1998; Xu et al., 2001;). This, as well as the fact that the K_{ATP} channels are responsible for membrane potential and cellular excitability, indicates that these channels are the major target of intra- and extracellular signaling molecules and act on the control of vascular tones and regional blood flow. Therefore, the identification of the molecular identity of these channels may allow an intervention to the vascular tones by manipulating these channels.

The Kir6.1 and SUR2B heteromeric channels show several properties similar to the vascular K_{ATP} channels including pharmacological properties and the sensitivity to NDPs (Zhang & Bolton, 1996; Yamada et al., 1997; Satoh et al., 1998). Northern blot and RT-PCR evidence also suggest that the combination of Kir6.1 and SUR2B is likely to be the K_{ATP}

channel isoform in vascular smooth myocytes (VSMs) (Inagaki et al., 1995; Isomoto et al., 1996). However, it remains unclear how the Kir6.1 and SUR2B are expressed in various blood vessels; whether they are expressed in large, intermediate and/or small arteries; and whether they are present in other vasculatures such as capillaries, veins and venules. Since Kir6.1 antibodies are not available, in situ hybridization histochemistry was chosen to address these questions. We studied most representative blood vessels in the heart, kidney, brain and skeletal muscles. We also examined blood vessels such as the aorta, vena cava, basilar artery, vertebral artery, and mesenteric artery. Our results indicate that Kir6.1 and SUR2B are differentially expressed in these tissues with the most abundant expression in small arteries and capillaries.

Materials and Methods

Chemicals

Digoxigenin (DIG)-11-UTP, T7 RNA polymerase, protease K, anti-DIG antibody conjugated with alkaline phosphatase (AP), nitrobule tetrazolium chloride (NBT), and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) were purchased from Roche Diagnostics (Indianapolis, IN). Other chemicals including bovine serum albumin, dextran sulfate, polyvinylpyrrolidone, Ficoll 400, yeast tRNA, salmon sperm DNA, deionized formamide and levamisole were purchased from Sigma-Aldrich (St Louis, MO). All proteins and other macromolecules were purchased as molecular biology grade and RNase free.

Tissue Preparation

Adult Sprague-Dawley rats (120–150 g) were used in the experiments. All procedures were conducted in accordance with the National Institutes of Health Guidance for the Care and Use of Laboratory Animals and approved by the Georgia State University Institutional Animal Care and Use Committee. The rats were deeply anesthetized by inhalation of isoflurane, and then perfused transcardially with 200 ml 0.9% saline followed by 300 ml 4% paraformaldehyde in phosphate-buffer saline (PBS, 0.1 mM, pH 7.4). The tissues to be studied were removed, post-fixed in the same fixative solution for another 2 h at 4°C, and then dehydrated in a gradient of 10%, 20% and 30% sucrose at 4°C overnight. All solutions were sterile-filtered and treated with 0.1% diethyl pyrocarbonate (DEPC).

Riboprobe Synthesis

The Kir6.1 and SUR2B were cloned into pBluescipt SK (−) for the antisense and sense riboprobe synthesis. The cDNAs of Kir6.1 was linearized at the *Xho*I and *EcoR*I sites for sense and antisense riboprobes, respectively. Similarly, the SUR2B was linearized at *Xba*I and *Xho*I sites for sense and antisense riboprobe syntheses. The antisense and sense riboprobes were produced by in vitro transcription using T7 and T3 polymerase, respectively, and labeled with DIG using a riboprobe labeling kit (Roche Diagnostics). The length of cRNAs was confirmed to be 250–350 bp with electrophoresis, in which only RNAs with sharp bands and the correct size were chosen for further studies. The riboprobes were diluted with double-distilled water treated with 0.1% DEPC to 10 ng/μl and stored in aliquots at −80°C. Before hybridization, the riboprobes were diluted with the hybridization buffer to 200 ng/ml, well mixed and denatured at 80°C for 10 min.

Tissue Sections and Hybridization

Tissues were embedded with TBS tissue-freezing medium (Ngle Biomedical Sciences, Durham, NC), and frozen to −20°C. 12-μm sections were cut using a cryostat at −21°C, and thaw-mounted onto 1% gelatin-coated slides. The sections were dried overnight at 42°C and stored at −70°C.

The sections were thawed at 42°C over 2 h and incubated in PBS for 5 min. The tissue-endogenous phosphatases were inactivated with a treatment of 0.2 M HCl for 8 min followed by two PBS washes (5 min each). The sections were permeabilized with 1 μg/ml RNase-free protease K in TE buffer (100 mM Tris-HCl, 50 mM EDTA, pH 8.0) at 37°C for 30 min, and post-fixed with 4% paraformaldehyde in PBS at 4°C for 5 min. To acetylate the sections, the sections were placed in a jar with 0.1 M triethanolamine containing 0.25% (v/v) acetic anhydride at pH 8.0 for 10 min on a shaking platform. The sections were then dehydrated using 100% ethanol for 15 min and incubated with the pre-hybridization buffer for 2 h at 42°C. After the pre-hybridization buffer was drained, the sections were overlaid with 35 μl hybridization buffer containing 200 ng/ml denatured cRNA antisense and sense riboprobes at 42°C. After overnight hybridization, the sections received high-stringency wash with 2× SSC, 1× SSC at 52°C followed by NTE buffer (500 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8.0) containing 20 μg/ml RNaseA for 30 min. Each section was washed with 0.1× SSC at 52°C for 60 min followed by 2 washes in Buffer 1 (100 mM Tris, 150 mM NaCl, pH 7.5). Then sections were incubated with 1:1000 AP-conjugated sheep anti-DIG antibody for 2 h. The unbound antibodies were washed away by Buffer 1 and Buffer 2 (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5). Finally, the sections were incubated with pre-mixed NBT/BCIP at 37°C until the optimal staining was obtained.

Positive stains were examined under a bright-field microscope and digit-imaged. Expression of Kir6.1 and SUR2B was judged according to the relative optical intensity of the stain.

Results

Expression of Kir6.1 and SUR2B mRNAs in Small and Intermediate Arteries

The distribution of the Kir6.1 and SUR2B mRNAs in various blood vessels was studied using antisense riboprobes. Side-by-side comparison of the expression was performed in two consecutive sections. The coexpression of Kir6.1/SUR2B mRNAs was observed in intermediate arteries, including basilar, vertebral, mesenteric, coronary, renal arteries, etc. (Fig. 1). The expression level of Kir6.1 and SUR2B was similar and both mRNAs were found in the smooth muscle layer. Also, we observed Kir6.1/SUR2B expression in small arteries and arterioles in the heart, brain, kidney and skeletal muscles (Figs. 2 and 3). The caliber of these arteries ranges from 20 μm to 200 μm (Table 1). In these tissues, the Kir6.1/SUR2B expression pattern showed a clear overlap.

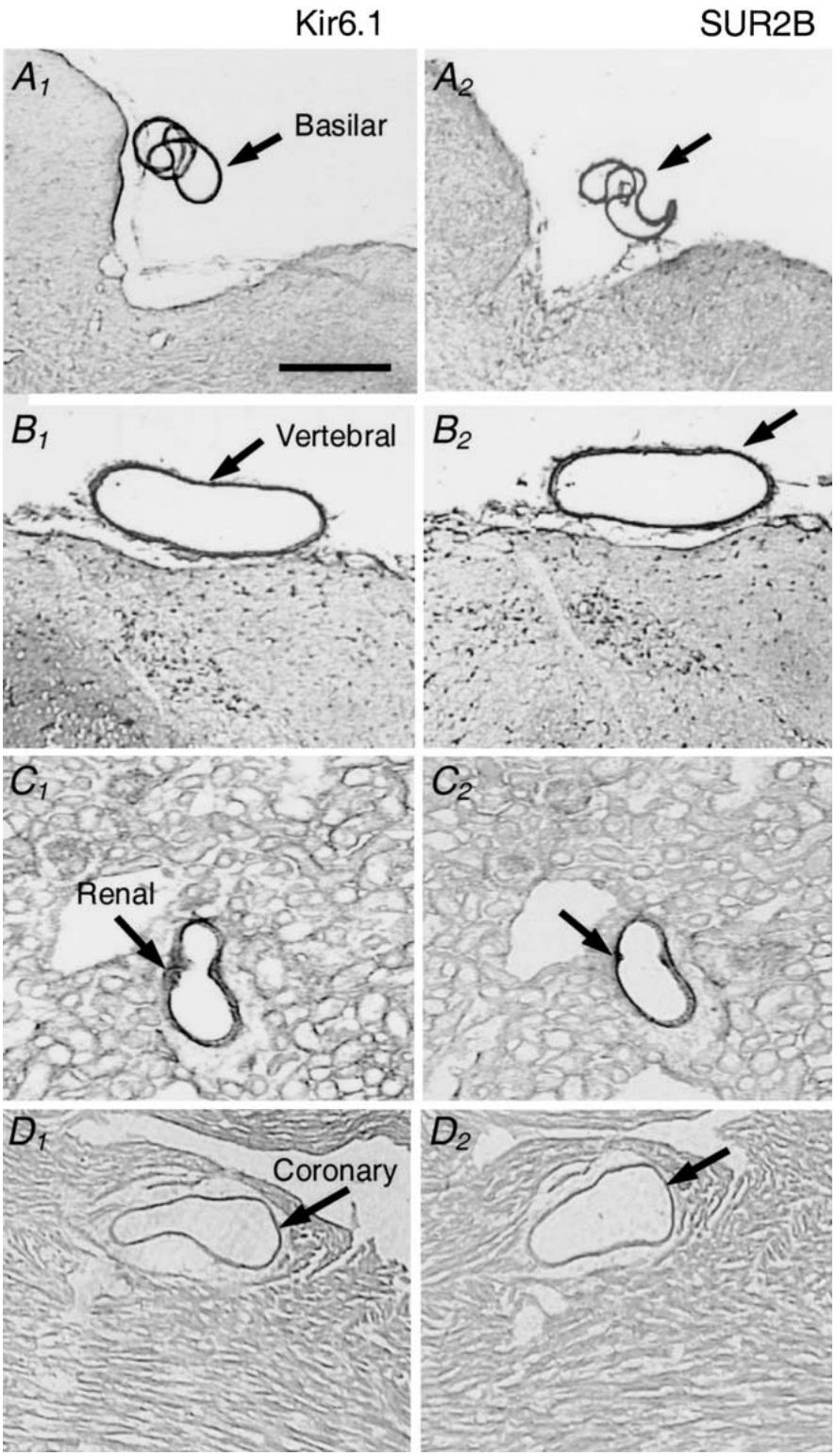

Fig. 1. Expression of Kir6.1 (*left column*) and SUR2B (*right column*) in vascular smooth muscles of intermediate-size arteries. In situ hybridization histochemistry was performed in the brain, kidney and heart using antisense riboprobes labeled with digoxigenin. Strong staining was seen in basal (*A*), vertebral (*B*) and renal arteries (*C*), while the coronary artery showed moderate labeling (*D*). Bar = 400 μm for *A* and *B*, 200 μm for *C* and *D*.

The coexpression of Kir6.1 and SUR2B was also identified in the branches of the basilar and vertebral arteries, suggesting that they may form K_{ATP} channels with these subunits. The labeling of the antisense riboprobe displayed distinct intensity in different tissues. Moderate stains were observed in the coronary and quadriceps arteries, and strong stains were seen in renal arteries and their branches. These arteries, known as the resistance arteries, play an important role in the regulation of the artery caliber and regional blood flow, suggesting the involvement of K_{ATP} channels in the regulation of blood circulation.

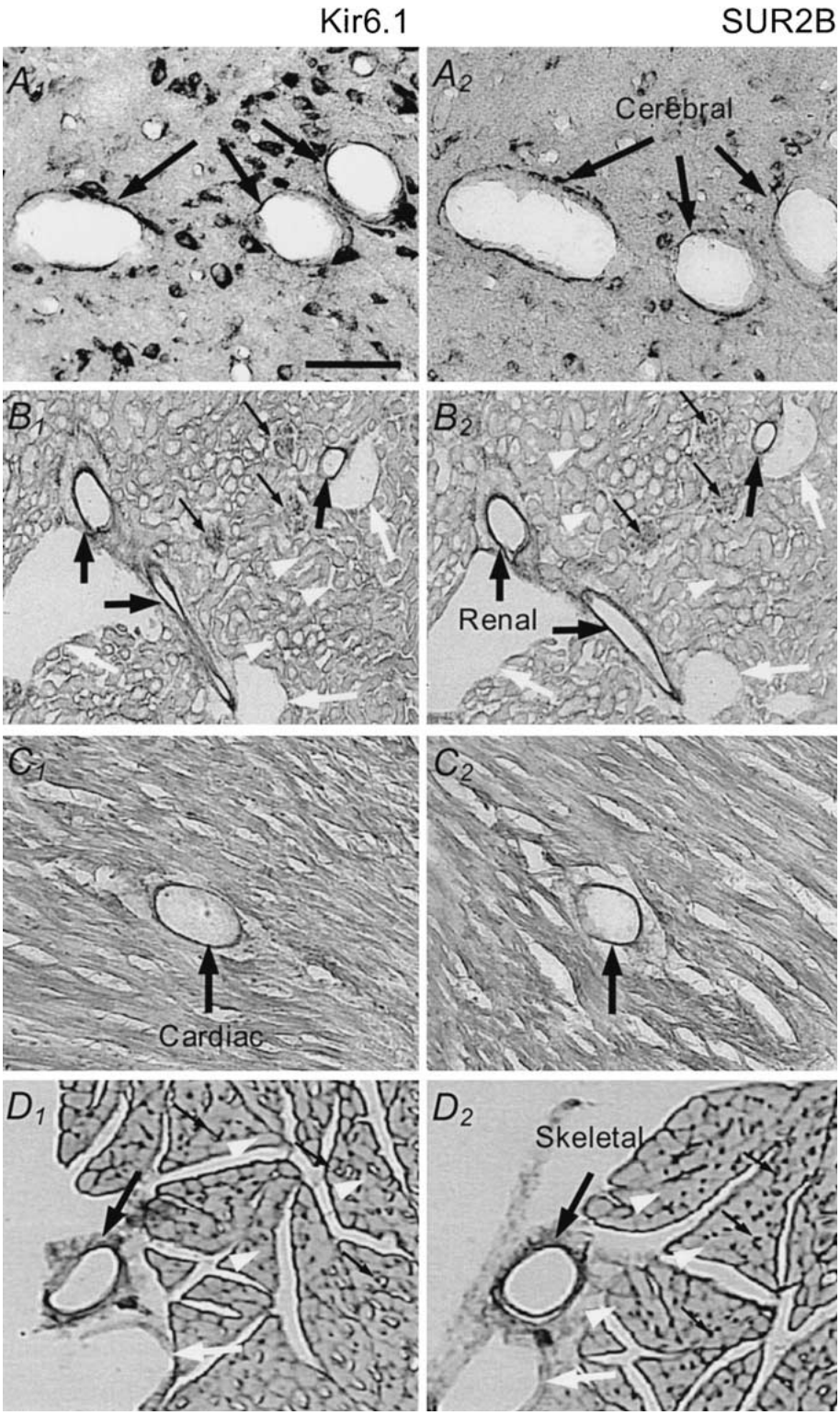

Fig. 2. The Kir6.1 and SUR2B expression in small arteries of the brain, kidney, heart and skeletal muscle. Strong labeling was seen in the cerebral (*A*) and renal arteries (*B*), and moderate labeling in the cardiac (*C*) and skeletal arteries (*D*). *Left column*, Kir6.1; *right column*, SUR2B. *Thick black arrow*, small arteries; *thin black arrow*, renal glomerulus (*B*) and the capillaries in the quadriceps (*D*); *white arrow*, small veins; *white arrow head*, renal tubules (*B*) and skeletal muscle fibers (*D*). Bar = 100 μm for *A*, and 200 μm for all others.

Several control experiments were performed to examine the specificity of the antisense probes. These included: 1) Kir6.1 and SUR2B sense riboprobes failed to show positive labeling in tissues such as in the kidney (*see* Fig. 5*D*); 2) cardiac myocytes and skeletal muscles, which are known to express Kir6.2 and SUR2A, were not labeled (*see* below); and 3) unlabeled antisense riboprobes did not produce any positive staining in these blood vessels (*not shown*).

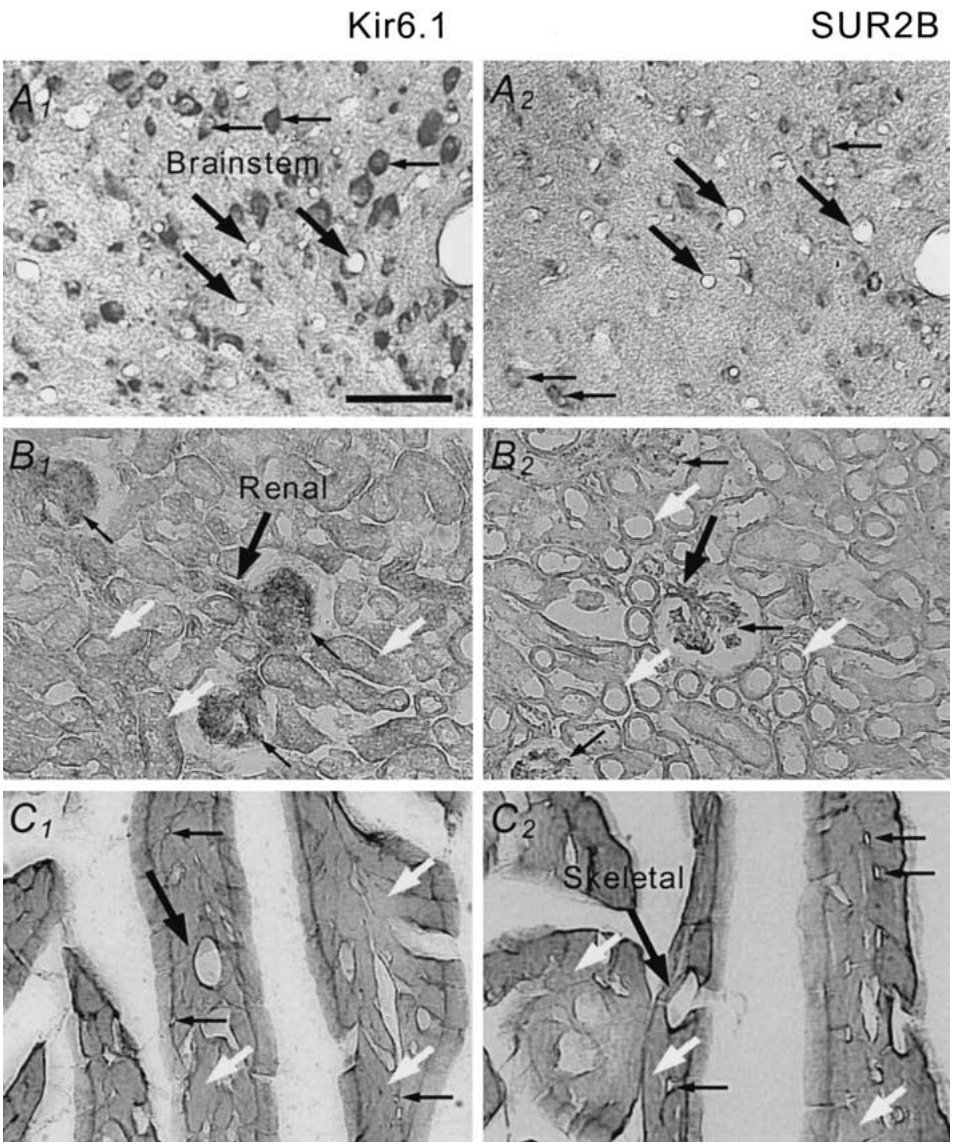

Fig. 3. Labeling of Kir6.1 and SUR2B transcripts in arterioles of the brain, kidney, and skeletal muscle. The arterioles in these tissues were differentially stained with strong labeling in the brain (*A*) and kidney (*B*), and moderate staining in the skeletal muscle (*C*). Neurons (*A*), renal glomerulus (*B*) and skeletal capillaries (*C*) were stained (*thin black arrow*). Kir6.1 and SUR2B expressions were very low in renal tubules (*B*), and barely detectable in arterioles of skeletal muscle fibers (*C*, *white arrow*). Bar = 100 μm.

Evidence for the Presence of Kir6.1 and SUR2B Transcripts in Capillary Endothelium

The lack of Kir6.1 and SUR2B expression in skeletal muscle fibers allowed us to see their expression in capillaries, as strong labeling was observed around the muscle fibers (Fig. 2*D*). Our detailed studies indicated that the microvascular endothelial cells were stained by the antisense riboprobes of Kir6.1 and SUR2B, especially in the skeletal muscle fibers of the quadriceps (Fig. 3*C*, 4*A*). In the kidney, the glomerulus was positively labeled (Figs. 2*B*, 3*B*). The labeling appeared to occur in glomerular capillaries rather than in the podocytes, as the parietal layer of the glomerulus did not show such labeling (Figs. 3*B*, 4*B*). Moderate stains were found in capillaries in the brain despite the relatively small size of the capillary endothelium. The clear visibility of the staining in the rather small vasculature indicates that the Kir6.1 and SUR2B expression density is high in capillaries.

Scarce Expression of Kir6.1 and SUR2B in Aorta, Vena Cava and Other Veins

Similar studies were performed in the aorta, vena cava, veins and venules. In contrast to the small arteries and capillaries, the Kir6.1 and SUR2B expression level was extremely low in the aorta, and appeared in only a small number of the arterial smooth myocytes (Fig. 5*A*). Positive labeling of the Kir6.1 and SUR2B mRNAs was not detected in the vena cava, veins and venules in the kidney and skeletal muscle tissues (Figs. 2, 5).

Table 1. Summary of the distribution of Kir6.1 and SUR2B mRNAs in vasculature

Tissue	Kir6.1	SUR2B	Diameter (μm)
Intermediate Artery			
Basal	+	+	350–400
Vertebral	+	+	250–320
Renal	+	+	130–150
Coronary	+	+	120–150
Mesenteric	+	+	100–180
Small Artery			
Kidney	+	+	70–90
Heart	+	+	60–80
Skeletal muscle	+	+	60–80
Arteriole			
Brain	+	+	30–40
Kidney	+	+	25–35
Skeletal muscle	+	+	20–30
Capillary			
Glomerulus	+	+	6–8
Skeletal muscle	+	+	6–8
Brain	+	+	8–10
Aorta	±	±	1,500–2,500
Vein			
Vena cava	–	–	2,000–3,000
Vein	–	–	20–100
Venule	–	–	8–10

Note that the + indicates positive stain with antisense riboprobes, the ± shows low labeling, and the – represents no detection of the mRNAs.

Kir6.1 and SUR2B mRNAs in Renal Tubular Epithelium, Myocardium and Skeletal Muscles

The transcripts of the Kir6.1 and SUR2B, though low, were detectable in renal tubular cells. In contrast, the expression of Kir6.1 and SUR2B was rather high in certain neurons (Figs. 2, 3). However, no labeling was detectable in cardiac myocytes and skeletal muscles. Thus, these observations suggest that the K_{ATP} channels recorded previously from these tissues seem to consist of different isoforms of Kir6.x and SURx.

Discussion

K_{ATP} channels are well known to play an important role in the regulation of vascular tone according to metabolic need and energy supply. The current studies provide evidence for the expression of the vascular isoform of the K_{ATP} channels in various blood vessels including arteries, arterioles, veins, venules and capillaries. To our knowledge, this is the first systematic study of the distribution of Kir6.1 and SUR2B in blood vessels.

The K_{ATP} channel was first identified in the heart and later found in almost all tissues (Noma, 1983; Terzic, Jahangir & Kurachi, 1995; Quayle et al., 1997; Seino, 1999). Molecular cloning over the past decade has shown that two Kir6.x and at least three SURx subunits exist in nature. The molecular identity of the K_{ATP} channels enabled detailed studies of the molecular mechanisms underlying the regulation of the Kir6.2 channel and its potential functions in the pancreas and heart (Ashcroft, 1988; Seino, 1999). Similar studies, however, have rarely been done on the Kir6.1 channel. One major reason is that tissue-specific expression of the Kir6.1 has not been well demonstrated. Based on the logical reasoning that they are expressed in all tissues and that blood vessels are everywhere, it has been supposed that Kir6.1 forms K_{ATP} channels with the SUR2B subunit in blood vessels. Some evidence came from pharmacological studies. Glibenclamide, a sulfonylurea K_{ATP} channel blocker, has been shown to inhibit relaxation of vascular smooth muscles of the mesenteric, coronary and vertebral arteries (Eckman, Frankovich & Keef, 1992; Murphy & Brayden, 1995; Nagao et al., 1996). However, sulfonylureas are known to inhibit other ion channels as well (Konstas et al., 2002; Zhou, Hu & Hwang, 2002). Therefore, the pharmacological evidence is still not conclusive. Better evidence was found in from previous electrophysiological studies showing a vascular K_{ATP} channel in mesenteric artery and portal veins that has a single-channel conductance of 35 pS and is inhibited by intracellular ATP in the presence of nucleotide diphosphates (Nelson & Quayle, 1995; Cao et al., 2002). By heterologous co-expression of Kir6.1 and SUR2B in HEK-293 cells, Yamada et al. (1997) demonstrated a K_{ATP} channel with pharmacological properties and nucleotide sensitivity similar to the vascular K_{ATP} channel. Consistently, our in situ hybridization studies reveal the expression of the Kir6.1 and SUR2B in vascular smooth muscles, thus providing the first morphological evidence for their vascular expression and distribution. Indeed, recent studies in our laboratory have shown a vascular K^+ current with ATP sensitivity, pH sensitivity and single-channel properties identical to the cloned Kir6.1/SUR2B, demonstrating the link of the Kir6.1/SUR2B expression to functional vascular K_{ATP} channels (Wang et al., 2003).

These in situ hybridization studies allow us to appreciate the differential expression of the Kir6.1 and SUR2B subunits in various blood vessels. Our results have shown that the Kir6.1 and SUR2B are selectively expressed in the smooth muscle layer of arterioles, as well as small and intermediate arteries. These blood vessels are known to determine the circulation resistance and blood pressure (Wilson, 1989). Thus, the expression of Kir6.1 and SUR2B in these blood vessels suggests that K_{ATP} channels composed of Kir6.1 and SUR2B are the potential mediators of vascular diameter in accordance with the change of physiological and pathophysiological conditions.

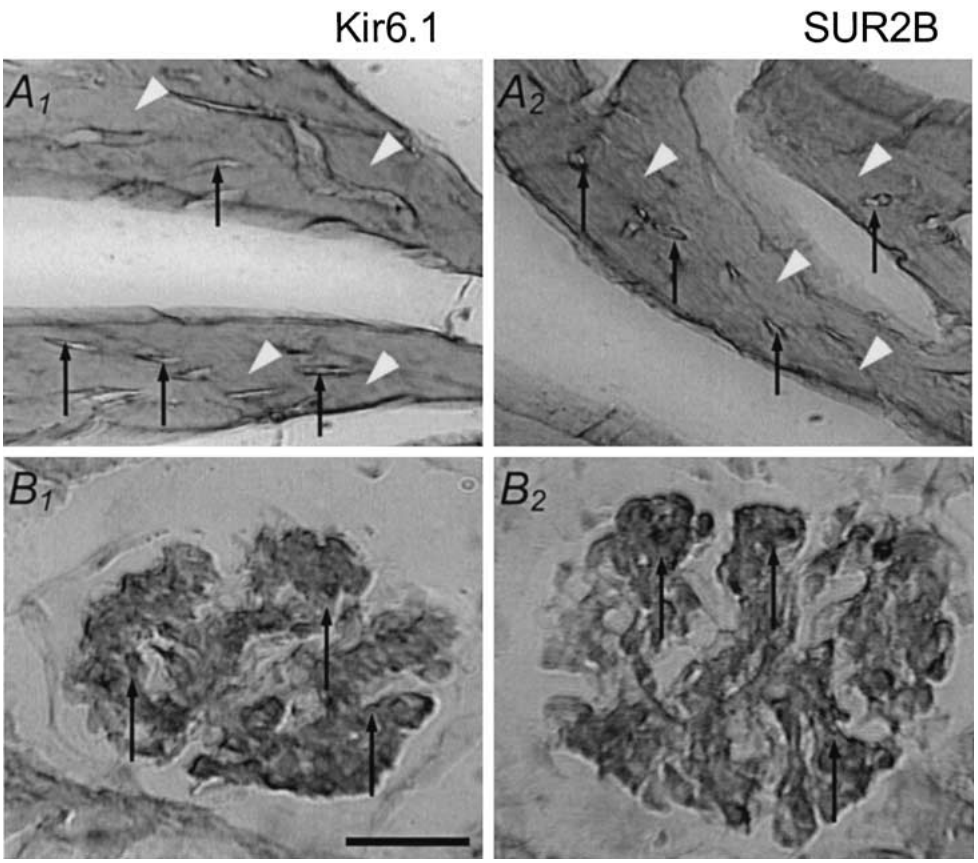

Fig. 4. The presence of Kir6.1 and SUR2B in capillary endothelium. With higher magnification than in Figs. 2 and 3, the capillaries in the quadriceps (*A*) and renal glomerulus (*B*) showed positive stain with these antisense riboprobes, as indicated by *black arrows*. *White arrow head*, skeletal muscle fibers. Bar = 50 μm.

Previous electrophysiological studies have shown that capillary endothelial cells possess certain ion channels that are believed to be responsible for the fast endothelial response to intracellular Ca^{2+} signals and mediate the release of endothelium-derived relaxing factor. However, the molecular identity of these ion channels remains largely unknown (Brayden, 1990; Nilius & Droogmans, 2001). Our studies suggest that one of these ion channels appears to be made of the Kir6.1 and SUR2B because their mRNAs are present abundantly in the capillaries of the skeletal muscle and renal glomerulus. It is possible that the K_{ATP} channel along with other ion channels functions in the regulation of the membrane potential in capillary endothelial cells. These ion channels may allow the membrane potential to change according to the concentration of energy substrates in these cells, leading to the release of endothelium-derived relaxing factor, and perhaps the alteration of the endothelial permeability as well. Therefore, the finding of the capillary K_{ATP} channel may provide the molecular basis for further understanding of the physiological role of these channels in the capillaries.

Kir6.2 and SUR2A have been known to be expressed in myocardium and skeletal muscles, whereas Kir6.1, Kir6.2 and SUR2 were reported to be present in renal tubular epithelium (Noma, 1983; Quast, 1996; Quayle et al., 1997). Our data are consistent with these previous results. The distribution of Kir6.1, Kir6.2 and SUR2 in renal tubular epithelium suggests there is more than one K_{ATP} channel in the kidney. The lack of expression of Kir6.1 and SUR2B mRNAs in the myocardium and skeletal muscle indicates that the K_{ATP} isoforms are unlikely to be made of Kir6.1 and SUR2B in these tissues. Concerning the high sequence homology between Kir6.1 and SUR2B with Kir6.2 and SUR2A, the negative staining with the Kir6.1 and SUR2B riboprobes indicates that their positive labeling in the blood vessels is specific.

The expression of Kir6.1 and SUR2B suggests that they play a role in the regulation of vascular tone. Indeed, Kir6.1 has been demonstrated to do so, as Kir6.1-null mice have a high rate of sudden death associated with spontaneous electrocardiographic ST elevation followed by atrioventricular block (Miki et al., 2002). The K_{ATP} channels consisting of Kir6.1 and SUR2B are inhibited by intracellular ATP at near millimolar concentrations and are stimulated by NDP (Larsson et al., 1993; Wang et al., 2003). These

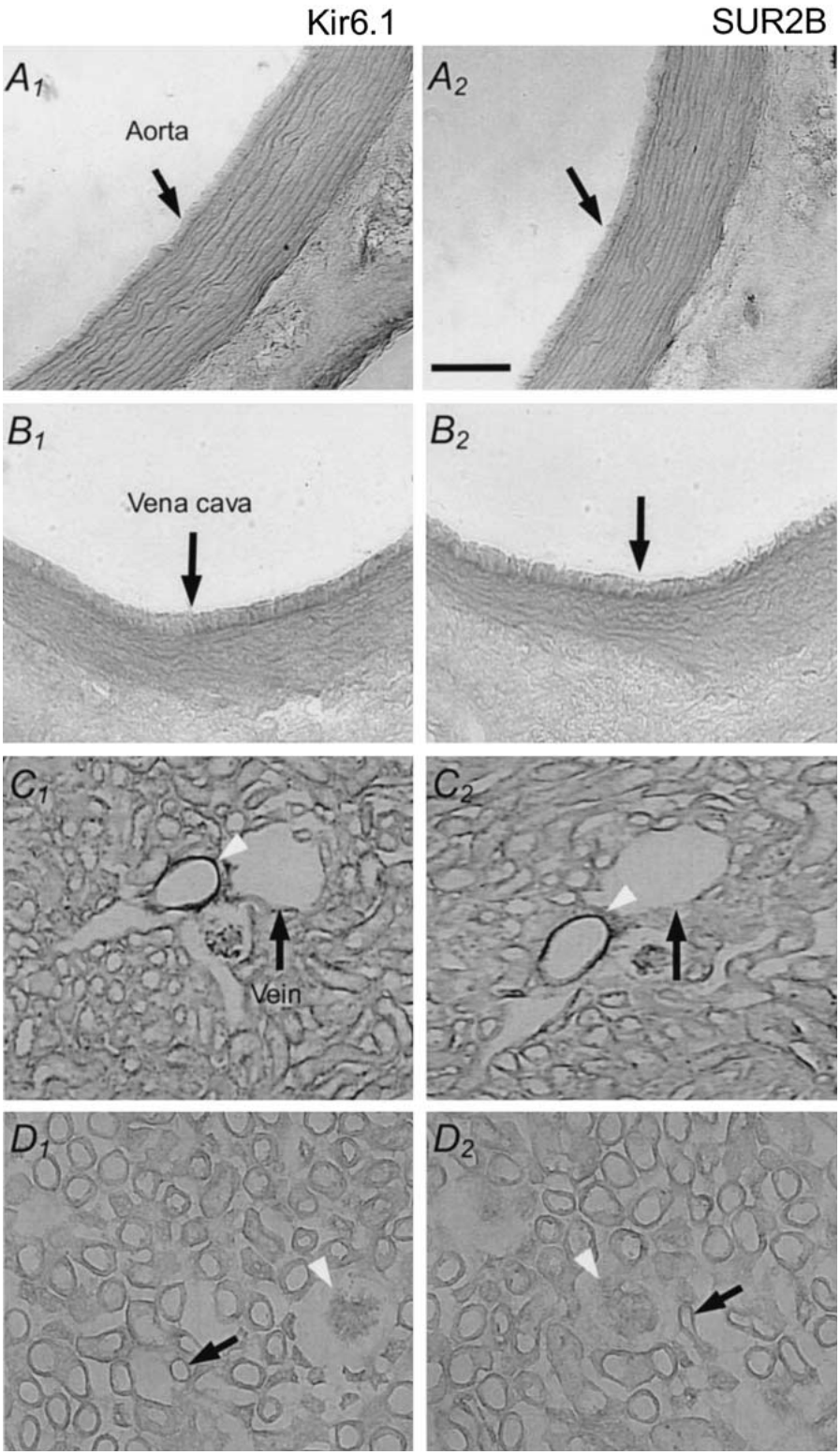

Fig. 5. The Kir6.1 and SUR2B mRNAs (*left* and *right columns*, respectively) were barely detectable in the aorta (*A*), vena cava (*B*) and small veins in the kidney (*C*), as shown by black arrows. In contrast, the renal arteries were strongly stained (*white arrow head* in *C*). Control experiments were done using the sense riboprobes. None of the Kir6.1 and SUR2B sense probes yielded positive labeling of the renal artery (*black arrow*) and glomerulus (*white arrow head*). Bar = 200 μm for *A*, *B* and *C*, 100 μm for *D*.

properties allow a direct coupling of the channel activity to the cellular metabolic state, making the vascular K_{ATP} channels to be readily activated during metabolic stress such as hypoxia, ischemia and hypoglycemia. Activation of these K^+ channels results in hyperpolarization and a decrease in cellular excitability. In turn, the voltage-activated Ca^{2+} channels are inhibited with hyperpolarization, lowering intracellular Ca^{2+} concentration. As a result the VSM contractility decreases. The vasodilation allows a better perfusion in the local tissues and reverses the hypoxic ischemia. It is clear that the Kir6.1

and SUR2B are important players in the feedback regulation of vascular tone. Thus, the demonstration of their expression pattern in various blood vessels provides useful information for the understanding of vascular regulation under a variety of physiological and pathophysiological conditions.

Special thanks to Dr. S. Seino for providing the Kir6.1 cDNA and Dr. Kurachi for the SUR2B cDNA. This work is supported by the NIH (HL058410, HL067890) and the American Diabetes Association (1-01-RA-12). CJ is a Career Investigator of the American Lung Association.

References

Ashcroft, F.M. 1988. Adenosine 5′-triphosphate-sensitive potassium channels. *Annu. Rev. Neurosci.* **11:**97–118

Ashcroft, F.M., Gribble, F.M. 1998. Correlating structure and function in ATP-sensitive K^+ channels. *Trends Neurosci.* **21:** 288–294

Baukrowitz, T., Schulte, U., Oliver, D., Herlitze, S., Krauter, T., Tucker, S.J., Ruppersberg, J.P., Fakler, B. 1998. PIP2 and PIP as determinants for ATP inhibition of K_{ATP} channels. *Science* **282:**1141–1144

Brayden, J.E. 1990. Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am. J. Phy siol.* **259:**H668–H673

Cao, K., Tang, G., Hu, D., Wang, R. 2002. Molecular basis of ATP-sensitive K^+ channels in rat vascular smooth muscles. *Biochem. Biophys. Res. Commun.* **296:**463–469

Eckman, D.M., Frankovich, J.D., Keef, K.D. 1992. Comparison of the actions of acetylcholine and BRL 38227 in the guinea-pig coronary artery. *Br. J. Pharmacol.* **106:**9–16

Gribble, F.M., Ashcroft, F.M. 2000. Sulfonylurea sensitivity of adenosine triphosphate-sensitive potassium channels from beta cells and extrapancreatic tissues. *Metabolism* **49:**3–6

Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M., Seino, S. 1995. Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J. Biol. Chem.* **270:**5691–5694

Isomoto, S., Kondo, C., Yamada, M., Matsumoto, S., Higashiguchi, O., Horio, Y., Matsuzawa, Y., Kurachi, Y. 1996. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J. Biol. Chem.* **271:**24321–24324

Konstas, A.A., Dabrowski, M., Korbmacher, C., Tucker, S.J. 2002. Intrinsic sensitivity of Kir1.1 (ROMK) to glibenclamide in the absence of SUR2B. Implications for the identity of the renal ATP-regulated secretory K^+ channel. *J. Biol. Chem.* **277:** 21346–21351

Larsson, O., Ammala, C., Bokvist, K., Fredholm, B., Rorsman, P. 1993. Stimulation of the K_{ATP} channel by ADP and diazoxide requires nucleotide hydrolysis in mouse pancreatic beta-cells. *J. Physiol.* **463:**349–365

Loussouarn, G., Pike, L.J., Ashcroft, F.M., Makhina, E.N., Nichols, C.G. 2001. Dynamic sensitivity of ATP-sensitive K^+ channels to ATP. *J. Biol. Chem.* **276:**29098–29103

Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H., Seino, S. 2002. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat. Med.* **8:**466–472

Murphy, M.E., Brayden, J.E. 1995. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *J. Physiol.* **486:**47–58

Nagao, T., Ibayashi, S., Sadoshima, S., Fujii, K., Fujii, K., Ohya, Y., Fujishima, M. 1996. Distribution and physiological roles of ATP-sensitive K^+ channels in the vertebrobasilar system of the rabbit. *Circ. Res.* **78:**238–243

Nelson, M.T., Quayle, J.M. 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* **268:**C799–C822

Nilius, B., Droogmans, G. 2001. Ion channels and their functional role in vascular endothelium. *Physiol. Rev.* **81:**1415–1459

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148

Quast, U. 1996. ATP-sensitive K^+ channels in the kidney. *Naunyn Schmiedebergs Arch. Pharmacol.* **354:**213–225

Quayle, J.M., Nelson, M.T., Standen, N.B. 1997. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol. Rev.* **77:**1165–1232

Samaha, F.F., Heineman, F.W., Ince, C., Fleming, J., Balaban, R.S. 1992. ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. *Am. J. Physiol.* **262:**C1220–C1227

Satoh, E., Yamada, M., Kondo, C., Repunte, V.P., Horio, Y., Iijima, T., Kurachi, Y. 1998. Intracellular nucleotide-mediated gating of SUR/Kir6.0 complex potassium channels expressed in a mammalian cell line and its modification by pinacidil. *J. Physiol.* **511:**663–674

Seino, S. 1999. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu. Rev. Physiol.* **61:**337–362

Song, D.K., Ashcroft, F.M. 2001. ATP modulation of ATP-sensitive potassium channel ATP sensitivity varies with the type of SUR subunit. *J. Biol. Chem.* **276:**7143–7149

Terzic, A., Jahangir, A., Kurachi, Y. 1995. Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am. J. Physiol.* **269:**C525–C545

Wang, X., Wu, J., Li, L., Chen, F., Wang, R, Jiang, C. 2003. Hypercapnic acidosis activates K_{ATP} channels in vascular smooth muscles. *Circulation Research*, **92:**1225–1232

Wilson, C. 1989. Inhibition by sulphonylureas of vasorelaxation induced by K^+ channel activators in vitro. *Auton. Pharmacol.* **9:**71–78

Xu, H., Cui, N., Yang, Z., Wu, J., Giwa, L.R., Abdulkadir, L., Sharma, P., Jiang, C. 2001. Direct activation of cloned K_{ATP} channels by intracellular acidosis. *J. Biol. Chem.* **276:**12898–12902

Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y., Kurachi, Y. 1997. Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. *J. Physiol.* **499:**715–720

Zhang, H.L., Bolton, T.B. 1996. Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. *Br. J. Pharmacol.* **118:**105–114

Zhou, Z., Hu, S., Hwang, T.C. 2002. Probing an Open CFTR Pore with Organic Anion Blockers. *J. Gen. Physiol.* **120:**647–662