Localization of the Sulphonylurea Receptor Subunits, SUR2A and SUR2B, in Rat Renal Tubular Epithelium

MING ZHOU,1 HUI-JING HE,1 OSAMU TANAKA,2 RYOJI SUZUKI,1 MASAKI SEKIGUCHI,2 YUKIKO YASUOKA,3 KATSUMASA KAWAHARA,3 HIDEAKI ITOH4 and HIROSHI ABE1

1Department of Anatomy, Akita University School of Medicine, Akita, Japan
2Department of Morphology, Tokai University School of Medicine, Isehara, Japan
3Department of Physiology, Kitasato University School of Medicine, Sagamihara, Japan
4Department of Material-process Engineering and Applied Chemistry for Environment, Akita University Faculty of Engineering and Resource Science, Akita, Japan

ATP-sensitive K⁺ (K\textsubscript{ATP}) channels in the kidney are considered to play roles in regulating membrane potential according to changes in the intracellular ATP concentration. They are composed of two types of subunits; the pore subunits (Kir6.1, Kir6.2), which are members of the inwardly rectifying K⁺ channel family, and the regulatory subunits, the sulphonylurea receptors, which belong to the ATP-binding cassette (ABC) superfamily. The sulphonylurea receptors (SURs) are receptors of sulphonylureas widely used for the treatment of type 2 diabetes mellitus. The SURs are divided into two isoforms, SUR1 and SUR2, the latter was further divided into SUR2A and SUR2B. In the present study, we have investigated the mRNA expression by RT-PCR assay, and protein expression profiles by immunoblotting, immunohistochemistry, and immunoelectron microscopy with anti SUR2A and anti SUR2B antibodies. RT-PCR detected the presence of mRNA transcripts of the SUR2A and SUR2B, while SUR1 mRNA was barely detected. In immunoblotting, SUR2A protein was detected distinctly in the microsomal fraction, weakly in the mitochondrial fraction and at negligible level in the cell membrane fraction. In contrast, the SUR2B protein was detected intensely in the microsomal fraction, with a low level in the mitochondrial fraction and scarcely in the cell membrane fraction. In immunohistochemistry SUR2A and SUR2B proteins were widely distributed in renal tubular epithelial cells, glomerular mesangial cells, and the endothelium and the smooth muscle of blood vessels. In immunoelectron microscopy, the immunoreactivity was localized in the endoplasmic reticulum and mitochondria throughout the epithelial cells for SUR2A, and dominantly in the apical cytoplasm of the cells for SUR2B. In conclusion, the regulatory subunits of the K\textsubscript{ATP} channel in the rat kidney are SUR2A and SUR2B; they also are candidate regulatory subunits for the mitochondrial K\textsubscript{ATP} channel. ATP-sensitive K⁺ channel; sulphonylurea receptor; immunohistochemistry; immunoelectron microscopy; kidney.

Received November 27, 2007; revision accepted for publication January 23, 2008.
Correspondence: Ming Zhou, M.D., Ph.D., Department of Anatomy, Akita University School of Medicine, Hondo 1-1-1 Akita, 010-8543, Japan.
e-mail: mzhou@med.akita-u.ac.jp

© 2008 Tohoku University Medical Press
In animals and human beings, the kidney clears the metabolic waste products from the blood and regulate the concentrations of many constituents of body fluids. The ionic balance of blood is maintained through various ionic channels that are localized in renal tubular epithelial cells. A variety of potassium channels in renal tubular epithelial cells contribute to renal K⁺ handling with pumps and co-transporters to control body fluid volume, maintaining the membrane potential, secreting and recycling K⁺, and regulating the cell volume (Kawahara and Anzai 1997; Noulin et al. 2001). Some of the K<sub>ATP</sub> channels have been cloned by expression and/or PCR techniques, which have clarified their amino acid sequences and molecular topology in the cell membrane. Immunohistochemical studies have shown the channels to be localized to the luminal and/or basolateral membranes of a certain nephron segment (Kawahara et al. 1987; Noulin et al. 1999). Examples include the ROMK family located in the apical membrane (Boim et al. 1995; Lee and Hebert 1995) and K<sub>ATP</sub>-2 (Kir4.1) in the basolateral membrane (Ito et al. 1996) of the distal segment of the convoluted tubules and various regions of collecting ducts. They belong to the superfamily of inwardly rectifying K⁺ channel clones, with common structural features of two putative membrane spanning domains and one pore-forming domain, and with the inward rectification capacity (Kubo et al. 1993).

ATP-sensitive K⁺ (K<sub>ATP</sub>) channels, have been discovered originally by a patch-clamp technique in the cardiac muscle (Noma 1983). These channels possess ATP sensitivity and weak inward rectification capacity (Aguilar-Bryan et al. 1998). They are sensitive to the change of intracellular ATP concentration even to a level of 1 mM or to the ratio of ATP/ADP, and they then close or open (Noma 1983). The pore-forming subunits of the K<sub>ATP</sub> channel, belonging to the members of the inwardly rectifying K⁺ channel (Kir) superfamily have been cloned and later called Kir6.1 and Kir6.2 (Inagaki et al. 1995a, b; Sakura et al. 1995; Isomoto et al. 1996).

Sulphonylurea receptors (SURs) belong to the ATP-binding cassette superfamily and are regulatory subunits of K<sub>ATP</sub> channels (Chutkow et al. 1996, 2002). The functional K<sub>ATP</sub> channels need both the Kir6.x and SURs subunits to form a heter-octameric compound consisting of four molecules of Kir6.x (Kir6.1 or Kir6.2) for the pore-forming subunits, and four molecules of SURs (SUR1, SUR2A, or SUR2B) for the regulatory subunits (Clement et al. 1997).

Up to now, little is known about the cellular and subcellular localization, the role, and the regulation of SUR2A and SUR2B in the renal tubules, although a great deal of data have shown that the genes of Kir6.1 and SUR2B are ubiquitously expressed in different tissues and cells, including the kidney (Inagaki et al. 1995b; Isomoto et al. 1996). Moreover, the SUR possesses not only a plasma membrane type but also a mitochondrial type (Szewczyk et al. 1997; Bajgar et al. 2001). Thus, it is important to determine which type of SUR specifically localizes in the renal tubular epithelium, especially in terms of its apical or basolateral cell membranes, or mitochondrial or endoplasmic reticulum (ER) membranes.

With the questions noted above in mind, the present study was attempted to clarify the expression and localization of the SURs in rat kidney by RT-PCR and immunohistochemistry at light and electron microscopic levels and to gain further insight into the functional roles of K<sub>ATP</sub> channels in the renal function.

**Materials and Methods**

Animals and tissue preparations

Male Wistar rats (4 to 6 weeks, n = 10) were used (Japan SLC; Hamamatsu). The protocols for animal experimentation described herein were approved by the Animal Research Committee, Akita University; all subsequent animal experiments adhered to the “Guidelines for Animal Experimentation” of the University.

Rats were anesthetized through peritoneal injection of pentobarbital sodium (Abbott Lab., Chicago, IL, USA) at 50 mg per kilogram body weight. Specimens taken out for reverse transcriptase polymerase chain reaction (RT-PCR) were quickly put into liquid nitrogen and stored at -80°C until use. Specimens for immunoblotting were cut into pieces and homogenized in a buffer as described previously (Zhou et al. 2007b) and stored at
Channel in Rat Kidney

ATP-Sensitive K⁺ Channel in Rat Kidney

-80°C until use. Specimens for immunohistochemistry were fixed by perfusion through heart with 4% paraformaldehyde (PFA) buffered at pH 7.4 by 0.1 M phosphate buffered saline (PBS). The excised kidneys were cut into thin slices and placed into the same fixative at 4°C over night and subsequently transferred into 30% sucrose in PBS at 4°C. Cryosections were cut at a thickness of 8-10 μm and thaw-mounted on MAS-coated glass slides (Matsunami Glass Ind. Ltd., Kishiwada).

**RT-PCR**

Total RNAs were extracted from the cortex and medulla of kidneys or hearts using an RNaseasy mini Kit (QIAGEN GmbH, Hilden, Germany). Reverse-transcription reactions (20 μl volume) were carried out with Oligo (dT) 12-18 Primer (Invitrogen, Tokyo) according to the manufacturer’s instructions. Briefly, 1 μl of Oligo (dT) 12-18 primer, 4 μl of dNTP mixture (2.5 mM each), with 1 μg of total RNA and add sterile distilled water (sdw) added to 12 μl. After heating the mixture at 65°C for 5 min, it was quickly put on ice, add 4 μl of 5 × First-Strand buffer, 2 μl of 0.1 M DTT and 1 μl of sdw were added, gently mixed then incubated at 42°C for 2 min, then 1 μl of SuperScript II RT (Invitrogen, Tokyo) was added. After mixing gently with pipetting, the specimens were incubated at 42°C for 50 min, and at last heated at 70°C for 15 min. This product was used as the cDNA for the PCR reaction.

PCR was performed with primers for the rat SURs (see below) as SUR1 (GenBank accession No. L40624), SUR2A (GenBank accession No. D83598) and SUR2B (GenBank accession No. AF019628) or the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (G3PDH, GenBank accession No. AF019628) or the housekeeping SUR2A (GenBank accession No. D83598) and SUR2B (see below) as SUR1 (GenBank accession No. L40624), 94°C for 1 min, then subjected to 30 cycles of denaturation (94°C, 30″), annealing (30°; 55°C for SUR2A special and G3PDH; 60°C for SUR1; 58°C for SUR2A and SUR2B), and extension (72°C, 30″). A final extension phase (72°C, 3-5 min) was included for all samples. PCR products were separated on 1.5% agarose gels and visualized by ethidium bromide staining under ultraviolet light. To test for any residual contamination of the RNA samples by genomic DNA, control RT-PCR reactions were performed in an identical manner to that described above, but with the omission of the reverse-transcriptase enzyme. All PCR products were extracted from the gel, and purified with a Gel Extraction Kit (Omega Bio-tek, Doraville, GA, USA), then labeled with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed by sequencing test with an ABI Prism 3100-Avant Genetic Analyzer (AB Applied Biosystems, Hitachi) to confirm correct amplification.

**Kₐₜ channel primers**

Primers for SUR1 were designed to reside 558 bp from 3019 to 3576, 5′-TGCCAGCTCTTTGAGCATTTG-3′ forward, and 5′-AGGATGATACGGTTGAGCAGG-3′ reverse. Special primers for SUR2A (set 1) were designed to reside 155 bp from 4646 to 4800, 5′-TTGTTCGAAGAAGAGCAGCATAC-3′ forward, and 5′-GCCCCATCCATAATAGAGG-3′ reverse. Primers for both SUR2A and SUR2B (set 2) were designed to reside 328-bp as SUR2A from 4646 to 4973 and reside 152 bp as SUR2B from 4646 to 4797, 5′-TTGTTCGAAGAAGAGCAGCATAC-3′ forward, and 5′-AGCAGTCAGAATGTTGGAACC-3′ reverse. PCR conditions and agarose gel electrophoresis are described above.

**Anti-SUR2 antisera**

Rabbit polyclonal antisera were raised against a synthetic 19-mer peptide, NH₂-PNLLQHKNGLFSTLVMTNKC-OH (Biologica Co., Nagoya), which corresponds to the amino acid residues 1526-1544 of the C-terminal peptides of rat SUR2A and a synthetic 14-mer peptide, NH₂-(C) MKRGNILEYDTPSE-OH (Hokkaido System Science Co., Sapporo), which corresponds to the amino acid residues 1515 -1528 of the C-terminal peptides of rat SUR2B. These antisera were obtained from Hokkaido System Science and Biologica (Nagoya). The antisera were purified by immunoaffinity column chromatography for SUR2A and protein-A chromatography column for SUR2B before immunoblotting and immunohistochemical assay. The specificities of these antisera were confirmed (Zhou et al. 2007a).

**Subcellular fractionations**

All procedures were carried out at 0-4°C. The kidneys were immediately excised from anesthetized rats and quickly washed with 0.9% NaCl solution. They were then cut into small pieces and homogenized with protein extraction buffer. Subcellular fractions were extracted as described elsewhere (Itoh et al. 2002).
Briefly, after centrifugation at 600 g for 10 min, the precipitate was discarded, and the 600 g supernatant was centrifuged at 7,000 g for an additional 10 min. The 7,000 g precipitate (P1) was re-dissolved in the buffer and centrifuged at 5,000 g for 10 min, and the 5,000 g precipitate was used as the mitochondrial fraction. The 7,000 g supernatant (S1) was centrifuged at 54,000 g for 60 min, and the supernatant (S2) was centrifuged at 105,000 g for an additional 60 min. The 54,000 g precipitate was used as the cell membrane fraction, the 105,000 g precipitate as the microsomal fraction, and the supernatant as the cytoplasmic fraction. Those fractions were confirmed as previously (Zhou et al. 2005, 2007b).

**Immunoblotting**

Proteins of the whole rat kidney and cellular fractions were denatured in a modified sample buffer (125 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% Bromophenol blue and 10% 2-mercaptoethanol). Electrophoresis was performed on 8% or 10% SDS-polyacrylamide gels. The proteins were subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane (NEN™ Life Science, Boston, MA, USA) using a semi-dry transfer unit (Hoefer TE70 series, Amersham Pharmacia Biotechnology, Little Chalfont, Buckinghamshire, England) according to the manufacturer’s instruction. The transferred PVDF membranes were then blocked with 5% Blot-QuickBlocker (Chemicon International, Inc., Temecula, CA, USA) in PBS over night at room temperature. After washing with PBS-T (PBS containing 0.1% Tween-20), the PVDF membranes were incubated with rabbit anti-rat SUR2A, or SUR2B antisera diluted to 1:500 or 1:1000, respectively, for 1 hr at room temperature. After rinsing with PBS-T, they were then reacted to HRP-conjugated donkey anti-rabbit IgG (Amersham Pharmacia Biotech.) diluted to 1:3,000 for 30 min at room temperature. The antigen-antibody complexes were visualized with ECL Western blotting detection reagents (Amersham Pharmacia Biotech.) according to the manufacturer’s instructions.

**Immunohistochemistry**

Cryosections of kidney were kept in PBS containing 0.3% Tween-20 for 45 min. Prior to incubation with the first antisera, sections were treated with 0.3% H2O2/methanol solution and ABC blocking kit (Vector Lab., Inc., Burlingame, CA, USA) to reduce the endogenous peroxidase reaction as well as non-specific binding with avidin-biotin complex. After incubation with 5% normal goat serum for 1 hr, the sections were reacted with antibodies of rabbit anti-SUR2A, or rabbit anti-SUR2B at a dilution of 1:500 for 12 hrs at room temperature. After thorough rinsing with PBS containing 0.05% Tween-20, the sections were then treated with diluted (1:200) biotinylated goat anti-rabbit IgG (BA-1000, Vector Lab. Inc.) for 30 min, and then with ABC complex (Vectastain ABC kit, Vector Lab., Inc.) according to the manufacturer’s instructions. Reactivity was visualized by incubating the sections in 0.001~0.005% DAB (3,3’-diaminobenzidine tetrahydrochloride) reaction with a 0.003% H2O2, and counterstaining with methyl green.

**Immunoelectron microscopy**

For electron microscopy, the same procedure as that for immunohistochemistry at light microscopy was performed. Tissue sections that showed immunoreaction to anti-SUR2A, or anti-SUR2B antibodies were post-fixed in 1% osmium tetroxide for 30 min, dehydrated in a graded series of ethanol, and embedded in Quetol 812. Thin sections were cut and observed under an electron microscope without uranyl acetate and lead citrate staining.

**RESULTS**

**RT-PCR**

During the course of the studies, reactions were replicated twice. The PCR products were sequenced and positive control (heart) was also used. RT-PCR of rat kidney RNA using the primer set1 for SUR2A generated a 155 bp product special for SUR2A, and using the primer set 2 for both SUR2A and SUR2B, generating a 328 bp product for SUR2A and a 152 bp product for SUR2B (Fig. 1). There was no SUR1 detected, suggesting that only SUR2A and SUR2B are expressed in the rat kidney.

**Immunoblotting**

In kidney fractions, SUR2A, an approximate 130 kDa band, was detected distinctly in the microsomal fraction (Fig. 2A, lane ms), weakly in the mitochondrial fraction (Fig. 2A, lane mit), and at a negligible level in the cell membrane fraction (Fig. 2A, lane cm).

SUR2B, an approximate 120 kDa band, was detected prominently in the microsomal fraction.
ATP-Sensitive K⁺ Channel in Rat Kidney

Fig. 1. Reverse transcriptase polymerase chain reaction (RT-PCR) of rat total RNA. PCR analysis of amplified total RNA from rat kidney cortex and medulla as well as positive control heart. The 1.5% agarose gels show bands of DNA fragments amplified with primer pairs specific for SUR1, SUR2A, for both SUR2A and SUR2B, as well as glyceraldehydes-3-phosphate dehydrogenase (G3PDH). RT-PCR products amplified from the rat heart (H), kidney cortex (Kc) and medulla (Km) RNA. Primers were tested to amplify fragments of 558 bp for SUR1, 155 bp for SUR2A (primers for SUR2A only), 328 bp for SUR2A and 152 bp for SUR2B (primers for both SUR2A and SUR2B). The result shows that SUR2A and SUR2B, but not SUR1, are expressed in kidney cortex and medulla, compared with the positive control of heart RNA.

(Fig. 2B, lane ms) and at a negligible level in the cell membrane fraction (Fig. 2B, lane cm), while a short band (approximately 50 kDa) and a weak large band (approximately 120 kDa) were detected in the mitochondrial fraction (Fig. 2B, lane mit). These detected signals disappeared with pre-absorption with corresponding antigen peptides (Fig. 2A, B, +Ag).

Fig. 2. Immunoblot analysis of SUR2A and SUR2B from cellular fractions of rat kidney. (A) In the three lanes on the left, the anti-rat SUR2A antibody recognized a prominent band of ~130 kDa in the microsomal (lane ms), and mitochondrial (lane mit) fractions, and a negligible signal was seen in the cell membrane (lane cm) fraction. In the three lanes on the right, preincubation with immunizing peptide prevented the appearance of these bands (+Ag). (B) In the three lanes on the left, the anti-rat SUR2B antibody recognized a prominent band of ~120 kDa in the microsomal (lane ms) fraction, weak in the mitochondrial (lane mit) fraction with a short, approximately 50 kDa band, and in the cell membrane fraction (lane cm) the signal was negligible. In the three lanes on the right, preincubation with immunizing peptide prevented the appearance of these bands (+Ag).
Localization of SUR2A and SUR2B

In immuno-light microscopy, SUR2A- and SUR2B-immunoreactivities were widely localized from the renal cortex to medulla (Figs. 3A and 4A, 4B). These proteins were distinctly in the proximal convoluted tubules, thick portions of proximal and distal tubules as well as distal convoluted tubules in the renal cortex (Figs. 3B and 4C) and also in the cortical collecting ducts (Figs. 3C, D and 4D). These proteins were also located in presumptive mesangial and podocytes of glomeruli (Figs. 3B and 4C). Immunoreaction products for SUR2A were found rather evenly throughout the cytoplasm without any clear polarity and the nucleus was free of the immunoreaction (Fig. 3B). On the other hand, immunoreaction products for SUR2B were found more intensely in the apical cytoplasm of the renal tubular epithelium (Fig. 4C). Both SUR2A and SUR2B were also localized in the endothelial cells and smooth muscles of blood vessels (Figs. 3E and 4E). No immunoreaction could be detected when the antibodies were absorbed with corresponding peptide antigens (Figs. 3E and 4E, insets).

In immunoelectron microscopy, the immunoreaction products for SUR2A were associated with endoplasmic reticulum and mitochondria as well as some apical canaliculi (Fig. 5A, 5B and 5C) throughout the cytoplasm of epithelium, while those for SUR2B were associated with apical canaliculi and vacuoles/vesicles, endoplasmic reticulum as well as mitochondria (Fig. 6A, B) in the apical cytoplasm of the epithelium. No distinct immunoreaction products for SUR2A or SUR2B were found along apical or basolateral cell membranes, except some weak immunoreaction of SUR2B in the microvilli (Fig. 6A).

Fig. 3. Immunohistochemistry on cryosections of rat kidney exhibiting expression of SUR2A protein. (A) Immunoreactivity with anti-SUR2A antibody is seen widely in the rat kidney in the outer renal cortex (Oco), inner renal cortex (Ico), outer medulla (Omd) and inner medulla (Imd). In the renal cortex (B) the proximal tubules (PCT) show SUR2A immunoreactivity, while the distal tubules (DCT) show SUR2A weakly. SUR2A is also expressed in mesangial cells (arrows) and podocytes (arrowheads) of the glomerulus (Glo). Immunoreactivity was localized in the cytoplasm of the renal tubules rather than in the basolateral membrane (B, C and D). In the renal medulla SUR2A is also localized in the collecting ducts (C and D). Smooth muscle and endothelium of blood vessel (BV) show immunoreactivity to SUR2A (E). No prominent immunoreactivity of SUR2A was seen following pre-absorption with immunizing peptide antigen (inset). Scale bars: A = 100 μm; B, C and D = 20 μm; E = 50 μm
Fig. 4. Immunohistochemistry on cryosections of rat kidney exhibiting expression of the SUR2B protein. Immunoreactivity with anti-SUR2B is seen widely in the renal cortex (A) and medulla of the rat kidney (B). (C) High magnification of the rectangle in Fig. A showing SUR2B immunoreactivity in the proximal tubules of the renal cortex. SUR2B is expressed in mesangial cells (arrows) and podocytes (arrowheads) of the glomerulus (Glo). (D) High magnification of the rectangle in Fig. B in the renal medulla showing SUR2B is also localized in the collecting ducts. (E) Smooth muscle and endothelium of blood vessels (BV) display immunoreactivity to SUR2B. No prominent immunoreactivity of SUR2B is seen following pre-absorption with immunizing peptide antigen (inset). Scale bars: A, B = 100 μm; C, D = 20 μm; E = 50 μm.

Fig. 5. Immunoelectron micrographs showing SUR2A protein in subcellular structures of the rat proximal tubule. (A) Apical portion of the proximal tubule with long, regularly oriented, and closely packed microvilli (Mv). SUR2A can be observed in the small vesicles and apical canaliculi (arrowheads). (B) Immunoreactivity for SUR2A can be seen in the mitochondria (Mit). (C) In the endoplasmic reticulum (arrowheads) the immunoreactivity for SUR2A is clearly seen. Scale bars: 1 μm.
DISCUSSION

As the combination of channel and regulatory molecules of $K_{\text{ATP}}$ channels, several examples have been reported as follows: Kir6.2 with SUR1 in pancreatic $\beta$ cells (Inagaki et al. 1995a), Kir6.2 with SUR2A in cardiac muscle (Inagaki et al. 1996), or Kir6.1, Kir6.2, SUR1, SUR2A and SUR2B in cardiac muscles (Lacza et al. 2003; Seino and Miki 2004; Zhou et al. 2007a), and Kir6.1 with SUR2B in the smooth muscle of blood vessels (Yamada et al. 1997; Seino and Miki 2003). RT-PCR analysis showed SUR2B mRNA to be ubiquitously expressed in diverse tissues, including the kidney (Isomoto et al. 1996), and also to be localized in rat vascular smooth muscle (Wang et al. 2003) and rat mesangial cells (Szamosfalvi et al. 2002). The SUR2 protein was shown to be expressed during development of the rat kidney (Hernandez-Sanchez et al. 1997). Although SUR2B has been reported to be a regulatory subunit of the $K_{\text{ATP}}$ channels in the rodent kidney (Beesley et al. 1999), the present study for the first time shows in immunohistochemistry as well as western blotting that both SUR2A and SUR2B are the regulatory subunits of $K_{\text{ATP}}$ channels in the rat kidney, which in accord with a previous finding of rabbit kidney in western and northern blottings (Brochiero et al. 2002). Since such wide distribution of SUR2A and SUR2B in rat kidney was quite similar to that of Kir6.1 (Zhou et al. 2007b), it is suggested that both SUR2A and SUR2B combine with Kir6.1 to form functional $K_{\text{ATP}}$ channels in the renal tubular epithelium.

The wide localization of SUR2A and SUR2B is quite different from other $K^+$ channels previously observed in specific nephron segments, such as ROMK (Kir1.1) specifically located in the apical membrane in the entire cell domain of the distal convoluted tubules and of the collecting ducts (Boim et al. 1995; Ito et al. 1996), and K$_{\text{ATP}}$-2 (Kir4.1) localized in the basolateral membrane of the distal convoluted tubule (DCT) (Ito et al. 1996). In contrast to the previous findings and the general understanding that, based on their localization in the plasma membranes, the $K_{\text{ATP}}$ channels in the renal epithelia are supposed to contain four functions such as the maintenance of cell negative potential, the $K^+$ recycling at the cell membrane, the $K^+$ secretion, and the regulation of cell volume (Kawahara and Anzai 1997), the pres-
ent study stressed the intracellular localization of SUR2s, probably in combination with Kir6.1 as functional K\textsubscript{ATP} channels with a suggestion of their possible roles related to mitochondria and endoplasmic reticulum. In addition, the shifted localization to the subsurface region of apical cytoplasm in the thick portion of the renal tubules is shown to be characteristic of SUR2B, while no such a shifted localization is seen for SUR2A in the present study. These features suggest that K\textsubscript{ATP} channels composed of SUR2B and Kir6.1 are more intimately involved in handling of potassium ions through the intracellular membranes, in the apical cell domain than the remainder domain. It remains to be elucidated how such intracellularly shifted handlings of potassium affects the functions of the renal epithelium including the urine formation.

Acknowledgments

This study is dedicated to Dr. Hisatake Kondo on his retirement from the position of Professor, Department of Cell Biology, Graduate School of Medicine, Tohoku University on March 31, 2008. We thank the staff of Bioscience Research-Education Center of Akita University School of Medicine for their useful help and Mr. Nibe for his assistance in this study. This work was supported by part of Research Grants of Akita University to H.A. and by part of the Research Grants of Kitasato University School of Medicine to K.K. Pacific Edit reviewed the manuscript prior to submission.

References


Noulin, J.F., Brochiero, E., Coady, M.J., Laprade, R. &
M. Zhou et al.


