Localization of sulphonylurea receptor proteins SUR2A and SUR2B and/or SUR1 in rat kidney

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Abstract: To further explore precise expression and localization of sulphonylurea receptor isoforms SUR2A and SUR2B (SUR1) in rat kidney, total RNA was isolated from the kidney tissue using the TRIzol kit. Three different primer sets designed against SUR isoforms were used in reverse transcriptase reactions. Western blotting was done on membrane fractions obtained from kidney tissues using the primary antisera for SUR2A and SUR2B (SUR1). Paraformaldehyde fixed kidney sections were immunostained with SUR2A and SUR2B (SUR1) primary antisera. Sections were developed with DAB as a chromogen. RT-PCR results demonstrated mRNA consistent with SUR1 isoform to be the only identifiable transcript. Western blotting could not identify any protein consistent with SUR2A or SUR2B (SUR1) but recognized instead a smaller 55kD protein of unknown identity. Immunohistochemistry demonstrated a differential staining pattern whereby SUR2A was localized to the mesangial cells, intra- and extrarenal blood vessels and smooth muscles. In contrast, SUR2B (SUR1) was localized only to distal nephron epithelia. Intense immunoreactivity was localized to the thick ascending limb and as well as in the outer and inner medullary collecting ducts, both. Our results demonstrate differential and highly localized expression pattern of sulphonylurea receptor proteins SUR2A and 2B (SUR1) in rat kidney with implications for drug design.

Keywords: Sulphonylurea; ABC proteins; K_{ATP} channels; Immunocytochemistry; SUR.

INTRODUCTION

Potassium channels form a very diverse group and are classified mainly on the basis of electrical rectification properties and ligand binding. One type of potassium channels, the ATP-sensitive K^+-channels (K_{ATP} channels) exist as tetramers of pore forming subunit that constitute the inwardly rectifying potassium channel (Kir 6.x), and a regulatory subunit made up of a 140-170 kD membrane protein, the sulphonylurea receptor (SUR) (Skeer *et al*., 1994; Inagaki *et al*., 1995a; Ammala *et al*., 1996a; Clement *et al*., 1997). SUR belongs to the ATP-binding cassette family that also includes other proteins like the cystic fibrosis transmembrane conductance regulator (CFTR) and multidrug resistance (MDR) protein (Seino, 1999; Bryan *et al*., 2005; Burke *et al*., 2008). First described in cardiac myocytes (Noma, 1983), K_{ATP} channels were subsequently discovered and described in a wide variety of tissues. They have a high open probability and are inhibited by intracellular ATP thus linking membrane potential to cellular metabolism (Inagaki *et al*., 1995b; Liss and Roeper, 2001; Standen, 2003).

In the pancreatic β-cell, an increase in glucose triggers insulin release through blocking of the K_{ATP} channel by an increase in the ATP (Seino, 1999; Bryan *et al*., 2005). Glibenclamide, an antidiabetic sulphonylurea drug used to treat type II non-insulin dependent diabetes mellitus, selectively blocks K_{ATP} channels by binding with high affinity to SUR (Challinor-Rogers and McPherson, 1994; Burke *et al*., 2008). On the other hand cromakalim, pinacidil and diazoxide act as potassium channel openers (Inagaki *et al*., 1996; Moreau *et al*., 2005). Several isoforms of SUR exist and splice variants of SUR1 and SUR2 showing cell specific expression have also been described (Gros *et al*., 2002; Hambrook *et al*., 2002). The consensus to date is that Kir6.2/SUR1 presumably forms the pancreatic β-cell K_{ATP} channel while Kir6.2/SUR2A and Kir6.2/SUR2B make up the cardiac type and vascular smooth muscle type K_{ATP} channels respectively (Aschroft and Gribble, 2000; Standen, 2003).

Because kidneys maintain long-term K^+ balance, nephrons possess a large number of transport pathways for K^+. Renal K^+ channels on the whole perform two major transport processes; K^+ recycling on basolateral membranes and K^+ secretion on the apical membranes.

Besides several different kinds of K^+ channels which are distributed throughout the nephron, there has been growing interest in ATP sensitive K^+ channels due to their physiologic importance and as they show biophysical properties similar to the renal outer medullary collecting duct potassium (ROMK) channels. Molecular data show that K_{ATP} channels are found on the basolateral surface of proximal tubule, apical membranes of distal nephron segments, the thick ascending limb (TAL) and the cortical collecting duct (CCD) (Giebisch and Wang, 2000).

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The importance of SUR in the kidney can be envisaged from the fact that apical K^+ channels are also a site of action of sulphonylurea drugs; namely, glibenclamide which causes natriureteric diuresis via inhibition of K^+ channels (Wang *et al*., 1997). Moreover, ^{3}H-glibenclamide labelling has detected sulphonylurea binding sites in rat isolated glomeruli (Metzger *et al*., 1997) and a low level expression of SUR mRNA has been demonstrated in the mouse kidney by RT-PCR (Inagaki *et al*., 1996; Isomoto *et al*., 1996) and solution hybridization /RNase protection assay (Hernandez-Sanchez *et al*., 1997). In our earlier study we demonstrated SUR expression in the mouse kidney at mRNA and protein level (Beesley *et al*., 1999). A similar isoform specific precise localization pattern of SUR in rat kidney is unclear and needs further elaboration. The present investigation was therefore aimed at collecting first hand data about localized expression pattern of SUR in the rat kidney using the reverse transcriptase polymerase chain reaction (RT-PCR), Western blotting and immunocytochemistry.

MATERIALS AND METHODS

Animals

Male rats (Wistar strain; adults; mean body weight 250 g; n=25) were obtained from the Animal House Facility of Biomedical Science Department, Sheffield University, United Kingdom. They were provided standard rat feed and drinking water. All animal handling procedures were according to the guidelines provided by the Animal Scientific Procedures Act 1986, United Kingdom. Animals were sacrificed as humanely as possible to minimize suffering.

RNA extraction and RT-PCR for Detection of SUR mRNA

Total RNAs were extracted from whole kidney and heart (as positive control) using the TRIzol Reagent (GIBCO-BRL). Reverse-transcription reactions contained 2 μg of total RNA, 2.5 μM oligo-(dT), 200 μM dNTPs, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT and 300 units M-MLV reverse transcriptase (Promega, UK). RNA denaturation was done at 90^{O}C for 2 min, primer annealing for 10 min on ice, while reverse transcription was at 35^{O}C for 1 hr. The reaction was ended after final heating at to 95^{O}C.

PCR reactions were performed with isoform specific primers for rodent SUR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as housekeeping gene. PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 μM mixed dNTPs, 3 mM $MgCl_2$, 2.5 units *Taq* DNA polymerase (Promega, UK), 200 nM of primer and 2.5 μl of the RT product. Samples underwent heating to 94^{O}C for 5min, 35-40 cycles of denaturation (94^{o}C, 1 min), annealing (58^{o}C, 1 min), and extension (72^{o}C, 1.5 min) and a final for 5-10 min at 72^{O}C. PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining using the Gel Documentation System (BIORAD, UK). Control RT-PCR reactions to detect contamination by genomic DNA were performed without reverse-transcriptase enzyme.

Three sets of primers (P1, P2 & P3) were designed (described elsewhere, Beesley *et al*., 1999) against the published sequences of mouse SUR2A and 2B (Genbank D86037 and D86038), rat and hamster SUR1 (Genbank L40624 and L40623), and rat GAPDH (Genbank X02231). Following were the primer sequences: SUR2A (P1): ACGGTCG TAACCATAGCT (sense, product size 555bp); SUR2B (P1): CTTAAGGCTGCTAGCATG (antisense, product size 379bp; SUR2A (P2): TCCATC GACATGGCCACGGA (sense, product size 291bp; SUR2B (P2): CCAGGTCAGCAGTCAGAATG (antisense, 111bp). SUR1 (P3): AGACAGCGGCCGATT CGGAT (sense); P3 (antisense) CTCAGGACCAGGGC ACTGT rat & *hamster sequences*, 865bp); glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as house keeping gene: CGGCAAGTTCAACGGCACA GTCA (sense); GGTTTCTCCAGGCGGCATGTCA (antisense, 597bp).

Western blotting

Kidneys were homogenized in an Ultra-Turrax homogenizer (Janke & Kunkel, Germany) in ice cold hypotonic lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM EDTA) supplemented with protease inhibitors (1.2 mM Pefabloc, Boehringer Mannheim, Germany), 1 mM orthovanadate (Sigma, UK) and 5 μl/ml protease inhibitor cocktail (Sigma, UK). Cell lysates were centrifuged at 300*g* for 5 min at 4°C to remove debris. The supernatants were centrifuged at 193000*g* in an ultracentrifuge (Beckman L7-65; 50.2 TI rotor) at 4°C for 30 min, and the pellets were resuspended in ice-cold PBS containing 2% Triton X-100, 1.5 mM Pefabloc and 1 mM orthovanadate. The protein concentration was determined using the detergent-compatible protein assay (BIORAD, UK). Samples were electrophoresed in 2× concentrated loading buffer (250 mM Tris (pH 10.9), 2% 2-mercaptoethanol, 2% SDS, 20% glycerol, 0.01% bromophenol blue) and boiled for 5 min. Electrophoresis was performed on a 7% SDS-polyacrylamide gel with 15 μg of protein loaded in each lane. The proteins were blotted onto a 0.45μm nitrocellulose membranes (BIORAD, UK) using the wet blotting method. Membranes were blocked in PBS containing 0.1% Tween (PBS-T) and 5% milk powder for 1 hr at room temperature. Subsequent antiserum incubations and washes (3 × 10 min between each step) were performed in PBS-T containing 1% milk powder. The membrane blots were incubated firstly with anti-SUR primary antisera (1:3000 for both types) overnight at 4°C, and then with a 1:2500 dilution of donkey anti-rabbit secondary antibody conjugated to horseradish peroxidase (Amersham, UK) for 1 hr at room temperature. All antibody dilutions were prepared in PBST containing 1% milk to block non-specific binding of primary antibodies. ECL system was used to visualize on hyperfilm antiserum binding to the blots (Amersham, UK). Polyclonal antisera were as described above for immunocytochemistry. Control membrane blots were incubated with SUR antisera adsorbed with immunizing peptides that were applied to the blots at the same concentration as used for immunohistochemistry and diluted 1:3000 in PBST containing 1% milk.

Immunocytochemistry

Rats were anaesthetized by intraperitoneal injection (i.p) of 60 mg/kg-sodium pentobarbitone (Sagatal, RMB Animal Health Ltd, UK). Transcardiac perfusion was performed with Mg^{2+} and Ca^{2+}-free phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Kidneys were removed, sliced and embedded in molten paraffin wax.

Immunocytochemistry was performed using a conventional avidin-biotin method on 5μm thick sections which were pre-treated with 0.3% hydrogen peroxide in methanol for 30 min to block the endogenous peroxidase activity. Sections were washed in PBS for 30 min with three 10 min changes. To permeabilize the membranes, tissue sections were treated with 0.2% Triton X-100 in PBS for 20 min. Sections were subsequently washed in PBS for 30 min with three changes of 10 min each. They were then incubated for 30 min in normal goat serum to block non-specific antigens (ABC Kit, Vector Labs. UK). To block endogenous biotin, sections were additionally treated with avidin D for 15 min, rinsed briefly with PBS and treated with biotin for 15 min (Avidin-Biotin Blocking Kit, Vector Labs. UK).

Rabbit polyclonal antisera (gift from Professor Susumo Seino, Chiba University, Japan) raised against the C-terminal peptides of SUR1 (rat & hamster sequence) and SUR2A (rat & mouse sequence) and IgG-purified with HiTrap affinity columns (Pharmacia Biotech) were used. Due to sequence homology at this region, anti-SUR1 antiserum is also predicted to recognize the terminal peptide sequence of mouse SUR2B and is therefore referred to hereafter as anti-SUR2B (SUR1) antiserum.

Dilutions of primary antibodies were prepared in 1% bovine serum albumin in PBS. Specific dilutions were determined by antibody titration method in serial dilutions ranging from 1:100 to 1:3000. Anti-SUR2A produced optimal staining at 1:500 dilution and anti-SUR2B (SUR1) showed specific staining reaction at 1:1000 dilution.

Sections were incubated with primary antisera SUR2A and SUR2B (SUR1) overnight at 4^0C under humidified conditions. After incubation with the primary antibody, sections were washed 3x in PBS for 5 min each and incubated for 1 hr with a biotinylated goat anti-rabbit peroxidase conjugated secondary antibody diluted in PBS (Vectastain ABC Kit, Vector Laboratories, UK). Sections were washed again for 5 min each and incubated for 1 hr with avidin-biotin complex (Vectastain ABC Kit). After washing in PBS, they were developed with AEC substrate (Aminoethylcarbazole Peroxidase Substrate Kit, Vector Laboratories, UK) for 15-20 min, rinsed first in PBS and then distilled water for 5 min. Sections were counter-stained with 1% hematoxylin for 2 min, rinsed in tap water and mounted in glycergel (Dako, UK). They were observed and photographed with an Olympus BH-2 photomicroscope using Fuji 100 ASA color films. Finally, they scanned with software, Adobe Photoshop (Version 7, Inc. Chicago, Illinois, USA) and presented as photoplates.

Controls for antibody staining

Control sections were run in parallel. They were incubated with antibodies that had been pre-adsorbed by co-incubation with respective immunizing peptides. The immunizing peptides were prepared in PBS at the concentration of 1mg/ml. The immunizing peptides for SUR2A and SUR2B (SUR1) were co-incubated with respective SUR2A and SUR2B (SUR1) antisera at concentrations of 5μl (0.1μg of immunizing peptide protein): 5μl (antiserum), and 10μl (0.2 μg of immunizing peptide protein): 5μl (antiserum) respectively for 6-8 hrs. Before applying to the tissue sections, antibody and immunizing peptide mixtures were diluted 1:1000 for SUR2A and 1:500 for SUR2B (SUR1) in 1% BSA in PBS. Other controls: omitting both the primary and/or secondary antibodies at the first and second incubation steps were also performed.

RESULTS

mRNA expression of SUR2A and SUR2B/SUR1

Only the primer set 2 (P2, SUR1 specific oligo-dT primers) yielded a 111bp product in rat kidney (K in lane 2), while the same primers yielded two bands of 291bp and 111bp from rat heart RNA (H in lane 3) consistent with the product sizes for rat SUR2A and SUR1 respectively. DNA ladder (M) is shown on the left (fig. 1). The plasmid vector containing gene for rat SUR1 served as positive control which generated a product as predicted for rat SUR1 (lane 1). Primer sets 1 and 3 corresponding to SUR2A and SUR2B failed to amplify any product from kidney or heart. GAP primers led to a successful amplification of a 597 bp product from rat kidney (lane 4) and heart (lane 5) demonstrating that RNA was not degraded (fig. 1).

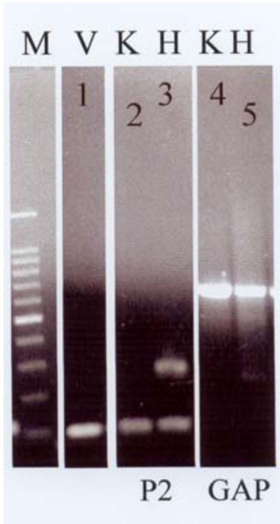

Fig. 1: Only the primer set 2 yielded a 111bp product in rat kidney (K in lane 2), while the same primers yielded two bands of 291 bp and 111 bp from rat heart (H in lane 3) consistent with the product sizes for rat SUR2A and SUR1 respectively. DNA ladder is shown on the left (M). The plasmid containing gene for rat SUR1 served as positive control which generated a product as predicted for rat SUR1 (lane 1).

Primer sets 1 and 3 corresponding to SUR2A and SUR2B failed to amplify any product from kidney of heart. GAP primers yielded a 597 bp product from both rat kidney and heart (lane 4 and 5 respectively).

Expression of SUR proteins
Neither anti-SUR2A nor SUR1 (SUR2B) antisera could detect appropriate size protein(s). However, both antisera recognized 55kD bands from lysates of total kidney and membrane fractions (fig. 2) which were not abolished by preadsorption of anti-SUR2A and anti-SUR2B (SUR1) antisera with immunizing peptides.

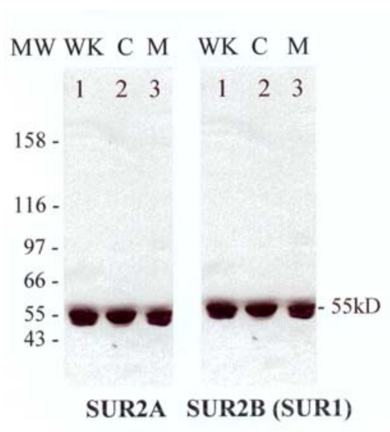

Fig. 2: Both SUR2A and SUR2B antisera detected 55kD bands in total kidney as well as cortex and medulla.

Immunolocalization of SUR
Intense immunoreactivity with anti-SUR2A antiserum was found localized to the large and smaller extraglomerular blood vessels, smooth muscles (fig. 3A-B) and a less intense but prominent staining was encountered in glomerular mesangial cells (fig. 3C). No immunoreactivity was detected in any portion of nephron epithelia. The staining was abolished in control sections incubated with preadsorbed anti-SUR2A antiserum with respective immunizing peptide (fig. 3D).

Anti-SUR2B (SUR1) immunoreactivity was localized to the distal nephron segments thick ascending limb and collecting ducts (fig. 4A) whereby, a dense and localized staining was observed in epithelia of distal tubules, cortical and both outer and inner medullary collecting ducts (fig. 4B-C). Proximal tubules also showed a low level immunoreactivity while thin limbs, glomeruli, intra or extra blood vessels did not show any labelling with this antiserum. The staining was completely abolished in control sections co-incubated with immunizing peptide for SUR2B (SUR1) antiserum (fig. 4D).

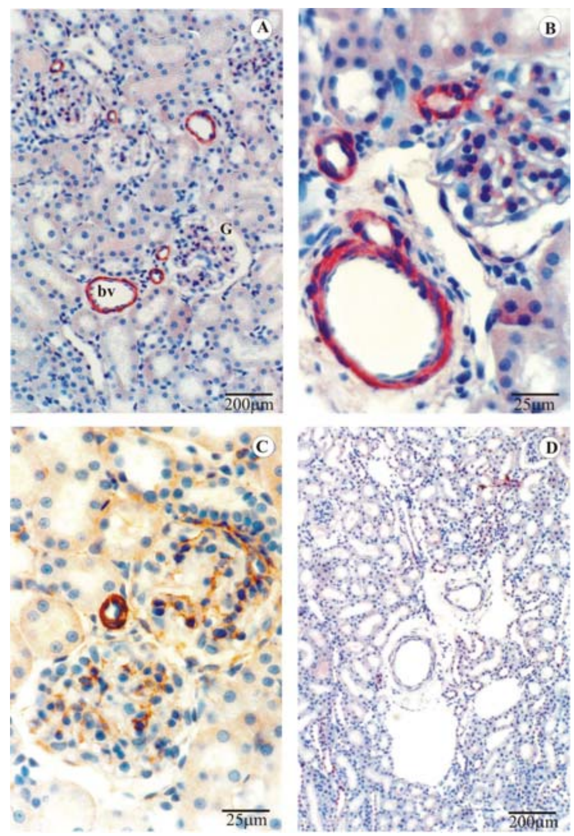

Fig. 3: Rat kidney sections immunostained with anti-SUR2A. A-B. Low and high magnification photomicrographs showing intense immunoreactivity in the extraglomerular blood vessels (bv) near glomeruli (G) and in the larger blood vessels present at some distance. C. Note intense immunoreactivity in glomerular mesangial cells. D. Control section showing abolition of immunoreactivity following preadsorption of antiserum with the immunizing peptide.

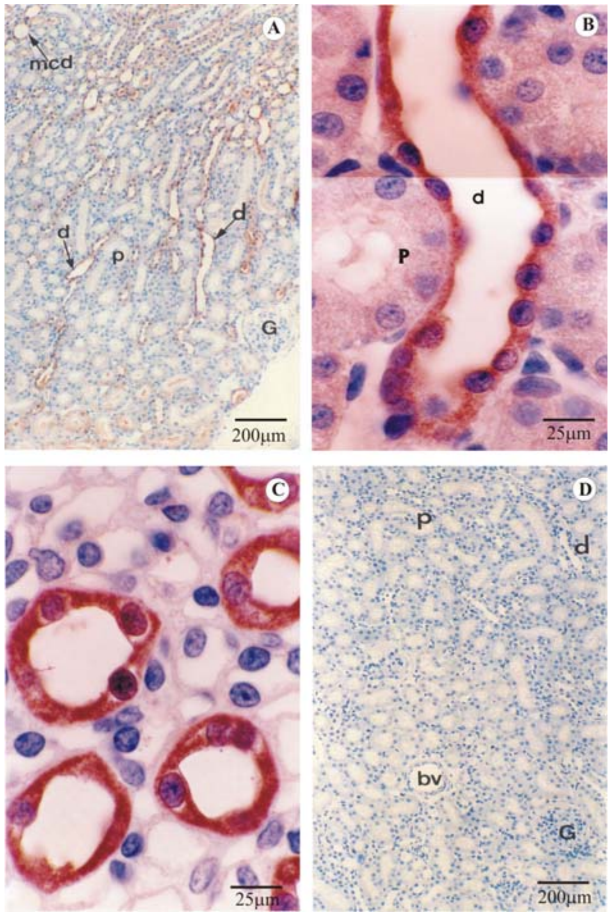

Fig. 4: Rat kidney sections immunolabelled with anti-SUR2B (SUR1). A-C. Photomicrograph taken at low and high magnification showing immunoreactivity in distal tubules (d) present near glomeruli (G) and in the medullary collecting ducts (mcd). Proximal tubules (P) also show low level staining. D. Control section showing complete abolition of immunoreactivity following adsorption of the antiserum with immunizing peptide, Bv (blood vessel).

DISCUSSION

Beesley *et al*. (1999) demonstrated that SUR2B (SUR1) is the only SUR isoform which is expressed in mouse kidney both at the level of mRNA and protein and that it is localized to the collecting ducts. Present immunocytochemistry on rat kidney sections with anti-SUR2A and anti-SUR2B (SUR1) antisera demonstrated a differential protein recognition, whereby SUR2A appears to be localized to the intrarenal blood vessels and resistance vessels the afferent and efferent arterioles; while SUR2B (SUR1) is expressed only in the distal nephron thick ascending limb epithelia and cortical and medullary collecting ducts. Thus, across species only collecting ducts represent those parts of nephron which express SUR2B (SUR1) in both mouse and rat kidney. Consistent with Isomoto *et al*. (1996) who have previously demonstrated SUR1 mRNA expression in the rat kidney; in our laboratory, RT-PCR conducted on rat kidney although demonstrated the presence of SUR1 and SUR2A mRNA but the proteins consistent with these isoforms could not be identified on Western blots. Only SUR1 specific primers could amplify a product consistent with the presence of an SUR1 isoform. In contrast in rat heart, these primers in addition to SUR1 also detected another product which was consistent with SUR2A isoform suggesting that it might have been due to oligo-dT primers because they amplify products randomly from whole of the mRNA. Moreover, Western blotting performed on rat kidney did not detect any protein(s) consistent with either SUR1 (SUR2B) or SUR2A; instead both antisera detected 55kD bands of an unknown protein in whole rat kidney, cortex and medulla. This observation is consistent with Beesley *et al*. (1999) who showed the existence of a similar 55 kD protein in mouse kidney, however its identity remained unknown.

As we performed stringent controls we are confident that the immunoreactivity observed presently was due to SUR2A and SUR2B (SUR1) and not due to background staining. Support to our observation comes from fact that the immunoreactivity appears to be cell specific and is localized to those kidney structures which are known to possess K_{ATP} channels (Wang *et al*., 1997; Giebisch and Wang, 2000) reiterating that the antisera we used recognized SUR protein (s). Double immunofluorescence using cell-specific markers should resolve cellular expression of SUR.

Our observations are little contradictory to those of Zhou *et al*. (2008) who showed mRNA transcripts for both SUR2A and SUR2B in renal epithelial cells, while SUR1 mRNA was barely detected. On immuoblots, they detected SUR2A and SUR2B in microsomal fractions. Contrary to their results, we could only detect mRNA transcript of SUR1. Zhou *et al*. (2008) also showed immunocytochemical expression of both SUR2A and SUR2B proteins to be widely distributed in renal tubular epithelial cells, glomerular mesangial cells, and the endothelium and smooth muscle of blood vessels. However, our results appear to be more precise in that they demonstrate distinct localization of SUR2A and SUR2B whereby, SUR2A was distinctively localized to mesangial cells, smaller and larger resistance vessels and smooth muscles, while SUR2B was localized only to the tubular epithelial cells.

Across species, the amino acid sequence of mouse SUR2B shares an overall 74% identity with rat SUR1 and 33% identity with mouse SUR2A but shows 97% identity with rat SUR2 but rat and mouse SUR2A are highly homologous to each other specifically in the C-terminus region (Isomoto *et al*., 1996). Sequence homologies performed by Isomoto *et al*. (1996) show that mouse

SUR2A and SUR2B are highly homologous to rat SUR2A and SUR1. Although the immunoreactivity observed presently is specific and localized, it appears difficult at this stage to attribute immunoreactivity to these isoforms. Given the existence of several splice variants of both isoforms (Bryan *et al*., 2005); involvement of other SUR isoforms sharing gene sequences is likely. Our study however stands in contrast with Li *et al*. (2003) who, by using in situ hybridization histochemistry have demonstrated in a wide variety of vasculature of different tissues of rat that it is the Kir6.1/SUR2B which is expressed in renal arteries and arterioles.

The immunoreactivity recognized by anti-SUR2A antiserum in glomerular mesangial cells of rat kidney appears to be consistent with a report that both high and low affinity SUR binding sites are expressed in rat glomeruli (Metzeger *et al*., 1997). The staining in particular in the extraglomerular blood vessels and glomerular mesangial cells seems to be of particular importance as regards renin release by mesangial cells and overall modulation of the renin-angiotensin aldosterone system by the juxtaglomerular apparatus to regulate glomerular capillary pressure. The present report also confirms observations by Asano *et al.* (1999) who demonstrated that a type 2 sulphonylurea receptor (SUR2) resides in rat mesangial cells but they did not show whether it was SUR2A or 2B. Present immunocyto-chemistry has however revealed that it quite possible that SUR2A receptor isoform is expressed in mesangial cells. Because mesangial cells bear prime importance as regards fibrogenic response during diabetic nephropathy, sulfonylureas may have beneficial effects in renal disease (Yee and Szamosfalvi, 2002). Li *et al*. (2003) however reported extremely low level expression of Kir6.1/SUR2B in renal tubular cells of rat kidney. On the other hand we observed strong immunoreativity of SUR2B in tubular cells, but only in the distal nephron segments, whereas low level staining was observed in proximal tubules with both SUR2A and 2B.

Regardless of the fact that more comprehensive and isoform specific studies are required to decipher these discrepancies, the expression of SUR2B (SUR1) as has been presently observed in distal nephron segments raises the possibility of its interaction with renal epithelial K^+ channels. The expression of SUR in the kidney further indicates the existence of K_{ATP}-SUR complexes in intrarenal blood vessels and that SUR may regulate renal hemodynamics.

Current observation as regards expression of SUR2A in resistance vessels of rat kidney is of clinical importance because the kidney is directly involved in the pathophysiology of hypertension (Ito, 1997). Moreover, treatment of non-insulin dependent diabetes mellitus with sulphonylurea drugs is known to cause hypertension via a vasoconstriction effect due to blocking of vascular K_{ATP} channels that coincidently elevates peripheral resistance (Standen, 2003) raising the possibility that the blood vessels might be the intrarenal site of action of both potassium channel inhibitors as well openers. Because vascular K_{ATP} channels normally exist in a closed state and are particularly more sensitive to potassium channel openers than to inhibitory sulphonylureas, an opening of K_{ATP} channels during cardiac ischemia or hypoxia of coronary arteries leads to K^+ efflux that results into vasodilation and a reduction in blood pressure, which ultimately decreases the kidney perfusion rate (Giebisch and Wang, 2000).

Extensive micropuncture, microperfusion and micro-catheterisation data have shown that these nephron segments play a role in regulating the final ionic composition of urine (Giebisch and Wang, 2000). Presently observed localization of SUR2B in collecting ducts together with the known data that SUR can interact with ROMK channels (Ammala *et al*., 1996b) raises the possibility that SUR may be involved in regulation of K^+ secretion by the distal nephron. It is equally possible that SUR may regulate other proteins such as epithelial sodium channels (ENaC) and CFTR (McNicholas *et al*. 1996). Since SUR is known to regulate the activity of K^+ channels in different tissues and because it is expressed in the same tubular regions that express K^+ channels sensitive to sulphonylureas, it is tempting to speculate that it may have a role in renal salt handling and/or K^+ recycling as well as K^+ secretion.

In conclusion, the present investigation on rat kidney provides the evidence that SUR isoforms (SUR2A and 2B) are differentially expressed in intrarenal blood vessels and distal nephron, and may have distinctive or overlapping expression pattern across species.

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