1-1-1999

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Recommended Citation

Asano K, Cortes P, Garvin JL, Riser BL, Rodríguez-Barbero A, Szamosfalvi B, Yee J. Characterization of the rat mesangial cell type 2 sulfonylurea receptor. Kidney International 1999; 55(6):2289-2298.

Kidney International, Vol. 55 (1999), pp. 2289–2298

Characterization of the rat mesangial cell type 2 sulfonylurea receptor

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Characterization of the rat mesangial cell type 2 sulfonylurea receptor.

Background. Sulfonylurea receptors are classified as either high-affinity type 1 (SUR1) or low-affinity type 2 receptors (SUR2), and the gene expression of SURs has recently been demonstrated in kidney. However, functional data regarding a renal SUR are lacking. We previously demonstrated that mesangial cell (MC) gene and protein expression of extracellular matrix components were up-regulated by the sulfonylurea, tolazamide. After noting this biological response, we next sought to investigate the presence of a sulfonylurea receptor in rat MCs.

Methods. Equilibrium binding studies employing [^{3}H]glibenclamide as a ligand were performed on crude MC membrane preparations. Gene expression for SUR was explored by Northern analysis of cultured MCs and whole kidney tissue. The effect of sulfonylurea on intracellular Ca^{2+} in MCs was assayed by spectrofluorometry, and glibenclamide-induced changes in the contractility of MCs were assessed.

Results. MCs bound [^{3}H]glibenclamide with a K_D of 2.6 μM and a B_{max} of 30.4 pmol/mg protein as determined by Scatchard analysis. Three SUR2 transcripts were detected in MCs. A major transcript was detected at 5.5 kb and minor transcripts at 7.5 and 8.6 kb. Following sulfonylurea treatment of MCs, real-time videomicroscopy revealed intense MC contraction, coinciding with oscillatory increments of intracellular Ca^{2+} concentration. Further evidence of sulfonylurea-induced MC contraction was demonstrated by glibenclamide-induced deformation of a silicone rubber substrate.

Conclusions. These results demonstrate that SUR2 resides on MCs. Functional activation of this receptor by sulfonylurea induces Ca^{2+} transients that result in MC contraction.

Sulfonylurea compounds, commonly used in the treatment of non-insulin-dependent diabetes mellitus [1, 2], exert an antihyperglycemic effect primarily by acting on pancreatic β cells as insulin secretagogues [3]. However, in some extrapancreatic tissues, including hepatocytes, myocytes, and adipocytes, sulfonylureas directly augment glucose uptake [4–6]. These effects and those in β cells are mediated by specific receptors that bind sulfonylurea compounds, such as glibenclamide and tolazamide. High- and low-affinity sulfonylurea receptors have been identified and are classified, respectively, as SUR1 and SUR2 [3, 7, 8]. SUR1 has been localized to cardiac, neuronal and smooth muscle tissues, brain microsomes, and pancreatic β cells.

SURs also bind the nonsulfonylurea hypoglycemic agents, linogliride and meglitinide, and several K^+-channel openers, including diazoxide, minoxidil, nicorandil, pinacidil, and cromakalim [9–12]. Recently, naturally occurring ligands for SUR, termed endosulfines α and β, have been chromatographically isolated and purified from ovine and porcine brain [13, 14]. The α isoform is structurally related to the porcine cAMP-regulated phosphoprotein. However, the function of endosulfines remains speculative.

SUR1 has been cloned recently, and its primary structure and membrane topology place it among members of the adenosine triphosphate (ATP)-binding cassette transporter/channel superfamily [15]. This diverse group of transporters totals more than 100 members. Included among these are the human P-glycoprotein (P-gp), which confers multiple resistance to chemotherapeutic agents, Pgh1, which accords chloroquine resistance to malaria-transmitting *Falciparum* species, and cystic fibrosis transmembrane conductance regulator (CFTR) [16]. All ABC transporters have two cytosolic nucleotide (ATP/ADP)-binding and multiple transmembrane-spanning domains. The entire complex confers substrate specificity to ATP-regulated solute transport. Dissimilar to other ABC transport proteins, CFTR is an ion channel, thus broadening the scope of the ABC transporter family.

The ATP-sensitive sulfonylurea-sensitive K^+ channels (K_{ATP}) in smooth muscle are comprised of two subunits.

Key words: tolazamide, glibenclamide, mesangial cell contraction, non-insulin dependent diabetes, glucose uptake, hypoglycemia.

Received for publication April 3, 1998
and in revised form November 6, 1998
Accepted for publication January 6, 1999

These are the regulatory subunit SUR and the pore-forming subunit that derives from the Kir inward rectifier gene family. Increasing the cellular ATP/ADP ratio or exposure to sulfonylurea inactivates K_{ATP} channels, thereby decreasing potassium efflux. Engagement of SUR1 by sulfonylureas on pancreatic β cells induces membrane depolarization and transiently augments Ca^{2+} influx through L-type calcium channels and accelerates insulin secretion [17–20]. Inadvertent interactions of normal pancreatic SUR1 with compounds structurally related to sulfonylurea compounds, such as sulfonamide antibiotics, have resulted in "therapeutic misadventure" with serious episodes of hypoglycemia [3]. Moreover, mutations of the SUR1 gene locus have been predictably associated with disorders of glucose homeostasis, including persistent hyperinsulinemic hypoglycemia [21–23].

The rat SUR isoform SUR2 is highly expressed in skeletal muscle and heart [24]. The murine isoform, SUR2A, is exclusively expressed by the heart, whereas the more ubiquitous SUR2B has been localized to skeletal and intestinal smooth muscle, as well as neuronal tissue. Not surprisingly, the intrinsic functions of these tissues, that is, force generation, peristalsis, and impulse conduction, are coupled to their resting membrane electropotentials [17, 25]. To date, renal K_{ATP} channel activity has been documented by Ho et al and Wang [26, 27], and SUR2 gene and protein expression in whole mouse kidney has been demonstrated by Chutkow et al [24]. We have recently observed that the sulfonylurea compound tolazamide enhances cytochalasin B-inhibitable 2-deoxyglucose uptake by mesangial cells (MCs) and gives rise to marked alterations of extracellular matrix metabolism [28]. Collectively, these data suggest that these metabolic effects of sulfonylurea may be mediated by a SUR residing on the MC surface. To explore this hypothesis, we initiated a series of experiments to characterize the putative MC SUR.

METHODS

Reagents

RPMI 1640 medium and RNA markers were purchased from Life Technologies (Gaithersburg, MD, USA). Nu-Serum and insulin-transferrin-selenium (ITS) were obtained from Becton Dickinson (Bedford, MA, USA). Glibenclamide, dimethylpolysiloxane, antibiotics, morpholino-(propane)-sulfonic acid (MOPS) buffer, and MOPS-ethylenediaminetetraacetic acid (EDTA)-sodium acetate (MESA) buffer were purchased from Sigma Co. (St. Louis, MO, USA). [^{3}H]glibenclamide (50 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were purchased from Dupont NEN (Boston, MA, USA). Bicinchoninic acid proteins assay kits were obtained from Pierce (Rockford, IL, USA). Random oligomer priming kits were obtained from Boehringer Mannheim (Indianapolis, IN, USA). Rat SUR2 cDNA (pJGAR7) was a generous gift of Dr. C. Burant (University of Chicago, Chicago, IL, USA) [24]. QuikHyb solution was obtained from Stratagene (La Jolla, CA, USA). Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). Nytran membranes were purchased from Schleicher & Schuell (Keene, NH, USA).

Tissue culture

The MCs used for all studies are from the previously characterized rat cell line $16KC_2$, originally cloned from glomerular explants of Fischer rat glomeruli [28–30]. These spindle-shaped cells form hillocks in nonconfluent tissue cultures and do not exhibit dome formation. The cells stain positively for vimentin, desmin, and α-smooth muscle actin, but not for cytokeratin or factor VIII. Cellular contraction occurs following exposure to either angiotensin II or arginine vasopressin. These cells also bear ANP receptors on their surfaces as well as the Thy-1 antigen. The SV40-transformed hamster insulinoma tumor β cell line (HIT) was obtained from the American Type Culture Collection (Cat. No. CRL-1777; Rockville, MA, USA) [31]. HIT cells have SUR1 receptors and exhibit ATP-sensitive K^+ channel activity following their exposure to sulfonylurea at nanomolar concentrations [3, 9]. The fibroblast RNA used in Northern analysis was obtained from SJL mouse cortical tubulointerstitial fibroblasts (TFBs), as previously described by Alvarez et al [32, 33]. Except where noted, MCs were seeded at a density of 1.5×10^5 cells per 150 mm diameter culture dish and were then grown for seven days to near confluence in RPMI 1640 medium containing 8 mM glucose, 20% Nu-Serum, and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). The culture medium was changed at two- or three-day intervals. HIT cells and TFB cells were passaged weekly and cultured in a similar medium, except that they contained 10% fetal calf serum (FCS) instead of Nu-Serum, as previously described [7].

Cell membrane preparation

Cells were scraped into an ice-cold homogenizing buffer (pH 7.4) containing 20 mM HEPES, 2 mM EDTA, 250 mM sucrose, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μM pepstatin, and 1 μM leupeptin. Cells were immediately homogenized by 20 strokes in a glass dounce with a matched Teflon pestle. The homogenate was centrifuged for 10 minutes at 760 *g*. The supernatant was pooled and centrifuged at 100,000 *g* for 60 minutes in a Beckman model L5-65 ultracentrifuge (Beckman Instruments, Palo Alto, CA, USA). The pelleted crude membrane preparation was resuspended in a buffer containing 20 mM MOPS and 0.1 mM PMSF, pH 7.4, and was aliquoted and stored at −70°C in preparation for binding studies. Prior to binding studies, the membrane preparation was briefly rehomogenized, and the mem-

brane protein concentration was assayed by the bicinchoninic acid method, using bovine serum albumin (BSA) as the standard.

Equilibrium binding assays

For high-affinity SUR receptor-binding studies, 200 μg protein fractions of cell membranes were incubated with [^{3}H]glibenclamide (specific activity, 50 μCi/nmol). Incubation mixtures contained final concentrations of 0 to 10 nM [^{3}H]glibenclamide, 50 mM MOPS, and 0.1 mM PMSF (pH 7.4). Incubations were carried out at 25°C for 60 minutes before their rapid termination by filtration through Whatman GF/F filters and immediate washing two times with 4 ml of chilled 80 mM MOPS (pH 7.4) containing 1% BSA. Filtration and washing were completed within 10 seconds. The amount of radioactivity retained by filters was determined by liquid scintillation counting in a Beckman model LS 3801 counter. For the high-affinity SUR receptor, nonspecific binding was determined by adding 1 μM nonradioactive glibenclamide to the incubation solution. For the low-affinity receptor, 500 μg of cell membrane samples and 0 to 4 μM [^{3}H]glibenclamide (specific activity, 2 μCi/nmol) were used, and all other steps were carried out as described earlier here. In this case, nonspecific binding was determined by adding 400 μM of nonradiolabeled glibenclamide to the incubation solution. The low solubility of glibenclamide precluded the use of higher concentrations to determine nonspecific binding. Scatchard analysis of the data was performed with commercial software (Prism vs. 2.0.3; GraphPad, San Diego, CA, USA).

Northern analysis

Total RNA was extracted from Fischer rat (Charles River Labs, Long Island, NY, USA) brain, heart, and kidney. Organs were immediately snap frozen in liquid N_2, pulverized to powder in a liquid N_2-cooled mortar, and homogenized in RNA Stat-60 (Tel-Test, Friendswood, TX, USA). RNAs were then extracted with a modified guanidinium thiocyanate method [33, 34]. Cultures of MCs and TFBs were washed twice with ice-cold Hank's buffered saline solution prior to RNA extraction. Experimental and control RNA species were vacuum dried and denatured at 70°C in 2 × MESA buffer containing 2.4 M formaldehyde, 15% formamide, 0.8 mg/ml ethidium bromide, and 10 mM EDTA. RNAs were fractionated on 1% agarose gels containing 1 × MESA and 0.4 M formaldehyde. RNA species were transferred by downward capillary action to Nytran membranes. A 4.1 kbp Eco RI restriction fragment of the rat SUR2 cDNA was radiolabeled in the presence of [α-^{32}P]dCTP by the random oligomer priming method [32]. Membranes were prehybridized at 68°C for 20 minutes in QuikHyb solution. Hybridization was carried out at 68°C for 14 hours (1×10^6 cpm/ml). Membranes were then washed twice in 2 × SSC (sodium chloride sodium citrate: 0.3 M sodium chloride and 0.03 M sodium citrate) containing 0.1% sodium dodecyl sulfate for 15 minutes at 25°C and once in 0.1 × SSC containing 0.1% sodium dodecyl sulfate for 30 minutes at 60°C. Autoradiography with intensifying screens was carried out for 16 to 72 hours at −70°C.

Spectrofluorometric measurement of Ca^{2+}

Mesangial cells were plated on cover slips and cultured for three to four days until a confluence of 60 to 70% was achieved. A 10 μM solution of Fura-2 AM dye in dimethylsulfoxide (DMSO) was added to a perfusion solution containing 120 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 2 mM Na_2HPO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 8 mM glucose, and 1% FCS, pH 7.4. MCs were loaded with dye and then placed in an environment of 95% air and 5% CO_2 for 60 minutes at 37°C. After incubation, cover slips were placed for 20 minutes in a temperature-regulated chamber and mounted on a Diaphot-TMD inverted microscope (Nikon, Tokyo, Japan) that was continuously irrigated with the perfusion solution at 1 ml/min. The intensity of fluorescence was measured during alternating excitations of the fluor at 340 and 380 nm wavelengths. Fluorescent images were filtered by a high-pass 510 nm filter, and measurements were conducted at 20-second intervals. Autofluorescence of cells was negligible. Fluorescence was digitally acquired with an image intensifier at a magnification of ×100 (Video Scope International, Herndon, VA, USA) and a CCD camera (Hamamatsu, Hamamatsu City, Japan). Digitized images of the spectrofluorometric ratio $A_{340/380}$ were analyzed by the Image One MetaFluor system (Universal Imaging, West Chester, PA, USA). After three control measurements, 10 mM of glibenclamide in DMSO was added to the perfusion solution to achieve a final concentration of 3 μM within five minutes. An increase in the fluorescence ratio $A_{340/380}$ signified an increase of the intracellular Ca^{2+} concentration [35, 36].

Silicon membrane preparation

A thin silicon rubber substratum for cell contraction experiments was prepared using a modification of the protocol of Harris, Wild, and Stopak [37]. One hundred microliters of dimethylpolysiloxane (60,000 cS) were applied as a thin layer to the surface of round glass cover slips (18 mm diameter). Grid markings were engraved on these coverslips to assist in the identification and location of cultured MCs. To polymerize the substrate, the dish was placed in a Hummer V sputter coater chamber (Technics Inc., Baltimore, MD, USA), 4 cm from a gold-palladium target [38, 39]. Air was evacuated from the chamber by gradual application of vacuum to 0.1 torr. The vacuum was continued at this level until air bubbles were no longer present on the surface of the

substrate. Next, the sputter coating chamber was flushed with argon gas until a pressure of 0.2 torr was achieved. A vacuum was gradually reapplied until the pressure declined to 0.1 torr, and this sequence was repeated a total of three times. Argon plasma was produced by the introduction of gas into the chamber during application of a steady 20 mA current for 16 to 20 seconds.

Mesangial cell contraction

Mesangial cells were grown on the coated coverslips described earlier at a density of 2×10^4 cells/cm^2 in RPMI 1640 containing 20% Nu-Serum for two days. Twenty-four hours before experiments, the culture media were replaced with RPMI 1640 containing 0.1% FCS and an ITS culture supplement to provide per liter the following: 6.3 mg insulin, 6.3 mg transferrin, 6.3 μg selenious acid, 1.3 g bovine serum albumin, 5.4 mg linoleic acid. Prior to the experiments, cells were incubated for 60 minutes in a perfusion solution similar to that used during intracellular Ca^{2+} measurements, but containing 0.1% FCS. For experiments, coverslips were placed in a temperature-regulated observation chamber and observed with a Hoffman contrast-modulation optical system. Images were obtained at a magnification of ×100 with a CCD camera and a videocassette recorder (Sony Corp., Tokyo, Japan). The temperature and pH of the perfusion solution were 37.0 ± 0.5°C and 7.45 ± 0.05, respectively. In eight independent experiments, 30 to 50 cells per experiment were located for observation with help of the coverslip grid markings. The perfusion solution in the chamber was then replaced with the same solution, but containing 5 μM glibenclamide or the vehicle used for its dissolution (DMSO). After 20 minutes, images of the same cells were recorded again. A change in the cell contractile force was determined by an increase or decrease in the number of wrinkles surrounding each cell. Each cell was then classified as having either "contracted," "no change," or "relaxed" from its original morphology, according to the appearance of wrinkles in the neighboring substratum. In addition, during the experimental period some cells underwent detachment. Differences between the numbers of cells classified in each category, before and after exposure to glibenclamide or its vehicle, were analyzed by a two-tailed unpaired *t*-test. Comparisons were considered significant at the level of $P < 0.05$.

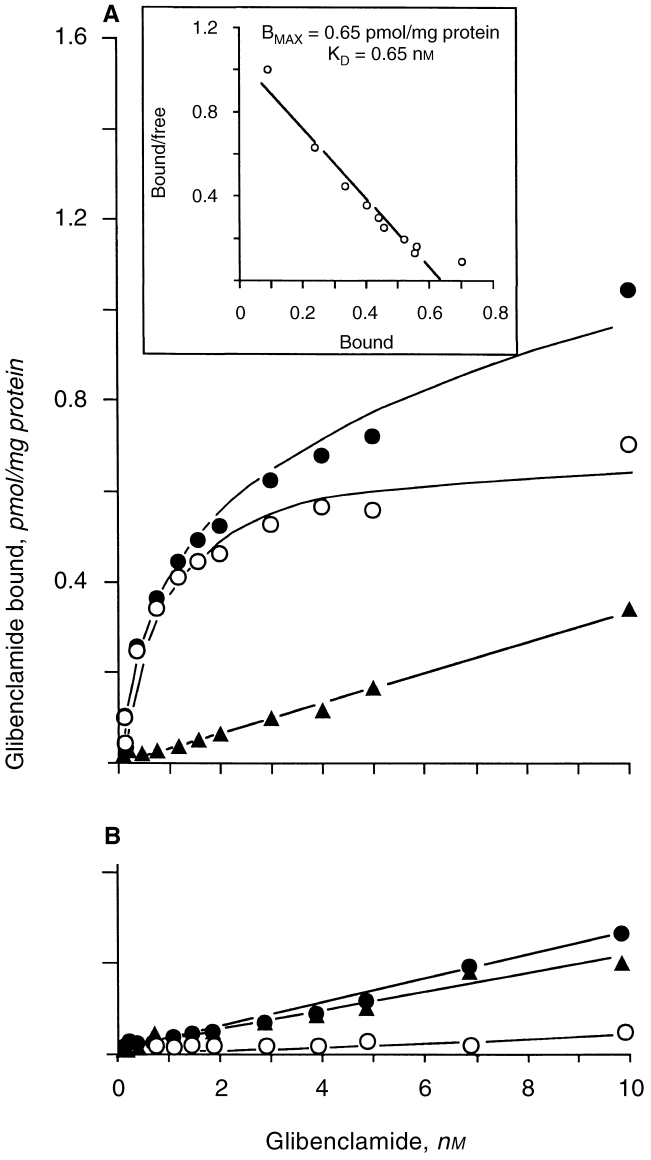

Fig. 1. Identification of a high-affinity, hamster insulinoma tumor β cell line (HIT) cell sulfonylurea receptor. Two hundred micrograms of HIT and mesangial cell (MC) membrane proteins were incubated at 25°C for 60 minutes with increasing amounts of [^{3}H]glibenclamide at a constant specific activity of 50 μCi/nmol during equilibrium radioligand binding studies. The results of a representative experiment are shown ($N = 2$). Specific binding of ligand (○) was calculated following the subtraction of nonspecific binding (▲) from total binding (●). Specific saturable binding of glibenclamide to HIT cell membranes is depicted (*A*) with a Scatchard plot of the binding data (*inset*). Nonspecific binding to MC membranes (*B*) is evident over the concentration range shown ($N = 2$). However, no specific binding (○) to MC membranes is demonstrated.

RESULTS

Identification of sulfonylurea receptors

To determine whether sulfonylurea receptors were present on MCs, we performed equilibrium binding studies on crude membrane preparations. Using pancreatic HIT cells as a positive control, we first validated our methodology by demonstrating the binding of [^{3}H]glibenclamide to HIT membranes ($N = 2$). At nanomolar concentrations of sulfonylurea, specific and saturable binding of the radioligand to HIT membranes was documented over the concentration range of 0 to 10 nM (Fig. 1A). Scatchard analysis of these data established an apparent K_D of 0.65 nM and B_{max} of 0.65 pmol/mg protein,

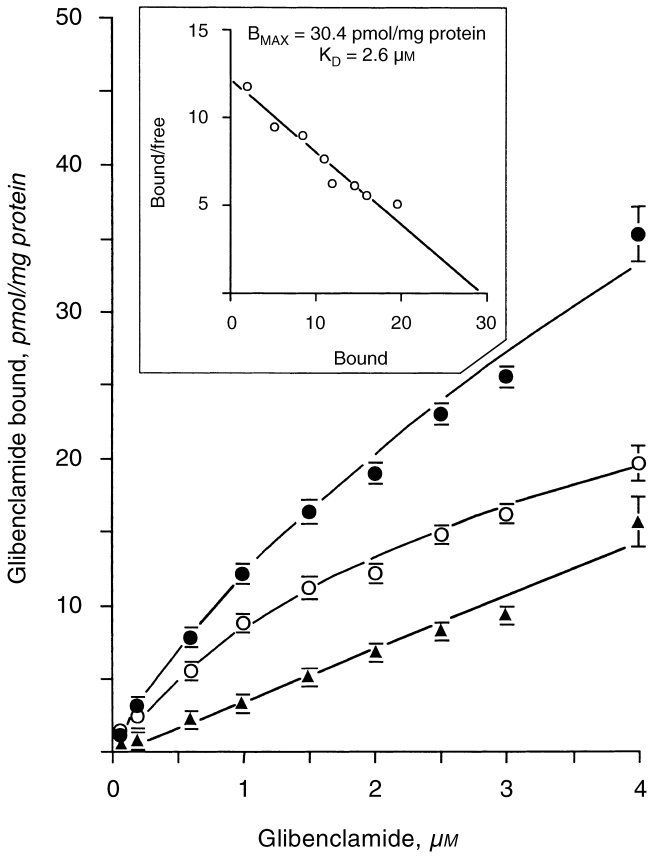

Fig. 2. Identification of a low-affinity mesangial cell sulfonylurea receptor. Five hundred microgram samples of MC membrane proteins were used for binding studies, using increasing concentrations of glibenclamide according to the methodology described in Figure 1, except that the specific activity of the sulfonylurea was 2 μCi/nmol. Total binding (●), nonspecific binding (▲), and specific saturable binding (○) of [^{3}H]glibenclamide to MC membranes are shown. A Scatchard plot of the binding data and apparent values for binding parameters are shown (*inset*). Each data point represents the pooled results obtained in four independent experiments. In each experiment, samples were tested in duplicate. Values are expressed as means ± SE. Except for the lowest amount tested (0.2 μM), differences between specific and nonspecific binding are statistically significant ($P < 0.05$) at all concentrations.

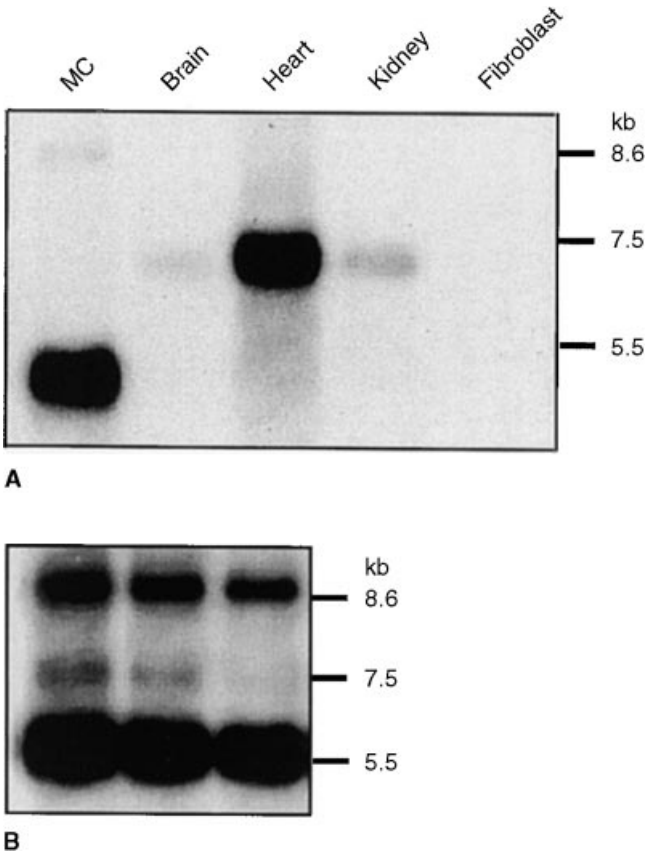

Fig. 3. Mesangial SUR2 gene expression. In Northern analysis, 20 μg quantities of total RNAs extracted from rat MCs, brain, heart, and kidney and mouse kidney fibroblasts were probed with rat SUR2 cDNA (*A*). In brain, heart, and kidney, hybridization at 7.5 kb was detected after 16 hours of autoradiography. In MCs, a major transcript appeared at 5.5 kb and a minor transcript at 8.6 kb. SUR2 transcripts were not detected in renal fibroblasts. In a separate experiment (*B*), after 72 hours of autoradiographic exposure, we reproducibly hybridized three mesangial transcripts at 5.5, 7.5, and 8.6 kb with the rat SUR2 cDNA used in (A).

values consonant with those previously reported for high-affinity SUR receptors (Fig. 1A, inset). To establish the presence of a high-affinity SUR in MCs, we performed similar binding studies on MC membranes. However, in contradistinction to our HIT cell results, there was no specific or saturable binding of radioligand to MC membranes at the nanomolar concentrations tested (Fig. 1B). To determine whether a low-affinity SUR receptor resided on MCs, we subsequently increased final glibenclamide concentrations to 0.2 to 4.0 μM. Apparent equilibrium binding was attained within 30 minutes of incubation and did not change afterward (unpublished data). In four independent experiments in which samples were tested in duplicate, we demonstrated specific binding of glibenclamide to MC membranes (Fig. 2). The Scatchard analysis of binding data yielded an apparent KD of 2.6 μM and a B_{max} of 30.4 pmol/mg protein (Fig. 2, inset).

SUR2 gene expression

To determine whether the SUR2 gene was expressed by rat MCs and whether this receptor was homologous to that previously described in extrapancreatic tissues, we probed total RNAs extracted from cultured MCs and rat brain, heart, and kidney. In Northern analysis, we probed for the SUR2 message with a 4.1 kbp Eco RI fragment of the rat SUR2 cDNA. Under high-stringency conditions, we demonstrated hybridization to a 7.5 kb transcript from whole brain and kidney, after autoradiography for 16 hours (Fig. 3A). However, this hybridization signal was much stronger in cardiac tissue. In total RNA from MCs, a signal equal in intensity to that of the heart 7.5 kb transcript was apparent at 5.5 kb, and a fainter

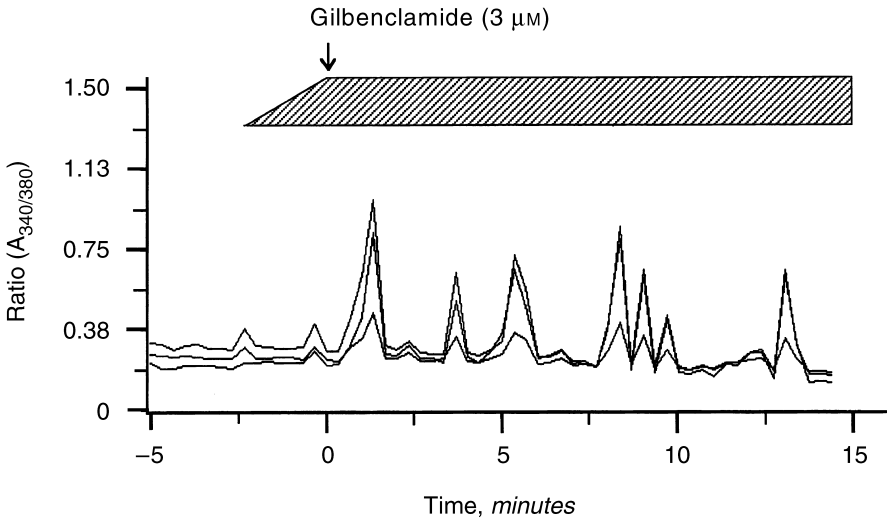

Fig. 4. Glibenclamide-induced Ca^{2+} transients in mesangial cells. The changes in the mesangial cell fluorescence ratio $A_{340/380}$ over a 15-minute time course are shown. Following loading of subconfluent mesangial cell cultures with fura-2 dye, cells were incubated at 37°C in an incubation/observation chamber irrigated by a perfusion solution containing 1% FCS. A representative experiment is shown in which three cells were marked, and then spectrofluorometric measurements were obtained after the addition of glibenclamide to the perfusion reservoir at $t = -5$ minutes. At $t = -2.5$ minutes, small initial spikes representing increases in Ca^{2+} concentration were demonstrated in all three cells, as glibenclamide entered the incubation/observation chamber. Glibenclamide concentration in the chamber's incubation solution reached a maximum 2.5 minutes later at $t = 0$ min (*arrow*). Ca^{2+} transients in all observed cells oscillated synchronously at two- to three-minute intervals following exposure to glibenclamide until the end of the observation period.

signal was detected at 8.6 kb. In addition, because fibroblasts share phenotypic similarities to activated MCs, we attempted to discern if there was SUR2 gene expression by TFB. SUR2 transcripts were undetectable from these renal fibroblasts, despite probing up to 40 μg of total RNA [32, 33]. Following 72 hours of autoradiography, we reproducibly detected two minor transcripts at 7.5 and 8.6 kb in replicate studies (Fig. 3B).

Glibenclamide-induced elevation of intracellular Ca^{2+}

Because cell depolarization is associated with SUR receptor binding and this results in the generation of intracellular Ca^{2+} ion transients in pancreatic β cells, we hypothesized that exposure of MCs to sulfonylurea would induce alterations of their intracellular Ca^{2+} levels. To explore this issue, we treated fura 2-loaded MCs with either a therapeutically relevant concentration of glibenclamide (3 μM) or the vehicle used to dissolve it and then continuously monitored their respective fluorescence ratios ($A_{340/380}$) in a perfusion/observation chamber over a period of 15 minutes. Glibenclamide, incorporated into the perfusate's reservoir, reached the perfusion/observation chamber within 2.5 minutes, achieving its maximum concentration of 3 μM 2.5 minutes later. Coincident with the initial appearance of glibenclamide in the observation chamber, oscillations in the Ca^{2+} concentration were apparent (Fig. 4). As glibenclamide reached its final maximal concentration of 3 μM, intracellular Ca^{2+} concentrations markedly increased simultaneously in all cells under observation (Fig. 4). These elevations returned to baseline after approximately 60 seconds. Thereafter, oscillatory elevations of intracellular Ca^{2+}, as indicated by an increase in their respective fluorescence ratios, occurred at two- to three-minute intervals, the magnitude of elevation varying among cells. Notably, changes in intracellular Ca^{2+} were frequently accompanied by alterations of cell morphology due to cell contraction (unpublished data). In some instances, the cells contracted with sufficient force to cause their total detachment from the coverslip.

Mesangial cell contraction

To demonstrate further that MC contraction was attributable to Ca^{2+} elevations mediated through sulfonylurea stimulation, we assessed the tensile forces generated by cells cultured on the silicon rubber substrate. In these cultures, tensile forces generated at points of MC adhesion are visually evident as a series of wrinkles in the elastic substrate. Exposure of MCs to glibenclamide at a final concentration of 5 μM resulted in cell contraction, evident as "new" wrinkle formations (Fig. 5), in a significant number of cells, as compared with vehicle-treated cells in which only infrequent spontaneous contractions were detected (39 vs. 6%; Fig. 6). In addition, a significant number of cells underwent detachment in the presence of glibenclamide (Fig. 6). As previously observed during the intracellular Ca^{2+} studies, this detachment is likely related to the generation of large increments in tensional forces by some cells. Only a few cells in either vehicle- or glibenclamide-treated groups demonstrated relaxation from their baseline states.

DISCUSSION

These studies represent the initial demonstration of a functional sulfonylurea receptor in MCs. We have shown

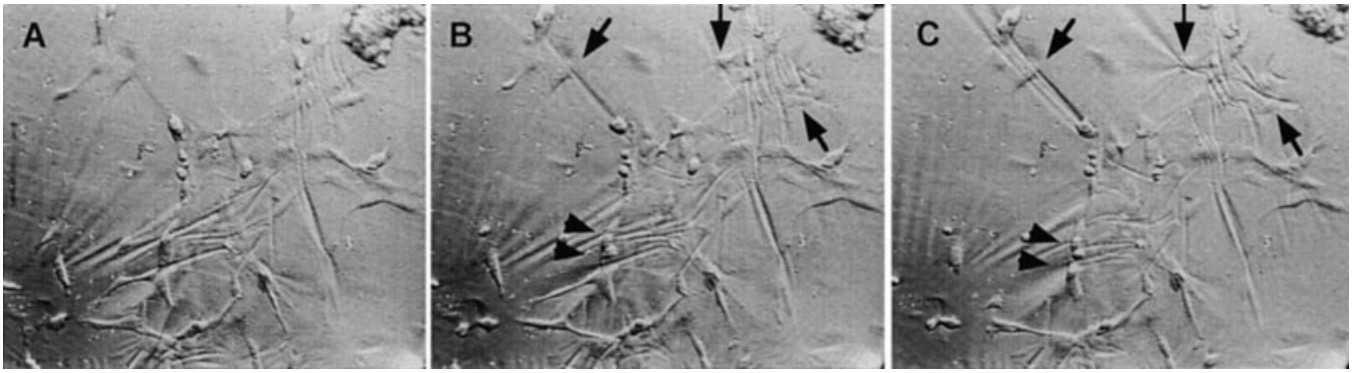

Fig. 5. Glibenclamide-induced mesangial cell contraction. Subconfluent cultures of mesangial cells were grown on polymerized dimethylpolysiloxane membranes and were then serum deprived for 24 hours at 37°C. Cells were then perfused with glibenclamide in 0.1% FCS to achieve a final concentration of 5 μM during continuous videomicroscopic recording. Cell contractions (arrowheads) and new glibenclamide-induced wrinkle formations that deformed the silicon rubber substrate (arrows) are shown at 0 (*A*), 10 (*B*), and 20 minutes (*C*).

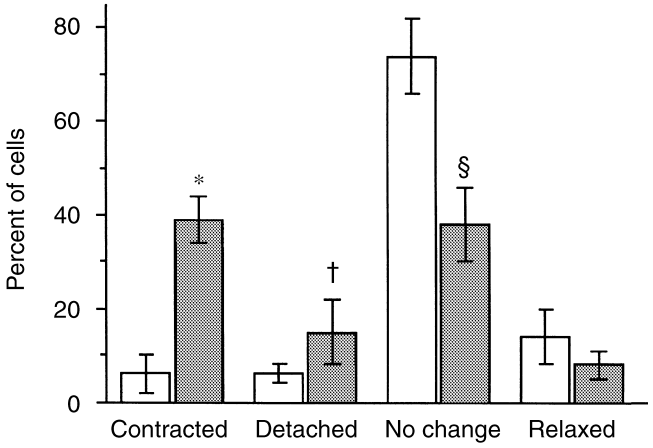

Fig. 6. Degree of glibenclamide-induced mesangial cell contraction. Cells were grown on grid-marked cover slips coated with a dimethylpolysiloxane substrate under the same conditions as in Figure 5. In each experiment, the exact locations of 30 to 50 cells were identified, and the corresponding degree of wrinkling produced by each cell was quantitated at baseline and after 20 minutes of exposure to 5 μM glibenclamide ($N = 8$). Control (□, $N = 5$) and glibenclamide-treated cells (■, $N = 8$) were classified as contracted, detached, no change, or relaxed after comparison to baseline. *$P < 0.05$; †$P < 0.005$; §$P < 0.002$.

that MC membrane preparations bind glibenclamide at concentrations typical for those of lower affinity SURs [3, 8]. The binding kinetics imply that receptor binding may occur at the micromolar concentrations that may be encountered during therapeutic administration of sulfonylurea compounds [40]. In this study, gene expression of the putative mesangial SUR2 was constitutive, and highly abundant levels of a 5.5 kb transcript were detected by Northern blotting. In addition, the functionality of this mesangial SUR2 is exemplified by the oscillatory Ca^{2+} transients and intense cell contraction that occur following the exposure of MCs to glibenclamide.

The detection of competitive binding of sulfonylurea agents to membrane preparations from various tissues has been used extensively to demonstrate and characterize the presence of SUR receptors in these tissues. SUR1 receptor is a high-affinity 140 kDa receptor with K_D of 0.05 to 1.0 nM. SUR2 is a 170 kDa receptor with an apparent K_D of 0.6 μM for glibenclamide [7, 8]. Northern analysis of rat tissues has documented constitutive expression of a 7.5 kb SUR2 transcript in heart, skeletal muscle, kidney, brain, and testis [24]. In addition, a SUR2 isoform has more recently been localized in rat brain by Inagaki et al [8]. The 5.5 kb transcript detected in our studies has not been previously described in rat, although a homologue may exist in human skeletal muscle that expresses transcripts of 5.6, 8.6, and 9.4 kb [24]. Our results suggest that the rat mesangial SUR2 represents an alternatively spliced variant of SUR2 that has not been previously described in kidney. The differential gene expression of the mesangial 5.5 kb transcript, in comparison to those found in whole rat kidney, insinuates that there is differential gene regulation of SUR2 among different nephronal compartments.

Characteristically, ligation of sulfonylureas by their receptors closes K_{ATP} channels [3, 8, 12]. By contrast, members of this channel family are activated by potassium channel openers such as diazoxide and pinacidil that are operative at a locus distinct from the binding site of sulfonylureas [9, 10]. Physiologically, K_{ATP} channels are reversibly inhibited by increasing cellular energetics, that is, elevated intracellular (ATP/ADP) ratio. Therefore, channel activation and ion transport are functionally linked to cellular metabolism. Currently, SURs and Kir6.0-type K^+ channels, respectively, comprise the regulatory and ion pore permeation subunits of a structurally and functionally engaged hetero-octameric K_{ATP} complex $(Kir6.2:SUR)_4$ [41, 42]. Evidence for a func-

tional interaction between Kir and SUR is found in studies involving Kir6.2 where C-terminus truncation mutants exhibit ATP sensitivity, but neither sulfonylurea (tolbutamide) nor diazoxide sensitivity in the absence of SUR1 coexpression [43]. Furthermore, Inagaki et al generated a glibenclamide-inhibitable potassium current in CV-1 origin of SV40 (COS) cells, presumably following generation of K_{ATP} channel activity (K_i = 1.8 nM) after functional coexpression of Kir6.2 and SUR1 [43]. Moreover, the authors also noted the presence of distinctive K_{ATP} channel activity following reconstitution of Kir6.2 and SUR2A [8].

Multiple K^+ channels have been described in the kidney. Among these are the Kir1.1/ROMK channels found in the distal nephron [44]. However, dissimilar to classic K_{ATP} channels, the Kir1.1 channel subfamily does not exhibit ATP sensitivity. Interestingly, Ämmälä et al conferred sulfonylurea sensitivity to HEK 293 cells by coupling SUR1 to either Kir1.1 or Kir6.1, with a consequent blockade of whole-cell currents following exposure to glibenclamide, implying the promiscuous nature of such receptor–channel associations [11]. In addition, SUR2B may also form an ATP-regulated, sulfonylurea-inhibitable complex after cotransfection with Kir6.1 [43]. Although the notion that mesangial SUR2 is functionally coupled to an inwardly rectifying K^+ channel may be extrapolated from our results, the exact nature of such a channel in MCs remains speculative.

The influx of Ca^{2+}, perhaps through opening of voltage-gated L-type channels, is coupled to cell contraction in our studies. However, the cytosolic Ca^{2+} elevations may not solely originate from entry of extracellular Ca^{2+} but may also derive from intracellular or nucleoplasmic stores [36, 45–49]. This speculation may be particularly relevant because sulfonylurea binding to an intracellular membranous component has been demonstrated, in addition to its known plasma membrane binding [3]. Oscillations of intracellular Ca^{2+} similar to those recorded in our experiments have been previously confirmed in hepatocytes, pancreatic cells, myocytes, and MCs [50–53]. In the latter, the frequency of such transients has been diminished by arginine vasopressin administration and augmented via phorbol ester-induced protein kinase C activation [53]. Aside from their obvious effects on intracellular Ca^{2+}, the exact mechanisms through which sulfonylureas induce their functional and metabolic effects on MCs remain unclear. It has been surmised that the modulation of K_{ATP} channel activity occurs by alternation of key phosphorylation/dephosphorylation sites on inwardly rectifying K^+ channels [44, 54].

In the aggregate, we have demonstrated the presence of a membrane-associated sulfonylurea-binding protein in which the binding characteristics parallel those of the SUR2 subclass of ABC channel/transporters. Ligation of mesangial SUR2 by glibenclamide mediates cell contraction via elevations of intracellular Ca^{2+}. The importance of this newly described receptor and its pathophysiological relevance will require further studies to elucidate whether SURs participate in other MC functions, including regulation of extracellular matrix metabolism. Nevertheless, it is intriguing to speculate the role that sulfonylureas might play in diabetic renal disease, wherein glomerular hyperfunction has been considered a significant contributor to the development of glomerulosclerosis. Because this hyperfunction is attributed, in part, to diminished mesangial contractility, the restoration and normalization of MC contractility by sulfonylureas may exert a salutary effect on glomerular injury [55]. The question of whether the summation of metabolic and functional consequences of sulfonylurea administration is beneficial or deleterious in the evolution of diabetic renal disease represents an appealing avenue of future inquiry.

ACKNOWLEDGMENTS

Dr. Pedro Cortes was supported in part by the National Institutes of Health (Grant No. RO1 DK2081). Dr. Kenichiro Asano was supported by the National Kidney Foundation of Michigan (Grant No. 1F). Dr. Jeffrey L. Garvin was supported in part by the National Institutes of Health (Grant Nos. B10514 and HL 28982) and is a recipient of a Research Career Development Award (HL 02891). A portion of this study was presented at the American Society of Nephrology, San Antonio, Texas, USA (November 1997) and the Third Annual Astra Merck Young Investigators' Forum, Washington, D.C., USA (September 1997). We also wish to acknowledge the expert technical assistance of Dr. Betty Liu and Mr. Wayne Pitchford in the performance of mesangial contraction experiments.

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