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Pharmacological and molecular characterization of ATP-sensitive K^+ channels in the TE671 human medulloblastoma cell line

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Abstract

ATP-sensitive K^+ (K_{ATP}) channels in the human medulloblastoma TE671 cell line were characterized by membrane potential assays utilizing a potentiometric fluorescent probe, *bis*-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$), and by mRNA analysis. Membrane potential assays showed concentration-dependent and glyburide-sensitive changes in fluorescence upon addition of (−)-cromakalim, pinacidil, diazoxide and P1075. The rank order of potency for these openers was P1075 > (−)-cromakalim ~ pinacidil > diazoxide. Additionally, glyburide and glipizide inhibited P1075-evoked responses in TE671 cells with half-maximal inhibitory concentrations of 0.22 and 14 μM, respectively. The rank order potencies of both openers and inhibitors were similar to those observed in the rat smooth muscle A-10 cell line. In contrast, in the rat pancreatic insulinoma RIN-m5F cell line, only diazoxide was effective as an opener. Reverse transcription–polymerase chain reaction (RT–PCR) studies detected sulfonylurea receptors SUR2B and SUR1 mRNA in TE671 cells whereas only SUR2B and SUR1 mRNA were, respectively, detected in A-10 and RIN-m5F cells. The inward rectifier Kir6.2 mRNA was detected in all three cell types whereas Kir6.1 was detected only in A-10 cells. Collectively, the molecular and pharmacologic studies suggest that K_{ATP} channels endogenously expressed in TE671 medulloblastoma resemble those present in the smooth muscle.

Keywords: K^+ channel, ATP-sensitive; TE671 medulloblastoma cell; Glyburide; Sulfonylurea receptor; K^+ channel, inward rectifying

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels, members of the inward rectifier K^+ channel subfamily, couple the metabolic state of the cell to membrane potential and thereby membrane excitability. These channels, inhibited by micromolar concentrations of intracellular ATP, were first identified in the heart (Noma, 1983). Subsequently, K_{ATP} channels have been reported in numerous tissues including the brain, pancreas, skeletal and smooth muscles (Ashcroft and Ashcroft, 1990; Quayle et al., 1997). For example, in pancreatic β-cells K_{ATP} channel inhibition by intracellular ATP and accompanying depolarization lead to activation of voltage-gated calcium channels and secretion of insulin (Ashcroft and Rorsman, 1989; Bryan and Aguilar-Bryan, 1997; Rorsman, 1997). The function of K_{ATP} channels can also be modulated pharmacologically by K^+ channel openers including cromakalim, nicorandil, pinacidil and diazoxide, and by sulfonylurea inhibitors such as glyburide, glipizide and tolbutamide (Gopalakrishnan et al., 1993; Lazdunski, 1994).

Recent molecular cloning and heterologous expression studies have revealed that the K_{ATP} channel is a heteromeric complex composed of at least two subunits: an inward rectifier channel belonging to the Kir6.0 subfamily and the sulfonylurea receptor (SUR), a member of the ATP-binding cassette superfamily (Inagaki et al., 1995; Clement et al., 1997). The sulfonylurea receptors SUR1, SUR2A and SUR2B in combination with the Kir6.2 subunit form K_{ATP} channels typical of pancreatic β-cell, cardiac/skeletal and smooth muscle, respectively, whereas the SUR2B-Kir6.1 combination resembles the nucleotide

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diphosphate-dependent smooth muscle K^+ channel (Inagaki et al., 1995, 1996; Isomoto et al., 1996; Yamada et al., 1997). The observation that channels containing either SUR1 and SUR2B, but not SUR2A, are activated by diazoxide suggests that the highly homologous C-terminal 42-amino acid residues of SUR1 and SUR2B may be important for activation by diazoxide (Isomoto et al., 1996). In contrast, compounds such as pinacidil and (−)-cromakalim activate both SUR2A-Kir6.2 and SUR2B-Kir6.2 subunit combinations (Inagaki et al., 1996; Isomoto et al., 1996). SUR1- and SUR2-containing channels are also distinguished by their differential sensitivities to glyburide (Inagaki et al., 1995, 1996; Isomoto et al., 1996). These observations collectively suggest that the tissue-selective pharmacology may largely be attributed to ligand interactions with the SUR subunit of the K_{ATP} channel.

In contrast to pancreatic and cardiovascular K_{ATP} channels, neuronal K_{ATP} channels are less clearly defined. Several reports have demonstrated the presence of K_{ATP} channels in the brain that exhibit diverse pharmacologic properties distinct from those described in pancreas and vascular tissues (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990; Freedman and Lin, 1996). Previous studies have shown that glyburide-sensitive $^{86}Rb^+$ efflux can be evoked in the human medulloblastoma TE671 following metabolic inhibition by 2-deoxy glucose and oligomycin treatment (Daniel et al., 1991) suggesting the presence of a K_{ATP} channel. In this study, we have utilized membrane potential assays and mRNA analysis techniques to examine in detail the pharmacology and molecular composition of native K_{ATP} channels of the TE671 human medulloblastoma cell line. Further, comparisons have been made with K_{ATP} channels endogenously expressed in rat smooth muscle A-10 and rat insulinoma RIN-m5F cells.

2. Materials and methods

2.1. Materials

The human TE671 medulloblastoma, rat A-10 smooth muscle and rat RIN-m5F pancreatic insulinoma cell lines were purchased from American Type Tissue Collection (Rockville, MD). Culture media and supplements were purchased from Life Technologies (Gaithersburg, MD). Diazoxide, pinacidil, glyburide and glipizide were purchased from RBI (Natick, MA) or Sigma (St. Louis, MO). (−)-Cromakalim, P1075 and Bay X 9228 were synthesized at Abbott Laboratories, Abbott Park, IL. Stock solutions of compounds were prepared in 100% dimethylsulfoxide (DMSO) and diluted in buffer prior to use. *Bis*-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$) was purchased from Molecular Probes (Eugene, OR). Black 96-well plates with clear bottoms were purchased from Packard Instruments (Meriden, CT). RNA isolation and SuperScript II cDNA synthesis kits were purchased from Life Technologies. Rat and human poly $(A)^+$ RNA were from Clontech (Palo Alto, CA). Taq polymerase was purchased from Perkin Elmer (Norwalk, CT).

2.2. Cell culture

TE671 and A-10 cells were cultured in media (Dulbecco's Modified Eagle Medium with high glucose, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, 0.25 μg/ml amphotericin B) supplemented with 10 and 20% heat inactivated fetal bovine serum, respectively. RIN-m5F cells were cultured in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum. Cultures were maintained in a humidified cell incubator containing 5% CO_2 at 37°C. For membrane potential assays cells were plated in black 96-well plates with clear bottoms and cultured to form a confluent monolayer.

2.3. Membrane potential assays

Confluent monolayers of cells cultured in 96-well plates were rinsed twice with 200 μl assay buffer (mM: *N*-2-hydroxyethylpiperazine-*N*′-[2-ethanesulfonic acid] (HEPES), 20; NaCl, 120; KCl, 2; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 5; pH 7.4 at 25°C) containing 5 μM $DiBAC_4(3)$ and incubated with 180 μl of the buffer in a CO_2 incubator for 30 min to ensure equilibrium of $DiBAC_4(3)$ distribution across the plasma membrane. The cell culture plate was then transferred to the temperature-controlled (37°C) compartment of the Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices, Sunnyvale, CA) where $DiBAC_4(3)$ fluorescence can be measured from the 96 wells simultaneously at excitation and emission wavelengths of 488 and 520 nm, respectively (Schroeder and Neagle, 1996). Assay runs were initiated to collect temporal updates of fluorescence at 30 s intervals for 5 min prior to and for at least 25 min after simultaneous addition of test compound to each of the 96 wells. All assays were carried out at 37°C. In studies where sensitivities to sulfonylurea analogs were evaluated, additions of K^+ channel openers and inhibitors were made simultaneously.

Changes in fluorescence evoked by test compounds were normalized to the response evoked by a reference opener, P1075 (10 μM). Sigmoid curves were fitted to concentration-response data by nonlinear regression analysis using the four parameter logistic equation (GraphPad Prism, San Diego, CA) to obtain EC_{50} or IC_{50} values as appropriate. Wherever plateau concentration response curves were not attainable, the EC_{50} values were estimated. Data are expressed as the mean ± S.E.M.

2.4. RNA preparation and analysis

Cells (typically 10×10^6) were harvested by trypsinization and washed once with phosphate buffered saline prior to RNA isolation. Total RNA from TE671, A-10 and

Table 1
Sequences of oligonucleotides used in RT–PCR studies

Subunit	Species	Primer sequence (location)	Size (bp)
Kir6.1	Human	F: 5′-GACGGAGAGGCAGGTGAGAG-3′ (−53– − 34)	
		R: 5′-CTGGATGCGCACAGAGGCAC-3′ (684-665)	737
	Rat	F: 5′-GGGCTATGCTGAAAGGAAG-3′ (−19– − 1)	660
		R: 5′-TCACCCACCCGGAACATGAAG-3′ (641–621)	
Kir6.2	Human	F: 5′-ATGCTGTCCCGCAAGGGCATC-3′ (1–21)	636
		R: 5′-GCTGATGATCATGCTCTTGC-3′ (636–617)	
	Generic	F: 5′-GGCTCCTAGTGACCTGCACCA-3′ (810–830)	316
		R: 5′-CCACAGCCACACTGCGCTTGCG-3′ (1126–1105)	
SUR1	Human	F: 5′-GCGTGCAAAAGCTAAGCGAG-3′ (1817–1836)	134
		R: 5′-GACGCTTGCGGTTCACAAC-3′ (1951–1933)	
	Rat	F: 5′-GCGACTCTGCTTGGCCTTCG-3′ (792–811)	229
		R: 5′-GAAACTGTGTCTTGGGCTGG-3′ (1021–1002)	
SUR2A/B	Generic	F: 5′-GCTGAAGAATATGGTCAAATCTC-3′ (4278–4300)	A: 451
		R: 5′-CGGAGTGTCGTATTCCAAAATA-3′ (4590–4569)	B: 312

F and R represents forward and reverse directions, respectively. The location(s) are indicated considering the ATG codon at position 1.

RIN-m5F cells were isolated using TRIzol reagent according to the manufacturer's instructions. First strand synthesis of cDNA was carried out by incubating 1–2 μg of DNase I-treated total RNA isolated from cells or rat/human heart poly $(A)^+$ RNA with random hexamers at 70°C for 10 min followed by incubation in PCR buffer (20 mM Tris HCl pH 8.4, 50 mM KCl) containing 2.5 mM $MgCl_2$, 1 mM each deoxyribonucleoside 5′-triphosphate (dNTP) and 10 mM dithiothreitol at 25°C for 5 min. The reverse transcription reaction was initiated by the addition of SuperScript II RT (200 U) at 25°C for 10 min followed by incubation at 42°C for 50 min. The reaction was terminated by incubation at 70°C for 15 min prior to chilling on ice. The polymerase chain reaction was performed using 2–4 μl of cDNA in a 50 μl volume containing 0.4 mM forward and reverse primers, 200 mM each dNTP, and 2.5 U of Taq polymerase for 40 cycles (95°C for 24 s, 55°C for 22 s, 72°C for 78 s) in a thermal cycler (GenAmp PCR System 9600, Perkin Elmer, Norwalk, CT). An aliquot (30 μl) of the reverse transcription–polymerase chain reaction (RT–PCR) product was analyzed on a 10% polyacrylamide/Tris-Borate-ethylenediaminetetraacetic acid (EDTA) gel (Bio-Rad, Hercules, CA).

The sequence of the oligonucleotide primers for the various subunits and the expected PCR product sizes are shown in Table 1. The primers were designed using nucleotide sequence data from each subunit obtained from Genbank. The accession numbers are as follows: Kir6.1 (rat, D42145; human, D50312); Kir6.2 (rat, D86039; human, D50582; mouse, D73626); SUR 1 (rat, L40624; human, L78207) and SUR2 (mouse 2A, D86037; 2B, D86038; rat, D83598; human, unpublished data). Notably, due to the high sequence homology between the subunits examined, some of the primer sets were designed based upon a consensus sequence between human and rat nucleotide information which successfully maintained subunit specificity.

3. Results

Activation of endogenous K_{ATP} channels expressed in human medulloblastoma TE671 cells was studied using the voltage-sensitive fluorescent probe $DiBAC_4(3)$. Distribution of this anionic fluorescent probe across the cell membrane has previously been shown to be dependent on membrane potential (Epps et al., 1994). With depolarization, $DiBAC_4(3)$ further partitions into the cell, whereupon fluorescence increases due to interactions of the dye with intracellular proteins and lipids; conversely, hyperpolarization results in dye extrusion and thus, a decrease in fluorescence.

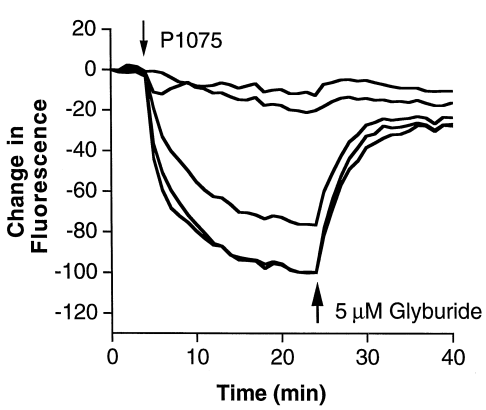

Fig. 1. Changes in membrane potential responses evoked by the K^+ channel opener P1075 in TE671 cells and its reversal by glyburide as measured by FLIPR. Shown are concentration-dependent changes in $DiBAC_4(3)$ fluorescence elicited by varying concentrations of P1075 (0.003, 0.03, 0.3, 3 and 30 μM) and subsequent reversal of these responses by the addition of 5 μM glyburide (indicated by arrows). Data averaged from duplicate determinations are normalized to the maximal response evoked by 10 μM P1075 (indicated as −100% along the Y-axis). The maximal fluorescence changes were ca. 4000 units.

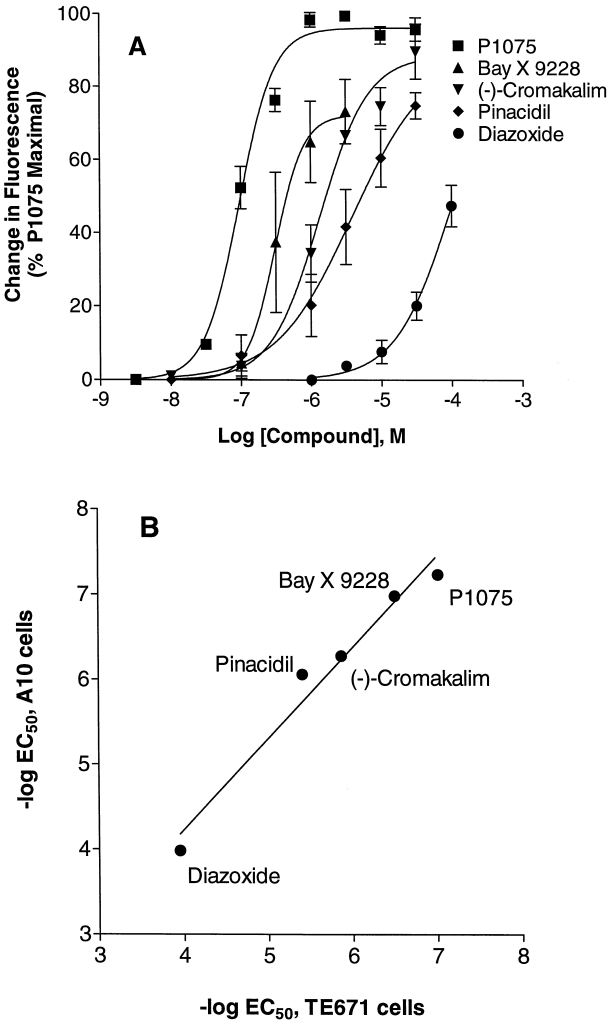

Fig. 2. (A) Concentration dependence of opener-evoked changes in membrane potential in TE671 cells. Each data point represents the mean ± S.E.M. of changes in $DiBAC_4(3)$ fluorescence expressed as a percentage of response evoked by 10 μM P1075 (indicated as 100%). Data are mean ± S.E.M. of at least 3 determinations each performed in duplicate. (B) Correlation of K^+ channel opener potencies (EC_{50} values) between TE671 medulloblastoma cells and A-10 smooth muscle cells ($r = 0.97$). The EC_{50} values were determined as described in Section 2.

3.1. Effect of K^+ channel openers

Fig. 1 shows the changes in membrane potential response evoked by the pinacidil analog P1075. Addition of glyburide (5 μM) reversed the effect suggesting the presence of functional K_{ATP} channels. Glyburide (5 μM) alone did not evoke fluorescence changes (data not shown). Other prototypical K^+ channel openers such as (−)-cromakalim, pinacidil, Bay X 9228 and diazoxide also evoked membrane potential changes in a concentration-dependent manner (Fig. 2A). The following rank order of potencies was observed: P1075 (95.9 ± 8.4 nM) ~ Bay X 9228 (311 ± 19 nM) > (−)-cromakalim (1340 ± 197 nM) ~ pinacidil (3890 ± 990 nM) > diazoxide (111 ± 5.2 μM). At the maximal concentration tested (100 μM), diazoxide evoked only 48 ± 5.7% response and compound solubility precluded establishing plateau responses. The vehicle employed, DMSO (up to 0.1%), did not affect $DiBAC_4(3)$ fluorescence.

The effects of K^+ channel openers were also examined in A-10 smooth muscle cells. A good correlation ($r = 0.97$) was observed between the EC_{50} values for evoking membrane potential changes in A-10 cells and in TE671 cells (Fig. 2B). The maximal efficacies of these compounds to evoke membrane potential changes were also similar between the cell types (Table 2).

The profile for K_{ATP} channel activation by K^+ channel openers in RIN-m5F insulinoma cells was distinct from those observed with TE671 and A-10 cells. In RIN-m5F cells, diazoxide (30 and 100 μM) evoked changes in fluorescence responses that were reversed by addition of glyburide. Both P1075 and (−)-cromakalim were inactive at the maximal concentrations tested (30 μM; data not shown); solubility limitations precluded testing at higher concentrations.

3.2. Inhibition by sulfonylurea analogs

K^+ channel opener-evoked membrane potential responses in TE671 cells were inhibited in a concentration-dependent manner by sulfonylurea analogs glyburide and glipizide (Fig. 3). The IC_{50} values for inhibition of P1075 (10 μM) responses by glyburide and glipizide were 219 ± 1.7 nM and 14.1 ± 0.3 μM, respectively ($n = 3$–4). A-10 cells revealed similar sensitivities to these sulfonylureas (glyburide, $IC_{50} = 588 \pm 111$ nM and glipizide, $IC_{50} = 39.4 \pm 2.4$ μM; $n = 3$–4). The potency of glyburide to

Table 2
Comparison of the pharmacological profile of K^+ channel openers in TE671 and A-10 cells

Compound	TE671		A-10	
	EC_{50} (M)	Efficacy (%)	EC_{50} (M)	Efficacy (%)
P1075	$9.59 \pm 0.84 \times 10^{-8}$	99.6 ± 0.3	$5.86 \pm 1.42 \times 10^{-8}$	103.7 ± 2.3
Bay X 9228	$3.11 \pm 0.19 \times 10^{-7}$	73.3 ± 8.8	$1.04 \pm 0.16 \times 10^{-7}$	99.9 ± 1.6
(−)-Cromakalim	$1.34 \pm 0.20 \times 10^{-6}$	89.8 ± 7.6	$5.28 \pm 1.22 \times 10^{-7}$	103.0 ± 7.3
Pinacidil	$3.89 \pm 0.99 \times 10^{-6}$	74.9 ± 3.6	$8.66 \pm 1.68 \times 10^{-7}$	83.7 ± 3.6
Diazoxide	$1.11 \pm 0.05 \times 10^{-4}$	47.5 ± 5.7[a]	$1.04 \pm 0.28 \times 10^{-4}$	52.6 ± 7.0[a]

Values shown are mean ± S.E.M. of 3–10 observations and were analyzed as described under Section 2. The efficacy values are expressed as % change relative to the response evoked by 10 μM P1075.

[a] For diazoxide, plateau responses were not attained and the efficacy values indicated are responses observed at 100 μM; EC_{50} values are estimated.

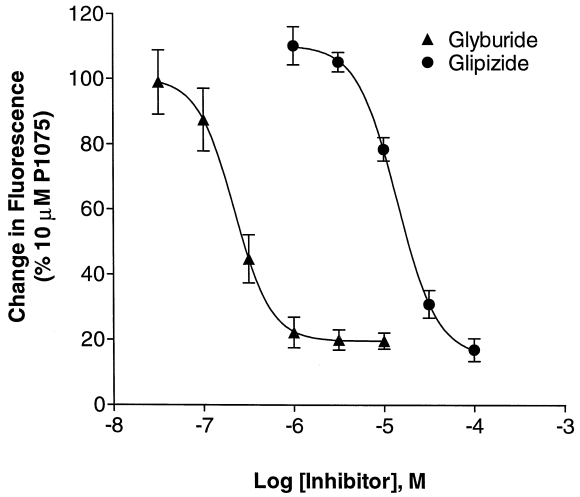

Fig. 3. Concentration-dependence for inhibition of P1075-evoked responses by sulfonylureas in TE671 cells. Each data point represents changes in $DiBAC_4(3)$ fluorescence following simultaneous addition of 10 μM P1075 and varying concentrations of sulfonylureas. Data are mean ± S.E.M. of 3–4 determinations each performed in duplicate. Values are expressed as percent relative to the response evoked by 10 μM P1075.

inhibit K^+ channel opener responses in both TE671 and A-10 cells is lower than those reported for inhibition of the pancreatic β-cell K_{ATP} channel ($IC_{50} \sim 1–10$ nM; Ashcroft and Rorsman, 1989; Inagaki et al., 1995), but is within the range reported for inhibition of smooth muscle K_{ATP} channels (Quayle et al., 1997).

3.3. Expression of K_{ATP} channel subunits

The observation that the effects of K^+ channel openers in TE671 cells were inhibited by glyburide suggests the presence of K_{ATP} channels. The molecular nature of these channels were further studied by RT–PCR analysis. Total RNA isolated from TE671 cells was employed for detection of mRNA for the K_{ATP} channel subunits Kir6.1, Kir6.2, SUR1, SUR2A and SUR2B. RT–PCR yielded products of expected sizes (see Table 1) for each of the subunit-specific primer sets when message was detected. In TE671 cells, mRNA corresponding to SUR2B, SUR1 and Kir6.2 was detected whereas in A-10 cells, SUR2B, Kir6.1 and Kir6.2 messages were found (Fig. 4). In contrast, in RIN-m5F cells, only SUR1 and Kir6.2 were present (Fig. 4).

Heart cDNA was used as a positive control for the detection of SUR2A and SUR2B mRNA since this tissue is known to contain both these splice variants. Primers designed to identify both variants in the heart, yielded PCR products of 451 and 312 base pairs corresponding to SUR2A and B, respectively (Fig. 4, lane 5), which were confirmed by sequence analysis. Plasmid DNA encoding each of the subunits or cDNA isolated from a tissue known

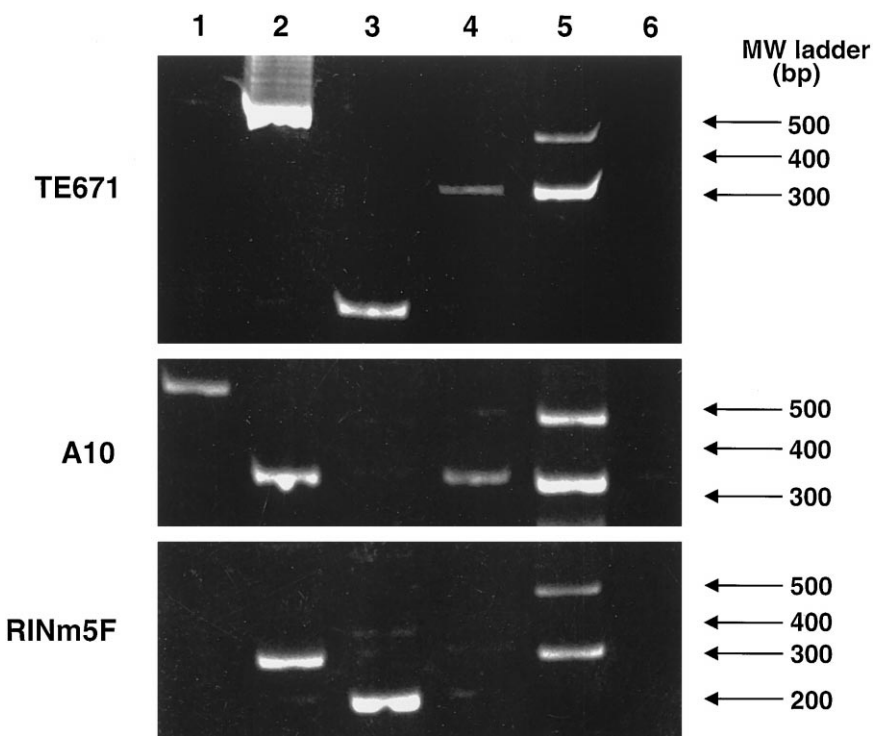

Fig. 4. Expression of K_{ATP} channel subunits in TE671, A-10 and RIN-m5F cells. RT–PCR was performed on cDNA prepared from total RNA isolated from TE671 (upper panel), A-10 (middle panel) and RIN-m5F (lower panel) cells using subunit specific oligonucleotide primers as described under Section 2. RT–PCR products were analyzed on 10% polyacrylamide gels and visualized by staining with ethidium bromide. Lanes 1–4 represents products corresponding to Kir6.1, Kir6.2, SUR1 and SUR2B. Lane 5 shows SUR2A and SUR2B from heart and lane 6 represents the negative control (no reverse transcriptase) for SUR2. Expected bands corresponding to the 1 kb DNA ladder (GIBCO/BRL) are indicated on the right.

to contain the subunit of interest were employed as positive controls to demonstrate subunit specificity of the primers (data not shown). Additionally, negative controls lacking reverse transcriptase during the cDNA synthesis step did not yield products.

4. Discussion

In the present study, the functional pharmacology of K_{ATP} channels expressed in human medulloblastoma TE671 was characterized with FLIPR-based assays of membrane potential using the potential-sensitive fluorescent probe $DiBAC_4(3)$. A set of structurally diverse K^+ channel openers were shown to evoke concentration-dependent membrane potential responses which were reversed by the sulfonylurea analogs glyburide and glipizide. Further, RT–PCR studies were employed to assess the molecular identity of sulfonylurea receptor and Kir6.0 subunits. Reported here is the first detailed pharmacological and molecular characterization of the K_{ATP} channel endogenous to the medulloblastoma TE671 cells. Additionally, these properties have been compared to those of K_{ATP} channels endogenously expressed in A-10 smooth muscle and RIN-m5F pancreatic cell lines.

The potencies of the K^+ channel openers Bay X 9228, P1075, (−)-cromakalim, pinacidil and diazoxide observed with TE671 cells were similar to those of rat smooth muscle A-10 cells (this study) and are consistent with those reported for activation of K_{ATP} currents in rabbit mesenteric artery smooth muscle cells where the relative potencies were cromakalim (1.9 μM) ~ pinacidil (0.6 μM) ≫ diazoxide (37 μM) (Quayle et al., 1995). Furthermore, TE671 and A-10 cells exhibited similar potencies for inhibition of K_{ATP} channel responses by glyburide and glipizide. The observed potency of glyburide (0.2 μM) is 20–200 fold lower than those reported at the pancreatic β-cell K_{ATP} channel (Ashcroft, 1988; Inagaki et al., 1995). In RIN-m5F cells, diazoxide evoked membrane potential responses whereas (−)-cromakalim and P1075 were ineffective at the maximal concentration (30 μM) tested in our studies. This result is consistent with previous observations in which diazoxide was more effective than (−)-cromakalim in activating currents and membrane potential responses in RIN-m5F cells or in human embryonic kidney 293 cells transfected with Kir6.2 and SUR1 subunit combinations (Dunne et al., 1990; Gopalakrishnan et al., 1998). Thus, the pharmacology for activation and inhibition of the native K_{ATP} channel expressed in TE671 cells resembles that of the smooth muscle channel and is clearly distinguishable from that of the pancreatic β-cell type.

Previous studies suggest that tissue-selective pharmacology of K^+ channel openers could largely be attributed to ligand interactions with the SUR subunit of the complex whereas Kir6.2 may be responsible for ion permeation (Inagaki et al., 1996; Isomoto et al., 1996). The subunit composition of K_{ATP} channels endogenously expressed in TE671 cells was explored by RT–PCR analysis for the SUR and Kir6.0 families. In TE671 cells, mRNA corresponding to SUR1, one of the sulfonylurea receptors described in pancreatic β-cells and neurons (Inagaki et al., 1995; Bryan and Aguilar-Bryan, 1997), SUR2B, a widely distributed SUR2 isoform with properties typical of smooth muscle K_{ATP} channels (Isomoto et al., 1996), and the inward rectifier Kir6.2 were detected. Although SUR1 mRNA was detected, it should be noted that [^{3}H]glyburide binding in TE671 membranes revealed a predominantly low affinity site ($K_d > 10$ nM; the high affinity binding site representing only ca. 2% of the total binding capacity; unpublished observations). This lack of high affinity binding suggests that, despite the presence of mRNA, SUR1 protein may not be expressed at significant levels to form functional K_{ATP} channels possessing this subunit in these cells. Thus, although in principle, SUR1 and SUR2B may form functional channels with Kir6.2, the pharmacological profile of both openers and inhibitors revealed from membrane potential studies together with the lack of high affinity [^{3}H]glyburide binding sites suggests that the SUR2B isoform is an apparent determinant of K_{ATP} channel function in TE671 cells. In contrast, the presence of SUR1 (and Kir6.2) mRNA in RIN-m5F cells agrees well with the presence of high affinity [^{3}H]glyburide binding sites and distinct pharmacology (Schmid-Antomarchi et al., 1987). In smooth muscle-derived A-10 cells, transcripts corresponding to SUR2B, Kir6.2 and Kir6.1, but not SUR2A or SUR1, were detected. Collectively, these observations are consistent with results from heterologous expression studies which suggest that tissue-selective pharmacology is largely determined by the SUR subunit of the K_{ATP} channel (Inagaki et al., 1996; Isomoto et al., 1996). It is to be noted that while both Kir6.1 and Kir6.2 mRNA were detected in A-10 cells, mRNA for Kir6.1 was not present in the two other cell lines studied. The significance of this is unclear at present although it has been suggested that Kir6.1 in combination with SUR2B resembles channels described in vascular smooth muscles that are activated by nucleotide diphosphates (K_{NDP} channels) (Yamada et al., 1997).

Emerging evidence suggests that neuronal K_{ATP} channels exhibit pharmacologic properties distinct from those described in pancreas and vascular tissues, as for example, the differential pharmacology of K^+ channel openers and sulfonylureas in modulating GABA release from the substantia nigra (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990), the higher sensitivity of tolbutamide compared to glyburide for inhibition of dopaminergic agonist evoked channel activity in corpus striatal neurons (Lin et al., 1993) and the atypical pharmacology reported in glucose-receptive hypothalamic neurons in which activation by leptin leads to hyperpolarization (Sellers et al., 1992; Spanswick et al., 1997). Clearly, there is a need for identifying cell lines as model systems for characterizing the functional

roles and pharmacologic properties of neuronal K_{ATP} channels as their molecular nature and diversity continue to be clarified. Although studies with tumor cell lines remain to be related to normal cells, the present molecular and functional studies indicate that this approach will be useful in systematic analysis of K_{ATP} channels. Our present analysis indicate that the endogenous channels expressed in the human medulloblastoma TE671 may represent a smooth muscle-type K_{ATP} channel.

References

Amoroso, S., Schmid-Antomarchi, H., Fosset, M., Lazdunski, M., 1990. Glucose, sulfonylureas and neurotransmitter release: role of ATP-sensitive K^+ channels. Science 247, 852–854.

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

Ashcroft, S.J., Ashcroft, F.M., 1990. Properties and function of ATP-sensitive K^+ channels. Cell Signal 2, 197–214.

Ashcroft, F.M., Rorsman, P., 1989. Electrophysiology of the pancreatic β-cell. Prog. Biophys. Mol. Biol. 54, 87–143.

Bryan, J., Aguilar-Bryan, L., 1997. The ABCs of ATP-sensitive potassium channels: more pieces of the puzzle. Curr. Opin. Cell Biol. 9, 553–559.

Clement, J.P. IV, Kunjilwar, K., Gonzalez, G., Schwanstecher, M., Panten, U., Aguilar-Bryan, L., Bryan, J., 1997. Association and stoichiometry of K_{ATP} channel subunits. Neuron 18, 827–838.

Daniel, S., Malkowitz, L., Wang, H.-C., Beer, B., Blume, A.J., Ziai, R., 1991. Screening for potassium channel modulators by a high throughput 86-rubidium efflux assay in a 96-well microtiter plate. J. Pharm. Methods 25, 185–193.

Dunne, M.J., Yule, D.I., Gallacher, D.V., Peterson, O.H., 1990. Comparative study of the effects of cromakalim (BRL 34915) and diazoxide on membrane potential, $[Ca^{2+}]_i$ and ATP-sensitive potassium currents in insulin-secreting cells. J. Membr. Biol. 114, 53–60.

Epps, D.E., Wolfe, M., Groppi, V., 1994. Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid) trimethine oxanol ($Dibac_4(3)$) in model systems and cells. Chem. Phys. Lipids 69, 137–150.

Freedman, J.E., Lin, Y.-J., 1996. ATP-sensitive potassium channels: diverse functions in the central nervous system. The Neuroscientist 2, 145–152.

Gopalakrishnan, M., Janis, R.A., Triggle, D.J., 1993. ATP-sensitive K^+ channels: pharmacologic properties, regulation, and therapeutic potential. Drug Dev. Res. 28, 95–127.

Gopalakrishnan, M., Molinari, E.J., Shieh, C.C., Monteggia, L.M., Roch, J.M., Scott, V.E.S., Anderson, K.L., Sullivan, J.P., Brioni, J.D., 1998. Pharmacological characterization of the human sulfonylurea receptor SUR1 and the inward rectifier Kir6.2 stably coexpressed in HEK-293 cells. 2nd International Conference on ATP-sensitive Potassium Channels and Disease. Abstr. 221, Giovanni Lorenzini Medical Foundation, Houston, TX.

Inagaki, N., Gonoi, T., Clement, J.P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., Bryan, J., 1995. Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 270, 1166–1170.

Inagaki, N., Gonoi, T., Clement, J.P., Wang, C., Aguilar-Bryan, L., Bryan, J., Seino, S., 1996. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 16, 1011–1017.

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.

Lazdunski, M., 1994. ATP-sensitive potassium channels: an overview. J. Cardiovasc. Pharmacol. 24, S1–S5.

Lin, Y.-J., Greif, G.J., Freedman, J.E., 1993. Multiple sulfonylurea-sensitive potassium channels: a novel subtype modulated by dopamine. Mol. Pharmacol. 44, 907–910.

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

Quayle, J.M., Bonev, A.D., Brayden, J.E., Nelson, M.T., 1995. Pharmacology of ATP-sensitive K^+ currents in smooth muscle cells from rabbit mesenteric artery. Am. J. Physiol. 269, C1112–C1118.

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.

Rorsman, P., 1997. The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint. Diabetologia 40, 487–495.

Schmid-Antomarchi, H., De Weille, J., Fosset, M., Lazdunski, M., 1987. The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. J. Biol. Chem. 262, 15840–15844.

Schmid-Antomarchi, H., Amoroso, S., Fosset, M., Lazdunski, M., 1990. K^+ channel openers activate brain sulfonylurea-sensitive K^+ channels and block neurosecretion. Proc. Natl. Acad. Sci. USA 87, 3489–3492.

Schroeder, K.S., Neagle, B., 1996. FLIPR: a new instrument for accurate, high throughput optical screening. J. Biomol. Screen. 1, 75–81.

Sellers, A.J., Boden, P.R., Ashford, M.L., 1992. Lack of effect of potassium channel openers on ATP-modulated potassium channels recorded from rat ventromedial hypothalamic neurones. Br. J. Pharmacol. 107, 1068–1074.

Spanswick, D., Smith, M.A., Groppi, V., Logan, S.D., Ashford, M.L., 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390, 521–525.

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.