Glibenclamide-Induced Apoptosis Is Specifically Enhanced by Expression of the Sulfonylurea Receptor Isoform SUR1 but Not by Expression of SUR2B or the Mutant SUR1(M1289T)

Annette Hambrock, Claudia Bernardo de Oliveira Franz, Sabrina Hiller, and Hartmut Osswald

Department of Pharmacology and Toxicology, Medical Faculty, University of Tübingen, Tübingen, Germany

Received October 20, 2005; accepted November 21, 2005

ABSTRACT

Sulfonylurea receptor 1 (SUR1) is the regulatory subunit of the pancreatic ATP-sensitive K⁺ channel (KATP channel), which is essential for triggering insulin secretion via membrane depolarization. Sulfonylureas, such as glibenclamide and tolbutamide, act as KATP channel blockers and are widely used in diabetes treatment. These antidiabetic substances are known to induce apoptosis in pancreatic β-cells or β-cell lines under certain conditions. However, the precise molecular mechanisms of this sulfonylurea-induced apoptosis are still unidentified. To investigate the role of SUR in apoptosis induction, we tested the effect of glibenclamide on recombinant human embryonic kidney 293 cells expressing either SUR1, the smooth muscular isoform SUR2B, or the mutant SUR1(M1289T) at which a single amino acid in transmembrane helix 17 (TM17) was exchanged by the corresponding amino acid of SUR2. By analyzing cell detachment, nuclear condensation, DNA fragmentation, and caspase-3-like activity, we observed a SUR1-specific enhancement of glibenclamide-induced apoptosis that was not seen in SUR2B, SUR1(M1289T), or control cells. Coexpression with the pore-forming Kir6.2 subunit did not significantly alter the apoptotic effect of glibenclamide on SUR1 cells. In conclusion, expression of SUR1, but not of SUR2B or SUR1(M1289T), renders cells more susceptible to glibenclamide-induced apoptosis. Therefore, SUR1 as a pancreatic protein could be involved in specific variation of β-cell mass and might also contribute to the regulation of insulin secretion at this level. According to our results, TM17 is essentially involved in SUR1-mediated apoptosis. This effect does not require the presence of functional Kir6.2-containing KATP channels, which points to additional, so far unknown functions of SUR.

Sulfonylurea receptors (SUR) are members of the ATP-binding cassette protein family and form important regulatory subunits of ATP-sensitive K⁺ channels (KATP channels). These channels are heterotetrameric complexes formed by SUR and the pore-forming Kir6.x subunit. Different combinations of these subunits (SUR1, SUR2A, or SUR2B and Kir6.1 or Kir6.2) form channels in various tissues with distinct pharmacological and electrophysiological properties. SUR2 is encoded by a different gene than SUR1. Alternative splicing of the SUR2 gene leads to expression of either SUR2A, predominantly found in heart and skeletal muscle, or SUR2B, typically occurring in smooth vascular muscle (Gribble and Reimann, 2003). To some extent, SUR1 and SUR2 show inverse pharmacological profiles; SUR1 exhibits high affinity for several sulfonylureas but low affinity for most K⁺ channel openers, whereas SUR2 shows lower affinity for sulfonylureas and high affinity for openers (Schwanstecher et al., 1998; Hambrock et al., 2002). In the pancreatic β-cell, KATP channels (constituted by SUR1 and Kir6.2) are essential for triggering insulin secretion via membrane depolarization. High blood glucose concentrations lead to an elevated ATP/ADP ratio and result in closure of KATP channels, opening of voltage-dependent Ca²⁺ channels, and release of insulin-containing vesicles (Ashcroft, 2000; Gribble and Reimann, 2003; Bryan et al., 2004). Several sulfonylureas and glinides act as synthetic K⁺ channel blockers and are widely used in the treatment of diabetes type 2 because of their insulinotropic effects (Doyle and Egan, 2003; Henquin, 2004).

Insulin secretion is also modulated and amplified by additional pathways that are not completely understood thus far. K⁺ channel-independent mechanisms involve, e.g., ele-
vated Ca\(^{2+}\) concentrations, regulation via incretin hormones or certain neurotransmitters or activation of the adenyllyl cyclase second messenger system or other kinases. There is also some evidence for a special role of SUR in controlling cAMP-dependent insulin secretion without necessarily involving the Kir6.2 subunit (Nakazaki et al., 2002; Eliasson et al., 2003; Shibasaki et al., 2004). These results are in contrast to other reports that doubt such a function of SUR (Nenquin et al., 2004).

According to recent studies, one further mechanism regulating the extent of insulin secretion on a different level is based on adaptive variation of \(\beta\)-cell mass by changes in cell proliferation or apoptosis. For example, an increasing demand for insulin can be compensated by a rise in \(\beta\)-cell mass in obese humans, whereas \(\beta\)-cell mass in patients suffering from diabetes (type 1 or 2) is often reduced in the course of the disease. However, the precise molecular mechanisms underlying \(\beta\)-cell-specific apoptosis are still unknown (Efanova et al., 1998; Mandrup-Poulsen, 2001; Donath and Halban, 2004; Ahren, 2005). In addition to glucotoxic effects, it is known that sulfonylureas, such as glibenclamide or tolbutamide, are able to induce apoptosis in \(\beta\)-cells or respective cell lines (Efanova et al., 1998; Iwakura et al., 2000; Rustenbeck et al., 2004a; Maedler et al., 2004, 2005) under certain conditions. Therefore, some concern is expressed regarding long-term treatment with sulfonylureas (Mandrup-Poulsen, 2001; Donath and Halban, 2004; Maedler et al., 2005), although a mechanistic explanation of the apoptotic effect of these drugs is lacking thus far.

The aim of this study was to evaluate whether SUR is involved in the induction of apoptotic processes by sulfonylurea compounds. As a model system, we employed recombinant HEK293 cells, which are widely used in the study of K\(_{ATP}\) channels or their subunits. HEK293 cells expressing either the SUR1 or the SUR2B isoform were analyzed after glibenclamide treatment. Cells expressing the mutant SUR1(M1289T) at which a single amino acid in transmembrane helix 17 (TM17) was exchanged by the corresponding amino acid of SUR2 were also investigated. It had been shown previously that a mutation at this position markedly influences some pharmacological properties of SUR (Moreau et al., 2000; Hambrock et al., 2004). For HEK293 cells stably or transiently expressing the different SUR forms, typical apoptotic parameters, such as cell detachment, nuclear condensation, DNA fragmentation, and enhanced caspase-3-like activity, were determined after glibenclamide treatment. To assess the extent of the glibenclamide effect, comparable experiments were performed in which etoposide, a classic inducer of apoptosis widely used in chemotherapy, was supplemented. In addition, cells were analyzed after coexpression of SUR1 and Kir6.2 to prove whether the apoptotic effect of glibenclamide requires the presence of intact K\(_{ATP}\) channels (Maedler et al., 2004, 2005) or whether it is also mediated by SUR in the absence of the Kir6.x subunit.

**Materials and Methods**

**Mutagenesis and Generation of Stably Transfected Cell Lines.** HEK293 cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO\(_2\), in minimal essential medium supplemented with glutamine, 10% fetal bovine serum, and 20 \(\mu\)g/ml gentamycin as described previously (Hambrock et al., 2002). Cells were transfected with pcDNA3.1 expression vector (Invitrogen, Karlsruhe, Germany) containing the coding sequences of SUR1 (GenBank X97279), SUR1(M1289T), or SUR2B (GenBank D86038). The mutant SUR1(M1289T) had been constructed from SUR1 using the QuikChange Mutagenesis System (Stratagene, Amsterdam, The Netherlands). Control cells were obtained by sham transfection with pcDNA3.1. Stably transfected cell lines were isolated as described previously (Hambrock et al., 2002) and cultured in the presence of 300 \(\mu\)g/ml geneticin.

**Transient Cotransfection of SUR1 and Kir6.2.** HEK293 cells at 60 to 80% confluence were transiently transfected either with SUR1 and Kir6.2 (GenBank D50581) plasmids or with SUR1 and pcDNA plasmids. Lipofectamine was applied as transfection reagent as described in Hambrock et al. (2002), and the plasmids were added in equimolar amounts.

**Treatment with Glibenclamide or Etoposide.** Cells were cultured in the absence of geneticin for at least 3 days. At 60 to 80% confluence (14–17 \(\times\) 10\(^5\) cells/9.5-cm-diameter culture dish), glibenclamide or etoposide was supplemented by adding 10 or 20 \(\mu\)g/ml, respectively, of a stock solution (100 mM glibenclamide in ethanol/ DMSO (50:50, v/v) or 50 mM etoposide in DMSO) to 10 ml of culture medium. In control experiments, 10/20 \(\mu\)l of solvent was added. In case of the transient transfections, glibenclamide was added 24 h after starting the transfection. Experiments with differently transfected cells were performed in parallel and repeated three to seven times. In each experiment, drug exposure was performed in two culture dishes, and solvent treatment took place in one dish.

**Quantification of Cell Detachment.** The numbers of adherent and detached cells were determined separately with a CASY TT Analyser System (Scharfe System GmbH, Reutlingen, Germany) according to the manufacturer’s instructions. After calibration with dead and vital HEK293 cells, cursor positions were set to 12.75 to 50.00 \(\mu\)m (evaluation cursor) and 7.63 to 50.00 \(\mu\)m (standardization cursor). From the supernatant of each culture dish, three 100-\(\mu\)l aliquots were withdrawn for measurement. The adherent cells were then rinsed off with CASY electrolyte solution and diluted if necessary. Again, 100-\(\mu\)l aliquots were analyzed in triplicate.

**Determination of Apoptotic Nuclei by Hoechst 33258 (Bisbenzimide) Staining.** After drug or solvent treatment, adherent cells were fixed with paraformaldehyde (3% in PBS) and stained with Hoechst 33258 (10 \(\mu\)g/ml in PBS) for 10 min. The cells were washed with PBS buffer (5 min), rinsed with distilled water, air-dried, and embedded in gelatin. Cells were evaluated using a Laborlux D fluorescence microscope (Leitz, Oberkochen, Germany) and an UV-2A filter system (excitation: 330–380 nm; emission: 420 nm). Microscopic images were captured with a digital CF20 DXC air CCD camera (Kappa-Messtechnik, Gießen, Germany).

**Analysis of DNA Fragmentation.** Cells from the different treatment groups were collected by combining adherent and detached cells of each culture dish. For isolation of cytoplasmic DNA, cells from each culture dish (9.4 cm diameter) were rinsed off separately with culture medium after different periods of treatment. Cells were pelleted by centrifugation (500 g, 6 min) and resuspended in 1 ml of PBS buffer. An aliquot of 300 \(\mu\)l was then withdrawn for determination of caspase activity. The remaining 700 \(\mu\)l was centrifuged again (200 g, 5 min), and cells were lysed in 300 \(\mu\)l of lysis buffer (0.5% Triton X-100, 5 mM Tris, and 20 mM EDTA, pH 7.4) for 20 min. Cell debris and nuclei were removed by centrifugation (14,000 g, 15 min, 4°C). Cytoplasmic DNA was extracted from the supernatant by phenol/chloroform extraction, precipitated with ethanol, and incubated with proteinase K and RNase A (for 60 and 30 min, respectively, at 37°C). Separation of the isolated DNA fragments or of a PEG 100-base pair DNA ladder Plus marker (Peglab, Erlangen, Germany) was performed in 1.5% agarose (30\(\mu\)g/ml) gels (Peqlab, Erlangen, Germany) was performed in 1.2% agarose gels. UV fluorescence of ethidium bromide-stained DNA was registered with a CCD video camera system (Raytest, Straubenhardt, Germany).
Determination of Caspase-3-Like (DEVD-Caspase) Activity.

Cells were prepared as described under “Analysis of DNA Fragmentation.” The aliquots withdrawn for caspase measurements were then processed according to the method of Wanke et al. (2004) using a Wallac 1420 Victor2 Multilabel Reader (PerkinElmer, Rodgau-Jugesheim, Germany). Cleavage of synthetic caspase-3-like substrate (DEVD-AFC) was monitored by fluorescence emission at 510 nm (excitation at 390 nm) in 20 min-intervals over a total period of 160 min. Caspase activities were calculated as increase in fluorescence per minute and normalized to the protein content of each sample.

Statistical Analysis. Data are presented as means from single experiments ± S.E.M. Statistical analyses were performed using Prism 4 software (GraphPad Software, San Diego, CA). For statistical comparisons, two-tailed Student’s paired t test or repeated measures one-way ANOVA analysis in combination with Tukey’s post hoc test were employed as appropriate.

Materials. All reagents used for cell culture were purchased from Invitrogen. Glibenclamide, Hoechst 33258, and AFC standards were from Sigma-Aldrich (Deisenhofen, Germany), DEVD-AFC caspase substrate was from Biomol (Hamburg, Germany), and etoposide from Calbiochem (Bad Soden, Germany).

Results

Cell Morphology after Glibenclamide Treatment of Different Cell Lines. HEK293 cells stably expressing either SUR1, SUR2B, or the mutant SUR1(M1289T) were incubated with glibenclamide concentrations (5–100 μM) over a 7-day culture period and compared with cells that were only treated with the same amount (0.1%) of solvent. In further controls, cells that were only sham-transfected with pcDNA3.1 expression vector (= pcDNA cells) were exposed to either glibenclamide or solvent. At the beginning of the experiments, all cells showed a vital appearance, exhibited a fibroblast-like morphology, and formed coherent monolayers with 60 to 80% confluence (Fig. 1). Normal cell growth within the next 7 days was not visibly influenced by solvent treatment. However, after continuous exposure to glibenclamide, many cells became roundish, detached themselves from the bottom of the culture dish, and went up into the supernatant. The extent of glibenclamide-induced detachment of SUR1 cells was much more pronounced than that of pcDNA cells. Cells expressing the mutant SUR1(M1289T) resembled pcDNA cells after glibenclamide treatment. Glibenclamide-treated SUR2B cells often showed an even smaller amount of detached cells than equally treated pcDNA controls.

Quantification of Cell Detachment after Treatment with Glibenclamide or Etoposide. Measurements of the number of adherent cells and of cells in the supernatant revealed an obvious correlation among concentration of glibenclamide, time of treatment, and degree of detachment. Exposure of SUR1 cells to 100 μM glibenclamide, for example, led to intensive cell detachment after 2 days; a similar effect was achieved by incubating the same cell line with 50 μM glibenclamide for 4 days. Differences between SUR1 and pcDNA cells concerning cell detachment were already seen at concentrations higher than 10 μM after 4 days of glibenclamide treatment (data not shown).

However, in further experiments, we applied 100 μM glibenclamide to see clear effects after a shorter incubation period that were not covered up by side effects like, e.g., cell aging, depletion of nutrients, or accumulation of toxic metabolites. Cell counting confirmed the results from microscopic investigation (Fig. 2). Glibenclamide treatment led to an...
enhanced number of cells in the supernatant compared with solvent-treated cells in the case of all cell lines. After glibenclamide treatment, the number of detached SUR1 cells was significantly (2.0–2.3-fold) higher than that of detached SUR2B or pcDNA cells, whereas there was no significant difference between SUR2B and pcDNA cells (Fig. 2A). For cells expressing SUR1(M1289T), the number of detached cells was reduced compared with SUR1 cells but slightly higher than the amount of detached pcDNA cells (Fig. 2B). However, these differences between SUR1(M1289T) cells and SUR1 or pcDNA cells were not significant. In contrast to glibenclamide treatment, all solvent-treated cell lines did not exhibit any significant differences.

Comparable experiments in which the anticancer drug etoposide was applied (in four series of parallel experiments) caused cell detachment with no significant differences between either all treated groups (P > 0.05) or all untreated groups (P > 0.05) (data not shown). Just as glibenclamide, etoposide was supplemented in the concentration of 100 μM, which is commonly used for apoptosis induction in cell culture. To directly compare the effects of glibenclamide and etoposide, the number of detached cells after drug treatment was expressed as the percentage of the number of detached cells after solvent treatment. Again, the degree of detached SUR1 cells after glibenclamide treatment (462 ± 82% of solvent control) was considerably higher than that of the other cells (pcDNA, 320 ± 66%; SUR1(M1289T), 262 ± 71%; and SUR2B, 254 ± 30%). The amount of glibenclamide-induced detachment of SUR1 cells was nearly the same as that of etoposide-induced cell detachment (pcDNA, 434 ± 75%; SUR1, 475 ± 127%; SUR1(M1289T), 488 ± 78%; and SUR2B, 389 ± 85%).

Effect of Coexpression with Kir6.2 on Detachment of SUR1-Expressing Cells. To examine whether the effect of glibenclamide on SUR1-expressing cells was modified by additional expression of Kir6.2, HER293 cells were transiently transfected with equimolar amounts of SUR1 and Kir6.2 plasmids or SUR1 and pcDNA plasmids (Fig. 3). Twenty-four hours after transfection, SUR1 and Kir6.2 were already well expressed as previous experiments had confirmed (data not shown). The cells were then treated with either 100 μM glibenclamide or solvent for up to 3 days. In all cases, the number of cells in the supernatant was higher for glibenclamide-treated cells than for the respective solvent controls. A direct comparison between cells expressing SUR1 alone and cells coexpressing SUR1 and Kir6.2 was difficult because, already after solvent treatment, cells expressing SUR1 and Kir6.2 showed a higher degree of detachment than SUR1 cells without Kir6.2. To assess the effect of glibenclamide in these experiments, the background of cell detachment due to solvent treatment was either subtracted from the total number of detached cells after glibenclamide treatment (Fig. 3B) or considered in its proportional contribution to the total amount (Fig. 3C). According to both evaluations, coexpression with Kir6.2 did not significantly alter the results obtained for cells that were transfected with SUR1 alone.

Nuclear Condensation and DNA Fragmentation after Glibenclamide Treatment. After glibenclamide treatment (100 μM glibenclamide, 48 h), staining with the DNA-binding dye Hoechst 33258 demonstrated the appearance of brightly stained, condensed, and fragmented nuclei, which is characteristic for apoptotic cells (Fink and Cookson, 2005). In SUR1 cells, apoptotic nuclei occurred to a much larger extent and were significantly more condensed and fragmented than in SUR2B (data not shown), SUR1(M1289T), or pcDNA cells (Fig. 4). After solvent treatment, however, all cell lines exhibited almost exclusively normal intact nuclei with no visible differences among the cell lines. DNA fragmentation after glibenclamide treatment was also confirmed by a visible DNA-laddering pattern typical of apoptotic cell death (fragment length: 400-1500 base pairs). Because this method does not allow any reliable quantification of isolated fragmented DNA, no direct comparison among the different glibenclamide-treated cell lines was possible. Nevertheless, all solvent-treated cells exhibited only very slight or no fragmentation.

Determination of Caspase-3-Like Activity after Glibenclamide Treatment. Initiation of apoptotic cell death is associated with sequential activation of a cascade of different caspases. Caspase-3 plays a key role within this signaling pathway in mammalian cells. Caspase-3 is able to specifically recognize the peptide sequence DEVD, but the cleavage of DEVD substrates by other enzymes with lower affinity cannot be excluded. Therefore, the turnover of DEVD substrates, strictly speaking, is due to caspase-3-like enzymes (Wanke et al., 2004). We quantified the activity of these enzymes by use of the synthetic fluorescent substrate DEVD-AFC after different intervals of glibenclamide or solvent treatment (12, 16, 24, and 48 h). However, solvent-treated cells showed a basal
amount of caspase-3-like activity that was lower than that of cells supplemented with 100 μM glibenclamide. The latter was slightly increased after 12 and 24 h of glibenclamide treatment but showed a marked increase after 48 h (approximately two and a half to three times higher compared with the beginning of the experiments, data not shown). After 48 h, caspase-3-like activity in SUR1 and pcDNA cells was significantly higher in the glibenclamide-treated groups than in the solvent-treated groups, but this difference was not observed in SUR1(M1289T) cells (Fig. 5). Enzyme activity after glibenclamide exposure was significantly enhanced in SUR1 cells compared with the other cell lines. SUR1(M1289T) cells showed even slightly lower activity than pcDNA controls after glibenclamide treatment, but this difference was not significant. Small differences were also detected among the solvent-treated groups. Caspase-3-like activity was a little higher in SUR1 cells than in pcDNA and SUR1(M1289T) cells in most experiments, and pcDNA cells for their part showed slightly but not significantly higher activity than SUR1(M1289T) cells. Yet, if total activity after glibenclamide treatment was referred to the respective solvent-dependent activity, the increase in caspase-3-like activity due to glibenclamide was still higher in SUR1 cells (323 ± 40%) compared with pcDNA cells (269 ± 37%) or SUR1(M1289T) cells (200 ± 41%).

**Discussion**

In this study, we were able to show that expression of SUR1 in HEK293 cells renders these cells more susceptible toward induction of apoptosis after exposure to glibenclamide. This was seen by an increase in total apoptotic parameters like cell detachment, nuclear condensation, DNA fragmentation, and caspase-3-like activity (Mandrup-Poulsen, 2001; Fink and Cookson, 2005), which rules out the involvement of necrotic cell death. A basal level of apoptosis was also present in sham-transfected control cells as well as in cells expressing the SUR2B isoform or the mutant SUR1(M1289T). However, in SUR1 cells, apoptotic processes were clearly enhanced after glibenclamide treatment.

These results are in accordance with previous studies reporting a negative impact of prolonged exposure to some sulfonylureas on islet cell function (Rustenbeck et al., 2004b; Del Guerra et al., 2005). In addition, the sulfonylureas glibenclamide and tolbutamide are known to elicit apoptosis in β-cells or pancreatic cell lines (Efano et al., 1998; Iwakura et al., 2000; Rustenbeck et al., 2004a; Maedler et al., 2004, 2005). However, a precise mechanistic explanation is still lacking. Mostly, it is assumed that the apoptotic effect of these secretagogues is mediated by the blockade of functional K<sub>ATP</sub> channels and depolarization-induced Ca<sup>2+</sup> influx into the cell. According to Miki et al. (2001), K<sub>ATP</sub> channels are important for β-cell survival, because apoptosis occurs more frequently in Kir6.2 knock-out or Kir6.2G132S-transgenic mice. Kim et al. (1999) on the other hand suggest a K<sub>ATP</sub>-channel-independent mechanism for glibenclamide-induced apoptosis in hepatoblastoma cells that involves inhibition of cystic fibrosis transmembrane conductance regulator channels.

In contrast to these findings, our data for the first time give evidence that expression of the SUR1 isoform can be specifically responsible for enhanced induction of apoptosis by glibenclamide. First, enhanced apoptosis induction is restricted to HEK293 cells expressing SUR1 and does not occur in SUR2B or control cells. Artifacts concerning the stably transfected SUR1 cell line can be excluded here, because similar results were obtained after transient transfections (data not shown). Second, a single mutation of SUR1 is able to abolish the SUR1-specific effect. Third, this effect does not require the presence of Kir6.2 and, furthermore, is not significantly enhanced by Kir6.2. At first glance, these results are surprising because assemblage of SUR and Kir6.2 is thought to be a prerequisite for correct trafficking of both subunits and formation of functional plasmalemmal K<sub>ATP</sub> channels (Neagoe and Schwappach, 2005). On the other hand, trafficking control can be dependent on factors, such as temperature, might...
differ between certain cell lines (Giblin et al., 2002) and could be overcome by recombinant overexpression (Mikhailov et al., 1998). There even might be diverse trafficking routes for K\textsubscript{ATP}, channel subunits within the same cell (Neagoe and Schwappach, 2005), as there are several reports that SUR or high-affinity sulfonylurea-binding sites in the β-cell are also located intracellularly, mainly in the insulin secretory granules (Carpentier et al., 1986; Ozanne et al., 1995; Barg et al., 1999; Geng et al., 2003). Even if Kir6.2 is required for correct targeting of SUR1 in the β-cell, glibenclamide-induced apoptosis would not require complete functional SUR1/Kir6.2 channels according to our data. Therefore, the results point to an additional function of SUR beyond being a regulatory subunit of K\textsubscript{ATP} channels. There are already some considerations that, in the β-cell, SUR1 plays a special role in cAMP-dependent exocytosis of insulin vesicles (Nakazaki et al., 2002; Eliasson et al., 2003; Shibasaki et al., 2004). Future work using SUR1-knockout mice might be suitable to confirm the involvement of the SUR1 isoform in β-cell apoptosis.

As in other studies (Efanova et al., 1998; Kim et al., 1999; Iwakura et al., 2000; Rustenbeck et al., 2004a; Maedler et al., 2004, 2005), we applied the sulfonylurea in rather high concentrations of 5 to 100 μM in our cell culture system to see clear rapid effects (see Results). However, the glibenclamide effect is not only dependent on drug concentration but also on cell pretreatment, cell density, and time of drug exposure. Perhaps because of these reasons, Del Guerra et al. (2005) did not notice a change in the apoptotic rate of human islets after 24 h of incubation with 10 μM glibenclamide. However, Maedler et al. (2005) observed that even low glibenclamide doses of 1 and 10 nM elicited clear effects (2–2.5-fold increase in apoptosis) in cultured human islets after 4 days. According to these observations, it has to be proven whether the apoptotic effects of glibenclamide could constitute problems in long-term treatment with therapeutic sulfonylurea concentrations and contribute to the phenomenon of secondary sulfonylurea failure.

Comparative experiments with equal concentrations of the chemotherapeutic agent etoposide demonstrate that the extent of apoptosis induced by glibenclamide in SUR1 cells is nearly the same as that induced by etoposide. In contrast to glibenclamide treatment, etoposide exposure does not lead to such pronounced differences among the cell lines. Apoptosis induction by specific substances depending on the SUR isoform and thereby on the type of cell or tissue might in this context provide a new perspective in cancer research. In that respect, the detection of other compounds that might be more potent than glibenclamide could be of interest.

The precise signal transduction pathway connected with the SUR1-specific apoptotic effect of glibenclamide remains to be elucidated. The intensive detachment of glibenclamide-treated SUR1 cells points to the involvement of “anoikis,” a special form of apoptosis characterized by extensive impairment of cell adhesion (Valentijn et al., 2004). Interestingly, an anoikis-like mechanism has been shown to be involved in early isolated islet apoptosis (Thomas et al., 2001). According to our results, caspase-3-like enzymes are activated after glibenclamide treatment. It still has to be noted that caspase-3-like activity in solvent-treated controls is slightly higher in SUR1 cells compared with pcDNA cells but lower in SUR1(M1289T) cells. However, this does not result in further damage of SUR1 cells in the absence of glibenclamide, because the other apoptotic parameters do not show any differences between all solvent-treated cell lines. Perhaps the expression of SUR1 per se (but not of SUR1(M1289T)) results in slightly higher caspase-3-like activity, which could be due to interaction of SUR1 with endogenous compounds or supplements in the culture medium, for example.

The clear SUR1-specific glibenclamide-induced apoptotic effect is completely abolished by exchanging a single amino acid in the carboxyl-terminal transmembrane helix TM17 by the corresponding amino acid of SUR2. In several transporters from the ATP-binding cassette protein family, the last carboxyl-terminal transmembrane helix plays an important role in binding of ligands and transport substrates (Oleschuk et al., 2003). SUR1(M1289T) is able to form functional plasma-membran channels with Kir6.2 according to electrophysiological experiments (Moreau et al., 2000) and thus possesses essential properties of an intact SUR protein. In radioligand-binding studies using [3H]glibenclamide, we were able to show that neither glibenclamide affinity (Hambrock et al., 2004) nor expression rate (S. Hiller, unpublished results) of the mutant is different from SUR1 wild type. However, the maximal amount of glibenclamide-binding sites is reduced at SUR1(M1289T) by approximately 30 to 50% at physiological MgATP concentrations (S. Hiller, unpublished results), which might explain the lower sensitivity of SUR1(M1289T) cells to glibenclamide-induced apoptosis. On the other hand, the maximal amount of binding sites (>200 fmol/mg membrane protein) is still quite high at the mutant. In contrast to this finding, glibenclamide-induced apoptosis in SUR1(M1289T) cells is at the same level as that of control cells (or is even lower for SUR1(M1289T) cells in case of caspase-3-like activity). For this reason, it is more plausible to assume that the specific integration of SUR1 in the relevant signaling pathway, perhaps via interaction with specific kinases, is disturbed by conformational changes due to the mutation in TM17. In this respect, SUR1(M1289T) is probably similar to the SUR2B isoform, which is also less effective in triggering glibenclamide-induced apoptosis. It has been shown previously that binding and effect of several K\textsubscript{ATP} channel openers are clearly enhanced in SUR1(M1289T) cells (Moreau et al., 2000; Hambrock et al., 2004). Taking into account that negative allosteric interactions exist between opener and glibenclamide binding (Schwanstecher et al., 1998; Hambrock et al., 2004) and that several openers exhibit antiapoptotic effects (Maedler et al., 2004; Teshima et al., 2003; Skak et al., 2004), TM17 is obviously involved in SUR1-specific apoptosis induction. Therefore, it will be an interesting task to investigate whether openers exert different protective effects in cells expressing SUR1, SUR1(M1289T), or SUR2.

In conclusion, glibenclamide induces apoptotic processes in HEK293 cells that are markedly enhanced by expression of SUR1 but not of SUR2 or the mutant SUR1(M1289T). Therefore, the SUR1 isoform can render cells more susceptible to glibenclamide and possibly also to other specific agents. SUR1 as a typical pancreatic protein for this reason might be involved in pancreatic β-cell apoptosis. Adaptive variation of β-cell mass is known to provide one mechanism in the regulation of insulin secretion. In this context, SUR1 could well influence insulin secretion by a second mechanism apart from regulating electrical activity as a K\textsubscript{ATP} channel subunit. These results could contribute to the knowledge of
Acknowledgments

We thank Dr. C. Derst (Jena, Germany) for kindly providing cDNA of SUR1 and Drs Y. Kurachi and Y. Horio (Osaka, Japan) for the generous gift of the SUR2B and Kir6.2 clones. We thank Dr. A. Buchmann, J. Mahr, and E. Zabinsky for friendly help with some of the apoptosis assays and C. Müller for excellent technical assistance in cell culture.

References


Buchmann, J. Mahr, and E. Zabinsky for friendly help with some of the apoptosis assays and C. Müller for excellent technical assistance in cell culture.


Ecoli 2002) The cytoplasmic C terminus of the sulfo-


