Aptosis of pancreatic β-cells is an important factor in the pathophysiology of diabetes. Previously, we have shown that the “phytoestrogen” resveratrol can induce β-cell apoptosis dependent on the expression of sulfonylurea receptor (SUR) 1, the regulatory subunit of pancreatic ATP-sensitive K⁺ channels. Here, we investigate whether 17β-estradiol also influences β-cell apoptosis in a SUR1-dependent manner. Therefore, islets from wild type or SUR1 knock-out mice, clonal β-cells, or HEK293 cells expressing different SUR forms were treated with 17β-estradiol or estrone. Different apoptotic parameters were determined and estrogen binding to SUR was analyzed. In murine islets, 17β-estradiol treatment resulted in significant apoptotic changes, which in their nature (either apoptotic or anti-apoptotic) were dependent on the age of the animal. These effects were not observed in SUR1 knock-out mice. Furthermore, 17β-estradiol, which specifically binds to SUR, induced enhanced apoptosis in SUR1-expressing HEK293 cells and clonal β-cells, whereas apoptosis in recombinant cells expressing SUR2A or SUR2B (cardiac or vascular SUR-isoforms) or sham-transfected control cells was significantly lower. The apoptotic potency of 17β-estradiol was much higher than that of resveratrol or estrone. SUR1-specific 17β-estradiol-induced apoptosis was either abolished by the mutation M1289T in transmembrane helix 17 of SUR1 or clearly enhanced by two apoptosis induced by these substances is not mediated by the electrical activity of KATP channels. As a pancreatic protein, SUR1 might therefore be involved in specific variation of pancreatic β-cell mass and could thus contribute to the regulation of insulin secretion also at this level.

Changes in the number of β-cells can be achieved via controlled apoptosis, proliferation, and/or neogenesis and are probably coupled to different developmental stages or varying metabolic demands (5–8). For instance, an adaptive increase in β-cell mass is often linked with obesity (9) or pregnancy (10, 11), whereas enhanced β-cell apoptosis has been shown to be involved in the pathophysiology of type 2 diabetes (9). However, more research is required to explore the numerous factors contributing to β-cell-specific cell death.

During pregnancy, 17β-estradiol concentrations are clearly elevated, especially during the third trimester. At this stage, gestational diabetes mellitus (GDM) is diagnosed in about 1–16% of pregnant women with the figures depending on the study conditions (12–14). Women with a history of GDM have an increased risk in developing type 2 diabetes later. The precise molecular mechanisms leading to GDM-related insulin resistance and β-cell failure, however, need to be determined (12).

Therefore, the aim of this study was to investigate whether 17β-estradiol specifically affects apoptotic cell death in β-cells.
17β-Estradiol Modulates β-Cell Apoptosis via SUR1

in a SUR1-dependent manner. Consequently, we analyzed different apoptotic parameters in isolated pancreatic islets of Langerhans from male and female wild type or SUR1 knock-out (SUR1KO) mice of different ages, in clonal β-cells, or in recombinant HEK293 cells (human embryonic kidney cells) expressing different forms of SUR after 17β-estradiol treatment. 17β-Estradiol acts as a $K_{ATP}$ channel blocker, closing pancreatic $K_{ATP}$ channels at physiological concentrations (15–17). However, to our knowledge no data exist as to whether 17β-estradiol is a ligand of SUR. To verify the direct interaction of this hormone with SUR, radioligand binding assays were performed. Comparable experiments were carried out with estrone, another endogenously occurring estrogen. In addition, we explored in which manner the action of 17β-estradiol was influenced by mutations (M1289T, R1379C, R1379L) in the SUR1 gene (ABCC8) that are of special importance for SUR function (18–20). The mutations at position 1379 are of special clinical interest because some of these mutants have been identified in diabetic patients (21, 22). By investigating the interaction of a potential endogenous ligand with SUR in this way, new insight into different factors involved in SUR1-specific modulation of apoptosis as well as β-cell death is provided.

EXPERIMENTAL PROCEDURES

Mutagenesis, Transfection, and Cell Culture—HEK293 cells (German Collection of Microorganisms and Cell Cultures, DSMZ, Braunschweig, Germany) were stably transfected with pcDNA3.1 expression vector (Invitrogen) containing the coding sequence of rat SUR1 (GenBank™ accession number X97279), SUR1(M1289T), SUR1(R1379C), SUR1(R1379L), murine SUR2A (GenBank D86037), SUR2A(Y1206S), murine SUR2B (GenBank D86038), or SUR2B(Y1206S), or they were transfected with empty pcDNA3.1 expression vector (Invitrogen). Transfections with these different SURs or co-transfections with SUR1 and murine Kir6.2 (GenBank D50581) were conducted according to Hambrock et al. (3). Site-directed mutagenesis as well as isolation and cultivation of the cell lines were also performed as described previously (3). HIT-T15 and RIN-m5F cells were purchased from the American Type Culture Collection (Manassas, VA) and grown as described (4).

Preparation of Pancreatic Islets—Pancreata were dissected from mice (wild type C57BL/6 mice, Charles River, Sulzfeld, Germany, or SUR1KO mice (23)). The experimental protocols were approved in accordance with the German Law on the Protection of Animals. After collagenase digestion of the pancreas, islets of Langerhans were microscopically selected and cultured in RPMI 1640 medium containing 5 mmol/liter glucose, 10% fetal calf serum, 2 mmol/liter l-glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Treatment with 17β-Estradiol or Estrone—At 60–80 (HEK293 cells) or 50–70% confluence (HIT-T15 and RIN-m5F cells), cells were supplemented with 17β-estradiol or estrone by adding 10 μl of a stock solution dissolved in ethanol/DMSO (50:50, v/v) or with the respective volume of solvent. Transient transfections were performed 24 h before treatment, isolation of islets preceded addition of substance by 6–24 h.

Quantification of Cell Detachment—Cell detachment was determined with a CASY TT Analyzer System (Innovatis, Reutlingen, Germany) according to Hambrock et al. (3). From the supernatant of each culture dish, three 100-μl aliquots were withdrawn for measurement. At least two culture dishes were tested per treatment group in a single experiment.

Determination of Apoptotic Nuclei—Condensed and fragmented nuclei were visualized by Hoechst 33258 staining after paraformaldehyde fixation as described previously (4). The number of total and apoptotic nuclei was quantified per randomly chosen 50 × 50-μm² areas (adherent cell culture cells) or per islet.

Determination of the Activities of Different Caspase Enzymes—The activities of caspases -3, -8, -9, and -12 were determined by cleavage of specific peptide substrates (DEVD, IETD, LEHD, and ATAD, respectively) conjugated to the fluorophore AFC (7-amino-4-trifluoromethyl coumarin). Enzyme activities were monitored by fluorescence emission at 510 nm (excitation at 390 nm) with a Wallac 1420 Victor2 Multilabel Reader (PerkinElmer Life Sciences) and were analyzed as described (4). Protein concentration of the samples was determined using Bio-Rad protein assay dye reagent (Bio-Rad) and bovine serum albumin as the standard.

Radioligand Binding Assays—Binding of 17β-estradiol (dissolved in ethanol/DMSO (50:50, v/v)) or estrone (dissolved in DMSO) to different SUR forms was determined in heterologous competition experiments using either [3H]glibenclamide or [3H]P1075 ([3H]N-cyano-N’-(1,1-dimethylpropyl)-N’-3-pyridyl-guanidine) as the radioligands (final label concentrations 1.0–3.0 nmol/liter). Membranes prepared from the respective HEK293 cell lines (0.01–0.30 mg of membrane protein/ml) were incubated at 37 °C for 15 ([3H]glibenclamide), 12 ([3H]P1075: SUR2A, SUR2A(Y1206S)), or 30 min ([3H]P1075: SUR2B, SUR2B(Y1206S)) and binding properties were analyzed according to Hambrock et al. (19). Nonspecific radioligand binding was determined in the presence of 10 μmol/liter unlabeled glibenclamide or 100 μmol/liter P1075, respectively, or at membranes from pcDNA cells.

Data Analysis—Data were calculated as mean ± S.E. from single experiments. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA). Two-tailed Student’s paired or unpaired t test and one-way analysis of variance in combination with Tukey’s post hoc test were employed as appropriate and a p value < 0.05 was considered statistically significant.

Equilibrium inhibition curves were analyzed as described by Hambrock et al. (4) using the logarithmic form of the Hill equation,

$$y = 100 - A(1 + 10^{nH p}x)^{-x}$$  \hspace{1cm} (Eq. 1)

A denotes the extent of inhibition, $n_H$ the Hill coefficient, and $IC_{50}$ the midpoint of the curve with $pIC_{50} = -\log IC_{50}$. $x$ is the concentration of the inhibitor, with $px = -\log x$. The $IC_{50}$ values were converted into inhibition constants ($K_I$) by correcting for the presence of the radioligand (L), according to the Cheng-Prusoff equation,

$$K_I = IC_{50}(1 + LK_E^{-1})^{-1}$$  \hspace{1cm} (Eq. 2)

where $K_E$ is the equilibrium dissociation constant of the radioligand. Fits of the equation to the data were performed using SigmaPlot software (SPSS, Inc., Chicago, IL).
Materials—Dulbecco’s modified Eagle’s medium was obtained from Cambrex Bio Science (Verviers, Belgium). All other reagents used for cell culture or molecular biology were purchased from Invitrogen. AFC standards, 17β-estradiol, estrone, Hoechst 33258, and resveratrol were from Sigma and DEVD- AFC from Biomol (Hamburg, Germany). [3H]Glibenclamide (specific activity 1.55 TBq) and Ultima Gold scintillant were purchased from PerkinElmer Life Sciences. [3H]P1075 (specific activity 4.26 TBq) was from GE Healthcare.

RESULTS

Aptotic Effect of 17β-Estradiol or Estrone in Recombinant SUR1-expressing HEK293 Cells—The apoptotic potency of 17β-estradiol or estrone in recombinant HEK293 cells expressing the pancreatic isofrom SUR1 (SUR1 cells) was evaluated by determination of cell detachment after treatment of SUR1-expressing cells (SUR1) or pcDNA control cells (pcDNA) with different substances (100 μmol/liter) or the respective amount of solvent (etoposide, resveratrol, DMSO; glibenclamide, 17β-estradiol, estrone, ethanol/DMSO (50:50, v/v)) for 24 h (or 48 h in case of glibenclamide) (4). In contrast to treatment with glibenclamide, resveratrol, 17β-estradiol, or estrone (chemical structures shown in B), exposure to the chemotherapeutic agent etoposide did not result in SUR1-specific enhancement of cell detachment. Data for 17β-estradiol and estrone are derived from a 9/4 series of experiments. Data for glibenclamide, resveratrol, and etoposide are from Refs. 3 and 4. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Effect of different substances on detachment of recombinant SUR1-expressing and pcDNA control cells. Cell viability was quantified by determination of cell detachment after treatment of SUR1-expressing cells (SUR1) or pcDNA control cells (pcDNA) with different substances (100 μmol/liter) or the respective amount of solvent (etoposide, resveratrol, DMSO; glibenclamide, 17β-estradiol, estrone, ethanol/DMSO (50:50, v/v)) for 24 h (or 48 h in case of glibenclamide) (4). In contrast to treatment with glibenclamide, resveratrol, 17β-estradiol, or estrone (chemical structures shown in B), exposure to the chemotherapeutic agent etoposide did not result in SUR1-specific enhancement of cell detachment. Data for 17β-estradiol and estrone are derived from a 9/4 series of experiments. Data for glibenclamide, resveratrol, and etoposide are from Refs. 3 and 4. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Activities of Different Caspase Enzymes in Recombinant SUR1-expressing Cells after 17β-Estradiol Treatment—To narrow down the broad spectrum of signaling pathways leading to the induction of apoptotic processes, the activities of different key caspase enzymes were determined. Accordingly, we measured the activity of caspase-8 (which mediates the death receptor pathway), caspase-9 (which is essentially involved in the mitochondrial apoptotic pathway), and caspase-12 (which triggers endoplasmic reticulum stress) after 17β-estradiol treat-
17β-Estradiol Modulates β-Cell Apoptosis via SUR1

FIGURE 2. Activities of different caspase enzymes in recombinant SUR1-expressing cells or pcDNA control cells after 17β-estradiol treatment. The activities of caspase-3 (A), caspase-8 (B), caspase-9 (C), and caspase-12 (D) were measured in SUR1-expressing cells (SUR1) or pcDNA control cells (pcDNA) after exposure to 100 μmol/liter 17β-estradiol (E2) or solvent (solv.) for 24 h. Enzyme activities were determined as an increase in fluorescence per minute normalized to the protein content of each sample (final concentration in the assay about 0.5–1.0 mg/ml). As cleavage of the respective peptide-substrate by other enzymes with lower affinity cannot be completely excluded, turnover of the specific substrate is, strictly speaking, due to the activity of “caspase-x-like” enzymes. Data, given as arbitrary units, have been determined in parallel experiments (n = 4–9). Please note that different scales are used in the figures. It has also to be taken into account that caspase-12 activity was determined via an experimental procedure different from that employed for caspases-3, -8, and -9 and therefore the arbitrary units shown in this case cannot directly be compared with the values shown for the other enzymes. Statistical differences between the different cell lines or treatment groups are given in the figures (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

FIGURE 3. Apoptotic effect of 17β-estradiol treatment on recombinant cells expressing different SUR isoforms or mutants. After treatment of SUR1, SUR1(M1289T), SUR2A, and SUR2B-expressing HEK293 cells (A and B) or of SUR1, SUR1(R1379C), and SUR1(R1379L) cells (C) with 17β-estradiol (100 μmol/liter, 24 h), cell detachment or changes in nuclear morphology were determined and compared with the results obtained with pcDNA control cells (please note the different scales in A and C). The extent of nuclear damage was quantified by determination of the percentage of apoptotic nuclei per total nuclei after Hoechst 33258 staining. The apoptotic parameters for all cell lines shown in one diagram were determined in 4–5 parallel series of experiments. For quantification of nuclear damage, 1000–2500 nuclei in 100–225 different fields (50 × 50 μm²) were evaluated per treatment group. Statistical differences between the different cell lines are given (*, p < 0.05; **, p < 0.01; ***, p < 0.001). The generally highly significant differences between 17β-estradiol-treated and solvent-treated cells (p < 0.001 or p < 0.01) are not shown here. No significant differences were detected among all the solvent-treated cell lines.

Effect of 17β-Estradiol Treatment on Apoptotic Processes in Pancreatic Islets from Mice of Different Age
17β-Estradiol Modulates β-Cell Apoptosis via SUR1

Groups—In contrast to the apoptotic effect of 17β-estradiol in SUR1-expressing HEK293 cells or in clonal β-cells, 17β-estradiol treatment of isolated islets of Langerhans from C57BL/6 mice resulted in either induction or prevention of apoptotic cell death dependent on the age of the animals (Fig. 5). The situation was complicated by the facts that (i) solvent-treated control islets from young mice (aged 5–7 weeks) and elderly mice (aged 20–32 weeks) exhibited different basal apoptotic levels, (ii) that these levels were not the same in male and female mice, and (iii) that the islets from young or old and/or male or female mice were influenced in a different way in the course of cultivation.

17β-Estradiol Treatment of Pancreatic Islets from Mice Aged 20–32 Weeks—Treatment of islets from female elderly mice (20–32 weeks, weight 22–46 g) with 100 μmol/liter 17β-estradiol induced a significant increase in the percentage of apoptotic nuclei compared with solvent-treated controls (Fig. 5B). The rate of apoptotic nuclei increased 2.0-fold after 17β-estradiol treatment for 18 h and 4.5-fold after treatment for 72 h. Significant effects were also observed with lower concentrations (Fig. 6). Exposure to 10 nmol/liter 17β-estradiol for 72 h already resulted in a clear increase in apoptosis (7.4 ± 0.6% apoptotic nuclei) compared with solvent-treated islets (3.4 ± 0.8%). The effects of 100 nmol/liter (9.6 ± 0.7%) and 1 μmol/liter 17β-estradiol (11.3 ± 0.8%) were even stronger. Concentrations >1 μmol/liter did not lead to a further increase in the apoptotic effect (10 μmol/liter, 9.8 ± 1.5%; 100 μmol/liter, 11.3 ± 1.3%).

In the islets from male mice, also a slight (1.4-fold) increase in the percentage of apoptotic nuclei was observed after 17β-estradiol treatment for 18 h, but no difference was found in the generally higher rate of apoptosis after 72 h.

17β-Estradiol Treatment of Pancreatic Islets from Mice Aged 5–7 Weeks—When islets from young male or female mice (5–7 weeks, weight 13–28 g) were treated with 100 μmol/liter 17β-estradiol for different intervals, the percentage of apoptotic nuclei was clearly reduced by a factor of 5.1 (18 h), 3.9 (24 h), or 5.6 (48 h) in female islets, but only by a factor of 1.5 (18 h) or 2.7 (24 h, 48 h) in male islets.

Generally, the percentage of apoptotic nuclei in solvent-treated islets from young mice was much higher than in elderly mice. In addition, apoptosis in islets from young female mice was significantly higher than in islets from young male mice after 18 h of solvent treatment. The rate of apoptosis decreased slightly in islets from young female mice during the next 30 h of cultivation, whereas it increased in young male mice.

Effect of 17β-Estradiol Treatment on Pancreatic Islets from SUR1KO Mice Versus Wild-type Mice—In contrast to wild type mice, no significant differences were observed between islets from SUR1KO mice treated with 17β-estradiol (100 μmol/liter) or with solvent (Fig. 5). This observation was made for female and male mice of both age groups. In some cases, especially in untreated/solvent-treated islets from young mice, which exhibit a high rate of apoptosis, the extent of apoptosis was somewhat lower in the islets isolated from SUR1KO mice than in the islets from wild type mice.

Binding of 17β-Estradiol or Estrone to Different SUR Isoforms or Mutants—To investigate whether 17β-estradiol was a specific SUR ligand, we performed heterologous competition experiments using the 3H-labeled KATP channel blocker glibenclamide, which binds to specific sites at SUR, as the radioligand and 17β-estradiol as the inhibitor (Fig. 7). Because binding of
17β-Estradiol Modulates β-Cell Apoptosis via SUR1

![Graph: Effect of different 17β-estradiol concentrations on isolated islets from female mice aged 20–32 weeks. Isolated islets from female wild type mice aged 20–32 weeks were treated with different concentrations of 17β-estradiol or solvent. Data are given as the mean ± S.E. *p < 0.05; **, p < 0.01; ***, p < 0.001.]

K$_{ATP}$ channel blockers or openers is influenced by nucleotides in different ways, the experiments were performed either in the presence or absence of MgATP.

At the physiological MgATP concentration of 1 mmol/liter, 17β-estradiol displaced specific [³H]glibenclamide binding in membranes from SUR1 expressing HEK293 cells to 55.1 ± 11.2% with an inhibitory constant ($K_i$) of 81.4 ± 26.3 μmol/liter (Table 1). Without MgATP, no displacement of [³H]glibenclamide binding was detectable. A comparable nucleotide dependence was also observed in the case of the mutant SUR1(M1289T) with binding curves being similar to those obtained for SUR1. Slight, but not significant differences were found concerning the $K_i$ value and amount (A) of displaced specific [³H]glibenclamide binding.

To be able to investigate 17β-estradiol binding properties in corresponding [³H]glibenclamide/17β-estradiol inhibition experiments also with the SUR2 isoforms, the mutation Y1206S was inserted in SUR2A and SUR2B. This mutation endows both isoforms, normally exhibiting a much lower glibenclamide affinity than SUR1, with a higher affinity toward this ligand (24, 25). 17β-Estradiol displaced [³H]glibenclamide binding at 1 mmol/liter MgATP to a large extent, but only weakly inhibited binding of [³H]glibenclamide without MgATP. Certain differences to the binding parameters of SUR1 were observed (Table 1), but again these differences were not significant.

In case of the different SUR2 forms, we also investigated the interaction between 17β-estradiol and the tritium-labeled opener P1075, a SUR-specific ligand showing high affinity to SUR2 with $K_j$ values in the low nanomolar range (26–28). In these experiments, a clear inhibition of [³H]P1075 binding by 17β-estradiol was seen with SUR2A(Y1206S) and SUR2B(Y1206S) as well as SUR2A and SUR2B. In these assays, only minor differences between the binding parameters were obtained for SUR2A and SUR2B(Y1206S) ($K_j = 20.6 ± 4.7$ and $27.8 ± 8.0$ μmol/liter, A = $37.6 ± 8.9/41.0 ± 13.2%$) or SUR2B and SUR2B(Y1206S) ($K_j = 17.4 ± 4.7$ and $16.1 ± 1.9$ μmol/liter, A = $69.5 ± 6.5/52.8 ± 8.1%$), which indicates that the mutation Y1206S did not visibly affect the interaction between 17β-estradiol and [³H]P1075.

In contrast to 17β-estradiol, estrone did not displace [³H]glibenclamide binding at SUR1 in the concentration range tested. In the case of SUR2B, no interaction between estrone and [³H]glibenclamide was discovered (using SUR2B(Y1206S)) and estrone displaced binding of [³H]P1075 to a much smaller extent (A = $19.7 ± 2.3%$) than 17β-estradiol (using SUR2B wild type). The $K_i$ value derived from [³H]P1075/estrone inhibition curves ($K_i = 6.7 ± 1.6$ μmol/liter) was slightly lower than in case of [³H]P1075/17β-estradiol inhibition.

DISCUSSION

Protection of pancreatic β-cells from apoptosis (e.g. after islet transplantation) is an attractive target for pharmacological
intervention in diabetes therapy (6, 7, 29). 17β-Estradiol has been proposed as one successful candidate for preservation of β-cell mass because of its protective effects on β-cells observed under certain experimental conditions (30–33). However, results obtained after 17β-estradiol treatment of different cells are often contradictory suggesting either anti-apoptotic or apoptotic effects of this substance (34–36).

Our experiments performed with recombinant HEK293 cells clearly show that 17β-estradiol can induce apoptotic processes specifically depending on the expression of the SUR1 isoform (but not of SUR2A or SUR2B) and not requiring the presence of the pore-forming K\textsubscript{ATP} channel subunit Kir6.x. This SUR1-dependent effect of 17β-estradiol is either abolished by mutation M1289T or enhanced by mutations R1379C or R1379L in SUR1. With 17β-estradiol, we have now identified an endogenous K\textsubscript{ATP} channel blocking agent that shows a much higher SUR1-specific apoptotic potency than the blockers glibenclamide (3) or resveratrol (4).

A clear apoptotic effect of 17β-estradiol is likewise found in clonal β-cells and in isolated pancreatic islets from elderly wild type mice aged 20–32 weeks, which all endogenously express the SUR1 isoform, but it is absent in isolated islets from SUR1KO mice of the respective age. In contrast, very clear anti-apoptotic effects are observed after exposure to the same 17β-estradiol concentration in young mice (5–7 weeks). Also this such as cytokines, streptozotocin, or H\textsubscript{2}O\textsubscript{2}. Our data now recommend a very cautious interpretation concerning definite statements about either protective or apoptotic effects of a certain substance and suggest that factors such as age and gender should also be considered. This is in accordance with a study by Maedler et al. (38) showing that aging correlates with changes in β-cell turnover and an enhanced sensitivity to glucose-induced β-cell apoptosis that could provide an explanation for the increased incidence of type 2 diabetes at an older age.

Many experiments were performed with the relatively high concentration of 100 μmol/liter 17β-estradiol (24 h) to be able to compare the results with previous experiments performed with substances such as etoposide, glibenclamide, or resveratrol (3, 4) and because of methodical requirements in the cell culture model (see “Results”). However, in isolated pancreatic islets, clear apoptotic effects were already observed after treatment with 10 nmol/liter 17β-estradiol for 3 days (female elderly mice). Obviously, these islets are much more sensitive to treatment with lower 17β-estradiol concentrations than recombinant SUR1-expressing HEK293 cells. In humans, an estradiol concentration of 10 nmol/liter is readily achieved in the later phase of pregnancy. During gestation, unconjugated serum effect of 17β-estradiol is SUR1-specific because it is not detectable in islets from SUR1KO mice.

Accordingly, age can be an important factor influencing the nature of 17β-estradiol-mediated effects. In young mice, untreated (or solvent-treated) islets are characterized by a high degree of apoptosis, probably due to intensive adaptive modulation of β-cell mass at this stage (37). Here, protective effects of 17β-estradiol are observed. Apoptosis in untreated/solvent-treated islets from elderly mice, however, is low, possibly because of less intensive β-cell turnover in adult individuals (6, 37). In this case, 17β-estradiol treatment promotes apoptotic processes, as it does in all SUR1-expressing cell culture cells tested in our experiments. Similar, but weaker effects were observed after treatment of female mice with resveratrol, again with an apoptotic action in elderly mice aged 20–32 weeks and an anti-apoptotic action in young mice aged 5–7 weeks.3

In those studies suggesting a protective effect of 17β-estradiol in β-cells (30–32), islets were isolated from young individuals and/or were pre-exposed to apoptotic stimuli intervention in diabetes therapy (6, 7, 29). 17β-Estradiol has

3 S. Ackermann, H. Osswald, and A. Hambrock, unpublished results.
TABLE 1

Binding parameters derived from the heterologous competition experiments using [3H]glibenclamide as the radioligand and 17β-estradiol as the competitor

| Parameter | SUR1 | SUR1(M1289T) | SUR2A(Y1206S) | SUR2B(Y1206S) |
|-----------|------|--------------|---------------|---------------|
|           | +MgATP | −MgATP | +MgATP | −MgATP | +MgATP | −MgATP | +MgATP | −MgATP |
| \( K_i \) (nmol/liter) | 81.4 ± 26.3 | 6.2 ± 2.3 | 6.2 ± 2.3 | 6.2 ± 2.3 | 81.4 ± 26.3 | 6.2 ± 2.3 | 6.2 ± 2.3 | 6.2 ± 2.3 |
| n | 11 | 6 | 6 | 4 | 7 | 7 | 7 | 7 |

a Binding parameters (\( K_i \), inhibition constant, A: amount of displaced specific radioligand binding, [B]) are means from the data determined in the individual [3H]glibenclamide inhibition experiments (n = number of experiments) as described in the legend to Fig. 7. For correction of the inhibition curves according to the Cheng-Prusoff equation (see Experimental Procedures) the following \( K_i = \bar{K}_i \) values for glibenclamide were taken into account (SUR1 + MgATP/−MgATP; 3.2/1.4 nmol/liter, SUR1(M1289T) + MgATP/−MgATP; 15.2/15.4 nmol/liter, SUR2A(Y1206S) + MgATP/−MgATP; 11.9/9.8 nmol/liter).

b In case of the experiments that were performed in the absence of MgATP, binding parameters were determined from the fits of the summarizing curves because under these conditions [3H]glibenclamide inhibition by 17β-estradiol was very weak.

17β-estradiol levels rise considerbly with concentrations of about 2–3 nmol/liter at the beginning of pregnancy (weeks 6–8) and concentrations between ~50 and 100 nmol/liter in the last trimester (39–41). At this point of time, maternal serum estradiol reaches values that can be more than 100 times higher than in nonpregnant states (follicular phase, ~0.1–1.0 nmol/liter; luteal phase, 0.5–2.0 nmol/liter) (42). Normally, the third trimester is the stage in which GDM is diagnosed. As several studies suggest, GDM is not exclusively caused by an increase in insulin resistance, but also by a spectrum of other factors (12). According to our data, 17β-estradiol-mediated changes in β-cell mass might also contribute to the etiology of GDM. This idea is congruent with the observation by van Assche et al. (11) that the number of β-cells in pregnant diabetic rats does not increase as it does in pregnant non-diabetic rats.

At concentrations of 0.1–1.0 nmol/liter, 17β-estradiol elicits a rapid insulinotropic effect by blocking pancreatic K\(_{\text{ATP}}\) channels (15–17, 43). It is still discussed whether 17β-estradiol directly interacts with K\(_{\text{ATP}}\) channels (17) or whether it binds to a so far unidentified “non-classical” plasmalemmal estrogen receptor that is linked to K\(_{\text{ATP}}\) channels via an intracellular signaling cascade (15, 16, 44). Our binding experiments performed with membranes from recombinant HEK293 cells, in which endogenous expression of the classical estrogen receptors ER\(_{\alpha}\) or ER\(_{\beta}\) is lacking (45) or very low (46, 47), show that 17β-estradiol displaces specific binding of [3H]glibenclamide (dependent on the presence of MgATP) and/or [3H]P1075 and thus clearly interacts with SUR. However, no clear differences in 17β-estradiol binding properties are discernible between SUR1 and the other forms of SUR, although cells expressing these different SUR forms are characterized by a very different susceptibility to 17β-estradiol treatment. In accordance with our previous results with resveratrol, no direct correlation exists between the quite low affinity of 17β-estradiol to SUR1 and its high potency in inducing apoptosis in SUR1-expressing cells. Estrone, another naturally occurring steroid hormone that differs from 17β-estradiol in a single substituent (Fig. 1B), does not displace [3H]glibenclamide binding and only weakly inhibits [3H]P1075 binding in the tested concentration range. Like 17β-estradiol, estrone induces enhanced apoptosis in SUR1-expressing cells, but to a much smaller extent.

Gluconorinated 17β-estradiol is a transport substrate of different multidrug resistance-associated proteins (MRPs, e.g. MRP1 or MRP3), which like SUR are members of the ATP-binding cassette subfamily C. Transport of 17β-estradiol 17β-glucuronide is markedly reduced by some mutations in transmembrane helix 17 (TM17) of these multidrug resistance-associated proteins (48, 49). The mutation M1289T, located in TM17 of SUR1, influences binding (19) and action (18) of several K\(_{\text{ATP}}\) channel openers. In the mutant SUR1(M1289T), a single amino acid in TM17 is exchanged by the corresponding amino acid of SUR2. (TM17 is highly conserved at SUR1 and SUR2 in its amino acid sequence except for five amino acids at positions 1285, 1289, 1290, 1291, and 1297, rat clone numbering.) The mutation M1289T completely abolishes the SUR1-specific apoptotic effects of K\(_{\text{ATP}}\) channel blockers glibenclamide (3) or resveratrol (4), or 17β-estradiol obviously without directly affecting binding of these substances to SUR1. These findings suggest an important role of (parts of) TM17 in the modulation of apoptotic processes, although this molecular region of SUR is probably not the only area involved.

To see whether SUR1-mediated apoptosis is linked with ATP hydrolysis, we explored the effects of mutations R1379C and R1379L in nucleotide binding fold 2 of SUR1 on 17β-estradiol action. Both mutations result in an increased ATPase activity of SUR (20). Interestingly, these mutations have been identified in patients with transient neonatal diabetes with some of them showing a family history in adult-onset type 2 diabetes or GDM (20–22). Our observation that these mutations clearly increase susceptibility of SUR1-expressing cells to 17β-estradiol-induced apoptosis points to an essential role of nucleotide binding fold function in this context.

With the visible enhancement of caspase-9 activity in SUR1-expressing cells after 17β-estradiol treatment, we have obtained an important hint to the signaling pathway(s) involved in SUR1-specific apoptosis modulation. Considering also our previous results observed after resveratrol treatment (4), these data point to a major role of the mitochondrial apoptotic pathway.

Taken together, 17β-estradiol can modulate apoptosis in pancreatic β-cells dependent on expression of SUR1, which obviously can act as a non-classical estrogen receptor. Future work will have to provide more information about the precise molecular mechanisms linking SUR function and the signaling cascades that modulate apoptotic cell death. Especially those factors determining whether protective or apoptotic effects are finally induced need to be identified. This is particularly important for the careful interpretation of studies suggesting a lower incidence of diabetes after estradiol replacement therapy (50, 51). Several mutations in the genes encoding both K\(_{\text{ATP}}\) channel subunits, SUR or Kir6.\(_x\), are known to confer an increased
risk for the development of diabetes because of defects in channel function (1, 2, 21). Yet, the observation that certain SUR1 mutations also affect the apoptotic action of substances like estrogen may provide a new context between single nucleotide polymorphisms in the SUR1 (ABCC8) gene and pathophysiological changes in β-cell mass finally leading to diabetes.

Acknowledgments—We are very grateful to Drs. Lydia Aguilar-Bryan and Joseph Bryan for providing the SUR1KO mice. We also thank Dr. Gisela Drews, Belinda Gier, and Stefanie Laucher for help with the SUR1KO or wild type mice and Dr. Hua Zhang for assistance with the isolation of pancreata. We acknowledge Dr. Christian Derst for kindly providing the cDNA of SUR1 and Drs. Yoshihisa Kurachi and Yoshiyuki Horio for the gift of the SUR2A, SUR2B, and Kir6.2 clones. We thank Claudia Müller for excellent assistance in isolation of islets and cell culture.

REFERENCES

1. Bryan, J., Muñoz, A., Zhang, X., Düfer, M., Drews, G., Krippeit-Drews, P., and Aguilar-Bryan, L. (2007) Pflugers Arch. Eur. J. Physiol. 453, 703–718
2. Ashcroft, F. M. (2007) Am. J. Physiol. 293, E880–E889
3. Hambrock, A., de OliveiraFranz, C. B., Hiller, S., and Osswald, H. (2006) J. Pharmacol. Exp. Ther. 316, 1031–1037
4. Hambrock, A., de Oliveira Franx, C. B., Hiller, S., Grenz, A., Ackermann, S., Schulze, D. U., Drews, G., and Osswald, H. (2007) J. Biol. Chem. 282, 3347–3356
5. Kahn, S. E., Hull, R. L., and Utschneider, K. M. (2006) Nature 444, 840–846
6. Butler, P. C., Meier, J. J., Butler, A. E., and Bhushan, A. (2007) J. Biol. Chem. 282, 1031–1037