Original Article

Dexamethasone Induces Cell Death in Insulin-Secreting Cells, an Effect Reversed by Exendin-4

Felicia Ranta,[1] Diana Avram,[1] Susanne Berchtold,[1] Martina Düfer,[2] Gisela Drews,[2] Florian Lang,[1] and Susanne Ullrich[1]

Glucocorticoid excess induces hyperglycemia, which may result in diabetes. The present experiments explored whether glucocorticoids trigger apoptosis in insulin-secreting cells. Treatment of mouse β-cells or INS-1 cells with the glucocorticoid dexamethasone (0.1 μmol/l) over 4 days in cell culture increased the number of fractionated nuclei from 2 to 7 and 14%, respectively, an effect that was reversed by the glucocorticoid receptor antagonist RU486 (1 μmol/l). In INS-1 cells, dexamethasone increased the number of transferase-mediated dUTP nick-end labeling–staining positive cells, caspase-3 activity, and poly-(ADP-) ribose polymerase protein cleavage; decreased Bcl-2 transcript and protein abundance; dephosphorylated the proapoptotic protein of the Bcl-2 family (BAD) at serine155; and depolarized mitochondria. Dexamethasone increased PP-2B (calcineurin) activity, an effect abrogated by FK506. FK506 (0.1 μmol/l) and another calcineurin inhibitor, deltamethrin (1 μmol/l), attenuated dexamethasone-induced cell death. The stable glucagon-like peptide 1 analog, exendin-4 (10 nmol/l), inhibited dexamethasone-induced apoptosis in mouse β-cells and INS-1 cells. The protective effect of exendin-4 was mimicked by forskolin (10 μmol/l) but not mimicked by guanine nucleotide exchange factor with the specific agonist 8CPT-Me-cAMP (50 μmol/l). Exendin-4 did not protect against cell death in the presence of cAMP-dependent protein kinase (PKA) inhibition by H89 (10 μmol/l) or KT5720 (5 μmol/l). In conclusion, glucocorticoid-induced apoptosis in insulin-secreting cells is accompanied by a downregulation of Bcl-2, activation of calcineurin with subsequent dephosphorylation of BAD, and mitochondrial depolarization. Exendin-4 protects against glucocorticoid-induced apoptosis, an effect mimicked by forskolin and reversed by PKA inhibitors. *Diabetes* 55:1380–1390, 2006

Glucocorticoids are widely used therapeutic tools, particularly in treatment for anti-inflammatory and immunomodulatory purposes. Side effects of glucocorticoid treatment include steroid diabetes (1,2). Glucocorticoid-induced hyperglycemia is partially due to increased hepatic glucose production and insulin resistance of peripheral tissues. Moreover, glucocorticoids are known to inhibit insulin secretion (3,4). The underlying mechanism involves increased α_2-adrenoceptor signaling (5), increased K_v channel activity (6), and impaired glucose metabolism (7,8). Although reduced insulin secretion during glucocorticoid treatment can be overcome by blocking adrenoceptor signaling or by inhibition of K_v channels, compelling evidence suggests that the proper functioning of β-cells also depends on cell survival (9). Accordingly, a reduction of β-cell mass in long-standing glucocorticoid therapy may contribute to the consecutive development of steroid diabetes.

Glucocorticoids are known to induce apoptosis and reduce proliferation in a variety of different cells, including muscle (10), bone cells (11), thymocytes (12), lymphoid cells (13), proliferative chondrocytes (14), and myeloma cells (15). Glucocorticoids have been shown to induce oxidative stress and the release of mitochondrial cytochrome c (16–20). On the other hand, glucocorticoids have been proposed to suppress prosurvival factors such as nuclear factor-κB (21,22). Thus, glucocorticoids seem to target distinct intracellular signaling pathways depending on the cell system, which finally leads to apoptotic cell death.

Apoptotic death of β-cells following exposure to fatty acids, H_2O_2, immunosuppressive drugs, streptozotocin, or staurosporine is blunted by substances that increase cytosolic cAMP levels and potentiate glucose-induced insulin release (23–27). Glucagon-like peptide 1 (GLP-1) receptor activation leads to G-protein–dependent stimulation of adenylyl cyclase, cAMP formation, and subsequent activation of PKA, guanine nucleotide exchange factor (GEF) and cAMP response element–binding protein. cAMP-dependent protein kinase (PKA) was found to phosphorylate the proapoptotic protein of the Bcl-2 family (BAD), which favors the binding of BAD to the chaperone protein 14-3-3, keeping BAD in the cytosol (28). Dephosphorylated BAD binds the antiapoptotic mitochondrial protein Bcl-2 and thus triggers opening of the mitochondrial permeability transition pore, cytochrome c release, and caspase activation.

The present study was performed to explore whether the glucocorticoid dexamethasone is able to induce apo-

From the [1]Institute of Physiology, University of Tübingen, Tübingen, Germany; and the [2]Department of Pharmacology, Institute of Pharmacy, University of Tübingen, Tübingen, Germany.

Address correspondence and reprint requests to Dr. Susanne Ullrich, Institut für Physiologie, Gmelinstrasse 5, D-72076 Tübingen, Germany. E-mail: susanne.ullrich@uni-tuebingen.de.

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S.B. is currently affiliated with the Medical Microbiology and Hygiene Department, University of Tübingen, Tübingen, Germany.

BAD, proapoptotic protein of the Bcl-2 family; DAPI, 4′6-diamidino-2-phenylindole; GEF, guanine nucleotide exchange factor; GLP-1, glucagon-like peptide 1; PARP, poly-(ADP-) ribose polymerase; PKA, cAMP-dependent protein kinase; PNA, *p*-nitroanilide; TUNEL, transferase-mediated dUTP nick-end labeling.

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ptosis of insulin-secreting cells and to possibly disclose mechanisms mediating and counteracting glucocorticoid-induced cell death.

RESEARCH DESIGN AND METHODS

Antibodies for poly-(ADP-) ribose polymerase (PARP) (no. 9,542) and BAD (no. 9,292) were from Cell Signaling Technology (Beverly, MA). Antibodies against 155-serine Phospho BAD (sc-12970-R), Bcl-2 (sc-7382), and β-tubulin–horseradish peroxidase (sc-5274HRP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and peroxidase-conjugated anti–guinea pig IgG were from Biomol. Enzymes for RT-PCR were from Qiagen (Hilden, Germany) and Roche Diagnostics (Mannheim, Germany). Cell culture media were purchased from GIBCO (Invitrogen, Karlsruhe, Germany) and FCS from Biochrom (Berlin, Germany). The protein assay was purchased from Bio-Rad Laboratories (München, Germany) and the protein ladder (no. 10748-010) and DNA ladder (no. 15613-011) from Invitrogen (Karlsruhe, Germany). H-89 was purchased from Calbiochem (Merck Biosciences, Bad Soden, Germany). The transferase-mediated dUTP nick-end labeling (TUNEL) assay kit (no. 1 684 795) was purchased from Roche Diagnostics. All other chemicals including 4′6-diamidino-2-phenylindole (DAPI), exendin-4, RU486, dexamethasone, δ-methrin, FK506, caspase-3, and calcineurin assays were from Sigma (Deisenhofen, Germany) and of analytical grade unless otherwise stated.

Cell culture. INS-1 cells (provided by C.B. Wollheim, University of Geneva, Geneva, Switzerland) derived from a rat insulinoma were cultured in HEPES-buffered RPMI-1640 supplemented with 10% FCS, 1 mmol/l HEPES, 1 mmol/l L-glutamine, 1 mmol/l Na pyruvate, 10 μmol/l β-mercaptoethanol, and antibiotics as described previously (6). Mouse islets (from C57BL/6 mice; Charles River Laboratories, Wilmington, MA) were isolated and single cells prepared as described previously using collagenase (Serva, Heidelberg, Germany) (6). They were cultured under the same conditions as INS-1 cells using RPMI-1640 without addition of β-mercaptoethanol. After 2 days, culture cells were treated with dexamethasone, 100 nmol/l dissolved in DMSO, for the indicated time period and proceeded further for DAPI or TUNEL staining as described below.

DAPI (DNA fluorescent) and TUNEL staining. Mouse β-cells and INS-1 cells, at a density of 2×10^5 cells/ml, were seeded onto poly-L-ornithine–coated glass cover slips in RPMI-1640 medium (6). They were cultured for 2 days and test substances added with fresh medium as indicated. After incubation for the appropriate time period, cells were fixed with ethanol (100%) for 5 min at room temperature and then incubated with DAPI (100 ng/ml ethanol) for 15 min at 37°C. After washing the cells with cold PBS, the morphology of stained nuclei was analyzed under an inverted fluorescence microscope (×40 objective; Nikon, Japan) using an excitation wavelength of 350 nm. Apoptotic nuclei were identified by fractionated and condensed chromatin. Between 100 and 200 cells per condition per experiment were counted and the percentage of apoptotic nuclei determined. For TUNEL staining, cells were fixed and processed as described by the protocol enclosed in the commercial kit.

Measurements of mitochondrial potential (ΔΨ). INS-1 cells were prepared as for DAPI staining and treated with 100 nmol/l dexamethasone for 1 day. Thereafter, cells were loaded with rhodamine-123, 10 μg/ml, for 10 min at 37°C. The fluorescence was excited at 480 nm, and the intensity of the emitted light (filtered through a long-pass filter of 515 nm) was measured using a digital camera and software provided by Till Photonics (Planegg, Germany).

Caspase-3 assay. Cells were seeded into culture dishes at a density of 4×10^6 cells/75-cm^2 dish and cultured for 3 days before adding test substances as indicated. Thereafter, culture medium was removed, and cells were washed once with ice-cold PBS and assayed for caspase-3 activity using a colorimetric assay (colorimetric caspase-3 assay kit; Sigma). Cells were lysed in lysis buffer containing 50 mmol/l HEPES, pH 7.4, 5 mmol/l 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, and 5 mmol/l dithiothreitol. Nuclei and organelles were removed by centrifugation at 20,000*g*. The supernatant was frozen in liquid nitrogen, and 50 μg of total cytosolic protein was used to assess cytosolic caspase-3 activity. To this end, cell homogenates were incubated up to 4 h at 37°C with caspase-3 substrate conjugated to the chromophore *p*-nitroanilide (Ac-DEVD-pNA; 1 mmol/l) in the presence and absence of the inhibitor Ac-DEVD-CHO (0.1 mmol/l). Cleavage of Ac-DEVD-pNA substrate by caspase-3 releases pNA, which was quantified spectrophotometrically at 405 nm using an enzyme-linked immunosorbent assay reader (Biorad Laboratories, München, Germany). The change in optical density is directly proportional to caspase-3 activity. The activity inhibited by the caspase-3 inhibitor was calculated as specific activity.

Measurements of phosphatase activity. INS-1 cells were prepared as for measurements of caspase-3 activity and treated with dexamethasone, 100 nmol/l, and RU486, 1 μmol/l, for 1 day. Thereafter, cells were lysed in 50 mmol/l Tris HCl, pH 7.0, supplemented with 0.1 mmol/l $CaCl_2$, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.5 mmol/l phenylmethylsulfonyl fluoride, and 0.5 mmol/l dithiothreitol. Phophatase activity in cytosolic lysate (4–8 μg/200 μl) was measured using 10 μg/ml calmodulin, 10 mmol/l $NiCl_2$, and 0.9 mg/ml *p*-nitrophenylphosphate as substrate with and without 100 μmol/l EGTA. Dephosphorylated *p*-nitrophenylphosphate (*p*-nitrophenol) was transformed into *p*-nitrophenolate with NaOH, a yellow-colored compound absorbing at 405 nm. The activity sensitive to EGTA was calculated as PP-2B specific activity.

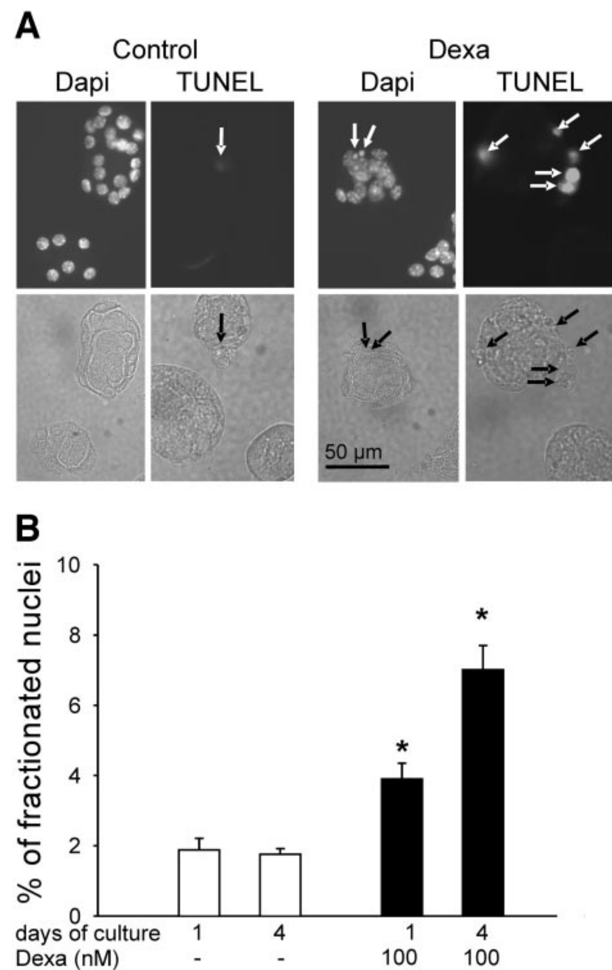

FIG. 1. Dexamethasone (Dexa)-induced cell death in mouse islet cells. Cells were cultured with 100 nmol/l dexamethasone for 1 and 4 days. *A*: Representative images of cells and fluorescent DAPI- and TUNEL-stained nuclei were taken after 4 days. Arrows indicate condensed nuclei. *B*: Cell death is expressed as percentage of condensed nuclei. *Denotes significant effect of dexamethasone versus control at the respective time point.

Western blotting. INS-1 cells, 4–5 × 10^6 cells/75-cm^2 culture dish, were cultured for 3 days. Thereafter, cells were treated with test substances as indicated. Culture medium was removed, and cells were lysed in a solution containing 125 mmol/l NaCl, 1% (vol/vol) Triton X-100, 0.5% sodiumdeoxycholate, 0.1% SDS, 10 mmol/l EDTA, 25 mmol/l HEPES, pH 7.3, 10 mmol/l NaPP, 10 mmol/l NaF, 1 mmol/l Na-vanadate, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, and 0.1 mmol/l phenylmethylsulfonyl fluoride. Total cellular protein, 75 μg, quantified by Coomassie blue G staining (Bradford dye assay; Bio-Rad Laboratories) was subjected to SDS-PAGE (8–12%) and transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). Blots were blocked for 3 h at room temperature with 5% nonfat dried milk. Blots were incubated overnight at 4°C with the respective antibody against PARP (diluted 1:1,000), Bcl-2 (1:250), BAD (1:1,000), P-BAD (1:250), and β-tubulin (1:2,000). Bound antibody was visualized using anti-mouse or anti-rabbit secondary antibody, dilution 1:1,000, coupled to horseradish peroxidase and developed using enhanced chemiluminescence reagents (Amersham Biosciences, Freiburg, Germany).

Real-time PCR. INS-1 cells (4–5 × 10^6 cells) were cultured in 75-cm^2 culture flasks and treated with dexamethasone (100 nmol/l) and RU486 (10 μmol/l) for

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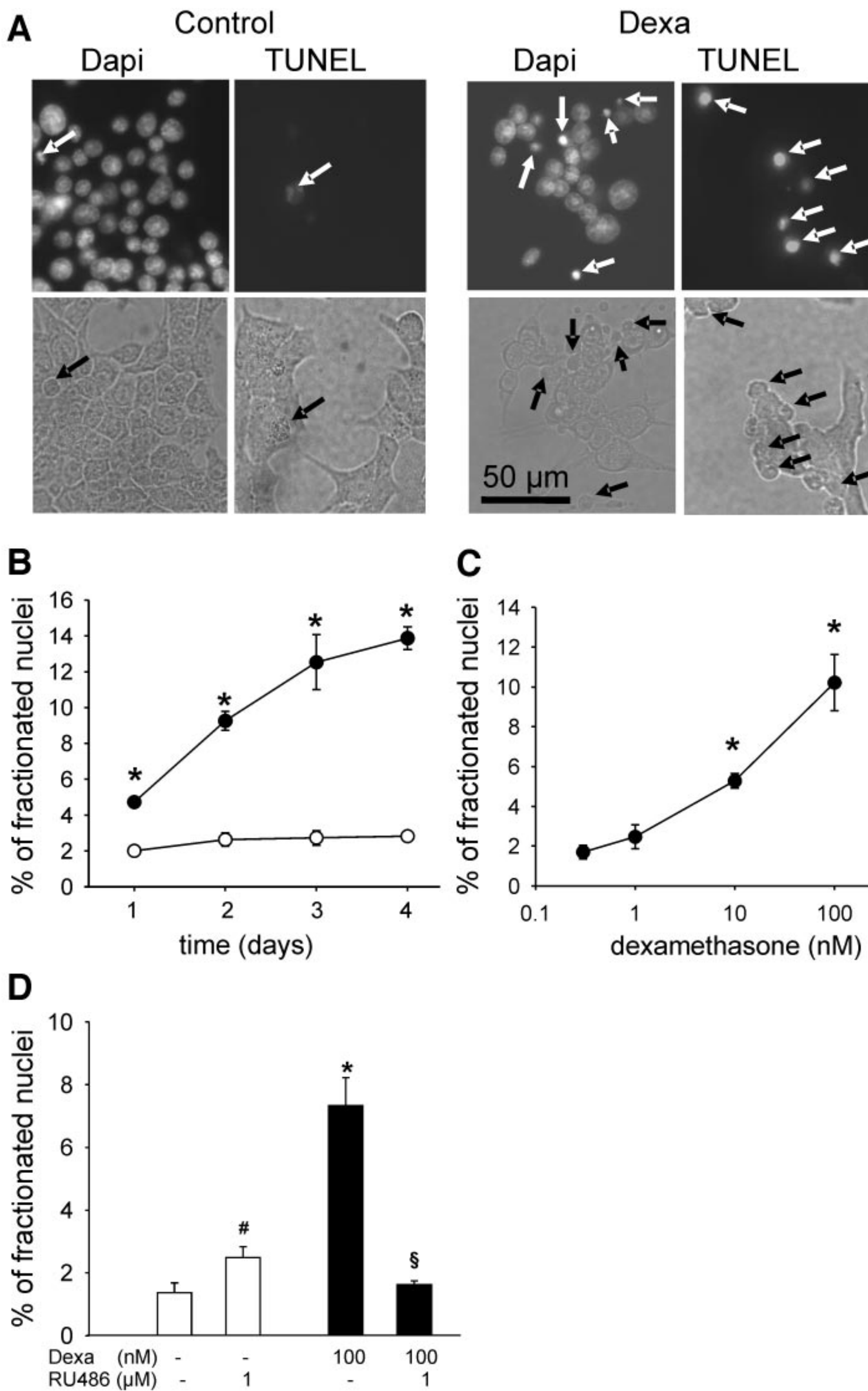

FIG. 2. Dexamethasone (Dexa)-induced cell death in INS-1 cells. Cells were incubated with 100 nmol/l dexamethasone and 1 μmol/l RU486 for the indicated time period. *A*: Fluorescent images of DAPI- and TUNEL-stained nuclei were taken after 4 days of control culture or dexamethasone treatment. Arrows indicate condensed nuclei. Time-dependent (*B*) and concentration-dependent (*C*) effect of dexamethasone after 4 days expressed as percentage of condensed nuclei ($n = 3$). *D*: Inhibition of dexa-induced cell death by RU486. *Denotes significant effect of dexamethasone versus control at the same time point; #denotes significance between control and RU486; §denotes significance between dexamethasone and dexamethasone plus RU486.

4 h. Cells were lysed, total RNA isolated (Mini kit; Qiagen), and 1 μg transcribed into cDNA using reverse transcriptase M-MuLV (Roche Diagnostics, Roche Applied Science, Mannheim, Germany). Aliquots of cDNA, corresponding to equal amounts of RNA, were used for quantification of mRNA. Specific primers used were for detection of rat Bcl-2 mRNA: left: 5′-GGAGATCGTGATGAAGTAC-3′, right: 5′-TCAGGTACTCAGTCATCCA-3′; for rat BAD: left: 5′-TGAGGAAGATGAAGGGATGG-3′, right: 5′-GCTTTGTCG CATCTGTGTTG-3′; for rat Bax: left: 5′-GGCGAATTGGAGATGAACTG-3′, right: 5′-TTCTTCCAGATGGTGAGCGA-3′.

Changes in mRNAs were quantified using a light cycler system (Roche Diagnostics, Roche Applied Science). PCRs were performed in a final volume of 20 μl containing 2 μl cDNA, 3 mmol/l $MgCl_2$, 0.5 μmol/l of each primer, 2 μl cDNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany). The transcript levels of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase of each sample were taken as reference using a commercial primer kit (Search, Heidelberg, Germany). Amplification of the target DNA was performed during 35 cycles, each 10 s at 95°C, 10 s at 68°C (declining by 0.5°C each cycle), and 16 s at 72°C. Melting curve, agarose gel analyses, and product sequences confirmed the specificity of amplified products (not shown). Results were calculated as a ratio of the target versus housekeeping gene transcripts.

Statistics. Data are provided as means ± SE; n represents the number of independent experiments. All data were tested for significance using ANOVA. Results with $P < 0.05$ were considered statistically significant.

RESULTS

Dexamethasone induces apoptosis through the glucocorticoid receptor in INS-1 cells and cultured mouse islet cells. To examine whether dexamethasone induces cell death of insulin-secreting cells, isolated

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mouse islet cells were cultured with 100 nmol/l of dexamethasone for 1–4 days. Programmed islet cell death was evaluated by the quantity of DAPI-stained condensed nuclei and TUNEL assay. The number of condensed nuclei was doubled from 1.9 ± 0.2 to 3.9 ± 0.45% ($n = 9$) after 1-day dexamethasone treatment. A 4-day dexamethasone treatment increased the number of condensed nuclei from 1.8 ± 0.2 to 7.0 ± 0.7% ($n = 5$; Fig. 1*A* and *B*), while under control conditions the number of dead cells did not exceed 2% at any time point. TUNEL staining, a more specific marker for apoptotic cell death, revealed that treatment with dexamethasone (100 nmol/l) for 4 days significantly increased the amount of TUNEL-positive cells (Fig. 1*A*).

Since the analysis of proteins that are involved in dexamethasone-mediated changes of signal transduction pathways requires the treatment of large quantities of cells, further experiments were performed on the insulin-secreting cell line INS-1. The amount of condensed nuclei in control INS-1 cell culture was almost constant over 4 days (2.0 ± 0.2 to 2.8 ± 0.2%; Fig. 2*B*). Treatment with dexamethasone (100 nmol/l) increased the number of condensed nuclei twofold after 1 day to sevenfold after 4 days of treatment (to 4.7 ± 0.12 and 13.9 ± 0.6%, respectively; Fig. 2*A* and *B*). In parallel, the number of TUNEL-stained cells increased dramatically after dexamethasone treatment but not in control INS-1 cells (Fig. 2*A*). The effect of dexamethasone was dose-dependent and significant at 10 nmol/l (Fig. 2*C*). Mifepristone (RU486), 1 μmol/l, an antagonist of the glucocorticoid receptor, abrogated dexamethasone-induced apoptosis (Fig. 2*D*).

Accordingly, dexamethasone triggered apoptotic cell death in insulin-secreting INS-1 and in mouse islet cells. Apoptotic markers, caspase-3 activity, and the cleavage of the caspase-3 substrate PARP were measured in INS-1 cells. After 2 days of dexamethasone treatment, caspase-3 activity increased threefold (from 30.5 ± 1.5 to 93.2 ± 4.6 μmol cleaved Ac-DEVD-pNA/mg protein), an effect antagonized by 1 μmol/l RU486 (Fig. 3*A*). Western blot analysis disclosed the significantly increased abundance of cleaved PARP (89 kDa) in INS-1 cell lysates after culture with dexamethasone as compared with control conditions (Fig. 3*B*). The effect of dexamethasone was inhibited by RU486. The amount of uncleaved PARP (116 kDa, Fig. 3*B*) remained high, reflecting the high amount (90%) of intact cells during dexamethasone treatment. These data indicate that dexamethasone induces apoptotic cell death in INS-1 cells via activation of the glucocorticoid receptor.

Dexamethasone reduces transcript and protein abundance of the anti apoptotic protein Bcl-2. Since activation of the glucocorticoid receptor leads to changes in nuclear transcription, further experiments were performed to analyze the effects of dexamethasone on mRNA levels in INS-1 cells. In other cell systems it has been shown that dexamethasone changes the transcription of proteins of the Bcl-2 family, which are involved in apoptosis (29,30). Thus, in the following the mRNAs of the early anti-apoptotic protein (Bcl-2) were analyzed using semi-quantitative real-time PCR. Already after 4 h of treatment with 100 nmol/l dexamethasone, the relative mRNA concentration of the anti-apoptotic marker Bcl-2 was reduced 3.5-fold (from 0.21 ± 0.04 to 0.06 ± 0.04 arbitrary units of mRNA; Fig. 4*A*). The effect of dexamethasone was blunted by 80% in the presence of RU486. According to Western blotting, this reduction of mRNA was paralleled by a decrease in protein abundance (Fig. 4*B*).

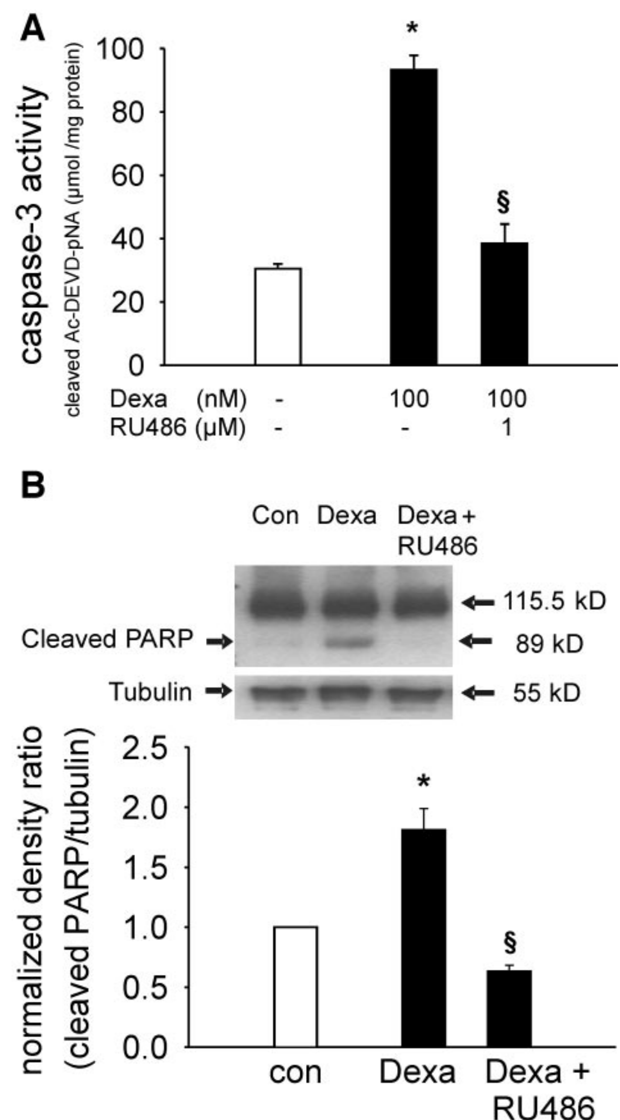

FIG. 3. Dexamethasone (Dexa)-induced apoptosis in INS-1 cells. Cells were treated for 2 days with dexamethasone and RU486 as indicated. Cytosolic caspase-3 activity ($n = 3$) (*A*) and Western blot representative for four experiments demonstrating protein abundance of cleaved PARP (89 kDa) (*B*). For quantification, the optical density ratio of the bands of PARP versus tubulin under control condition was set to one in each experiment. *Denotes significant effect of dexamethasone versus control; §denotes significance between dexamethasone and dexamethasone plus RU486.

Dexamethasone depolarizes the mitochondrial membrane. In a next step, we analyzed whether dexamethasone treatment affects the mitochondrial potential. Since glucose-induced $[Ca^{2+}]_i$ oscillations have been observed after dexamethasone treatment, (3,6) it is not surprising that glucose hyperpolarizes the mitochondrial membrane in 72% of control and 83% of dexamethasone-treated cells (Fig. 4*C*). However, the basal rhodamine-123 fluorescence in relation to the maximal possible depolarization induced by carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (1 μmol/l) was significantly lower in control than in dexamethasone-treated cells, indicating that mitochondria are depolarized in dexamethasone-treated cells as compared with control cells (Fig. 4*C*).

Dexamethasone induces cell death at least partially via activation of calcineurin and dephosphorylation

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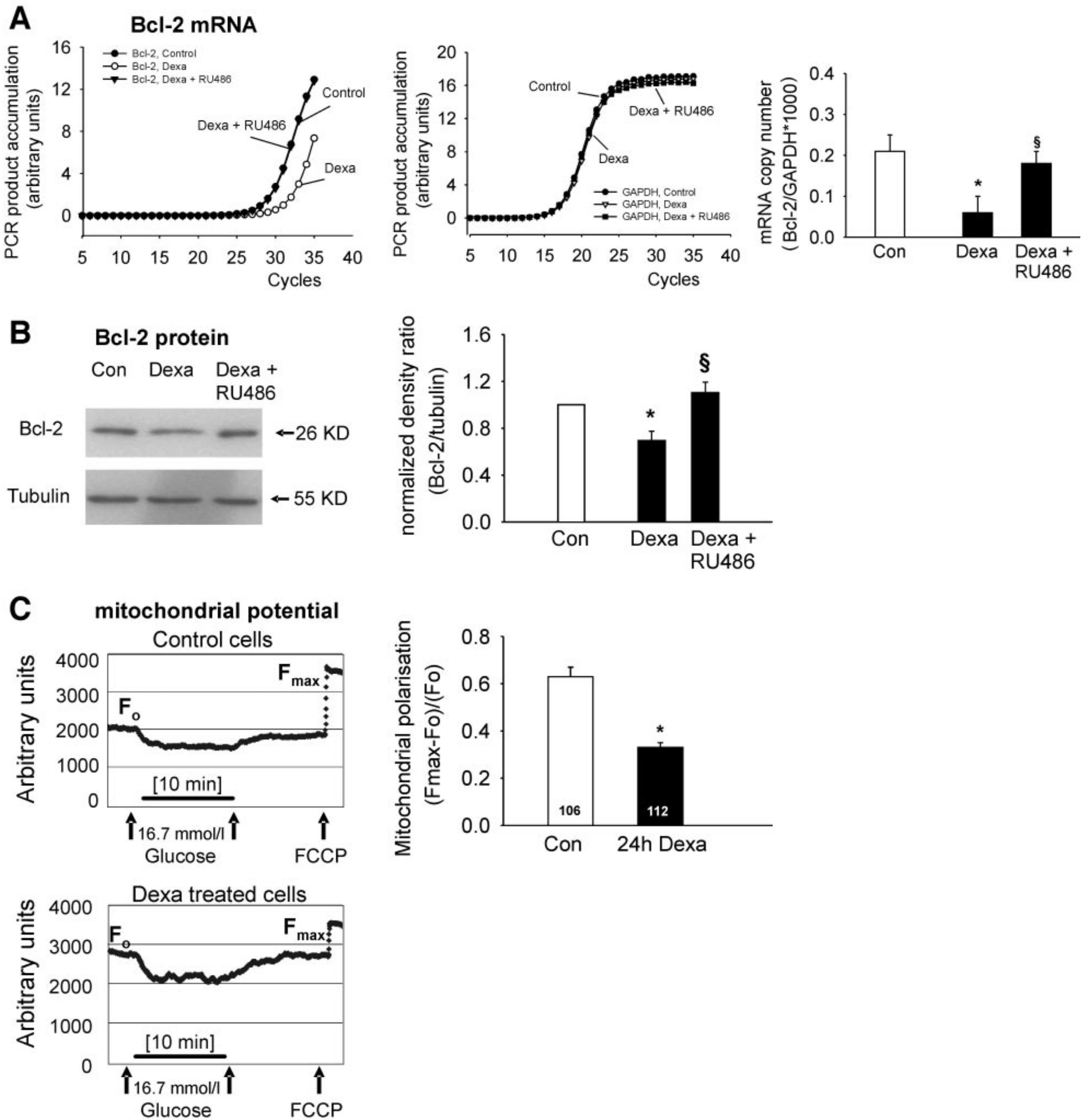

FIG. 4. Dexamethasone (Dexa)-induced mitochondrial apoptosis in INS-1 cells. Cells were cultured in the presence of 100 nmol/l dexamethasone and 1 μmol/l RU486 for 4 (*A*) and 24 h (*B* and *C*). *A*: Semiquantitative analysis of mRNA of Bcl-2 using real-time PCR. Relative amounts of real-time PCR products for Bcl-2 is expressed as ratio to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (*n* = 4). *B*: Western blot representative for four experiments demonstrating Bcl-2 protein abundance. For quantification, the optical density ratio of the bands of Bcl-2 vs tubulin under control condition was set to one in each experiment (*n* = 4). *C*: Representative traces of mitochondrial potential at 0.5 mmol/l (Fo) and 16.7 mmol/l glucose and after maximal depolarization (Fmax) with 1 μmol/l carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP). The ratio [(Fmax) − (Fo)]/(Fo) is expressed as means ± SE for 106 and 112 cells of three independent experiments. *Denotes significant difference between control and dexamethasone-treated cells; §denotes significance between dexamethasone and dexamethasone plus RU486.

of BAD. In a further series of experiments, we determined expression and phosphorylation of BAD. While Bax mRNA was unchanged by dexamethasone treatment (4 and 24 h), BAD mRNA was significantly increased after 24 h (from 9.9 ± 2.3 to 17.1 ± 3.1 arbitrary units of mRNA; Fig. 5*A* and *B*), an effect reversed by RU486. However, the protein concentration of BAD after 1 day's dexamethasone treatment was not significantly changed (Fig. 5*C*). Since only dephosphorylated BAD stimulates apoptosis, the amount of phosphorylated BAD was examined. Dexamethasone (100 nmol/l) for 1 day significantly reduced BAD phosphorylation at serine 155, an effect blunted by RU486 (*n* = 4; Fig. 5*D*). These results indicate that dexamethasone triggers dephosphorylation of BAD.

It has previously been proposed that glucocorticoids activate calcineurin, which in turn dephosphorylates the apoptotic protein BAD (31). Therefore, we analyzed whether dexamethasone-induced cell death is antagonized by calcineurin inhibitors. Since cyclosporine A does not only inhibit calcineurin but also binds to the mitochondrial

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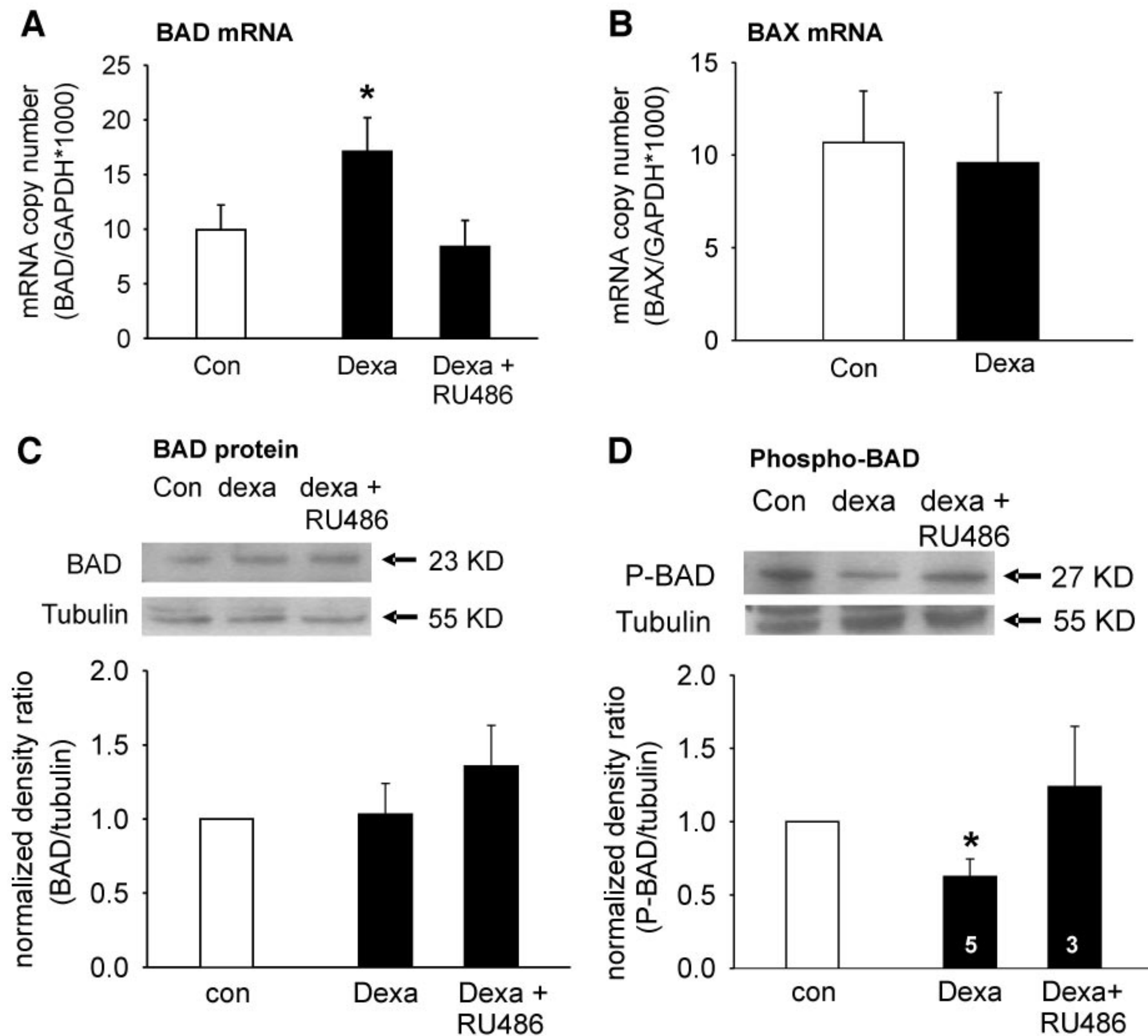

FIG. 5. Effects of dexamethasone (Dexa) on BAD and Bax in INS-1 cells. Cells were cultured in the presence of 100 nmol/l dexamethasone and 1 μmol/l RU486 as indicated for 1 day. *A* and *B*: Relative amounts of RT-PCR products for BAD ($n = 4$) and Bax ($n = 3$) to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *C* and *D*: Western blots representative for five similar experiments demonstrating BAD and phospho-BAD protein abundance. For quantification, the optical density ratio of the bands of BAD or P-BAD versus tubulin under control condition was set to one in each experiment. *Denotes significant effect of dexamethasone versus control.

transition pore, we used the calcineurin inhibitors FK506 and deltamethrin (32). Indeed, deltamethrin (1 μmol/l) and FK506 (0.1 μmol/l) significantly inhibited dexamethasone-induced cell death by 86 (Fig. 6*A*) and 44% (Fig. 6*B*), respectively. Next, we examined the effect of dexamethasone treatment on calcineurin activity. Ca^{2+}-dependent phosphatase activity was significantly increased threefold by dexamethasone, an effect blunted by RU486 (by 55%), deltamethrin (by 55%), and FK506 (by 100%) (Fig. 6*C*).

These data indicate that dexamethasone induced cell death in INS-1 cells at least partially through the activation of the glucocorticoid receptor with subsequent activation of calcineurin and dephosphorylation of BAD at serine 155. The effect favors the interaction of BAD with mitochondria, thus promoting the depolarization of the mitochondrial membrane. The effect is compounded by an inhibitory effect of dexamethasone on the transcript levels of anti-apoptotic Bcl-2.

Exendin-4 protects mouse islet cells and INS-1 cells against dexamethasone-mediated cell death via activation of PKA. Earlier studies (27,33–35) indicated that incretins protect islet cells against apoptosis. Thus, we examined whether exendin-4, a stable agonist of the GLP-1 receptor, protects mouse islet cells and INS-1 cells against dexamethasone-induced apoptosis. Exendin-4 (10 nmol/l) did not change the rate of cell death under control condition but significantly reduced dexamethasone-induced cell death by 82% (Fig. 7*A* and *B*). In INS-1 cells exendin-4 reduced dexamethasone-mediated cleavage of PARP (Fig. 7*C*). In addition, this effect was accompanied by a significant reduction of caspase-3 activation by 76% (Fig. 7*D*). To examine whether PKA is involved in the protective effects of exendin-4, two PKA inhibitors, H89 and KT5720, have been applied. H89 (10 μmol/l) increased cell death about twofold in control INS-1 cells (from 3.1 ± 0.3 to 6.0 ± 0.5%; Fig. 8*A*). In the presence of H89, exendin-4 did not reverse cell death in control cells nor did it protect against dexamethasone-induced cell death. Comparable results were obtained with the PKA inhibitor KT5720 (5 μmol/l; Fig. 8*B*). High concentrations of forskolin, 10 μmol/l, mimicked the protective effect of exendin-4. The effect was, again, reversed by PKA inhibition (Fig. 8*C*). To rule out whether GEF might be involved in exendin-4 effects, we used 8CPT-Me-cAMP (50 μmol/l) for specific

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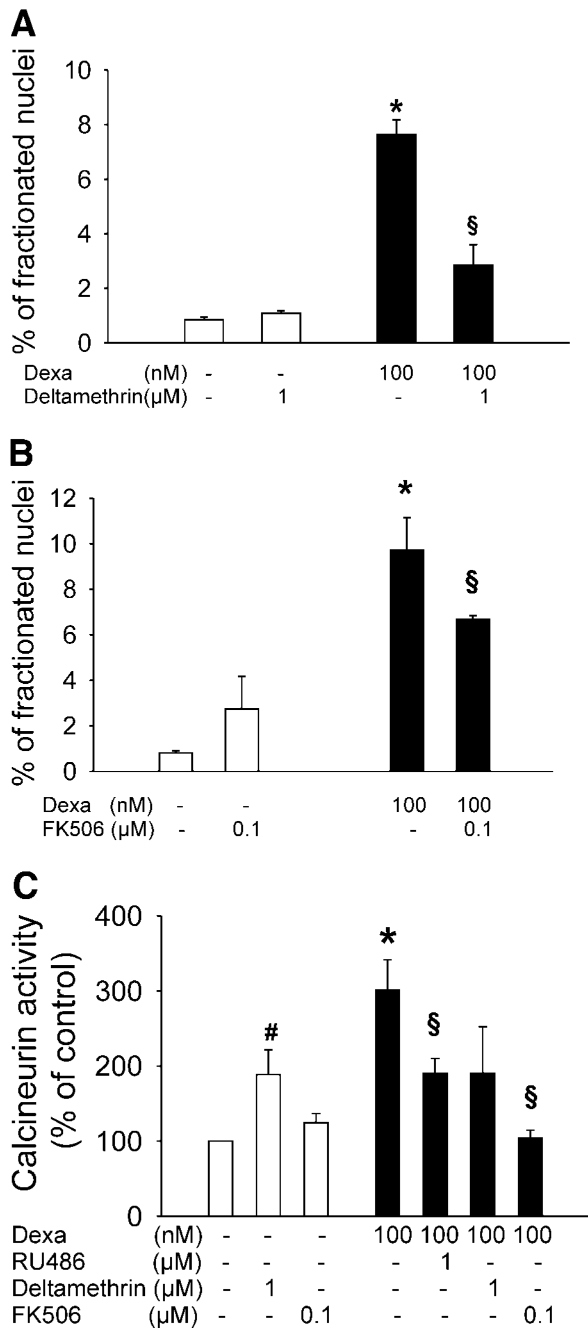

FIG. 6. Dexamethasone (Dexa)-activated calcineurin in INS-1 cells. Cells were incubated with test substances as indicated for 4 days (*A* and *B*) and 1 day (*C*). *A* and *B*: Condensed nuclei are expressed as means ± SE for n = 3. *C*: Ca^{2+}-dependent phosphatase activity (n = 3–4). *Denotes significant effect of dexamethasone versus control; §Denotes significance between dexamethasone and dexamethasone plus RU486 or deltamethrin or FK506; #significant difference between control and deltamethrin.

activation of GEF. Since the agonist did not affect dexamethasone-induced cell death (Fig. 8*D*), the stimulation of GEF seems not to mediate anti-apoptotic effects of exendin-4. These results further suggest that inhibition of PKA, which itself induces cell death, antagonizes the protective effect of exendin-4.

DISCUSSION

This study demonstrates that dexamethasone induces cell death in insulin-secreting INS-1 cells and mouse β-cells through activation of the glucocorticoid receptor. Cell death is accompanied by caspase-3 activation and increased accumulation of cleaved PARP, a caspase-3 substrate, indicating that apoptosis is activated by dexamethasone. This conclusion is further supported by the increased amount of TUNEL-positive cells after dexamethasone treatment. Interestingly, glucocorticoids have been described to induce apoptosis in prolactin-stimulated rat islets, a mechanism that might downregulate β-cell function during late pregnancy (36).

The reduction of the antiapoptotic protein Bcl-2, the stimulation of calcineurin, and the dephosphorylation of BAD indicate that dexamethasone activates a mitochondrial pathway of apoptotic cell death. Dexamethasone-induced cell death was indeed inhibited by calcineurin inhibitors. Furthermore, activation of PKA by GLP-1 receptors counteracted dexamethasone-induced cell death.

The Bcl-2 protein family comprises anti- and proapoptotic proteins, which are involved in programmed cell death. Some of these proteins (such as Bcl-2 and Bcl-XL) are antiapoptotic and located at the outer mitochondrial membrane, while others (such as BAD or Bax) are proapoptotic and cytosolic proteins. The proapoptotic proteins act as sensors of cellular damage or stress. BAD is bound to the cytosolic adaptor protein 14-3-3 in its phosphorylated form (Fig. 9). Cellular stress induced by irradiation, viral infection, or chemicals, e.g., dexamethasone, may result in an increase of cytosolic Ca^{2+} activity, which in turn activates the phosphatase calcineurin. Calcineurin is known to dephosphorylate BAD, which triggers its release from the cytosolic protein 14-3-3 (37). This enables BAD to bind to Bcl-2 at the surface of the mitochondrial membrane. The interaction between BAD as pro- and Bcl-2/Bcl-XL as antiapoptotic protein at the mitochondrial membrane disrupts the normal function of the antiapoptotic Bcl-2 proteins. The binding of BAD leads to the formation of permeability transition pores in the mitochondria and the release of cytochrome c and other proapoptotic molecules from the mitochondrial intermembrane space (37,38). This in turn leads to the formation of the apoptosome and the activation of the caspase cascade (39). Active cytosolic caspase-3 is a key enzyme in the apoptotic process, which accomplishes the degradation of nuclear DNA (40,41).

Dexamethasone-mediated cell death is known to activate the mitochondrial apoptotic pathway (16–20,42). However, dexamethasone has been found to have opposing effects in distinct cells. In granulosa cells, dexamethasone protects against serum deprivation– and cAMP-induced apoptosis and increases expression of Bcl-2 (43). In contrast, in LTR-6 cells (mouse M1 myeloid leukemia cells) dexamethasone induces cell death and reduces Bcl-2 expression. In thymocytes, lymphoid cells and airway epithelial cells apoptosis by dexamethasone is blocked by the overexpression of Bcl-2 (42,44,45). Indeed, the expression of Bcl-2 seems to determine the pro- or antiapoptotic effect of glucocorticoids (45). Thus, one prerequisite for dexamethasone-induced apoptosis might be the downregulation of Bcl-2 protein. In insulin-secreting cells, Bcl-2 overexpression has been described to protect against cytokine-induced cell damage (46). We show here that Bcl-2 expression is reduced by dexamethasone in INS-1 cells (Fig. 4*A* and *B*). The effect is antagonized by the glucocorticoid receptor antagonist RU486 and, thus, mediated by the glucocorticoid receptor. Activated glucocorticoid receptor enters the nucleus and modifies gene

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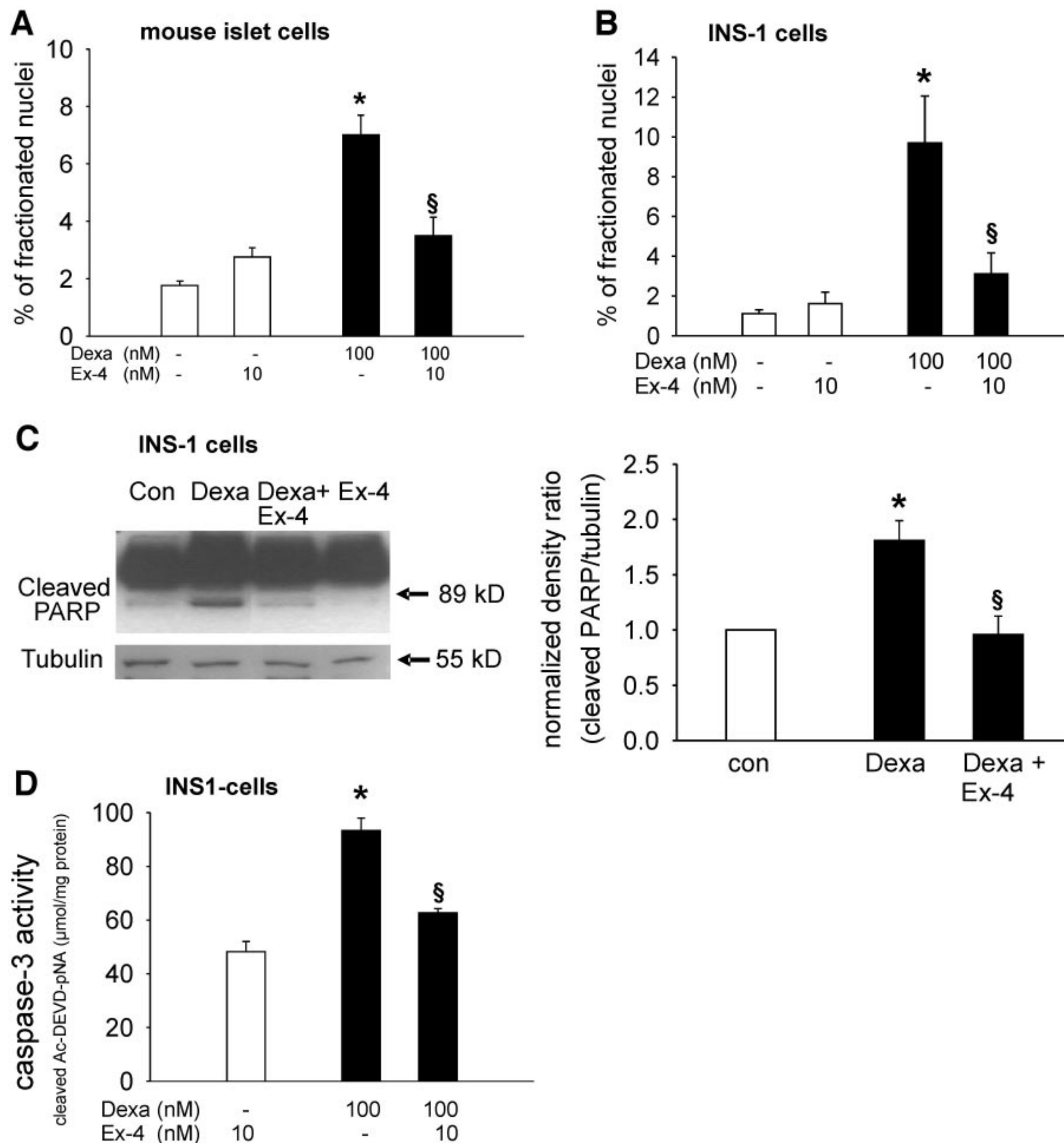

FIG. 7. Exendin-4 reversed dexamethasone (DEXA)-induced cell death in mouse islet and INS-1 cells. Cells were incubated with dexamethasone, 100 nmol/l and exendin-4, 10 nmol/l for 4 days as indicated. Percentage of DAPI-stained condensed nuclei of mouse β-cells (*A*) (n = 9) and INS-1 cell (*B*) (n = 6). *C*: Western blots representative for four similar experiments demonstrating protein abundance of cleaved PARP (89 kDa) (n = 4). For quantification, the optical density ratio of the bands of PARP versus tubulin under control condition was set to one in each experiment. *D*: Caspase-3 activity in INS-1 cells was measured after 2 days' treatment with dexamethasone and exendin-4 in cell culture (n = 3). *Denotes significant effect of dexamethasone versus control; §denotes significance between dexamethasone and dexamethasone plus exendin-4.

transcription by binding to glucocorticoid response element sites (47).

Glucocorticoid receptor activation by glucocorticoids results in release of HSP90 from the glucocorticoid receptor complex (48). HSP90 has been described to activate calcineurin (31). Calcineurin activation involves increases in $[Ca^{2+}]_i$ and Ca^{2+}-calmodulin–mediated activation of the enzyme independent of transcriptional changes (49). Dexamethasone stimulated calcineurin activity in INS-1 cells (Fig. 6*C*). The increased phosphatase activity was sensitive to chelating Ca^{2+} with EGTA and to FK506, a specific blocker of calcineurin activity (50). The effect of dexamethasone on calcineurin is reversed by the glucocorticoid receptor antagonist RU486. Thus, dexamethasone may activate calcineurin by a factor released from the glucocorticoid receptor complex, possibly HSP90.

Dexamethasone rather blunts glucose-induced $[Ca^{2+}]_i$ oscillations in insulin-secreting cells, making it unlikely that dexamethasone increases the $[Ca^{2+}]_i$ activity (3,6). The treatment of dexamethasone has been performed in cell culture in the presence of 11 mmol/l glucose, a glucose concentration that induces $[Ca^{2+}]_i$ oscillations in INS-1 cells. This condition mimics hyperglycemia induced by hepatic glucose mobilization during glucocorticoid treatment. Since inhibition of calcineurin counteracted dexamethasone-induced apoptosis in INS-1 cells (Fig. 6*A*), it can be concluded that glucocorticoid receptor–dependent activation of the enzyme is crucial for the apoptotic effect of dexamethasone.

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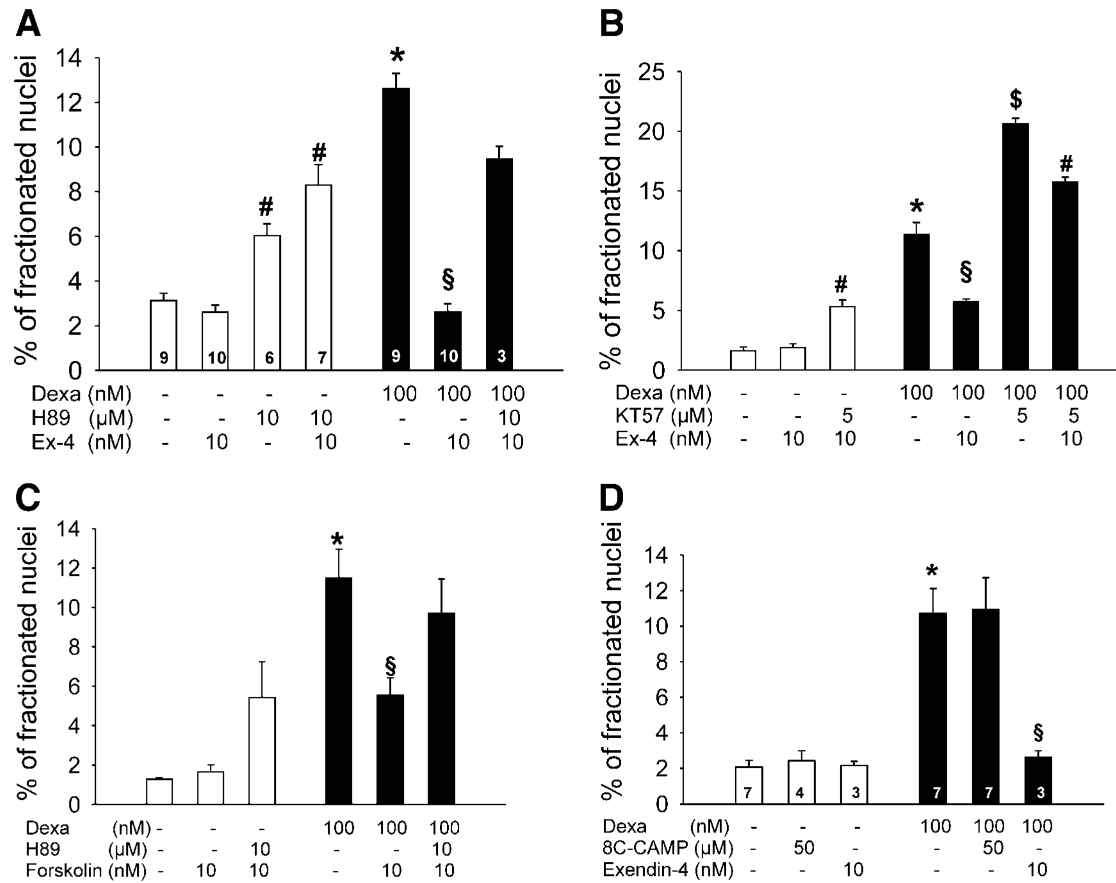

FIG. 8. Effects of PKA inhibition and GEF activation on dexamethasone (Dexa)-induced apoptosis. Before DAPI staining, cells were incubated with test substances as indicated for 4 days (*A–D*). Results are shown as means ± SE for the number (*n*) of independent observations indicated in each bar. *Denotes significant effect of dexamethasone versus control; §denotes significance between dexamethasone and dexamethasone plus exendin-4 or forskolin; #denotes significance between control and test.

In other cells, BAD and Bax were found to have an increased expression or a shift of the cellular location from a soluble to a membrane-bound form during dexamethasone-induced apoptosis (19,42). Three different phosphorylation sites of BAD have been described. Protein kinase B phosphorylates at serine 136, PKA at serine 155, and mitogen-activated protein kinase–AP-K1 at serine 112, preventing release of BAD from the chaperone protein 14-3-3 (28). Our data suggest that dexamethasone promotes dephosphorylation of BAD at serine155, while

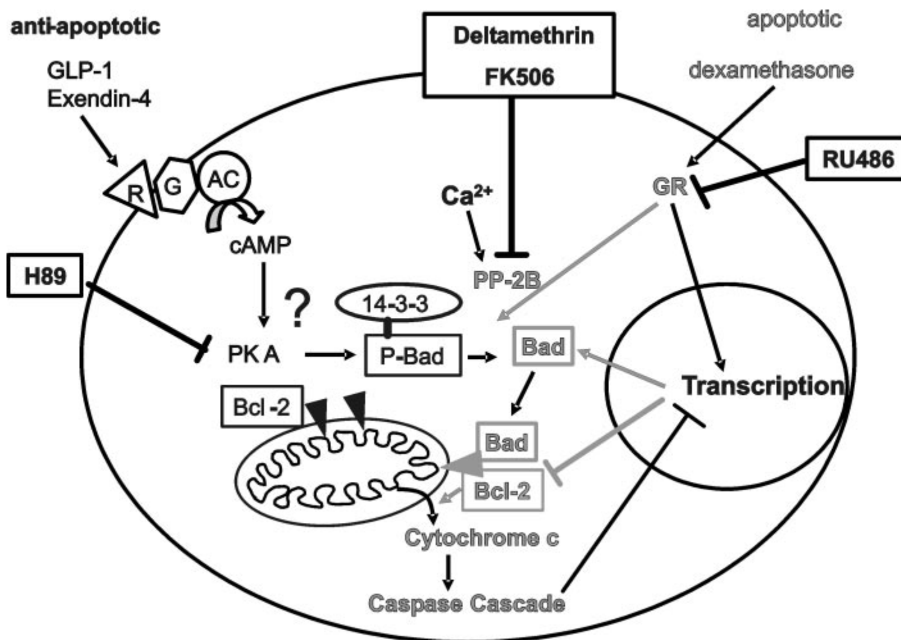

FIG. 9. Proposed signaling pathways of dexamethasone and GLP-1 receptor activation which affect apoptotic pathways in INS-1 cells. Dexamethasone affects transcription of BAD and Bcl-2 and activates PP-2B (calcineurin) through binding to the glucocorticoid receptor (GR). Decreased Bcl-2 expression and increased dephosphorylated BAD trigger apoptosis. In contrast, PKA-induced phosphorylation stabilizes BAD and counteracts dexamethasone-induced apoptosis.

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incretins might stabilize phosphorylation via activation of PKA in insulin-secreting cells.

Specific PKA-induced phosphorylation of BAD on serine 155 has been demonstrated to inhibit binding of Bcl-2 (28). We show here that the protective effect of exendin-4 against dexamethasone-induced apoptosis is antagonized by PKA inhibitors, which themselves promote apoptosis. However, unspecific effects of those inhibitors cannot be ruled out, and further experiments are needed to analyze the role of PKA-dependent phosphorylation of BAD, cAMP/PKA-dependent activation of Bcl-2 transcription via cAMP response element–binding protein, and cAMP-dependent activation of Akt/protein kinase B, which have been shown to be involved in incretin-mediated protection against apoptosis (23,24).

Our observations suggest that glucocorticoids may affect normal β-cell function not only by inhibition of insulin secretion but also by promotion of apoptosis, which may in the long term reduce β-cell mass (9,51). Calcineurin activation might play a key role in this process. The observed effects may well be involved in the development of steroid-induced diabetes, and GLP-1 receptor activation could be used for therapeutic protection.

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REFERENCES

1. Hoogwerf B, Danese RD: Drug selection and the management of corticosteroid-related diabetes mellitus. *Rheum Dis Clin North Am* 25:489–505, 1999
2. Schacke H, Docke WD, Asadullah K: Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 96:23–43, 2002
3. Lambillotte C, Gilon P, Henquin JC: Direct glucocorticoid inhibition of insulin secretion: an in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 99:414–423, 1997
4. Jeong IK, Oh SH, Kim BJ, Chung JH, Min YK, Lee MS, Lee MK, Kim KW: The effects of dexamethasone on insulin release and biosynthesis are dependent on the dose and duration of treatment. *Diabetes Res Clin Pract* 51:163–171, 2001
5. Hamamdzic D, Duzic E, Sherlock JD, Lanier SM: Regulation of α_2-adrenergic receptor expression and signaling in pancreatic β-cells. *Am J Physiol* 269:E162–E171, 1995
6. Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F: Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. *Diabetes* 54:1090–1099, 2005
7. Gremlich S, Roduit R, Thorens B: Dexamethasone induces posttranslational degradation of GLUT2 and inhibition of insulin secretion in isolated pancreatic β-cells: comparison with the effects of fatty acids. *J Biol Chem* 272:3216–3222, 1997
8. Ling ZC, Khan A, Delauny F, Davani B, Ostenson CG, Gustafsson JA, Okret S, Landau BR, Efendic S: Increased glucocorticoid sensitivity in islet β-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. *Diabetologia* 41:634–639, 1998
9. Rhodes CJ: Type 2 diabetes-a matter of β-cell life and death? *Science* 307:380–384, 2005
10. Oshima Y, Kuroda Y, Kunishige M, Matsumoto T, Mitsui T: Oxidative stress-associated mitochondrial dysfunction in corticosteroid-treated muscle cells. *Muscle Nerve* 30:49–54, 2004
11. Liu Y, Porta A, Peng X, Gengaro K, Cunningham EB, Li H, Dominguez LA, Bellido T, Christakos S: Prevention of glucocorticoid-induced apoptosis in osteocytes and osteoblasts by calbindin-D28k. *J Bone Miner Res* 19:479–490, 2004
12. Purton JF, Monk JA, Liddicoat DR, Kyparissoudis K, Sakkal S, Richardson SJ, Godfrey DI, Cole TJ: Expression of the glucocorticoid receptor from the 1A promoter correlates with T lymphocyte sensitivity to glucocorticoid-induced cell death. *J Immunol* 173:3816–3824, 2004
13. Malone MH, Wang Z, Distelhorst CW: The glucocorticoid-induced gene tdag8 encodes a pro-apoptotic G protein-coupled receptor whose activation promotes glucocorticoid-induced apoptosis. *J Biol Chem* 279:52850–52859, 2004
14. Chrysis D, Zaman F, Chagin AS, Takigawa M, Savendahl L: Dexamethasone induces apoptosis in proliferative chondrocytes through activation of caspases and suppression of the Akt-phosphatidylinositol 3′-kinase signaling pathway. *Endocrinol* 146:1391–1397, 2005
15. Frankfurt O, Rosen ST: Mechanisms of glucocorticoid-induced apoptosis in hematologic malignancies: updates. *Curr Opin Oncol* 16:553–563, 2004
16. Hegardt C, Andersson G, Oredsson SM: Spermine prevents cytochrome c release in glucocorticoid-induced apoptosis in mouse thymocytes. *Cell Bio Int* 27:115–121, 2003
17. Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E: Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochim Biophys Acta* 1642:115–123, 2003
18. Renner K, Kofler R, Gnaiger E: Mitochondrial function in glucocorticoid triggered T-ALL cells with transgenic bcl-2 expression. *Mol Biol Rep* 29:97–101, 2002
19. Tome ME, Lutz NW, Briehl MM: Overexpression of catalase or Bcl-2 alters glucose and energy metabolism concomitant with dexamethasone resistance. *Biochim Biophys Acta* 1693:57–72, 2004
20. Tonomura N, McLaughlin K, Grimm L, Goldsby RA, Osborne BA: Glucocorticoid-induced apoptosis of thymocytes: requirement of proteasome-dependent mitochondrial activity. *J Immunol* 170:2469–2478, 2003
21. Amsterdam A, Tajima K, Sasson R: Cell-specific regulation of apoptosis by glucocorticoids: implication to their anti-inflammatory action. *Biochem Pharmacol* 64:843–850, 2002
22. Kovalovsky D, Refojo D, Holsboer F, Arzt E: Molecular mechanisms and Th1/Th2 pathways in corticosteroid regulation of cytokine production. *J Neuroimmunol* 109:23–29, 2000
23. Hui H, Nourparvar A, Zhao X, Perfetti R: Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5′-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinol* 144:1444–1455, 2003
24. Wang Q, Li L, Xu E, Wong V, Rhodes C, Brubaker PL: Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. *Diabetologia* 47:478–487, 2004
25. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ: Glucagon-like peptide-1 receptor signaling modulates β cell apoptosis. *J Biol Chem* 278:471–478, 2003
26. Hennige AM, Ozcan U, Okada T, Jhala US, Schubert M, White MF, Kulkarni RN: Alterations in growth and apoptosis of IRS-1 deficient β-cells. *Am J Physiol Endocrinol Metab* 2005
27. Kwon G, Pappan KL, Marshall CA, Schaffer JE, McDaniel ML: cAMP dose-dependently prevents palmitate-induced apoptosis by both protein kinase A- and cAMP-guanine nucleotide exchange factor-dependent pathways in β-cells. *J Biol Chem* 279:8938–8945, 2004
28. Lizcano JM, Morrice N, Cohen P: Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. *Biochem J* 349:547–557, 2000
29. Almeida OF, Conde GL, Crochemore C, Demeneix BA, Fischer D, Hassan AH, Meyer M, Holsboer F, Michaelidis TM: Subtle shifts in the ratio between pro- and antiapoptotic molecules after activation of corticosteroid receptors decide neuronal fate. *FASEB J* 14:779–790, 2000
30. Smets LA, Van den BJ, Acton D, Top B, Van Rooij H, Verwijs-Janssen M: BCL-2 expression and mitochondrial activity in leukemic cells with different sensitivity to glucocorticoid-induced apoptosis. *Blood* 84:1613–1619, 1994
31. Tumlin JA, Lea JP, Swanson CE, Smith CL, Edge SS, Someren JS: Aldosterone and dexamethasone stimulate calcineurin activity through a transcription-independent mechanism involving steroid receptor-associated heat shock proteins. *J Clin Invest* 99:1217–1223, 1997
32. Enan E, Matsumura F: Specific inhibition of calcineurin by type II synthetic pyrethroid insecticides. *BiochemPharmacol* 43:1777–1784, 1992
33. Farilla L, Bulotta A, Hirshberg B, Li CS, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R: Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinol* 144:5149–5158, 2003
34. Lingohr MK, Dickson LM, Wrede CE, Briaud I, McCuaig JF, Myers MG Jr,

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Rhodes CJ: Decreasing IRS-2 expression in pancreatic beta-cells (INS-1) promotes apoptosis, which can be compensated for by introduction of IRS-4 expression. *Mo lCell Endocrinol* 209:17–31, 2003
35. Wrede CE, Dickson LM, Lingohr MK, Briaud I, Rhodes CJ: Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic β-cells (INS-1). *J Biol Chem* 277:49676–49684, 2002
36. Weinhaus AJ, Bhagroo NV, Brelje TC, Sorenson RL: Dexamethasone counteracts the effect of prolactin on islet function: implications for islet regulation in late pregnancy. *Endocrinol* 141:1384–1393, 2000
37. Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, Shibasaki F, McKeon F, Bobo T, Franke TF, Reed JC: Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* 284:339–343, 1999
38. Orrenius S: Mitochondrial regulation of apoptotic cell death. *Toxicol Lett* 149:19–23, 2004
39. Hui H, Dotta F, Di Mario U, Perfetti R: Role of caspases in the regulation of apoptotic pancreatic islet beta-cells death. *J Cell Physiol* 200:177–200, 2004
40. Nakano M, Matsumoto I, Sawada T, Ansite J, Oberbroeckling J, Zhang HJ, Kirchhof N, Shearer J, Sutherland DE, Hering BJ: Caspase-3 inhibitor prevents apoptosis of human islets immediately after isolation and improves islet graft function. *Pancreas* 29:104–109, 2004
41. Nagata S: Apoptotic DNA fragmentation. *Exp Cell Res* 256:12–18, 2000
42. Almawi WY, Melemedjian OK, Jaoude MM: On the link between Bcl-2 family proteins and glucocorticoid-induced apoptosis. *J Leukoc Biol* 76:7–14, 2004
43. Sasson R, Tajima K, Amsterdam A: Glucocorticoids protect against apoptosis induced by serum deprivation, cyclic adenosine 3′,5′-monophosphate and p53 activation in immortalized human granulosa cells: involvement of Bcl-2. *Endocrinol* 142:802–811, 2001
44. Dorscheid DR, Wojcik KR, Sun S, Marroquin B, White SR: Apoptosis of airway epithelial cells induced by corticosteroids. *Am J Respir Crit Care Med* 164:1939–1947, 2001
45. Huang ST, Cidlowski JA: Glucocorticoids inhibit serum depletion-induced apoptosis in T lymphocytes expressing Bcl-2. *FASEB J* 13:467–476, 1999
46. Tran VV, Chen G, Newgard CB, Hohmeier HE: Discrete and complementary mechanisms of protection of β-cells against cytokine-induced and oxidative damage achieved by bcl-2 overexpression and a cytokine selection strategy. *Diabetes* 52:1423–1432, 2003
47. Defranco DB: Role of molecular chaperones in subnuclear trafficking of glucocorticoid receptors. *Kidney Int* 57:1241–1249, 2000
48. Pratt WB, Toft DO: Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 18:306–360, 1997
49. Tong DC, Buck SM, Roberts BR, Klein JD, Tumlin JA: Calcineurin phosphatase activity: activation by glucocorticoids and role of intracellular calcium. *Transplantation* 77:259–267, 2004
50. Ivery MT: A proposed molecular model for the interaction of calcineurin with the cyclosporin A-cyclophilin A complex. *Bioorg Med Chem* 7:1389–1402, 1999
51. Donath MY, Halban PA: Decreased β-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* 47:581–589, 2004

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