Serum- and Glucocorticoid-Inducible Kinase 1 (SGK1) Mediates Glucocorticoid-Induced Inhibition of Insulin Secretion

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Glucocorticoid excess predisposes to the development of diabetes, at least in part through impairment of insulin secretion. The underlying mechanism has remained elusive. We show here that dexamethasone upregulates transcription and expression of the serum- and glucocorticoid-inducible kinase 1 (SGK1) in insulin-secreting cells, an effect reversed by mifepristone (RU486), an antagonist of the nuclear glucocorticoid receptor. When coexpressed in Xenopus oocytes, SGK1 increases the activity of voltage-gated K^+ channel $K_v1.5$. In INS-1 cells, dexamethasone stimulates the transcription of $K_v1.5$, increases the repolarizing outward current, reduces peak values of $[Ca^{2+}]_i$ oscillations, and decreases glucose-induced insulin release. The latter effect is reversed by K^+ channel blockers 4-aminopyridine and tetraethylammonium and by a more selective $K_v1.5$ channel inhibitor MSD-D. Dexamethasone also increases expression of $K_v1.5$ in mouse islets and reduces glucose-induced insulin secretion, an effect reversed by MSD-D. In islets isolated from wild-type but not SGK1 knockout mice, dexamethasone significantly blunted glucose-, forskolin-, and phorbol myristic acid–induced insulin release. In conclusion, dexamethasone stimulates the transcription of SGK1, which in turn upregulates the activity of voltage-gated K^+ channels. Increased K^+ channel activity reduces Ca^{2+} entry through voltage-gated Ca^{2+} channels and insulin release. *Diabetes* 54:1090–1099, 2005

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4-AP, 4-aminopyridine; GAPDH, glyceraldehyde-3-phsophate dehydrogenase; SGK1, serum- and glucocorticoid-inducible kinase 1; TEA, tetraethylammonium.

Glucocorticoids are known to induce diabetes (1–3). In addition to peripheral insulin resistance and increased hepatic glucose production by stimulating gluconeogenesis (4), glucocorticoids interfere with insulin secretion of pancreatic β-cells (5–7). Despite extensive (8–12) studies, the molecular mechanism is still a matter of debate. Increased expression of α_2-adrenoceptors has been proposed to account for dexamethasone-induced inhibition of insulin secretion (9). Thus, transgenic mice overexpressing glucocorticoid receptors in β-cells show 30% more UK14304 binding, a selective adrenoceptor agonist, than wild-type islets (2). These mice are glucose intolerant and have reduced plasma insulin levels. Since pertussis toxin and cAMP overcome dexamethasone inhibition of glucose-induced insulin release, decreased cAMP levels during dexamethasone treatment may be responsible for inhibition of secretion (6,13). Furthermore, dexamethasone was reported to decrease Glut2 protein abundance at the plasma membrane, a change that may contribute to impaired glucose-induced insulin secretion (8). Dexamethasone also downregulates glucokinase mRNA in an insulin-secreting cell line (14). Mifepristone (RU486), a nuclear glucocorticoid receptor antagonist, completely abolished dexamethasone-induced inhibition of insulin secretion (5,6), pointing to the involvement of glucocorticoid-dependent gene expression. Glucocorticoid-sensitive genes include the serum- and glucocorticoid-inducible kinase 1 (SGK1) (rev. in 15). The kinase is expressed in virtually all human tissues tested. Unlike its isoforms SGK2 and SGK3 and the related kinase protein kinase B, SGK1 is under strong transcriptional control of glucocorticoids (15) and mineralocorticoids (16). SGK1 has been shown to regulate a variety of ion channels including K^+ channels such as voltage-gated K_v channels (17).

Ion channel activity is in turn decisive for insulin secretion from pancreatic β-cells. Glucose stimulates insulin secretion by closing ATP-sensitive K^+ channels, which depolarizes the cells and activates voltage-gated Ca^{2+} channels with subsequent increase of cytosolic Ca^{2+} activity (rev. in 18). The increase of $[Ca^{2+}]_i$ is the primary signal for stimulation of insulin secretion and largely, although not solely, depends on membrane potential (19,20). The duration and magnitude of Ca^{2+} influx is

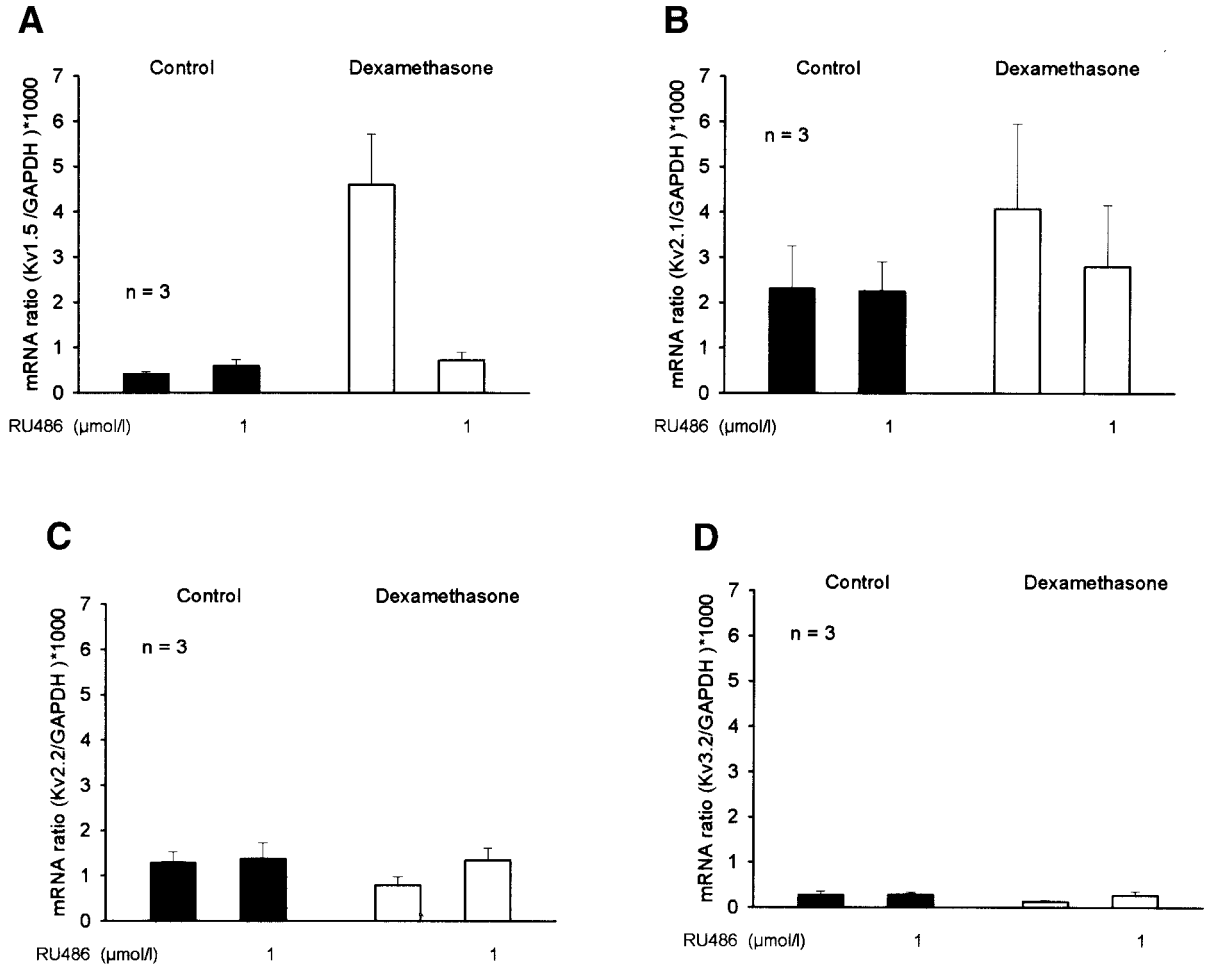


FIG. 1. Induction of K_v channels by dexamethasone in INS-1 cells. INS-1 cells were treated with 100 nmol/l dexamethasone or vehicle (DMSO) in culture for 4 h. Quantification of $K_v1.5$ (*A*), $K_v2.1$ (*B*), $K_v2.2$ (*C*), and $K_v3.2$ (*D*) mRNA was performed using the same RNA preparations as for the experiments described in Fig. 2*A*. Data are expressed as means ± SE of three independent preparations.

regulated by the open time of Ca^{2+} channels as well as by the activity of repolarizing K^+ channels including K_v, K_{Ca}, and ATP-sensitive K^+ channels (rev. in 21–23). Specifically, the outward-rectifying K_v channels are activated by voltages more positive than −40 mV and thus could serve to limit depolarization and subsequent activation of voltage-gated Ca^{2+} channels. Indeed, it has been shown that the blockade of K_v channels augments stimulated secretion while basal secretion is unaffected (22,24,25). Since a variety of different K_v channels are expressed in insulin-secreting cells, the contribution to membrane potential regulation of distinct channels is difficult to assess due to the lack of specific inhibitors and activators. Moreover, the molecular composition of functionally expressed channels is unknown. Rodent islets express mainly $K_v2.1$ but also several other K^+ channels including $K_v3.2$ and $K_v3.4$ (22, 26). In human islets and INS-1 cells, additional expression of $K_v1.5$ has been described (22,25,26). Overexpression of human $K_v1.5$ in transgenic mice reduced serum insulin levels threefold and decreased Ca^{2+} oscillations in stably transfected βTC3 insulinoma cells (27). Inhibition of $K_v2.1$ outward current has been shown to enhance glucose-induced insulin secretion (24). Little is known about regulation of K_v channel activities in normal β-cells. Previous experiments revealed that K_v currents are not modulated by cAMP or cAMP-dependent protein kinase in INS-1 cells (25). Glucocorticoids have been found to increase $K_v1.5$ channel activity in heart (28), probably due to a direct effect since the gene contains a glucocorticoid-responsive element (29).

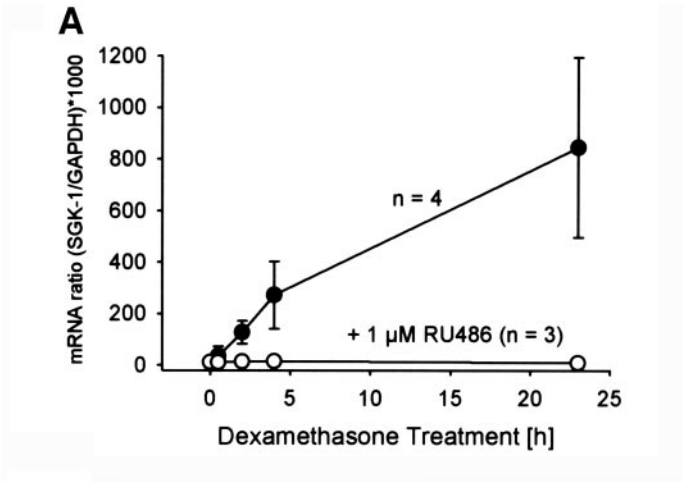
We show here that glucocorticoids augment $K_v1.5$ channel expression in insulin-secreting cells. Furthermore, glucocorticoids induce the expression of SGK1, a serine/threonine kinase known to regulate ion channel activities. Evidence is provided that SGK1 is involved in dexamethasone-induced inhibition of insulin secretion by increasing K_v channel activities.

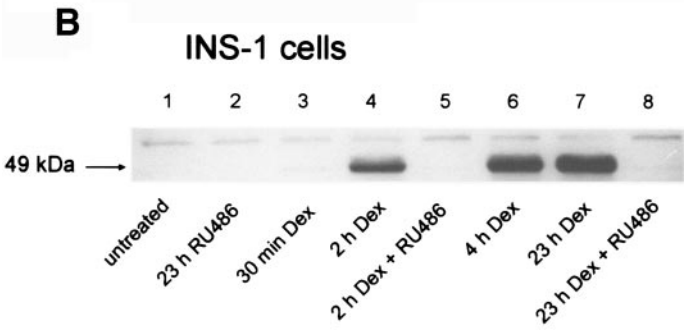
RESEARCH DESIGN AND METHODS

MSD-D, a cardiac $K_v1.5$ channel inhibitor (patent no. WO9818475), and full-length hKv2.1 and hKv3.1 plasmids suitable for expression in Xenopus oocytes were a generous gift of Aventis Pharma Deutschland (Frankfurt, Germany). All other chemicals were from Sigma (Deisenhofen, Germany) and of analytical grade, unless otherwise stated.

Generation of SGK1 knockout mice. Homozygous *SGK1*$^{(-/-)}$ and *SGK1*$^{(+/+)}$ littermate mice with 129/SvJ background were generated as previously described (30). Animal protocols were approved by the animal research committees of the respective institutions.

Cell culture and measurement of insulin secretion. INS-1 cells (kindly 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 (Biochrom, Berlin, Germany), 1 mmol/l HEPES,





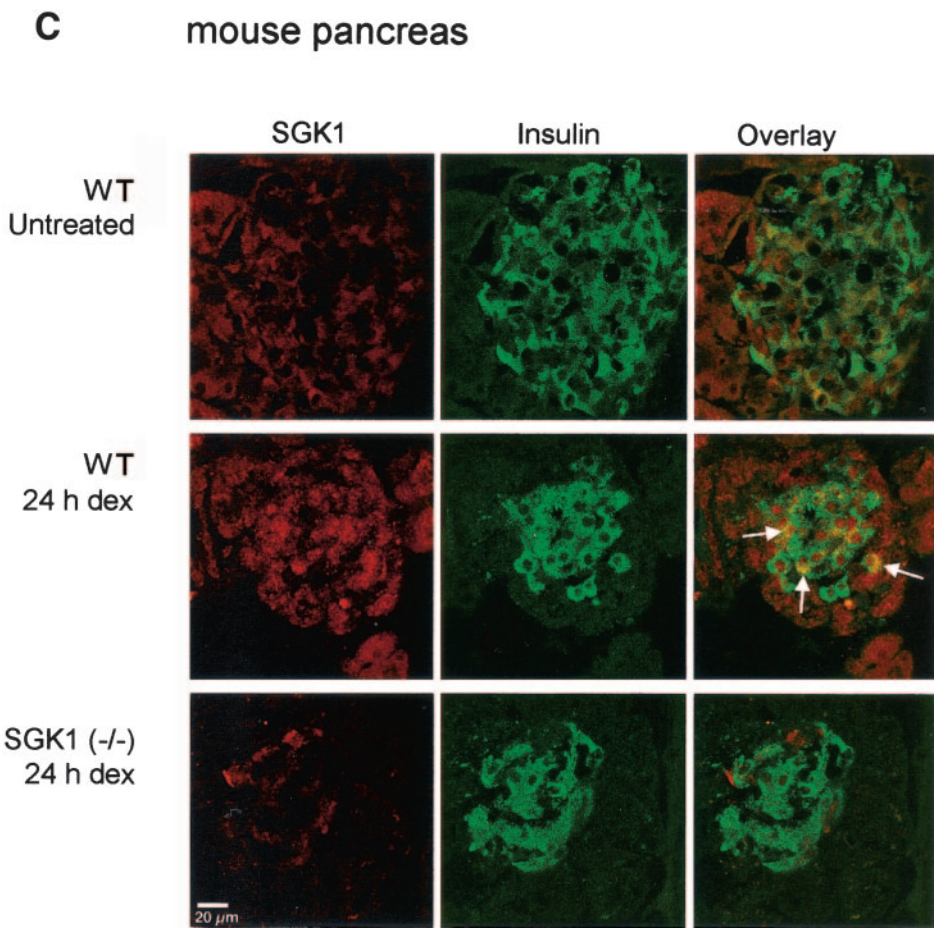


FIG. 2. Dexamethasone induces the expression of SGK1 in INS-1 cells (*A* and *B*) and mouse pancreas (*C*). INS-1 cells were treated with 100 nmol/l dexamethasone or DMSO (untreated) in culture. *A*: SGK1-specific PCR products expressed as means ± SE of the indicated number (*n*) of experiments. *B*: Representative Western blot for SGK1 (49-kDa band) out of three independent experiments. *C*: Immunostaining for SGK1 (red), insulin (green), and overlay (yellow) are shown on confocal images of pancreas from untreated *SGK1*$^{+/+}$ (wild type, WT) and 24-h dexamethasone-treated *SGK1*$^{+/+}$ (WT) and *SGK1*$^{-/-}$ mice.

1 mmol/l Na pyruvate, 10 μmol/l β-mercaptoethanol (Sigma, Munich, Germany), and antibiotics as described elsewhere (31,32). Cells were seeded at a cell density of 2.0–2.5 × 10^5 cells/ml in 24-well culture plates and cultured for 2 days before the experiment. Cells were washed twice with incubation buffer containing (in mmol/l) 140 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 0.5 glucose, 10 HEPES, and 5 g/l bovine serum albumin (fraction V; Sigma, Deisenhofen, Germany), pH 7.4, and preincubated for 30 min at 37°C. Thereafter, cells were incubated for 30 min at 37°C in fresh medium containing the test substances at the appropriate concentrations. Incubations were stopped on ice, and insulin released into the supernatant and insulin content after extraction with acid ethanol (1.5% [vol/vol] HCl/75% [vol/vol] ethanol) were measured by radioimmunoassay using rat insulin antiserum (Linco, Biotrend Chemikalien, Cologne, Germany), I^{125}-insulin (CIS Diagnostik, Dreieich, Germany), and rat insulin (Novo Nordisk, Mainz, Germany) as standard or an insulin enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden).

Islets were isolated by collagenase digestion (1 mg/ml; Serva, Heidelberg, Germany) for 10 min at 37°C and isolated from exocrine tissue under the dissection microscope. Islets were cultured overnight in RPMI 1640 containing 11 mmol/l glucose. Dexamethasone (100 nmol/l) or DMSO (control) were added during the last 4 h of culture. Culture islets were then preincubated for 1 h at 37°C in incubation buffer containing 2.8 mmol/l glucose. Thereafter, batches of five islets/0.5 ml were incubated for 30 min at 37°C in the presence of test substances as indicated for each experiment.

Measurement of membrane currents and $[Ca^{2+}]_i$. INS-1 cells and mouse islet cells after digestion of isolated islets with trypsin (1 mg/ml) were cultured for 2–4 days at subconfluent cell densities. The cells were superfused with incubation buffer containing 0.5 mmol/l glucose. For patch clamp experiments, pipettes (Clark-Medical, Reading, U.K.) with a resistance of 4–6 MΩ were pulled using a DMZ-universal puller (Zeitz, Augsburg, Germany). They were filled with an internal solution containing (in mmol/l) 30 KCl, 95 K^+ gluconate, 1 $MgCl_2$, 1.2 NaH_2PO_4, 4.8 Na_2HPO_4, 5 Na_2ATP, 1 Na_3GTP, and 5 EGTA, pH 7.2. An EPC9 patch clamp amplifier (Heka Electronic, Lambrecht, Germany) was used for current measurements. Only stable current measurements, i.e., when currents reached at least 90% of control current after removal of the respective inhibitory drug, were used for analysis. INS-1 cells were transfected with pIRES2SGK1 using a TransFast kit (Promega, Mannheim, Germany). One to 2 days after transfection, cells were treated with IGF-1 (50 ng/ml) for 4 h to activate SGK1 before patch clamp experiments were performed on green fluorescent protein–positive cells.

For $[Ca^{2+}]_i$ measurements, cells were loaded with fura-2/AM: 10 μmol/l, for 10 min at 37°C. Fluorescence measurements were performed using an inverted microscope (Axiovert 100 TV; Carl Zeiss Jena, Jena, Germany), a monochromator (Till Photonics, Martinsried, Germany), and a digital camera (Micromax 5 MHz; Princeton Instruments, Trenton, NJ). The fluorescence emission ratio at 340/380-nm excitation was calculated as a measure of $[Ca^{2+}]_i$.

Immunostaining of mouse islets. $SGK1^{+/+}$ and $SGK1^{-/-}$ littermates were treated with dexamethasone for 24 h by subcutaneous injection of 10 mg/kg body wt. Pancreata were fixed with 4% phosphate-buffered paraformaldehyde. Subsequent sections of tissue were stained for SGK1 and insulin with rabbit anti-hSGK1 antibodies (1:400) and guinea pig anti-insulin serum antibodies (1:100; Linco, St. Charles, MO; diluted with PBS containing 0.3% Triton-X and 1% DMSO). Binding of anti-SGK1 antibodies was visualized with anti-rabbit antibodies conjugated to Alexa546, and anti-insulin antibody binding was visualized with anti–guinea pig conjugated to Alexa488 (molecular probes). Sections were analyzed on a confocal laser-scanning microscope (Zeiss LSM 510) using a He/Ne at 543 nm and an Ar-laser at 488-nm excitation wavelengths with appropriate filter sets. Unspecific staining was controlled by omitting the primary antibody.

Real-time PCR. INS-1 cells were cultured in 75-cm (2) flasks and treated with dexamethasone and RU486 as indicated. Cells were lysed, total RNA was isolated (Mini kit; Qiagen, Hilden, Germany), and 2 μg were transcribed into cDNA using RT M-MuLV (Roche Diagnostics, Roche Applied Science, Mannheim, Germany). Aliquots of cDNA, corresponding to equal amounts of RNA, were used for quantification of mRNA. The following specific primers were used: for detection of rat $K_v1.5$ channel mRNA: sense: 5′-ACTTCGCAGAGGC AGACAATCA-3′; antisense: 5′-GGTTGCCTTGTTCTTCCTTCAG-3′. For rat $K_v2.1$ channel: sense: 5′-GGATCCCCCGAAAAGGCCAG-3′ and antisense: 5′-G TTGGTCTCCGTGAGCCTCC-3′; for rat $K_v2.2$ channel: sense: 5′-GAGGTTAG CCAAAAAGACTC-3′ and antisense: 5′-TTGCAAAGGACCATGGGAAG-3′; and for rat $K_v3.2$ channel: sense: 5′-GCAAGCTCACCTACATTTTG-3′ and antisense: 5′-TCCATCAGAAGCGCACGTGT-3′. Primers used for rat SGK1 mRNA quantification were sense: 5′-TTTTTTTTCCCAACCCTTGC-3′ and antisense: 5′-AATGAACAAAGGTTGGGGGG-3′. SGK1 and K_v channel mRNAs were quantified using a light cycler system (Roche Diagnostics, Roche Applied Science, Mannheim, Germany). PCR reactions 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, and 2 μl cDNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany). The transcript levels of the housekeeping gene glyceraldehyde-3-phsophate dehydrogenase (GAPDH) of each sample was taken as reference using a commercial primer kit (Search LC, Heidelberg, Germany). Amplification of the target DNA was performed during 35 cycles, each 10 s at 95°C, 10 s at 68°C, and 16 s at 72°C. Melting curve and agarose gel analyses confirmed the specificity of amplified products. Results were calculated as a ratio of the target versus housekeeping gene transcripts.

After cell culture with dexamethasone, mouse islets were collected and dissolved in lysis buffer (Mini kit; Qiagen, Hilden, Germany) and disrupted mechanically. RT-PCR for detection of SGK1 mRNA was performed using as sense primer: 5′-TGTCTTGGGGCTGTCCTGTATG-3′ and an antisense primer: 5′-GCTTCTGCT GCTTCCTTCACAC-3′.

Western blotting. INS-1 cells were cultured in 75-cm (2) flasks without (control) or with 100 nmol/l dexamethasone for the indicated period of time. Cells were lysed in a solution containing 300 mmol/l NaCl, 20 mmol/l Tris HCl, pH 7.4, 1% (vol/vol) Triton X-100, 1% sodiumdeoxycholate, 0.1% SDS, 2.5 mmol/l EDTA, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, and 0.1 mmol/l phenylmethylsulfonyl fluoride. Total cellular protein, 50 μg quantified by Coomassie blue G staining (Bradford dye assay; Biorad Laboratories, Munich, Germany), was subjected to SDS-PAGE (10%) and blotted onto a nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). Blots were incubated with antibodies against SGK1 (New England Biolabs, Beverly, MA). Bound antibody was visualized using a secondary antibody coupled to horseradish peroxidase.

Oocyte injection experiments. Dissection of Xenopus laevis ovaries has been described elsewhere (33). Oocytes were injected with 0.12 ng cRNA of human $K_v1.5$, $K_v2.1$, or $K_v3.1$ alone or together with 4 ng hSGK1 cDNA in a volume of 60 nl. Three days after injection, two-electrode voltage clamp recordings were performed at room temperature using a TurboTEC 10CX amplifier (NPI Electronics, Tamm, Germany). Currents were elicited with voltage steps from a holding potential of −60 to +60 mV for 3 s with an interval of 10 s. The data were filtered at 1 KHz and recorded with an interface DIGIDATA 1322a (Axon Instruments, San Diego, CA) and the software Clampex 9.0 (Axon instruments). The external control solution (superfusate/ND96) contained (in mmol/l) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, pH 7.4.

Statistics. Data are presented as means ± SE. ANOVA for multiple groups and Student's t tests were used for statistical analysis. P values <0.05 were accepted to indicate statistical significance.

RESULTS

Glucocorticoids induce expression of K_v channels and SGK1 in insulin-secreting cells. To examine dexamethasone-induced transcription in insulin-secreting cells by semiquantitative RT-PCR, we have chosen the clonal rat insulinoma cell line INS-1 (34). We first examined whether dexamethasone affects transcription of K_v channels. Previously, we have found that INS-1 cells express a variety of K_v channels, including $K_v1.5$, $K_v2.1$, $K_v2.2$, and $K_v3.2$. Real-time PCR reveals that dexamethasone (100 nmol/l) treatment increases the abundance of $K_v1.5$ mRNA approximately sevenfold (Fig. 1*A*). The expression of other K_v channels, $K_v2.1$, $K_v2.2$, and $K_v3.2$, was not significantly stimulated by dexamethasone (Fig. 1*B–D*). In mouse islets treated for 4 h with 100 nmol/l dexamethasone, $K_v1.5$ mRNA increased fourfold from 0.11 ± 0.02 to 0.41 ± 0.05 copies $K_v1.5$/GAPDH*1,000 ($n = 3$, $P < 0.005$).

Next, we searched for SGK1 transcripts in INS-1 cells. According to real-time PCR, the SGK1 transcript level is low in untreated INS-1 cells (Fig. 2*A*). However, incubation of INS-1 cells with 100 nmol/l dexamethasone for 2–23 h increased mRNA transcript levels, an effect that was completely abrogated by the glucocorticoid receptor antagonist RU486 (Fig. 2*A*). Within 23 h, dexamethasone increased the cellular SGK1/GAPDH mRNA ratio by a factor of 146 ± 58 (range 28- to 275-fold, $n = 4$). When SGK1 transcript levels were measured in isolated mouse islets, SGK1 mRNA increased by a factor of 13.6 ± 3.4 ($n =$

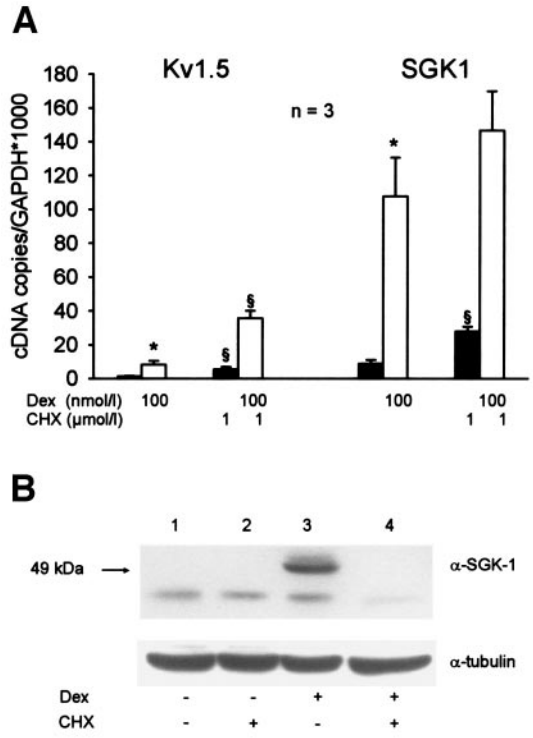


FIG. 3. Cycloheximide abolishes SGK1 synthesis but not dexamethasone-activated $K_v1.5$ channel transcription. INS-1 cells were treated for 20 h with 1 μmol/l cycloheximide (CHX). During the last 4 h, dexamethasone or DMSO was added. *A*: $K_v1.5$ and SGK1 PCR products of control cells (■) and after dexamethasone treatment (□) are given as means ± SE from three independent experiments. *B*: A representative Western blot for SGK1 (49 kDa) of three independent experiments is shown. *Denotes significant difference between the dexamethasone-treated cells and the respective control. §Denotes significant effect of cycloheximide.

6) following treatment with dexamethasone. This induction of SGK1 in mouse islets is larger than the sixfold increase of human SGK1 in epithelial cells (35).

To confirm the expression of SGK1 on protein level, Western blotting has been performed with INS-1. SGK1 protein was not detectable in untreated INS-1 cells but appeared within 2 h and increased further within the next 23 h of exposure to dexamethasone (100 nmol/l) (Fig. 2*B*). The increase in SGK1 protein was fully inhibited by RU486. Immunostaining of SGK1 in mouse pancreas revealed that SGK1 is expressed in insulin-containing cells of wild-type mice but not $SGK1^{-/-}$ mice after dexamethasone treatment (Fig. 2*C*). These experiments demonstrate that dexamethasone stimulates the expression of both $K_v1.5$ and SGK1 in insulin-secreting cells. To analyze whether changes in $K_v1.5$ mRNA depend on synthesis of SGK1 protein, INS-1 cells were incubated with protein synthesis inhibitor cycloheximide. Incubation of the cells with 1 μmol/l cycloheximide for 20 h inhibited SGK1 protein synthesis completely (Fig. 3*B*) but led to a further increase of mRNA levels for $K_v1.5$ and SGK1 (Fig. 3*A*). Dexamethasone increased $K_v1.5$ mRNA in the absence as well as in the presence of cycloheximide. Thus, expression of the SGK1 protein is not required for the transcriptional stimulation of $K_v1.5$, pointing to a more direct effect of glucocorticoids on $K_v1.5$ transcription. Indeed, the $K_v1.5$ gene contains a glucocorticoid response element (29).

SGK1 activates K_v channels when coexpressed in Xenopus oocytes. To examine whether SGK1 modulates K_v channel activities, we used the Xenopus leavis expression system. While water-injected Xenopus leavis oocytes did not show appreciable voltage-gated K^+ currents (data not shown), those currents were elicited in oocytes injected with 0.12 ng cRNA encoding $K_v1.5$ (Fig. 4*A*). Coexpression of SGK1 and $K_v1.5$ doubled $K_v1.5$-mediated current, pointing to upregulation of $K_v1.5$ channel activity by SGK1. As shown earlier, SGK1 similarly stimulates K^+ channels including K_v (17,36).

To characterize $K_v1.5$ currents in more detail, we used the K^+ channel inhibitor MSD-D (Fig. 4*B*). MSD-D dose-dependently inhibited $K_v1.5$ channel currents with a K_i of 0.16 ± 0.02 μmol/l. The drug (0.3 μmol/l) had no effect on currents of $hK_v2.1$ and $hK_v3.1$ channels (data not shown). Thus, MSD-D is indeed more selective for $K_v1.5$ than 4-aminopyridine (4-AP) and tetraethylammonium (TEA) and about ~1,000 times and 9,000 times more potent than 4-AP and TEA, respectively (25).

Dexamethasone increases K_v channel activity in INS-1 cells and mouse islet cells. To examine whether K_v channel activity is increased after dexamethasone

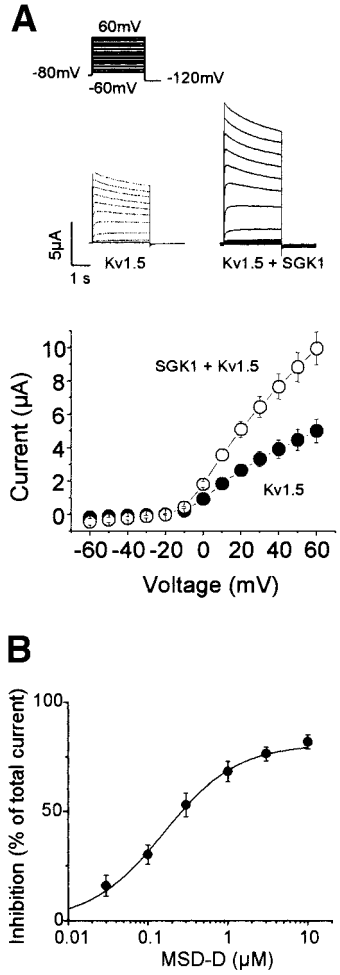


FIG. 4. Outward current induced by $K_v1.5$ channel expression in Xenopus oocytes is activated by SGK1 and inhibited by MSD-D. *A*: Shown are representative traces and means ± SE of $n = 11$ ($K_v1.5$, ●) and $n = 14$ ($K_v1.5$ + SGK1, ○) experiments. *B*: MSD-D effect on $K_v1.5$ channel-induced current is expressed as percent inhibition of total current. Shown are means ± SE of $n = 5$–10 measurements.

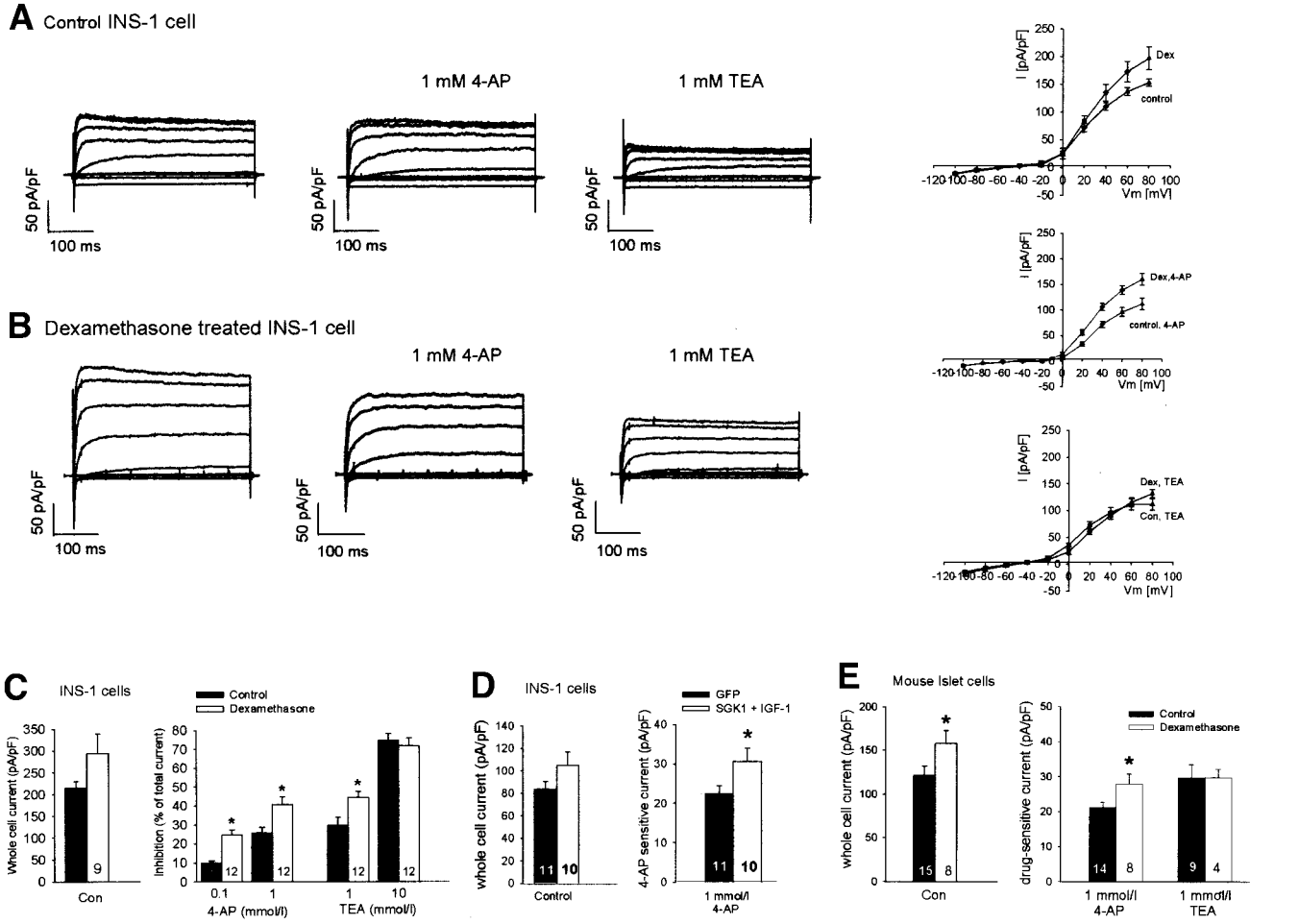


FIG. 5. Effect of dexamethasone on whole-cell outward current in INS-1 (*A–D*) and mouse islet cells (*E*). Representative traces and IV curves (n = 5–7) under control condition and in the presence of 1 mmol/l 4-AP or 1 mmol/l TEA of untreated INS-1 cells (*A*) and after dexamethasone-treatment (*B*) are shown. *C*: Effects of 4-AP (0.1 and 1 mmol/l) and TEA (1 and 10 mmol/l) in control cells (■) and after dexamethasone treatment for 4 h (□) are expressed as means ± SE for the indicated number of observations. *Denotes significance ($P < 0.05$) to current in control untreated cells at the same inhibitor concentration. *D*: INS-1 cells were transfected with hSGK1 as described in RESEARCH DESIGN AND METHODS. Cells expressing green fluorescent protein (GFP) cells were analyzed for 4-AP (1 mmol/l)-sensitive outward current. Shown are means ± SE for n = 10 experiments. *$P < 0.05$. *E*: Effect of 100 nmol/l dexamethasone for 4 h and 4-AP and TEA, each 1mmol/l, on outward current in mouse islet cells.

treatment, we analyzed outward currents in INS-1 cells, which are sensitive to TEA and 4-AP (25). The activation of $K_v1.5$ channels should result in a selective increase of 4-AP–sensitive outward current. As illustrated in Fig. 5*A–C*, treatment with dexamethasone increased 4-AP–sensitive voltage-gated outward current. In untreated cells, the K^+ channel blocker 4-AP inhibited 10% (at 0.1 mmol/l) and 28% (at 1 mmol/l) of outward current. Following a 4-h treatment with 100 nmol/l dexamethasone, the 4-AP–sensitive current increased to 28% (by 0.1 mmol/l 4-AP) and 40% (by 1 mmol/l 4-AP) of total current. TEA inhibited significantly more current at 1 mmol/l but not at 10 mmol/l after dexamethasone. The role of SGK1 was further examined in cells overexpressing hSGK1. INS-1 cells were transiently transfected with hSGK1 and the enzyme activated by 50 ng/ml IGF-1 (Fig. 5*D* and ref. 37). In cells expressing hSGK1, 1 mmol/l 4-AP inhibited 29.2% of whole-cell current, whereas in cells transfected with the empty GFP vector, it inhibited 26.7% of current ($P < 0.03$).

In mouse islet cells treated with dexamethasone, whole-cell current was significantly larger, and 1 mmol/l 4-AP inhibited 28% of outward current and 21% in control cells. These data suggest that parallel to increased expression, the activity of $K_v1.5$ channels is augmented by dexamethasone and activated by SGK1 in insulin-secreting cells.

$[Ca^{2+}]_i$ oscillations were blunted in dexamethasone-treated INS-1 cells. To examine whether dexamethasone alters $[Ca^{2+}]_i$, INS-1 cells were loaded with fura-2 after a 4-h treatment with 100 nmol/l dexamethasone or DMSO (0.1%, control). Average fluorescence ratio (340/380 nm) reflecting Ca^{2+} concentration was at 0.5 mmol/l glucose, 0.86 ± 0.03 in control cells, and not significantly modified by dexamethasone treatment (0.87 ± 0.04). The addition of glucose led to the appearance of irregular Ca^{2+} spikes, with a minor increase of average fluorescence ratio in both control (1.05 ± 0.05) and dexamethasone-treated (1.01 ± 0.01) cells. Peak values of Ca^{2+} oscillations (reaching values >1.2) were observed in 29.0 ± 2.8% of control (166 cells of $n = 3$ independent experiments) and in 19.7 ± 4.2% of dexamethasone-treated (145 cells of $n = 3$ independent experiments, Fig. 6) cells. Large magnitude $[Ca^{2+}]_i$ spikes (reaching a ratio value >1.4) were significantly more frequent in control (18.0 ± 2.3%) than in dexamethasone-treated (4.0 ± 1.8%) cells. This observation suggests that increased K_v channel activity results in a reduction of Ca^{2+}

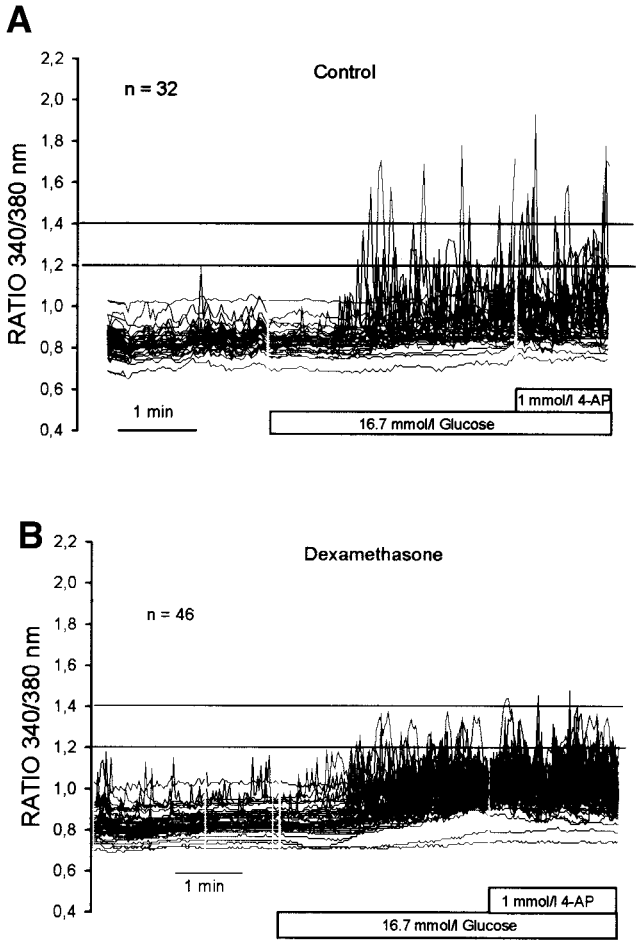


FIG. 6. Dexamethasone loweres $[Ca^{2+}]_i$ oscillations in INS-1 cells. After treatment of cells for 4 h with dexamethasone (100 nmol/l) or DMSO (0.1%), cells were first perfused with incubation solution containing 0.5 mmol/l glucose, followed by 16.7 mmol/l glucose and 1 mmol/l 4-AP as indicated. Horizontal lines depict values of a ratio >1.2 and >1.4, respectively.

peaks after dexamethasone. The difference was not reflected by changes of average Ca^{2+} concentrations.

K_v channel inhibitors antagonize dexamethasone-induced inhibition of insulin secretion. The following experiments were performed to elucidate the impact of K_v channels on the blunting of insulin release by dexamethasone. As illustrated in Fig. 7*A–C*, pretreatment of INS-1 cells with dexamethasone (100 nmol/l) significantly (*$P <$ 0.05) inhibited glucose-induced insulin secretion. This inhibition was reversed by K_v channel blockers 10 mmol/l TEA and 5 mmol/l 4-AP, suggesting that dexamethasone-mediated inhibition of insulin secretion depends on K_v channel activity (Fig. 7*A*). However, higher concentrations of K^+ channel inhibitors were required to counteract secretion than those significantly decreasing whole-cell current. Therefore, a more selective $K_v1.5$ channel inhibitor, MSD-D, was used in the following. The inhibition of $K_v1.5$ channel activity by 0.3 μmol/l MSD-D had no effect on secretion at low glucose but augmented glucose-stimulated insulin secretion (#$P = 0.008$). MSD-D reversed the inhibitory effects of dexamethasone on glucose-induced insulin release (§$P = 0.0002$, Fig. 7*B*). Next, insulin secretion was stimulated in the presence of 30 mmol/l KCl and diazoxide, thus bypassing K^+ channel regulation (Fig. 7*C*). Glucose at high KCl significantly (#$P = 0.004$) stimulated secretion to the same extent in control and dexamethasone-treated cells. These data support the idea that increased K_v channel activity is involved in dexamethasone-mediated inhibition of glucose-induced insulin secretion. Finally, we tested whether inhibition of K_v channels counteracts dexamethasone-induced inhibition of insulin secretion in mouse islets as it does in INS-1 cells. Glucose significantly (§$P = 0.0085$) stimulated secretion fourfold in untreated but not in dexamethasone-treated mouse islets. In the presence of MSD-D (0.3 μmol/l), glucose stimulated secretion 14-fold (#$P = 0.0028$). MSD-D completely reversed the inhibition of secretion by dexamethasone ($^aP = 0.0054$, Fig. 7*D*). Thus, as in INS-1 cells, inhibition of $K_v1.5$ channels improves glucose-induced insulin secretion in dexamethasone-treated mouse islets.

Dexamethasone does not inhibit insulin secretion in islets from mice deficient of SGK1. To estimate the contribution of SGK1 to the inhibitory effect of dexamethasone on insulin secretion, we studied the effects of dexamethasone in SGK1 knockout mice (*SGK1*$^{-/-}$) compared with that in wild-type littermates (*SGK1*$^{+/+}$). Without dexamethasone pretreatment, insulin secretion following exposure to glucose (16.7 mmol/l), activation of adenylate cyclase (5 μmol/l forskolin), or stimulation of protein kinase C (100 nmol/l phorbol myristic acid) was not significantly different in islets isolated from *SGK1*$^{-/-}$ and *SGK1*$^{+/+}$mice (Fig. 8*A* and *B*, *black bars*). Dexamethasone treatment, however, significantly decreased the stimulatory effect of glucose, forskolin, or phorbol myristic acid on insulin secretion in *SGK1*$^{+/+}$ islets but not in *SGK1*$^{-/-}$ islets. These data strongly suggest that SGK1 participates in the downregulation of insulin secretion by dexamethasone.

DISCUSSION

We demonstrate here that the synthetic glucocorticoid dexamethasone induces the expression of SGK1 in insulin-secreting cells. This kinase is only slightly expressed in untreated β-cells (Fig. 1), and low transcript levels have been reported previously for human pancreatic islets (38). The expression after glucocorticoid treatment of INS-1 cells is high; the amount of SGK1 mRNA reaches 80% of the abundance of GAPDH mRNA. Similarly, strong stimulation of SGK1 transcription by glucocorticoids was observed in other cell types (39,40). In native mouse islets, the copy numbers of SGK1 mRNA compared with GAPDH mRNA were much smaller. However, in both cell preparations, INS-1 cells and mouse islets, SGK1 protein was detectable after dexamethasone treatment. The amount of protein detected by western blotting also increased with time. This induction of SGK1 expression may contribute to the phenotype of impaired glucose-induced insulin secretion after glucocorticoid treatment and may contribute to the development of diabetes.

The endogenous cortisol plasma concentrations are governed by a circadian rhythm, reaching maximal concentrations of 700 nmol/l in the morning (41). Dexamethasone concentrations used for studying metabolic parameters range from 10 nmol/l to 1μmol/l, concentrations encountered in glucocorticoid excess (6,42,43). Dexamethasone is ~30 times more potent than cortisol.

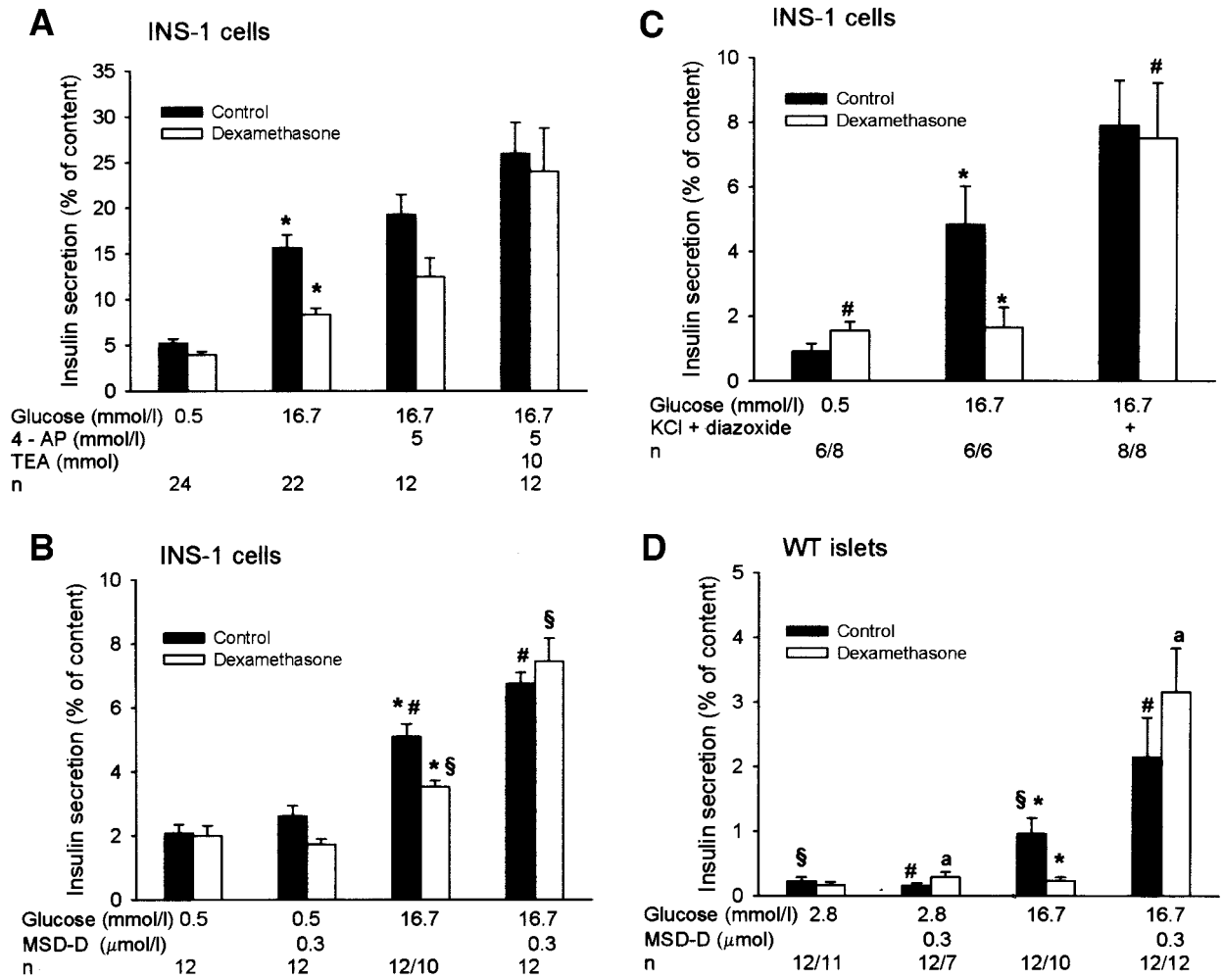


FIG. 7. K_v channel inhibition reverses dexamethasone-induced inhibition of insulin secretion in INS-1 cells (*A–C*) and mouse islets (*D*). Before measurements of insulin secretion after static incubations for 30 min, INS-1 cells (*A–C*) and islets (*D*) were treated for 4 h with 100 nmol/l dexamethasone or DMSO (0.1%). *C*: The concentrations used were 30 mmol/l KCl and 1 mmol/l diazoxide. Shown are means ± SE of $n = 12$ observations of three independent experiments. *P* values explained in RESULTS. WT, wild type.

We have chosen a concentration (100 nmol/l) that significantly and reproducibly inhibited insulin secretion in a variety of systems including INS-1 cells and isolated mouse islets (ref. 6 and Figs. 7 and 8).

The pleiotropic effects of glucocorticoids on insulin-secreting cells include an increase of α_2-adrenoceptor mRNA and binding sites (2,9) and a decrease of Glut2 transporter expression at the plasma membrane (8,11). Both proteins are regulated at a transcriptional and a posttranslational level. Here, we present two additional proteins, SGK1 and $K_v1.5$ channels, whose expression is regulated by dexamethasone at the transcriptional level. Both genes contain a glucocorticoid responsive element in their respective promoter region (15,29). Increased transcription is accompanied by increased translation of SGK1 as judged by Western blotting. $K_v1.5$ channels are minor components of plasma membrane proteins of insulin-secreting cells. Although the protein was detected by Western blotting, the abundance was too low to be quantified, since detection was largely limited by the quality of the antibody (data not shown). Functionally, 4-AP–sensitive outward currents were increased in INS-1 cells, suggesting increased $K_v1.5$ channel activity after dexamethasone treatment. $K_v1.5$ currents have been described to be insensitive to TEA (27). Since, under control conditions, the major part of voltage-activated outward current is TEA sensitive, it is afforded by $K_v2.1$ and/or $K_v2.2$. and $K_v3.4$ (22,24). The effect of dexamethasone was reflected by a decrease of Ca^{2+} spikes rather than a decrease of average Ca^{2+} concentration. It should be kept in mind that the local increase of Ca^{2+} and not the mean cytosolic Ca^{2+} concentration matters for insulin release (44,45). The mechanism by which such changes in $[Ca^{2+}]_i$ oscillations might affect insulin secretion remains to be elucidated. In mouse islets, $K_v1.5$ mRNA was indeed low under control conditions. A fourfold increase of $K_v1.5$ mRNA levels after dexamethasone treatment might, however, change the ratio of K_v channel expression and function. Changes in the function of K_v channels have previously been described to alter glucose-induced insulin release (24). We thus propose that changes in $K_v1.5$ channel function might contribute to impaired insulin secretion after dexamethasone treatment. Indeed, blocking K_v channels in INS-1 cells and mouse islets counteracts dexamethasone-induced inhibition of insulin secretion. The sensitivity of insulin secretion to 4-AP and TEA is lower than that of

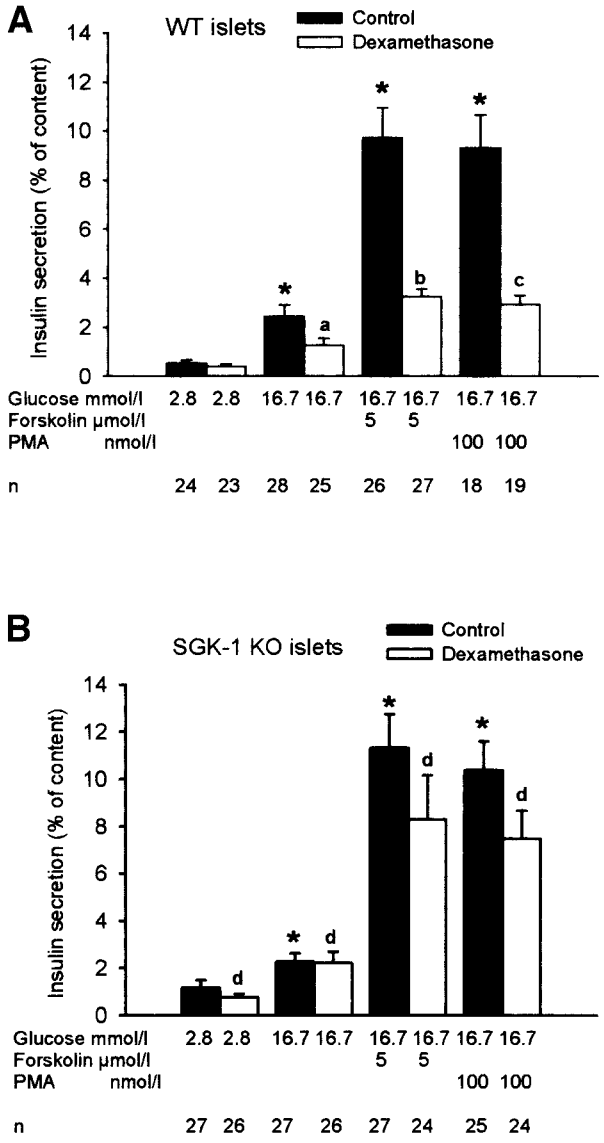


FIG. 8. Dexamethasone did not affect secretion from islets of SGK1 knockout (KO) mice. Isolated islets were cultured overnight, and dexamethasone (100 nmol/l) or DMSO (control) were added 4 h before the experiment. Insulin secretion was measured after static incubation of islets for 30 min. Shown are means ± SE for the indicated number of observations (*n*). *Denotes significant stimulation of secretion compared with respective control secretion at 2.8 mmol/l glucose. (*a–c*) indicates significant inhibition of secretion by dexamethasone compared with the respective secretion from untreated islets. (*d*) indicates significance between secretion of wild-type (WT) and *SGK1*$^{-/-}$ islets at the same condition.

whole-cell currents, indicating that other mechanisms may contribute to impairment of release. Dexamethasone did not inhibit secretion induced by 15 mmol/l glucose in the presence of 30 mmol/l KCl and 0.25 µmol/l diazoxide, i.e., when K^+ channel regulation was bypassed. This observation strongly suggests that dexamethasone influences insulin release indeed by modifying K^+ channel activity. Previously, Henquin et al. (6) described that dexamethasone inhibits secretion induced by glucose and KCl in mouse islets. Thus, under the experimental conditions of these previous experiments, dexamethasone may exert additional inhibitory effects on insulin release that are independent from K^+ channel activation. Whether these additional mechanisms are mediated by SGK1 cannot be answered from our observations.

Pertussis toxin and cAMP have been found to counteract dexamethasone inhibition of insulin secretion (6). Thus, incretins and glucagon, i.e., mediators increasing cAMP in β-cells might have a protective effect against glucocorticoid-induced diabetes. Whether an impaired function of incretins is responsible for increased susceptibility to glucocorticoid-induced diabetes especially in humans needs further examinations. Indeed, incretins not only potentiate glucose-induced insulin secretion but also activate cell proliferation and inhibit apoptosis (46).

The involvement of SGK1 in dexamethasone-induced inhibition of insulin secretion is suggested by experiments in SGK1$^{-/-}$ islets. Dexamethasone did not significantly affect secretion from islets of *SGK1*$^{-/-}$ mice, while secretion of the wild-type littermates was impaired. These results clearly show that SGK1 activation is involved in dexamethasone-induced inhibition of insulin secretion.

Impairment of insulin release contributes to but presumably does not fully account for the diabetic effect of glucocorticoid excess. The actions of glucocorticoids also include stimulation on hepatic glucose mobilization and peripheral insulin resistance (4).

In conclusion, the present experiments consider two proteins that are upregulated by glucocorticoids in insulin-secreting cells. The glucocorticoid dexamethasone enhances the transcription and expression of SGK1 and $K_v1.5$ channels. The kinase is able to activate $K_v1.5$ channels. Overexpression of K_v channels hyperpolarizes the β-cell plasma membrane, thus impeding the activation of voltage-gated Ca^{2+} channels. Accordingly, the kinase may contribute to the inhibition of insulin release during glucocorticoid excess and thus to the well-known aggravation of diabetes by stress conditions (47). Whether SGK1 is also involved in translational changes of α_2-adrenoceptors and Glut2 is an attractive hypothesis and should be examined.

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