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Dexamethasone suppresses phospholipase C activation and insulin secretion from isolated rat islets

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Abstract

Dexamethasone inhibits insulin secretion from isolated islets. In the present experiments, possible underlying biochemical mechanisms responsible for defective secretion were explored. Dexamethasone (1 μmol/L) had no immediate deleterious effect on 15 mmol/L glucose-induced insulin release from perifused rat islets. However, a 3-hour preincubation period with 1 μmol/L dexamethasone resulted in parallel reductions in both the first (64%) and second phases (74%) of 15 mmol/L glucose-induced insulin secretion monitored during a dynamic perifusion. When measured after the perifusion, there were no differences in insulin content or in the capacity of control or dexamethasone-treated islets to use glucose. Dexamethasone (1 μmol/L) preexposure also reduced phorbol ester– and potassium-induced secretion. In additional experiments, islets were labeled for 3 hours with ^{3}H-inositol in the presence or absence of 1 μmol/L dexamethasone. The steroid did not affect total ^{3}H-inositol incorporation during the labeling period. However, the capacity of 15 mmol/L glucose, 30 mmol/L KCl, and 100 μmol/L carbachol to activate phospholipase C (PLC), monitored by the accumulation of labeled inositol phosphates, was significantly reduced in dexamethasone-pretreated islets. Inclusion of the nuclear glucocorticoid receptor antagonist RU486 (mifepristone, 10 μmol/L) abolished the adverse effects of dexamethasone on both glucose-induced inositol phosphate accumulation and insulin secretion. Quantitative Western blot analyses revealed that the islet contents of PLCδ1, PLCβ1, β2, β3, and protein kinase C α were unaffected by dexamethasone pretreatment. These findings demonstrate that dexamethasone pretreatment impairs insulin secretion via a genomic action and that impaired activation of the PLC/protein kinase C signaling system is involved in the evolution of its inhibitory effect on secretion.

1. Introduction

Previous studies conducted by Fajans and coworkers [1,2] demonstrated the usefulness of prior dexamethasone treatment as a potential tool to identify those destined to develop diabetes. A direct effect of the steroid on the beta cell has been implicated in a number of studies [3,4]. A comprehensive report by Lambillotte and coworkers [5] with mouse islets explored the impact of dexamethasone treatment on multiple parameters of beta-cell activation that are thought to participate in the regulation of insulin secretion. Despite a pronounced inhibitory effect of dexamethasone pretreatment on glucose-induced insulin release, they did not find any obvious metabolic or calcium handling defect that might account for the altered secretory response. Cyclic adenosine monophosphate levels were unaffected as well. They did observe, however, that the phospholipase C (PLC)–mediated hydrolysis of ^{3}H-inositol–labeled phosphoinositide pools was reduced in parallel with secretion. They concluded that a classic genomic action of the steroid, resulting in the reduced synthesis of proteins necessary for insulin secretion, may be involved. The nature of the proteins was not identified, although the authors did conclude that the activation of information flow in the PLC/protein kinase C (PKC) signal transduction

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pathway was impaired and might account, in part, for the effect of dexamethasone.

More recent studies using islets and MIN6 cells [6,7] demonstrated further that dexamethasone impairs prolactin-induced up-regulation of islet function, a necessary adaptation to meet the increased insulin requirements of gestation. In this context, the steroid limits or restrains this adaptive response. We have suggested previously that phospholipase C and the distal activation of PKC participate in the regulation of glucose-induced biphasic insulin secretion from rat islets studied in vitro during a dynamic perifusion [8-11]. This suggestion was initially based on the observation that in rat islets, the pharmacological activation of PKC by phorbol esters resulted in a slowly rising insulin secretory response reminiscent, at least qualitatively, to the second phase of glucose-induced release [12,13]. Subsequent studies demonstrated that glucose induces rapid and significant changes in PLC activation, monitored by a number of different methodologies [14-18]. Glucose also promotes the translocation of PKCα from a predominantly cytosolic compartment to a membrane-associated one [19,20] and increases the phosphorylation state of an established PKC substrate [21]. Considering the involvement of the PLC/PKC signaling cascade in the physiological regulation of release and its potential disruption by dexamethasone, we decided to explore in more detail the impact of short- and long-term exposure to dexamethasone on insulin secretion from rat islets.

2. Materials and methods

The detailed methodologies used to assess insulin output from collagenase-isolated islets have been previously described [22,23]. Male Sprague-Dawley rats (weighing 300-450 g) were purchased from Charles River (Wilmington, MA) and used in all studies. All animals were treated in a manner that complied with the NIH Guidelines for the Care and Use of Laboratory Animals. The animals were fed ad libitum. After Nembutal-induced anesthesia (pentobarbital sodium, 50 mg/kg; Abbott, North Chicago, IL), islets were isolated by collagenase digestion and handpicked, using a glass loop pipette, under a stereomicroscope into Krebs-Ringer bicarbonate (KRB) supplemented with 3 mmol/L glucose. They were free of exocrine contamination. Some islets were directly perifused after isolation to assess the effects of dexamethasone on release. Other islets were loaded onto nylon filters (Tetko, Briarcliff Manor, NY) and incubated for 3 hours in 400 μL KRB supplemented with 5 mmol/L glucose ± 1 μmol/L dexamethasone. The steroid was dissolved in dimethyl sulfoxide (DMSO), and equivalent amounts of diluent (1 μL DMSO/999 μL KRB for a final concentration of 0.1%) were added to control islets. In some experiments, mifepristone (RU486, dissolved in DMSO) was added together with dexamethasone.

2.1. Perifusion studies

Islets were perifused in a KRB buffer at a flow rate of 1 mL/min for 30 minutes in the presence of 3 mmol/L glucose to establish basal and stable insulin secretory rates. After this 30-minute stabilization period, they were then perifused with the appropriate agonist or agonist combinations as indicated in the figure legends and Results. Perifusate solutions were gassed with 95% O_2/5% CO_2 and maintained at 37°C. Insulin released into the medium was measured by radioimmunoassay [24]. In some experiments, the islets were sonicated after the perifusion and analyzed for insulin content.

2.2. Islet labeling for inositol phosphate studies

Groups of 18 to 26 islets were loaded onto nylon filters and incubated for 3 hours in a *myo*-[2-^{3}H]-inositol–containing KRB solution made up as follows: 10 μCi of *myo*-[2-^{3}H]-inositol (specific activity, 16-23 Ci/mmol) were placed in a 10 × 75-mm culture tube. Two hundred fifty-five microliters of warmed (to 37°C) and oxygenated KRB medium supplemented with 5.0 mmol/L glucose ± 1 μmol/L dexamethasone were added to this aliquot of tracer. In some experiments, 10 μmol/L RU486 was added together with the dexamethasone. After mixing, 240 μL of this solution was gently added to the vial with islets. The vial was capped with a rubber stopper, gassed for 10 seconds with 95% O_2/5% CO_2, and placed in a metabolic shaker at 37°C. After 90 minutes, the vials were again gently oxygenated. After the labeling period, the islets were washed with 5 mL of fresh KRB and used for inositol phosphate (IP) measurements (see below). In some studies, after washing, total amount of ^{3}H-inositol incorporated into the islet was measured immediately after the 3-hour incubation period.

2.3. IP measurements

After washing to remove free labeled inositol, the islets on nylon filters were placed in small glass vials. We gently added to the vial 400 μL of warmed (to 37°C) KRB supplemented with 10 mmol/L LiCl to prevent IP degradation and the appropriate agonists as indicated. The vials were capped, and after 30 minutes, the generation of IPs was stopped by adding 400 μL of 20% perchloric acid. Total IPs were then measured using Dowex columns as described previously [25,26].

2.4. Glucose use rates

The use of glucose was measured by determining the rate of ^3H_2O formation from 5-^{3}H-glucose. After the 3-hour incubation in the presence or absence of 1 μmol/L dexamethasone, the islets were perifused and stimulated with 15 mmol/L glucose. At the termination of the perifusion, the islets were incubated in 125 μL of 15 mmol/L glucose supplemented with tracer 5-^{3}H-glucose. The ^3H_2O formed during the subsequent 1-hour incubation

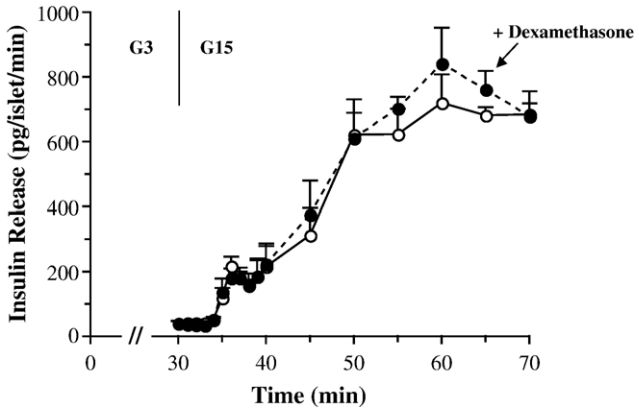

Fig. 1. Glucose-induced insulin secretion in the presence and absence of dexamethasone. Two groups of rat islets were isolated and perifused for 30 minutes with 3 mmol/L glucose (G3). They were then stimulated (onset indicated by the vertical line) for 40 minutes with 15 mmol/L glucose (G15) alone (open circles) or with 15 mmol/L glucose plus 1 μmol/L dexamethasone (Dexa) (closed circles). At least 3 experiments were conducted under each condition, and means ± SE are given. This and the subsequent perifusion experiments have not been corrected for the dead space in the perifusion apparatus, 2.5 mL or 2.5 minutes with a flow rate of 1 mL/min.

was separated from the unused ^{3}H-glucose as described previously [27].

2.5. Western blot analyses

Groups of islets were incubated for 3 hours in KRB medium together with DMSO alone or in DMSO supplemented with 1 μmol/L dexamethasone. At the end of the incubation, the islets were collected, pelleted by centrifugation, and suspended in 25 to 50 μL of homogenization buffer containing various protease inhibitors as described previously [28]. After sonication and centrifugation, triplicate aliquots were analyzed for protein content according to the Lowry method using bovine serum albumin as a standard. For the Western blots, 15 to 20 μg of protein sonicate from islets was boiled for 90 seconds in 4× Laemmli sample buffer and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using a 4% stacking gel and a 7% running gel run at 12 and 16 mA, respectively, as described in detail previously [28]. Gel-resolved proteins were electrotransfered onto Immobilon polyvinylidene fluoride (PVDF) membranes at 15 V (k) for 20 hours. The Immobilon was stained with Ponceau S solution for protein, washed, and blocked for 2 hours in Tris-buffered saline supplemented with 0.05% Tween 20 and 5% milk powder. For PLCδ1 determinations, the membranes were incubated for 150 minutes with the primary anti-PLCδ1 antibody (1.0 μg/mL dilution), washed, incubated for 60 minutes with horseradish peroxidase (HRP)–conjugated IgG, and washed again. For PLCβ determinations, the membranes were incubated for 60 minutes with the primary anti-PLCβ antibody (0.5 μg/mL dilution), washed, incubated for 45 minutes with HRP-conjugated IgG, and washed again. For PKCα determinations, the membranes were incubated for 45 minutes with the primary anti-PKCα antibody (0.5 μg/mL dilution), washed, incubated for 45 minutes with HRP-conjugated IgG, and washed again. The antigen-antibody complexes were visualized using the enhanced chemiluminescence system (Amersham, Arlington Heights, IL) and quantitated densitometrically

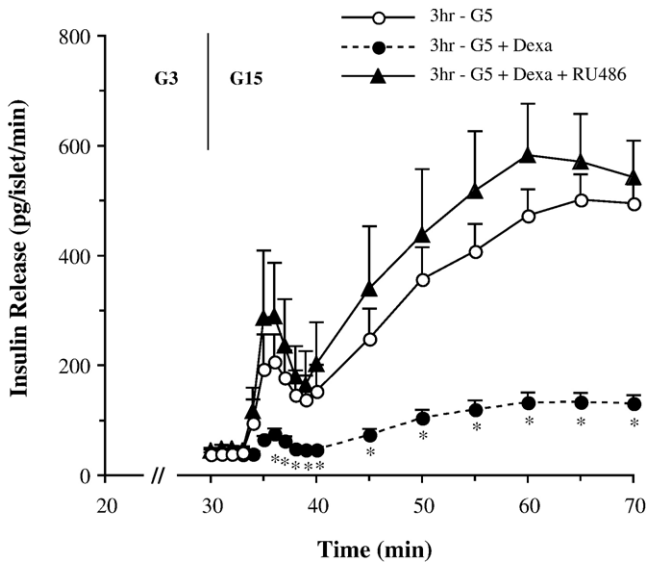

Fig. 2. Effects of a 3-hour prior exposure to dexamethasone (Dexa) alone or dexamethasone plus RU486 (mifepristone) on 15 mmol/L glucose-induced insulin secretion. Three groups of islets were isolated and incubated for 3 hours in a 5-mmol/L glucose–containing KRB solution alone (open circles), plus the addition of 1 μmol/L dexamethasone (closed circles, dashed line) or 1 μmol/L dexamethasone plus 10 μmol/L RU486 (closed triangles, solid line). After this, all groups of islets were perifused for 30 minutes with 3 mmol/L glucose (G3) and for an additional 40 minutes (onset indicated by the vertical line) with 15 mmol/L glucose (G15). At least 6 experiments were conducted under each setting, and means ± SE are given. Asterisk indicates a significant difference between islets incubated with 5 mmol/L glucose plus RU486 versus islets incubated in the presence of 5 mmol/L glucose alone (control) or 5 mmol/L glucose plus Dexa plus RU486.

using the Visage 2000 (Bio Image, Ann Arbor, MI). Control and dexamethasone-pretreated islets from the same animal were always run in parallel, and equivalent amounts of islet protein were analyzed. The density of control protein samples was taken as 100%. The density of dexamethasone-pretreated islets was expressed as a percentage of the control value. There was a strong linear correlation between the amount of islet protein analyzed and the optical density of the sample (unpublished observations).

2.6. Reagents

Hanks solution supplemented with 3 mmol/L glucose was used for the islet isolation. The KRB medium used for both the 3-hour incubation and the perifusions consisted of 115 mmol/L NaCl, 5 mmol/L KCl, 2.2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 24 mmol/L $NaHCO_3$, and 0.17 g/dL bovine serum albumin. Other compounds were added where indicated, and the solution was gassed with a mixture of 95% O_2/5% CO_2. The iodine 125–labeled insulin used for the insulin assay, 5-^{3}H-glucose, and ^3H_2O were purchased from Perkin-Elmer Life and Analytical Science (Billerica, MA) and the labeled *myo*-[2- ^{3}H]-inositol from Amersham. Bovine serum albumin (RIA grade), mifepristone (RU486), carbachol, dexamethasone, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), glucose, and the salts used to make the Hanks solution and perifusion medium were purchased from Sigma (St. Louis, MO). Rat insulin standard (lot 615-ZS-157) was the generous gift of Dr Gerald Gold, Eli Lilly (Indianapolis, IN). Collagenase (type P) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Antibody to PLCδ1 was purchased from Upstate Biotechnology (Lake Placid, NY). Antibodies to PLCβ1-3, PKCα, goat antimouse, and goat antirabbit IgG-HRP antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence reagents and films were from Amersham.

2.7. Statistics

Statistical significance was determined using the Student *t* test for unpaired data or analysis of variance in conjunction with the Newman-Keuls test for unpaired data. $P < .05$ was taken as significant. Values presented in the figures and Results represent means ± SE of at least 3 observations.

3. Results

3.1. Short- and long-term effects of dexamethasone on insulin secretion

Freshly isolated perifused rat islets respond to 15 mmol/L glucose stimulation with a biphasic insulin secretory response. The inclusion of 1 μmol/L dexamethasone during immediate stimulation with glucose had no adverse effect on insulin secretion during the ensuing 40 minutes of stimulation (Fig. 1). For example, 35 to 40 minutes after the onset of stimulation with 15 mmol/L glucose, insulin secretory rates from control islets averaged 685 ± 33 pg per islet per minute (n = 4). The secretory rate was 678 ± 77 pg per islet per minute (n = 4) in the additional presence of 1 μmol/L dexamethasone at this time.

A marked deviation in insulin secretory responsiveness to glucose was observed, however, in islets that had been preincubated with 1 μmol/L dexamethasone for 3 hours before stimulation with 15 mmol/L glucose. Although control islets again exhibited a biphasic response to glucose (Fig. 2), a marked reduction in both first- and second-phase responses were noted from the 1-μmol/L dexamethasone-pretreated islets. For example, peak first-phase secretion averaged 207 ± 49 pg per islet per minute (n = 17) from control islets, but only 75 ± 10 pg per islet per minute (n = 19) from islets exposed previously to 1 μmol/L dexamethasone. Release rates measured 35 to 40 minutes

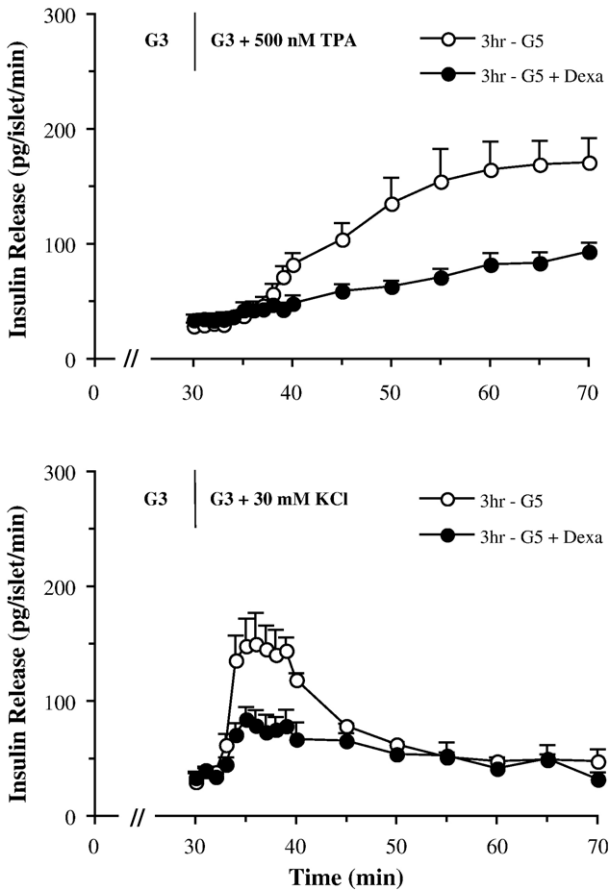

Fig. 3. Dexamethasone reduces TPA- and K-induced insulin secretion. Groups of islets were incubated for 3 hours in a 5-mmol/L glucose–containing KRB solution ± 1 μmol/L dexamethasone (Dexa). After this, all groups of islets were perifused for 30 minutes with 3 mmol/L glucose (G3). Top, For an additional 40 minutes (onset indicated by the vertical line), the islets were perifused with 500 nmol/L TPA in the continued presence of 3 mmol/L glucose. Release was significantly less from the dexamethasone-pretreated islets for the final 30 minutes of the perifusion. Bottom, For an additional 40 minutes (onset indicated by the vertical line), the islets were perifused with 30 mmol/L KCl in the continued presence of 3 mmol/L glucose. Release was significantly less from the dexamethasone-pretreated islets from minutes 34 to 40 minutes of the perifusion. At least 3 experiments were conducted under each setting, and means ± SE are given.

after the onset of stimulation with 15 mmol/L glucose averaged 495 ± 34 pg per islet per minute from control islets but only 131 ± 15 pg per islet per minute from islets exposed previously to 1 μmol/L dexamethasone.

3.2. Glucose use and insulin content of dexamethasone-pretreated islets

The impairment in the 15-mmol/L glucose–induced insulin secretion caused by dexamethasone could not be ascribed to any obvious reduction in glucose use rates by these islets. When measured after the 40-minute stimulation period with 15 mmol/L glucose, islet glucose use rates averaged 124.3 ± 6.5 or 119.4 ± 15.2 pmol per islet per hour in control or dexamethasone-pretreated islets, respectively. Moreover, and in agreement with 2 previous studies [3,5], dexamethasone pretreatment was without any adverse effect on islet insulin content measured after the perifusion. Insulin averaged 98 ± 10 or 96 ± 8 ng per islet from control or dexamethasone-pretreated islets, respectively.

3.3. Blockade of dexamethasone effects with mifepristone

In their report with mouse islets, Lambillotte and coworkers [5] demonstrated that mifepristone (RU486), a potent antiglucocorticoid [29], abolished the inhibitory effect of prior dexamethasone treatment on glucose-induced insulin secretion. We confirmed this observation with rat islets. Coincubation of rat islets with 10 μmol/L mifepristone abolished the adverse impact of 1 μmol/L dexamethasone on 15 mmol/L glucose-induced insulin secretion (Fig. 2).

3.4. Effects of dexamethasone on TPA- or potassium-induced release

In a previous study using mouse islets [5], dexamethasone impaired release in response to a variety of agonists. In agreement with this study [5], we confirmed using rat islets that both TPA- and potassium-induced insulin secretion were also impaired by prior exposure to dexamethasone (Fig. 3).

3.5. IP accumulation in control and dexamethasone-pretreated islets

Glucose-induced IP accumulation was impaired in mouse islets previously exposed to dexamethasone [5]. We confirmed this in rat islets (Fig. 4), which exhibit much greater responses than mouse islets, in both second-phase insulin secretion and the activation of PLC [22]. Although IP levels in the presence of 3 mmol/L glucose were slightly lower in the dexamethasone-pretreated islets, a significant reduction in this surrogate marker of PLC activation [30] was observed in dexamethasone-pretreated islets in response to 15 mmol/L glucose stimulation (Fig. 4). The addition of 10 μmol/L RU486 together with 1 μmol/L dexamethasone abolished the inhibitory effect of the glucocorticoid on glucose-induced IP accumulation (Fig. 4). In addition to glucose, dexamethasone also reduced IP accumulation in response to 100 μmol/L carbachol or 30 mmol/L potassium stimulation (Fig. 4).

The decrease in agonist-induced IP accumulation was not a consequence of altered label incorporation during the 3-hour labeling period. Control (44 936 ± 3505 cpm/40 islets) or 1 μmol/L dexamethasone-pretreated (44 639 ± 3255 cpm/40 islets) islets incorporated similar amounts of ^{3}H-inositol during the 3-hour labeling period.

3.6. PLC and PKC contents of control and dexamethasone-pretreated islets

As previously suggested by Lambillotte and coworkers [5], the time course of dexamethasone's inhibitory actions

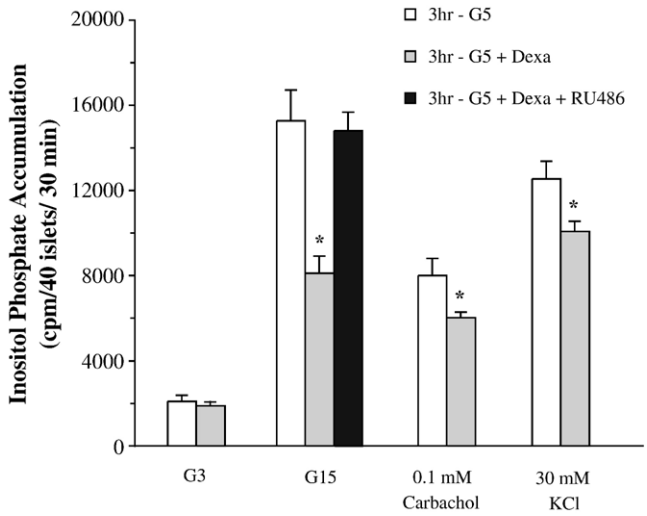

Fig. 4. Dexamethasone reduces agonist-induced IP accumulation in islets. Groups of islets were incubated for 3 hours in a 5-mmol/L glucose–containing KRB solution ± 1 μmol/L dexamethasone or 1 μmol/L dexamethasone plus 10 μmol/L RU486. Also included during the 3-hour incubation was *myo*-[2-^{3}H]-inositol to label phosphoinositide pools as indicated the Materials and Methods. After washing to remove unincorporated label, islets were incubated for an additional 30 minutes with the indicated agonists. Lithium chloride (10 mmol/L) was present during the 30-minute period to facilitate IP measurements. Asterisk indicates a significant difference from control islets labeled in the presence of 5 mmol/L glucose alone.

on beta-cell secretion, as well as the observation that its deleterious effects were abolished by the nuclear glucocorticoid antagonist mifepristone, suggests a genomic action of the steroid. In this context, the effect of dexamethasone pretreatment may be to reduce the synthesis of key proteins or cofactors involved with the regulation of insulin secretion. The present findings as well as those previously reported [5] identified the PLC/PKC signal transduction cascade as possible target of dexamethasone. In an attempt to isolate the protein(s) regulated by dexamethasone, we focused on this system. Islets were incubated for 3 hours in a 5-mmol/L glucose–containing KRB medium supplemented with DMSO alone or with the further addition of 1 μmol/L dexamethasone. Islets were then harvested and

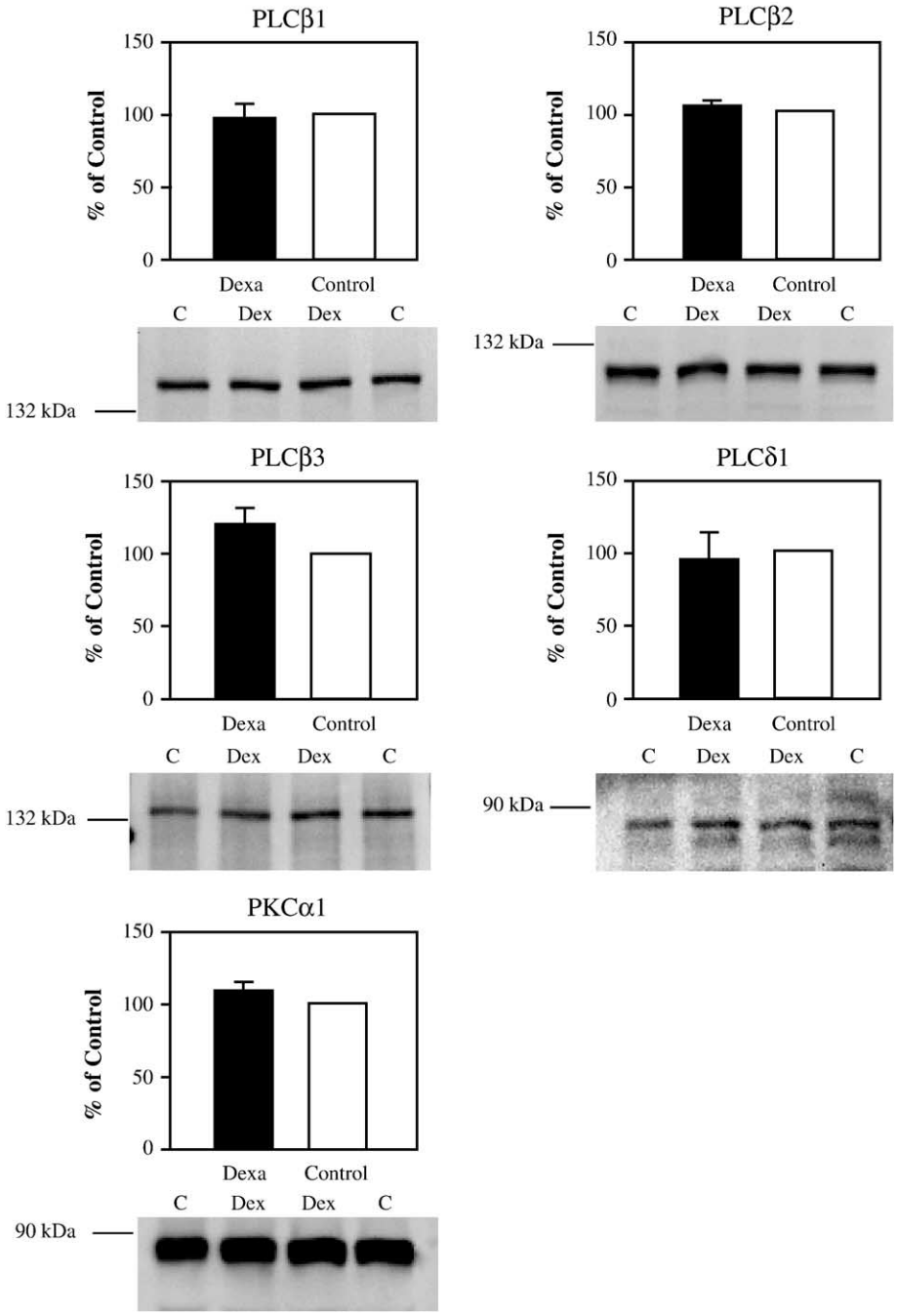

Fig. 5. Effects of dexamethasone pretreatment on rat islet content of PLC and PKC. Groups of islets were incubated for 3 hours in a 5-mmol/L glucose–containing KRB solution ± 1 μmol/L dexamethasone. Islets were then harvested, centrifuged, and extracted as indicated in Materials and Methods for the measurement of immunoreactive protein contents. The densitometric analyses of at least 3 separate paired blots are depicted here. Control expression levels were taken as 100%, and the dexamethasone-pretreated islets were expressed as percentage of control. Equivalent amounts of protein were used for the paired blots and examples of these are given below each graph. Dexamethasone pretreatment had no effect on islet levels of any of the PLCs measured or on PKCα content.

sonicated in extraction buffer as previously described [28]. Identical amounts of islet protein were probed with specific monoclonal or polyclonal antibodies using Western blot techniques coupled with densitometric analyses of the appropriate bands. Our findings are presented in Fig. 5. Exposure to 1 μmol/L dexamethasone for 3 hours had no effect on expression levels of PLCβ1, β2, and β3, PLCδ1, or PKCα.

4. Discussion

Our findings, as well as those of Lambillotte and coworkers [5] using the nuclear steroid antagonist mifepristone, support the concept that a genomic action of the steroid mediates its adverse effect on islet secretory responsiveness. In this context, at least several possibilities exist. Dexamethasone may inhibit the transcription of a key protein involved in the regulation of glucose-induced insulin secretion. If operative via this mechanism and considering the rapid onset (within several hours) of dexamethasone's inhibitory action, this protein must be labile in nature. Alternatively, dexamethasone may increase the transcription of a protein with the capacity to negatively affect secretion [31]. In view of these possibilities, further comment on our findings as well as those reported previously is warranted.

That prior glucocorticoid treatment influences islet function has been known for some time. For example, steroid administration in vivo has been used to stress the pancreatic beta cell, a manipulation used to identify those individuals with a propensity to develop diabetes [1,2]. In vitro, steroids exert a direct inhibitory effect on release [3]. In agreement with a recent report using mouse islets [5], the synthetic steroid dexamethasone had no immediate adverse effect on immediate glucose-induced insulin secretion from isolated perifused rat islets. However, a prior 3-hour exposure to the steroid resulted in parallel 60% to 75% reductions in both the first and second phases of 15 mmol/L glucose-induced insulin release from perifused rat islets. Similar observations have been made previously with rat islets [3]. The kinetics of glucose-induced insulin release were similar and biphasic in both control and dexamethasone-pretreated islets. This suggests that whatever the nature of the biochemical alteration produced by dexamethasone, the affected system participates in the regulation of both phases of insulin secretion from these islets.

The adverse effect of dexamethasone on insulin release could not be ascribed to any reduction in glucose use rates by the islet or their content of insulin measured after the perifusion. Thus, defects at the level of glucose transport, glucose phosphorylation, or glycolysis would appear to be eliminated as possible causes for impaired responsiveness to the hexose. However, significant reductions in glucose-induced IP accumulation accompanied the reduction in glucose-induced insulin secretion. These and other findings [11,32] reinforce our contention that PLC activation plays a particularly important role in glucose-induced insulin secretion. The lesion in PLC activation was not confined to glucose however. IP responses to both potassium and carbachol were also significantly reduced. Thus, irrelevant of the agonist used to activate PLC, the responsiveness of this signal transduction pathway was impaired.

Because of our interest in signaling via the PLC/PKC signaling system in the regulation of the time-dependent effects that glucose exerts on the beta cell including biphasic insulin secretion and time-dependent potentiation [8] and because previous results [5] suggested that dexamethasone adversely influences this system, we conducted additional experiments. Islet exposed to dexamethasone for 3 hours contained amounts of immunoreactive PLCδ1, PLCβ1-3, and PKCα comparable to control islets. Thus, the reduced IP response to glucose stimulation in dexamethasone-pretreated islets could not be attributed to any perceptible decline in the contents of these PLC isozymes. In addition, it was not the result of any obvious impairment in label incorporation used to monitor PLC activation under these conditions. In fact, a most recent study by Ullrich and coworkers [31] demonstrated quite convincingly that dexamethasone increases the expression of the K_v 1.5 voltage-gated potassium channel. A major consequence of this channel alteration was a reduction in peak values of intracellular calcium concentrations. Because the activation of all the PLC isozymes [33-35], as well PKCα [36,37], depends at least in part on cellular calcium levels, this ion channel change may explain why both PLC and PKC activation are impaired.

These findings emphasize the complexity of stimulus-response coupling in islets. Genomic manipulations used to alter insulin secretion may reflect alterations in the specific content of various proteins or the induction of unique proteins that possess the capacity to affect secretion in either a positive or negative manner. Our studies are consistent with the most recent report by Ullrich and coworkers [31]. The induction of a novel cation channel that impairs calcium signaling by the islet adequately explains impaired agonist-induced insulin release. Of particular importance, they also identify a lesion of the PLC/PKC signaling cascade as a important proximal component of impaired secretion and emphasize as well the involvement of this second messenger system in the physiological regulation of insulin secretion.

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References

[1] Fajans SS, Conn JW. The approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes 1954;3:296-304.

[2] Fajans SS, Conn JW. Comments on the cortisone-glucose tolerance test. Diabetes 1961;10:63-7.
[3] Pierluissi J, Navas FO, Ashcroft SJH. Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans. Diabetologia 1986;29:119-21.
[4] Grill V, Rundfeldt M. Abnormalities of insulin responses after ambient and previous exposure to glucose in streptozotocin-diabetic and dexamethasone-treated rats. Diabetes 1986;35:44-51.
[5] Lambillotte C, Gilon P, Henquin J-C. Direct glucocorticoid inhibition of insulin secretion. J Clin Invest 1997;99:414-23.
[6] Weinhaus AJ, Bhagroo NV, Brelje TC, et al. Dexamethasone counteracts the effects of prolactin on islet function: implications for islet regulation in late pregnancy. Endocrinology 2000;141:1384-93.
[7] Shao J, Qiao L, Friedman JE. Prolactin, progesterone, and dexamethasone coordinately and adversely regulate glucokinase and cAMP/PDE cascades in MIN6 β-cells. Am J Physiol 2004;286:E304-10.
[8] Zawalich WS. Modulation of insulin secretion from beta-cells by phosphoinositide-derived second-messenger molecules [Review]. Diabetes 1988;37:137-41.
[9] Zawalich WS, Zawalich KC, Rasmussen H. Control of insulin secretion: a model involving Ca^{2+}, cAMP and diacylglycerol. Mol Cell Endocrinol 1990;70:119-37.
[10] Zawalich WS. Regulation of insulin secretion by phosphoinositide-specific phospholipase C and protein kinase C activation. Diabetes Rev 1996;4:160-76.
[11] Zawalich WS, Zawalich KC. Regulation of insulin secretion by phospholipase C. Am J Physiol 1996;271:E409-16.
[12] Malaisse WJ, Sener A, Herchuelz A, et al. Insulinotropic effect of the tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate in rat pancreatic islets. Cancer Res 1980;40:3827-31.
[13] Zawalich W, Brown C, Rasmussen H. Insulin secretion: combined effects of phorbol ester and A23187. Biochem Biophys Res Commun 1983;117:448-55.
[14] Axen KV, Schubart UK, Blake AD, et al. Role of Ca^{2+} in secretagogue-stimulated breakdown of phosphatidylinositol in rat pancreatic islets. J Clin Invest 1983;72:13-21.
[15] Best L, Malaisse WJ. Stimulation of phosphoinositide breakdown in pancreatic islets by glucose and carbamylcholine. Biochem Biophys Res Commun 1983;116:9-16.
[16] Best L, Malaisse WJ. Nutrient and hormone-neurotransmitter stimuli induce hydrolysis of polyphosphoinositides in rat pancreatic islets. Endocrinology 1984;115:1820-31.
[17] Zawalich WS, Zawalich KC. Phosphoinositide hydrolysis and insulin release from isolated perifused rat islets. Studies with glucose. Diabetes 1988;37:1294-300.
[18] Vadakekalam J, Rabaglia ME, Chen Q-H, et al. Role for GTP in glucose-induced phospholipase C activation in pancreatic islets. Am J Physiol 1996;271:E85-E95.
[19] Ganesan S, Calle R, Zawalich K, et al. Glucose-induced translocation of protein kinase C in rat pancreatic islets. Proc Natl Acad Sci U S A 1990;87:9893-7.
[20] Ganesan S, Calle R, Zawalich K, et al. Immunocytochemical localization of α-protein kinase C in rat pancreatic β-cells during glucose-induced insulin secretion. J Cell Biol 1992;119:313-24.
[21] Calle R, Ganesan S, Smallwood JI, et al. Glucose-induced phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) in isolated rat pancreatic islets. J Biol Chem 1992;267:18723-7.
[22] Zawalich WS, Zawalich KC, Kelley GG. Regulation of insulin release by phospholipase C activation in mouse islets: differential effects of glucose and neurohumoral stimulation. Endocrinology 1995;136:4903-9.
[23] Zawalich WS, Zawalich KC. Species differences in the induction of time dependent potentiation of insulin secretion. Endocrinology 1996;137:1664-9.
[24] Albano JDM, Ekins RP, Maritz G, et al. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol 1972;70:487-509.
[25] Zawalich WS, Takuwa N, Takuwa Y, et al. Interactions of cholecystokinin and glucose in rat pancreatic islets. Diabetes 1987;36:426-33.
[26] Berridge MJ, Dawson RMC, Downes CP, et al. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem J 1983;212:473-82.
[27] Zawalich WS, Rognstad R, Pagliara AS, et al. A comparison of the utilization rates and hormone-releasing actions of glucose, mannose, and fructose in isolated pancreatic islets. J Biol Chem 1977;252:8519-23.
[28] Zawalich WS, Bonnet-Eymard M, Zawalich KC. Insulin secretion, inositol phosphate levels and phospholipase C isozymes in rodent pancreatic islets. Metabolism 2000;49:1156-63.
[29] Moguilewsky M, Philibert D. Potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J Steroid Biochem 1984;20:271-6.
[30] Ashkenazi A, Peralta EG, Winslow JW, et al. Functionally distinct G proteins selectively couple different receptors to PI hydrolysis in the same cell. Cell 1989;56:487-93.
[31] Ullrich S, Berchtold S, Ranta F, et al. Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. Diabetes 2005;54:1090-9.
[32] Rasmussen H, Zawalich KC, Ganesan S, et al. Physiology and pathophysiology of insulin secretion [Review]. Diabetes Care 1990;13:655-66.
[33] Rhee SG, Suh R-G, Ryu S-H, et al. Studies of inositol phospholipid-specific phospholipase C. Science (USA) 1989;244:540-50.
[34] Rhee SG, Choi KD. Regulation of inositol phospholipid-specific phospholipase C isozymes. J Biol Chem 1992;267:12393-6.
[35] Rhee SG, Bae YS. Regulation of phosphoinositide-specific phospholipase C isozymes. J Biol Chem 1997;272:15045-8.
[36] Nishizuka Y. Studies and perspectives of protein kinase C. Science 1986;233:305-12.
[37] Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation [Review]. Nature 1988;334:661-5.