Role of protein kinase C and Ca^{2+} in glucose-induced sensitization/desensitization of insulin secretion

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Abstract. The role of protein kinase C and Ca^{2+} in glucose-induced sensitization/desensitization of insulin secretion was studied. A 22–24 h exposure of mouse pancreatic islets to glucose (16.7 mmol/l) in TCM 199 culture medium, with 0.26 mmol/l or 1.26 mmol/l Ca^{2+}, reduced total islet protein kinase C activity to approx. 85% and 60% of control values, respectively.

At 0.26 mmol/l Ca^{2+} in TCM 199 medium, exposure to glucose (16.7 mmol/l) led to a potentiation of both phase 1 and phase 2 of glucose-induced insulin secretion, and caused a shift in the dose-response curve with 10 mmol/l and 16.7 mmol/l glucose exhibiting equipotent effects in stimulation of insulin secretion. In glucose-sensitized islets, the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (0.16 μmol/l) did not further potentiate induction of secretion by 10 mmol/l or 16.7 mmol/l glucose. At 3.3 mmol/l glucose, however, phorbol ester-induced secretion was augmented, and was characterized by a faster onset of secretion in glucose-sensitized islets relative to control islets. In contrast, a partial reduction in arachidonic acid (100 μmol/l)-induced insulin release was observed in glucose-sensitized islets in the absence of extracellular Ca^{2+}.

Increasing the Ca^{2+} concentration to 1.26 mmol/l in TCM 199 during the 22–24 h exposure to glucose (16.7 mmol/l) led to inhibition of phase 1 and abolition of phase 2 of glucose (10 mmol/l, 16.7 mmol/l)-induced insulin secretion. In addition, this treatment abolished phorbol ester-induced and arachidonic acid-induced insulin secretion at 3.3 mmol/l glucose. Altogether, these data suggest that sensitization of insulin secretion is caused by a preferential down-regulation of the inhibitory effects of protein kinase C, leading to an increased first phase, and an increased coupling of glucose to the stimulatory effects of protein kinase C during the second phase of glucose-induced insulin secretion. Desensitization of insulin secretion appears to be a consequence of sustained Ca^{2+} influx, inducing extensive down-regulation of protein kinase C and also causing deleterious effects on islet cell function in protein kinase C-deprived islets.

Key words. Pancreatic islet; β-cell; calcium; protein kinase C down-regulation; sensitization; desensitization; insulin secretion.

Stimulation of insulin secretion by glucose is characterized by a biphasic response consisting of a prompt first phase and a slower, progressively increasing, second phase of secretion. Some evidence suggests that phase 1 is initiated by Ca^{2+}, and that termination of phase 1 and induction of phase 2 is regulated by protein kinase C[1]. Thus down-regulation of protein kinase C by the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), or inhibition of protein kinase C by staurosporine, have been found to potentiate phase 1 and to inhibit phase 2 of glucose-induced insulin secretion in mouse pancreatic islets, which indicates that protein kinase C exerts a predominantly inhibitory effect during phase 1 of glucose-induced insulin secretion, and a predominantly stimulatory effect during phase 2[1]. Available data suggest that the inhibitory action of protein kinase C during phase 1 may involve an attenuating effect on cytosolic Ca^{2+} concentration[2] and on phosphoinositide hydrolysis[3], and that the stimulatory action during phase 2 probably involves stimulation of cyclic AMP accumulation[4]. In accordance with this view, a reduction in the negative control by protein kinase C might lead to an increased Ca^{2+} activity in pancreatic islets which, despite an immediate potentiation of Ca^{2+}-induced secretion, may show an ability to induce desensitization of secretion[1].

Two studies have recently shown that protein kinase C may have a role in the so-called priming effect of glucose[5,6]. According to these studies, performed with rat islets, either transient stimulation of phosphoinositide hydrolysis by glucose[5], or stimulation of protein kinase C by TPA[6], leads to a potentiation of a subsequent glucose stimulation of insulin secretion.

Other evidence has suggested that in biphasic insulin release induced by glucose, the second phase, i.e. the progressively increasing phase of insulin secretion, may be considered as the priming phase of secretion. Thus prior stimulation of islets with glucose leads to a potentiation of both phases of secretion, but when compared with control islets, the priming of the second phase is only seen at early time-points, and is gradually overcome by self-priming during phase 2 in control islets not previously exposed to glucose[7]. These results are consistent with the concept that protein kinase C stimulates the second phase of secretion and that this effect may be involved in glucose memory.

Recently, exposure of isolated rat pancreatic islets to glucose for a sufficient length of time was reported to result in a decreased sensitivity of the β-cells to glucose. This third phase of insulin secretion is characterized as a spontaneous decline of secretion during sustained expo-

sure to glucose or other secretagogues[8,9]. The mechanisms responsible for this phase of secretion are unknown, but it is known not to be due to exhaustion of islet insulin content[10].

The aim of the present study was to investigate the possible significance of protein kinase C down-regulation in glucose-induced sensitization/desensitization of insulin secretion.

Materials and methods

Islet culture. Islets were prepared by collagenase digestion of the pancreases of male albino mice (NMRI) (approx. 18–22 g b. wt) fed ad libitum on a standard laboratory diet. Islets were kept in tissue culture for 24–48 h in TCM 199 medium (1.26 mmol/l Ca^{2+}, 5.5 mmol/l glucose) supplemented with 10% (v/v) newborn-calf serum (Gibco), 20 mmol/l Hepes, 5 mmol/l $NaHCO_3$, 100 units/ml penicillin, 100 µg/ml streptomycin and further additions when indicated.

Phosphorylation assay. Groups of 50 islets were collected in 200 µl of 25 mmol/l Tes buffer, pH 6.90, containing 5 mmol/l $MgCl_2$, 1 mmol/l EGTA, 0.1 mmol/l dithiothreitol, 0.01% (w/v) leupeptin and 10 units/ml aprotinin. A homogenate was prepared by sonication (10 s, 40 W). For solubilization of membrane-bound protein kinase activity, the homogenate was incubated for 1 h at 4 °C in the above buffer supplemented with 0.125% Triton X-100. The solubilized homogenate was frozen in liquid N_2 and stored at − 80 °C until the next day.

Samples of homogenate were preincubated for 2 min in 200 µl reaction mixture containing 25 mmol/l Tes buffer, pH 6.90, 5 mmol/l $MgCl_2$, 1 mmol/l EGTA, 0.1 mmol/l dithiothreitol and 0.2 mg/ml lysine-rich histone, and further additions as indicated. The reaction was started by addition of [γ-^{32}P]ATP (500 c.p.m./pmol, final conc. 10 µmol/l) and terminated by transferring 150 µl of the incubation mixture to 1 ml of 25% (w/v) trichloroacetic acid containing 0.3 mg of bovine serum albumin/ml as a carrier protein. The acid-precipitable radioactivity was determined after three cycles of solubilization in 0.1 ml 1 mmol/l NaOH and precipitation with 2 ml of 25% trichloroacetic acid.

Glucose oxidation. Glucose oxidation was measured by determining the amount of CO_2 released from [U-^{14}C]glucose, as previously described[11].

Insulin release. Insulin release from islets was measured by perifusion in a non-circulating system with beads of polyacrylamide as a supporting medium as described[1]. The islets were perifused at 37 °C at a flow rate of 0.26 ml/min. The perifusion medium was Krebs-Ringer medium supplemented with 20 mmol/l Hepes, 5 mmol/l $NaHCO_3$, 2 mg/ml human serum albumin and 3.3 mmol/l glucose. Islets were perifused for 45 min with 3.3 mmol/l glucose to obtain a basal release rate and then challenged with different insulin secretagogues as indicated. The effluent medium was collected for periods of 5 or 10 min and assayed for insulin content by radioimmunoassay. The rate of insulin release was expressed as ng of insulin/min per 100 islets.

Results

Glucose-induced down-regulation of protein kinase C. Exposure of mouse islets for 22–24 h to 16.7 mmol/l glucose in TCM 199 culture medium led to a partial down-regulation of protein kinase C (table 1). Thus an increase in glucose from 5.5 mmol/l to 16.7 mmol/l reduced total islet protein kinase C activity to approximately 60% of control values. Glucose-induced down-regulation of protein kinase C was greatly Ca^{2+}-dependent, and after reduction in Ca^{2+} in TCM 199 from 1.26 mmol/l to 0.26 mmol/l by inclusion of EGTA, glucose (16.7 mmol/l) exposure only reduced protein kinase C to approx. 85% of control values.

In contrast, exposure of mouse islets to 0.16 µmol/l TPA for 22–24 h led to a total abolition of protein kinase C at Ca^{2+} concentrations of both 0.26 mmol/l and 1.26 mmol/l in TCM 199 medium (table 1).

Ca^{2+}-induced desensitization of insulin secretion in protein kinase C-depleted islets. In a recent study with mouse islets it was shown that Ca^{2+} influx during down-regulation of protein kinase C may lead to a total abolition of both phase 1 and phase 2 of glucose-induced insulin secretion[1]. Thus down-regulation of protein kinase C by TPA in normal TCM 199 culture medium (5.5 mmol/l

Table 1. Effects of TPA and glucose on protein kinase C down-regulation.

Culture Added agents	Protein kinase C activity (pmol/2 min per 50 islets)	P
–	17.05 ± 1.65(11)	
TPA (0.16 µmol/l)	0.28 ± 0.24(7)	< 0.001
Glucose (16.7 mmol/l)	9.74 ± 1.20(11)	< 0.001
EGTA (1 mmol/l)	16.21 ± 1.91(11)	NS
EGTA (1 mmol/l) + TPA (0.16 µmol/l)	0.37 ± 0.28(7)	< 0.001
EGTA (1 mmol/l) + Glucose (16.7 mmol/l)	13.99 ± 1.61(11)	< 0.025

Mouse islets were pretreated for 22–24 h in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) with other additions as indicated. Protein kinase C activity in islet homogenate was determined as described in methods. The activity in the presence of Ca^{2+} (100 µmol/l) + TPA (32 nmol/l) + phosphatidylserine (20 µg/ml), corrected for the activity in the sole presence of Ca^{2+} (100 µmol/l), is given. Results are means ± SEM (number of experiments). The statistical significance of the differences from control islets was assessed by using a paired t-test. NS, not significant ($p > 0.05$).

Table 2. Effect of TPA pre-exposure on glucose oxidation.

Culture Added agents	Glucose oxidation (pmol/2 h per 10 islets) Glucose (3.3 mmol/l)	P	Glucose (16.7 mmol/l)	P
4α-PDD (0.16 μmol/l)	209 ± 13		787 ± 82	
TPA (0.16 μmol/l)	228 ± 20	NS	611 ± 32	NS

Islets were pretreated in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) for 22–24 h in the presence of either 4α-PDD or TPA. Oxidation of [U-^{14}C]glucose to $^{14}CO_2$ was determined as previously described[11]. Results are means ± SEM (n = 7). Statistical evaluation of the data was made by a paired t-test. NS, not significant.

glucose, 1.26 mmol/l Ca^{2+}) led to a total abolition of glucose-induced secretion, whereas down-regulation of protein kinase C in TCM 199 with a reduced Ca^{2+} concentration (5.5 mmol/l glucose, 0.26 mmol/l Ca^{2+}) preserved and potentiated the phase 1 of glucose-induced secretion[1]. To investigate this relationship further, the possible toxicity of Ca^{2+} during down-regulation of protein kinase C was examined.

After a 22–24 h exposure in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) to TPA (0.16 μmol/l), islets exhibited a normal glucose oxidation rate as compared with that in islets pretreated in the presence of the inactive phorbol ester 4α-phorbol 12,13-didecanoate (4α-PDD) (table 2). The effect of TPA in abolishing secretion was partly reversible, as was shown by the reestablishment of both glucose-induced and TPA-induced insulin secretion after removal of TPA and incubation of these desensitized islets in normal TCM 199 for 24 h (fig. 1).

In other experiments, the ability of Ca^{2+} to induce desensitization in protein kinase C-depleted islets was investigated. Figure 2 shows that protein kinase C-depleted islets exhibit a greatly reduced response to repeated stimulation by glucose. In addition, Ca^{2+}-induced desensitization of insulin secretion is also observed when islets which have been depleted of protein kinase C activity, by incubation for 24 h in TCM 199 with 0.26 mmol/l Ca^{2+} (5.5 mmol/l glucose, 0.16 μmol/l TPA), are incubated for 24 h in TCM 199 with 1.26 mmol/l Ca^{2+} (5.5 mmol/l glucose, 0.16 μmol/l TPA) (results not given). These data suggest that Ca^{2+}-induced desensitization of glucose-induced insulin secretion occurs as a consequence of protein kinase C down-regulation.

Role of protein kinase C and Ca^{2+} in glucose-induced sensitization/desensitization of insulin secretion. In short-term perifusion experiments, it was not possible to detect a priming effect of glucose, even compared with the declining responsiveness of control islets not previously exposed to glucose (fig. 3).

To investigate the time dependency of glucose sensitization, the effect of prolonged glucose exposure of islets in

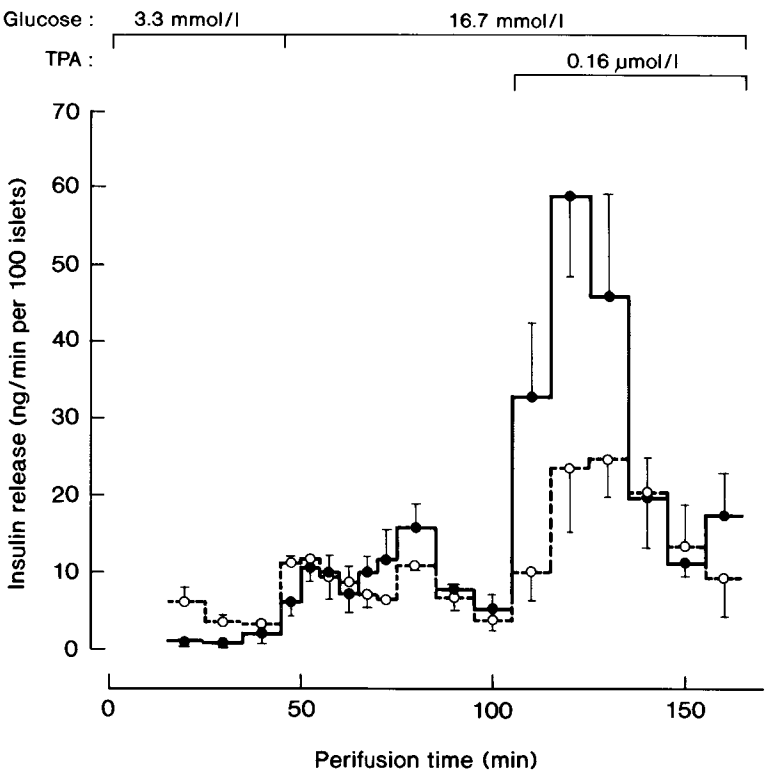

Figure 1. Reversibility of Ca^{2+}-induced desensitization of insulin secretion. Insulin release from column-perifused mouse islets in response to 3.3 mmol/l glucose, 16.7 mmol/l glucose and 16.7 mmol/l glucose + 0.16 μmol/l TPA was determined as described in methods. Islets, pretreated for 22–24 h with (o) or without (•) 0.16 μmol/l TPA in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}), were maintained for additional 22–24 h in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) culture medium. Results are means ± SEM (n = 3).

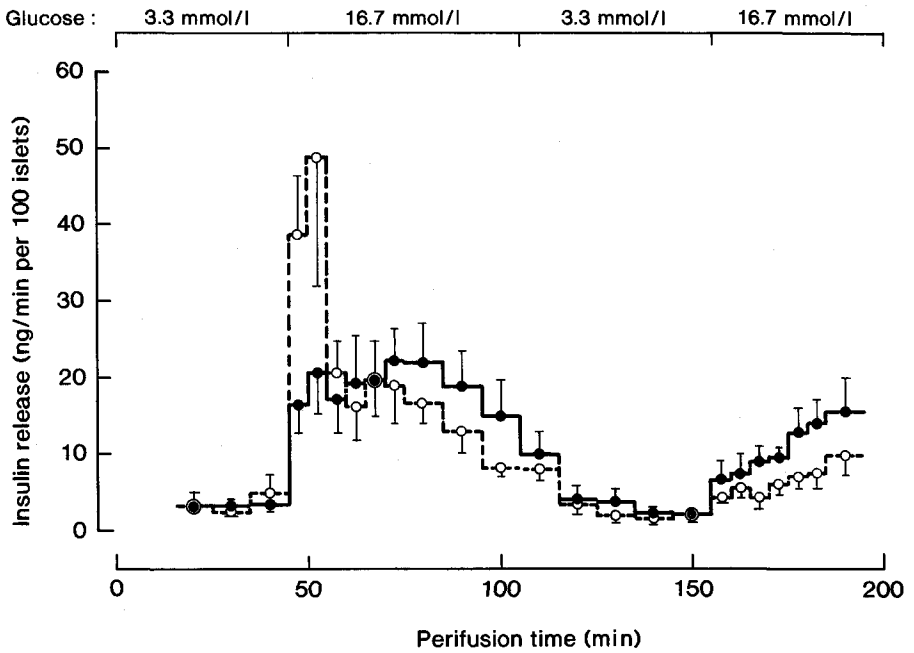

Figure 2. Effect of repeated glucose stimulation on insulin secretion from protein kinase C-depleted islets. Insulin release from mouse islets in response to 3.3 mmol/l glucose and 16.7 mmol/l glucose was determined as described in methods. Islets were pretreated with (o) or without (•) 0.16 µmol/l TPA for 22–24 h in TCM 199 (5.5 mmol/l glucose, 0.26 mmol/l Ca^{2+}) medium. Results are means ± SEM (n = 4).

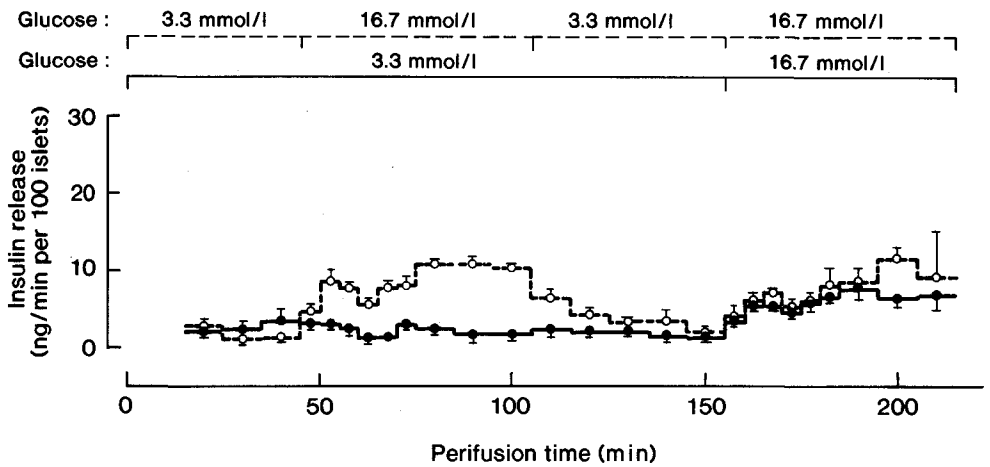

Figure 3. Effect of a preceding glucose stimulus on glucose-induced insulin secretion from mouse islets. The dynamics of insulin release in response to 3.3 mmol/l glucose and 16.7 mmol/l glucose from mouse islets maintained in TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) medium for 22–24 h were determined as described. Islets were perifused according to the protocol: (o) 0–45 min: 3.3 mmol/l glucose, 45–105 min: 16.7 mmol/l glucose, 105–155 min: 3.3 mmol/l glucose, 155–215 min: 16.7 mmol/l glucose (•) 0–155 min: 3.3 mmol/l glucose, 155–215 min: 16.7 mmol/l glucose. Results are means ± SEM (n = 4).

TCM 199 medium on subsequent glucose-induced insulin secretion was investigated. A 22–24 h exposure in TCM 199 medium with 0.26 mmol/l Ca^{2+} to glucose (16.7 mmol/l) was associated with a potentiation of both phases of glucose (16.7 mmol/l)-induced insulin secretion in a manner which was associated with a virtual neutralization of TPA potentiation of secretion (fig. 4a). In comparison, a 22–24 h exposure to glucose (16.7 mmol/l) in TCM 199 medium with 1.26 mmol/l Ca^{2+} led to a loss of both glucose (16.7 mmol/l)-induced and TPA (0.16 µmol/l)-potentiated insulin secretion (fig. 4b). Glucose sensitization caused a shift in the dose-response curve for glucose stimulation of secretion. Thus 10 mmol/l of glucose, which in control islets caused a modest increase in secretion, was equipotent to 16.7 mmol/l glucose in sensitized islets (figs 4a and 5a). Desensitiza-

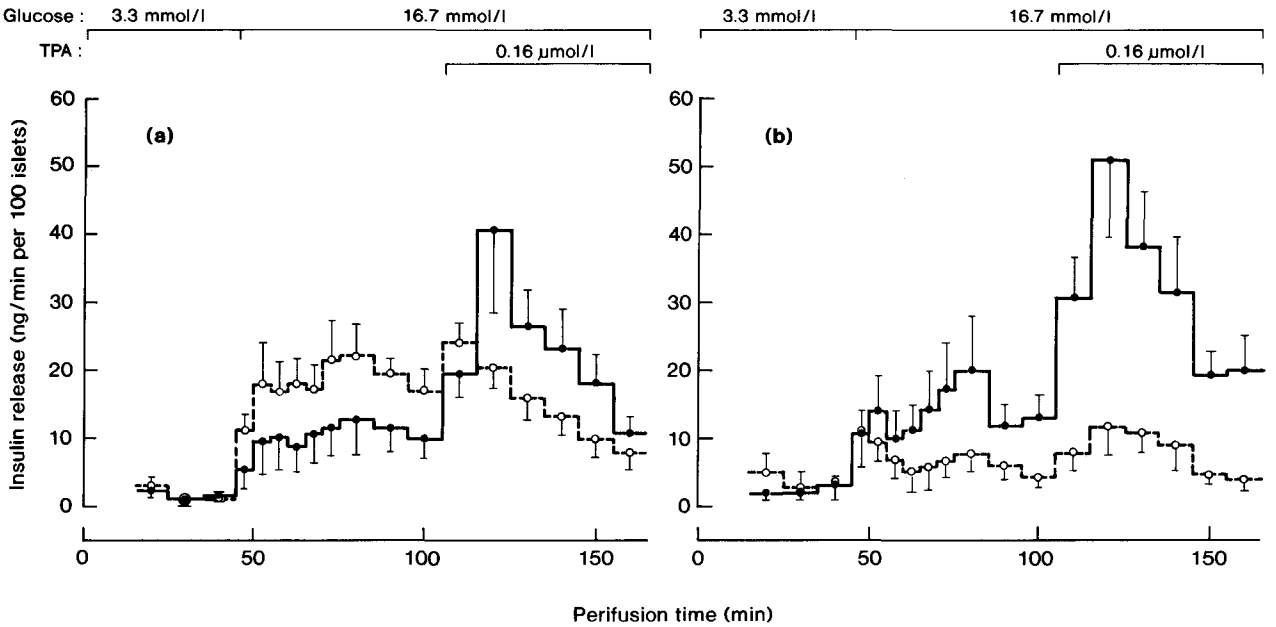

Figure 4. Effects of glucose pre-exposure on glucose-induced and TPA-induced insulin secretion. Insulin release from column-perifused mouse islets in response to 3.3 mmol/l glucose, 16.7 mmol/l glucose and 16.7 mmol/l glucose + 0.16 µmol/l TPA was determined as previously described. Islets were pretreated with 16.7 mmol glucose (o) or 5.5 mmol/l glucose (•) for 22–24 h in TCM 199 culture medium containing (a) 0.26 mmol/l Ca^{2+} or (b) 1.26 mmol/l Ca^{2+}. Results are means ± SEM (n = 6).

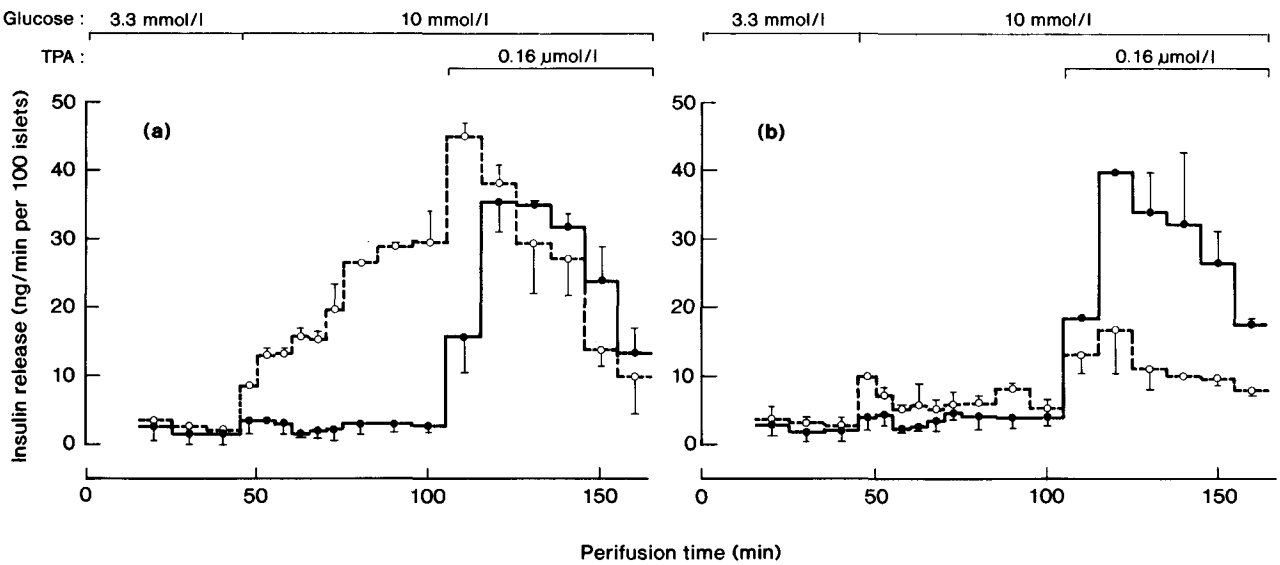

Figure 5. Effects of glucose pre-exposure on glucose-induced and TPA-induced insulin secretion. Insulin release from column-perifused mouse islets in response to 3.3 mmol/l glucose, 10 mmol/l glucose and 10 mmol/l glucose + 0.16 µmol/l TPA was determined. Islets were pretreated with 16.7 mmol/l glucose (o) or 5.5 mmol/l glucose (•) for 22–24 h in TCM 199 culture medium containing (a) 0.26 mmol/l Ca^{2+} or (b) 1.26 mmol/l Ca^{2+}. Results are means ± the range of 2 experiments.

tion of insulin secretion by Ca^{2+} led to a decrease in insulin secretion at both 10 and 16.7 mmol/l of glucose (figs 4b and 5b). However, when compared to control islets pretreated in normal TCM 199 medium, stimulation with 10 mmol/l glucose still potentiated the phase 1 response of insulin secretion (fig. 5b).

Likewise, glucose priming led to a potentiation of TPA (0.16 µmol/l)-induced insulin secretion at 3.3 mmol/l glucose (fig. 6a). Thus TPA-induced insulin secretion was characterized by a faster onset of secretion relative to control islets. Again, the ability of glucose to potentiate secretion was dependent on the Ca^{2+} concentration in

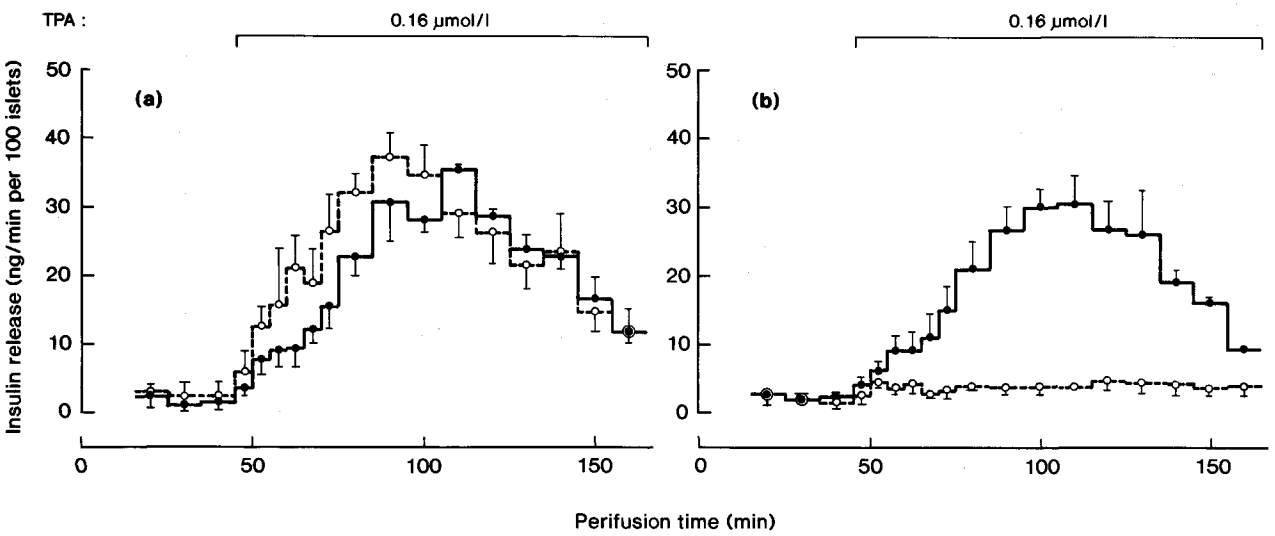

Figure 6. Effects of glucose pre-exposure on TPA-induced insulin secretion. The dynamics of insulin release from column-perifused islets in response to 0.16 µmol/l TPA was determined. Mouse islets were pretreated with 16.7 mmol/l glucose (o) or with 5.5 mmol/l glucose (•) for 22–24 h in TCM 199 medium containing (a) 0.26 mmol/l Ca^{2+} or (b) 1.26 mmol/l Ca^{2+}. The perifusion medium was Krebs-Ringer medium supplemented with 20 mmol/l Hepes, 5 mmol/l $NaHCO_3$, 2 mg/ml human serum albumin and 3.3 mmol/l glucose. Results are means ± SEM (n = 3).

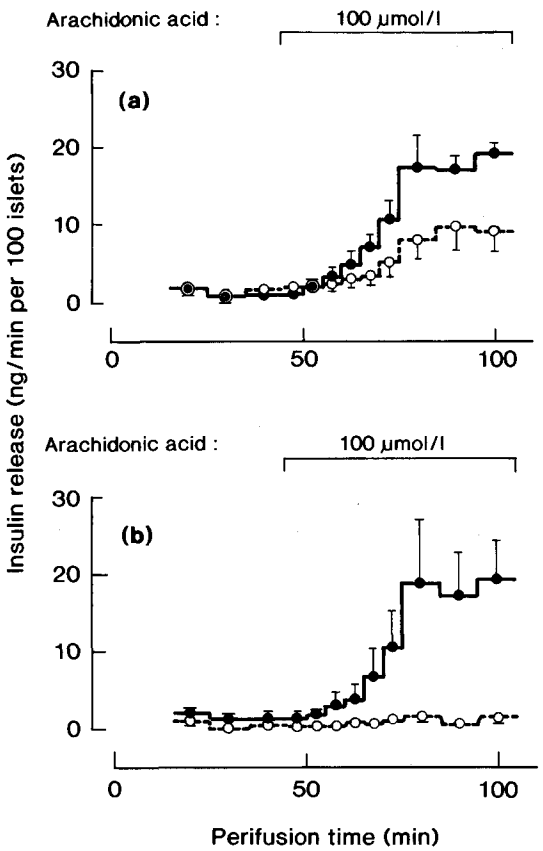

Figure 7. Effects of glucose pre-exposure on arachidonic acid-induced insulin secretion. Insulin release in response to 100 µmol/l arachidonic acid was determined. Mouse islets were pretreated with 16.7 mmol/l glucose (o) or with 5.5 mmol/l glucose (•) for 22–24 h in TCM 199 medium containing (a) 0.26 mmol/l Ca^{2+} or (b) 1.26 mmol/l Ca^{2+}. The perifusion medium was Ca^{2+} free Krebs-Ringer medium supplemented with 20 mmol/l Hepes, 5 mmol/l $NaHCO_3$, 3.3 mmol/l glucose and 0.2 mmol/l EGTA. Results are means ± SEM (n = 3–5).

the TCM 199 medium, and increasing the Ca^{2+} concentration to 1.26 mmol/l during glucose exposure led to a total abolition of TPA-induced secretion (fig. 6b).

Finally, the effect of glucose exposure on arachidonic acid-induced insulin release was investigated. Arachidonic acid stimulates insulin secretion in the absence of extracellular Ca^{2+} by a mechanism involving protein kinase C activation[12]. Indeed, both TPA-induced and arachidonic acid-induced insulin secretion are characterized by a slowly rising rate of secretion (figs 6 and 7) which is abolished after TPA-induced down-regulation of protein kinase C (results not given).

In agreement with the observations for glucose and TPA, glucose exposure in TCM 199 medium (1.26 mmol/l Ca^{2+}) led to a total abolition of arachidonic acid-induced insulin secretion (fig. 7b). In contrast to glucose-induced and TPA-induced insulin secretion, however, arachidonic acid stimulation of insulin secretion was also reduced after glucose exposure in TCM 199 medium containing 0.26 mmol/l Ca^{2+} (fig. 7a).

Discussion

A previous study comparing insulin secretion from rat and mouse pancreas failed to detect a priming effect of glucose in the perfused mouse pancreas[13]. In agreement with that study, it was not possible to demonstrate an immediate priming effect of glucose during perifusion of mouse islets. The reason for this difference between rat and mouse islets is not known, but based on the present experiments it seems possible that it may reflect different degrees of down-regulation of protein kinase C during the second phase of insulin secretion.

According to the present results sensitization of insulin secretion may occur as a consequence of protein kinase

C down-regulation. Thus both protein kinase C depletion after TPA exposure and partial protein kinase C down-regulation after glucose exposure are associated with subsequent potentiation of glucose-induced insulin release.

As noted, exposure to glucose (16.7 mmol/l) in TCM 199 medium (0.26 mmol/l Ca^{2+}) for 24 h decreased total islet protein kinase C activity to approximately 85% (table 1). In contrast to TPA, therefore, glucose only caused partial down-regulation of protein kinase C, and the ensuing secretory patterns after glucose and TPA exposure also appeared to be quite different. Thus whereas down-regulation of protein kinase C by TPA at 0.26 mmol/l Ca^{2+} resulted in a potentiation of phase 1 and a reduction of phase 2 of glucose-induced secretion (fig. 2), partial down-regulation of the kinase by glucose under similar conditions was associated with a potentiation of both phases of glucose-induced insulin secretion (fig. 4a). In accordance with the previous observation that protein kinase C inhibits phase 1 and stimulates phase 2 of glucose-induced secretion, these data therefore suggest that long-term exposure to glucose with partial down-regulation of protein kinase C results in both an increased first phase and an increased coupling of glucose to the stimulatory effects of protein kinase C during the second phase of insulin secretion. This is also indicated by the lack of potentiation of secretion by TPA during glucose stimulation in these islets (fig. 4a).

In agreement with this view, glucose priming led to a potentiation of TPA-induced secretion at 3.3 mmol/l glucose (fig. 6a). Thus TPA-induced insulin secretion was characterized by a faster onset of secretion relative to control islets. Furthermore, despite a slightly reduced protein kinase C content in these islets, the maximum secretory rate in glucose-primed islets reached a similar value as observed in control islets (fig. 6a). In contrast, arachidonic acid stimulation of insulin secretion was reduced in glucose-primed islets (fig. 7a), which on the other hand may reflect the overall decrease in protein kinase C in these islets. The reason for this deviation between TPA and arachidonic acid, which both appear to stimulate secretion by activation of protein kinase C, is not known. However, since arachidonic acid-induced insulin secretion was observed in Ca^{2+}-free medium, it seems possible that Ca^{2+} deprivation may obliterate the priming effect of glucose.

Altogether, it is tempting to speculate, therefore, that glucose priming is caused by a partial down-regulation of protein kinase C. Partial down-regulation of protein kinase C may lead to a preferential reduction in the negative modulatory effects of protein kinase C, which include inhibitory effects on cytosolic Ca^{2+} [2] and phosphoinositide hydrolysis [3]. Alleviation of the negative control by protein kinase C may then subsequently lead to increased formation of putative activators of protein kinase C, e.g. diacylglycerol, arachidonic acid and lysophospholipids by activation of Ca^{2+}-dependent phospholipid hydrolysis [14, 15], resulting in an increased coupling between glucose and the stimulatory effects of protein kinase C during insulin secretion.

The available data suggest that sustained Ca^{2+} influx secondary to protein kinase C down-regulation by TPA may lead to desensitization of glucose-induced insulin secretion. In a previous study, Ca^{2+} was identified as the cause of desensitization, since both Ca^{2+} uptake inhibitors and the Ca^{2+}-chelating agent EGTA, which themselves did not affect islet cell function after inclusion in TCM 199 medium, were found to prevent desensitization of secretion during down-regulation of protein kinase C [1]. According to the present results, Ca^{2+}-induced desensitization may occur as a consequence of protein kinase C down-regulation, since protein kinase C-deficient islets are subjected to desensitization upon incubation in normal TCM 199 medium (1.26 mmol/l Ca^{2+}) or upon repeated stimulation by glucose during perifusion. However, the mechanism underlying the ability of Ca^{2+} to induce a refractory state in protein kinase C-depleted islets remains to be established. The lack of responsiveness is not simply due to exhaustion of islet insulin stores [1]. Also, it does not appear to represent a toxic effect of Ca^{2+} during down-regulation of protein kinase C. Thus although the effect was associated with a considerable islet loss of approx. 50% as observed after 48 h of culture [1], this loss was not seen after 24 h, and islets pretreated with TPA in normal TCM 199 (5.5 mmol/l glucose, 1.26 mmol/l Ca^{2+}) for 24 h exhibited a normal glucose oxidation rate as compared with control islets. In addition, the effect of Ca^{2+} in abolishing secretion during down-regulation of protein kinase C was partly reversible.

To investigate whether the observation of Ca^{2+}-induced desensitization of insulin secretion in protein kinase C-depleted islets may be of relevance as a mechanism for the induction of a third phase of glucose-induced secretion, the effect of glucose exposure for 24 h in TCM 199 (1.26 mmol/l Ca^{2+}) culture medium on glucose-induced insulin secretion was investigated. A 22–24 h exposure in TCM 199 (1.26 mmol/l Ca^{2+}) culture medium to glucose (16.7 mmol/l) was associated with a loss in glucose-induced (figs 4b and 5b), TPA-induced (fig. 6b) and arachidonic acid-induced (fig. 7b) insulin secretion.

A 22–24 h exposure to glucose (16.7 mmol/l) in TCM 199 (1.26 mmol/l Ca^{2+}) decreased total islet protein kinase C activity to approx. 60% of control values (table 1). In accordance with the previous observations with TPA [1], these observations suggest that long-term exposure to glucose may cause down-regulation of protein kinase C which may subsequently lead to deleterious effects of Ca^{2+} on secretion.

As regards the influence of Ca^{2+} during glucose exposure, it is clear that Ca^{2+} caused a decrease in both the first and the second phases of glucose-induced insulin secretion. This can be seen by comparing glucose-induced insulin secretion in glucose-sensitized (fig. 4a) and

glucose-desensitized (fig. 4b) islets. A preferential down-regulation of phase 2 of secretion was, however, noted in these experiments, probably reflecting a further down-regulation of protein kinase C by glucose in the presence of Ca^{2+} (table 1). TPA did not induce insulin secretion at 3.3 mmol/l glucose in desensitized islets (fig. 6b), but TPA caused some potentiation of glucose (10 mmol/l, 16.7 mmol/l)-induced secretion in desensitized islets (figs 4b and 5b). Since glucose-induced, Ca^{2+}-dependent down-regulation of protein kinase C may be suspected to be restricted to β-cells, it seems possible that this potentiating effect of TPA in glucose-desensitized islets (figs 4b and 5b) may reflect TPA-induced glucagon release from α-cells to some degree[16].

According to the present experiments glucose priming causes a shift in the dose response curve for glucose in stimulation of secretion, leading to equipotent effects of 10 mmol/l and 16.7 mmol/l glucose. Desensitization of insulin secretion by Ca^{2+} led to a decrease in insulin secretion at both 10 and 16.7 mmol/l of glucose (figs 4b and 5b). However, when compared to controls pretreated in normal TCM 199, stimulation with 10 mmol/l glucose still exhibited a potentiated phase 1 response of insulin secretion (fig. 5b). Desensitization of glucose-induced secretion could therefore be overlooked, depending on the glucose concentration employed during stimulation of insulin secretion. These results confirm the observations by Grodsky[9] that glucose-induced desensitization of islets to stimulation by a specific secretagogue should be evaluated by comparison with the response to that particular secretagogue during maximal priming. In addition, these results suggest that the priming effect of glucose may be preserved in desensitized islets in that 10 mmol/l and 16.7 mmol/l glucose still elicited comparable responses in desensitized islets (figs 4b and 5b).

Along this line of evidence, the possibility exists that desensitization by Ca^{2+} in glucose-primed islets or protein kinase C-depleted islets may include Ca^{2+}-induced perturbation of islet phospholipid metabolism. Desensitization of insulin secretion has been associated with an impaired generation of phosphoinositide-derived second messengers[17], and may involve exhaustion of secretagogue-sensitive phosphoinositide stores in islets[18]. In addition, studies in other cell types have disclosed that continuous stimulation by Ca^{2+} may lead to a reduction in structural phospholipids like phosphatidylcholine, and an increased production of lysophospholipids like lysophosphatidylcholine[19]. Furthermore, recent observations with neonatal rat islets[20] have revealed that protein kinase C stimulates reacylation of lysophospholipids, suggesting that Ca^{2+} influx and Ca^{2+}-induced phospholipid hydrolysis in protein kinase C-deprived islets may lead to a massive perturbation of islet phospholipid composition.

In conclusion, the present results open the possibility that both sensitization as well as desensitization of insulin secretion may reflect the same phenomenon, i.e. down-regulation of protein kinase C, which initially may lead to potentiation of secretion but in the long run also to deleterious effects of Ca^{2+} on islet cell function.

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