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Divergent effects of epinephrine and prostaglandin E_2 on glucose-induced insulin secretion from perifused rat islets

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Abstract

The impact of the catecholamine epinephrine and the postulated inhibitory second messenger prostaglandin E_2 (PGE_2) on the kinetics and magnitude of glucose-induced insulin secretion were compared and contrasted. In agreement with a number of studies, epinephrine was a most effective antagonist of glucose-induced insulin secretion. Dose-response studies using 8 to 10 mmol/L glucose as stimulant established that levels as low as 1 to 10 nmol/L of the catecholamine were effective at inhibiting release. Glucose (20 mmol/L) caused an approximately 25-fold increase in insulin secretion, an effect that was completely abolished by 1 μmol/L epinephrine. Under conditions where it completely abolished 20 mmol/L glucose–induced insulin release, epinephrine (1 μmol/L) reduced, but did not abolish, the stimulatory effect of glucose on phospholipase C activation. Chronic 3-hour exposure to 10 mmol/L glucose alone desensitized the islet to subsequent stimulation by glucose. Despite its ability to completely suppress secretion to 10 mmol/L glucose, epinephrine failed to protect the islet from hyperglycemia-induced desensitization. In sharp contrast to epinephrine, PGE_2 at levels ranging from 1 to 10 μmol/L had no discernible adverse effect on 10 mmol/L glucose–induced secretion. These findings suggest that multiple mechanisms contribute to the inhibitory impact of epinephrine on release and, in conjunction with other studies, cast serious doubt on the concept that PGE_2 plays any significant inhibitory role in the regulation of glucose-induced secretion.

1. Introduction

The regulation of insulin secretion is a complex process that depends on the proper integration of a large number of factors. Although most experimental attention has been placed on those compounds such as glucose, glucagon-like peptide-1, and cholinergic agonists that positively impact on the secretory process, dramatic effects can also be induced by a number of inhibitors as well. We reported recently [1], in agreement with prior studies [2], that diazoxide, by maintaining the patency of the adenosine triphosphate–sensitive potassium channel, not only abolished the short-term impact of glucose on insulin secretion but also protected the beta cell from the adverse impact of chronic sustained hyperglycemia. We attributed this sparing effect of diazoxide to its ability to impair the activation of beta cell phospholipase C (PLC). Our interest in the identification of those pathways that positively or negatively affect the secretory process prompted us to address the issue as to whether other inhibitors of insulin secretion, in addition to diazoxide, might also spare the islet from glucose toxicity. Both epinephrine and prostaglandin E_2 (PGE_2) have been reported to possess the capacity to inhibit glucose-induced insulin release [3-6]. Experiments were designed to determine their impact on the short-term regulation of insulin secretion and, in the case of epinephrine, its effect on glucose-induced desensitization as well.

2. Materials and methods

The detailed methodologies used to assess insulin output from collagenase-isolated islets have been previously described [7,8]. 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 National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*. The animals were fed ad lib. After pentobarbital sodium (Nembutal, 50 mg/kg, Abbott, North Chicago, IL)–induced anesthesia, islets were

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isolated by collagenase digestion and handpicked by using a glass loop pipette under a stereomicroscope into Krebs-Ringer bicarbonate (KRB) solution supplemented with 3 mmol/L glucose. They were free of exocrine contamination.

2.1. Perifusion studies

After isolation, islets were loaded onto nylon filters (Sefar America, Kansas City, MO). Some islets were immediately perifused, whereas others were subjected to 3 hours incubation with various agonists before perifusion (see below). All islets were perifused with 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 section. Perifusate solutions were gassed with 95% oxygen/5% carbon dioxide and maintained at 37°C. Insulin released into the medium was measured by radioimmunoassay [9].

After being loaded onto nylon filters, other groups of islets were incubated for 3 hours. The filter, with islets attached, was placed in a small glass vial. KRB solution (400 μL), supplemented with the 5 to 10 mmol/L glucose, was then added. In some studies, 100 nmol/L epinephrine was added together with 10 mmol/L glucose. The vials were gently aerated for 10 seconds with 95% oxygen/5% carbon dioxide and maintained at 37°C. After 90 minutes they were aerated again for 10 seconds. After the 3 hours, they were then perifused as described above and in the figure legends.

2.2. Efflux 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. Added to this aliquot of tracer was 255 μL of warmed (to 37°C) and oxygenated KRB medium supplemented with 5 mmol/L glucose. In additional experiments, 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% oxygen/5% carbon dioxide, and incubated 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 solution and perifused. Samples (200 μL) were analyzed every 2 minutes for [^{3}H]inositol radioactivity from minutes 28 to 70 of the perifusion. Fractional efflux rates were calculated as described previously [10-12].

2.3. Reagents

Hanks solution was used for the islet isolation. The perifusion medium 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. The ^{125}I-labeled insulin for the insulin assay and the [^{3}H]inositol were purchased from PerkinElmer Life Sciences (Boston, MA). Bovine serum albumin (RIA grade), L-epinephrine bitartrate (cat no. E 4375), PGE_2 (cat no. P 5640), glucose, and the salts used to make the Hanks solution and perifusion medium were purchased from Sigma (St Louis, MO). The PGE_2 was dissolved in ethanol to make a stock solution of 10 mmol/L. Because the amount of alcohol used in these studies, 1 μL per 1000 μL of KRB solution, had no discernible effect on insulin secretion, control and diluent-treated islet data were pooled. Rat insulin standard (lot no. 615-ZS-157) was the generous gift of Dr Gerald Gold, Eli Lilly (Indianapolis, IN). Collagenase (type P) was obtained from Roche Diagnostics Corp (Indianapolis, IN).

2.4. Statistics

Statistical significance was usually determined by using the Student *t* test for unpaired data or analysis of variance. The data in Fig. 3 were analyzed using the *t* test for paired data. A *P* value of .05 or less was taken as significant. Values presented in the figures and results represent means ± SEs of at least 3 observations.

3. Results

3.1. Studies with epinephrine

In the initial series of experiments we confirmed the previous report by Axen et al [13] that 1 μmol/L epinephrine abolishes 20 mmol/L glucose–induced insulin release (Fig. 1). In these studies, control islet responses to 20 mmol/L glucose alone were characterized by a brisk insulin secretory response that, on average, was more than 30-fold greater than basal secretion rates measured in the

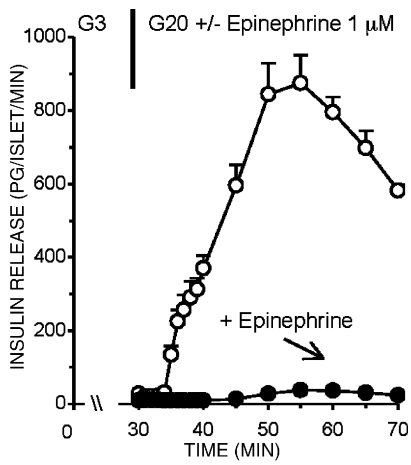

Fig. 1. Inhibition of 20 mmol/L glucose–induced insulin release by epinephrine. Groups of islets were perifused immediately after isolation. For the initial 30 minutes they were perifused with 3 mmol/L glucose (G3) to establish basal and stable rates of release. At this time (indicated by the vertical line) they were then stimulated for 40 minutes with 20 mmol/L glucose (open circles) or 20 mmol/L glucose plus 1 μmol/L epinephrine (closed circles). A minimum of 3 experiments was performed under each condition and mean values ± SEs are shown. This and subsequent figures 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.

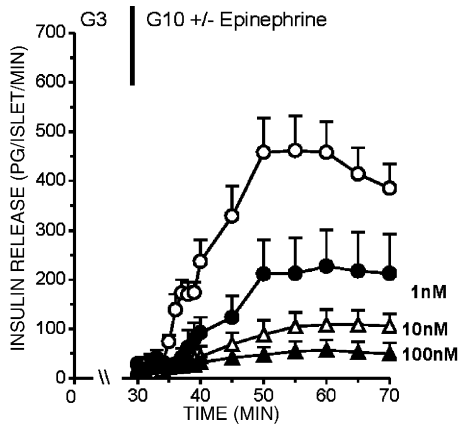

Fig. 2. Concentration-dependent impact of epinephrine on 10 mmol/L glucose–induced secretion. Groups of islets were perifused immediately after isolation. For the initial 30 minutes they were perifused with 3 mmol/L glucose (G3) to establish basal and stable rates of release. At this time (indicated by the vertical line) they were then stimulated for 40 minutes with 10 mmol/L glucose (open circles), 10 mmol/L glucose plus 1 nmol/L epinephrine (closed circles), 10 mmol/L glucose plus 10 nmol/L epinephrine (open triangles) or 10 mmol/L glucose plus 100 nmol/L epinephrine (closed triangles). A minimum of 3 experiments was performed under each condition and mean values ± SEs are shown.

presence of 3 mmol/L glucose. The addition of 1 μmol/L epinephrine together with 20 mmol/L glucose abolished the stimulatory effect of the hexose. Two important points were established in these experiments. First, the physiologic integrity of beta-cell sensitivity to glucose is retained during the collagenase isolation procedure. Second, epinephrine is most efficacious at inhibiting secretion to a maximally effective glucose stimulus. However, with the realization that neither 20 mmol/L glucose nor 1 μmol/L epinephrine would ever exist in vivo, additional studies with lower levels of both compounds were conducted.

After a 30-minute stabilization period with 3 mmol/L glucose, stimulation of collagenase-isolated islets with 10 mmol/L glucose resulted in a brisk secretory response. When compared with prestimulatory rates of approximately 30 pg per islet per minute, release rates after 35 to 40 minutes of stimulation increased about 12- to 15-fold to about 380 pg per islet per minute (Fig. 2). The inclusion of epinephrine dramatically altered this response. For example, the highest level (100 nmol/L) of epinephrine used in these initial studies virtually abolished the response to 10 mmol/L glucose. Even at 1 nmol/L of the catecholamine, a significant reduction in 10 mmol/L glucose–induced secretion was noted. An IC_{50} of approximately 5 nmol/L was calculated from the data by using 10 mmol/L glucose as stimulant.

Human plasma catecholamine (epinephrine plus norepinephrine) levels in the unstressed state average approximately 0.1 to 0.2 nmol/L and can increase about 3- to 5-fold with stress [14]. To add credence to the potential physiologic role of epinephrine in the regulation of insulin secretion, we decided to explore how even lower levels of the catecholamine affect secretion. We lowered the glucose level to 8 mmol/L in these studies to more readily uncover any inhibitory action of epinephrine. Control islets respond to 8 mmol/L glucose with about an 8- to 9-fold increase in insulin secretory rates. The inclusion of 0.5 nmol/L epinephrine to the perifusion medium, a concentration well within the high physiologic range that occurs with stress, during both the prestimulatory phase with 3 mmol/L glucose and during the stimulatory phase with 8 mmol/L glucose significantly reduced secretion for the final 25 minutes of 8 mmol/L glucose stimulation period (Fig. 3).

Our immediate interest in the role being played by epinephrine in the regulation of insulin secretion was initially motivated by our studies exploring how chronic hyperglycemia, often referred to as glucose toxicity, adversely impacts secretion. We have reported recently [1], in agreement with other studies [15-17], that sustained increases in the glucose level bathing the beta cell induces a defect in the subsequent insulin secretory response. Although our studies with diazoxide and carbachol suggest the proximal involvement of PLC in the secretory lesion, we decided to assess whether epinephrine altered the induction of desensitization. To this end, several series of studies were performed.

In the first set of experiments, we explored the potential effects of epinephrine, 0.1 to 1.0 μmol/L, on 20 mmol/L glucose–induced activation of PLC. After labeling their phosphoinositide pools with [^{3}H]inositol, islets were perifused and then stimulated with 20 mmol/L glucose. An increase in the fractional efflux rates of [^{3}H]inositol occurred contemporaneously with insulin secretion (Fig. 4), a finding made by others as well [13,18,19]. The addition of epinephrine abolished insulin secretion; however, a significant increase in PLC activation occurred in spite of the abolition of secretion.

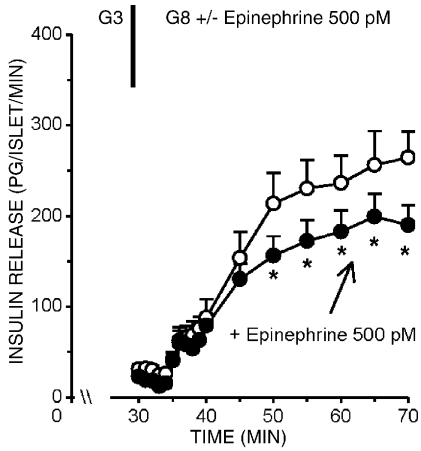

Fig. 3. Physiologic levels of epinephrine impair 8 mmol/L glucose–induced insulin secretion. Two groups of islets were studied. The first group was perifused for 30 minutes with 3 mmol/L glucose. For an additional 40 minutes (onset of stimulation indicated by the vertical line), these islets were stimulated with 8 mmol/L glucose (open circles). The second group of islets (closed circles) was similarly treated except that during the entire perifusion, 500 pmol/L epinephrine was included in the medium. Eight experiments were conducted under each condition. The asterisks indicates a significant difference between controls and epinephrine exposed islets at this time point.

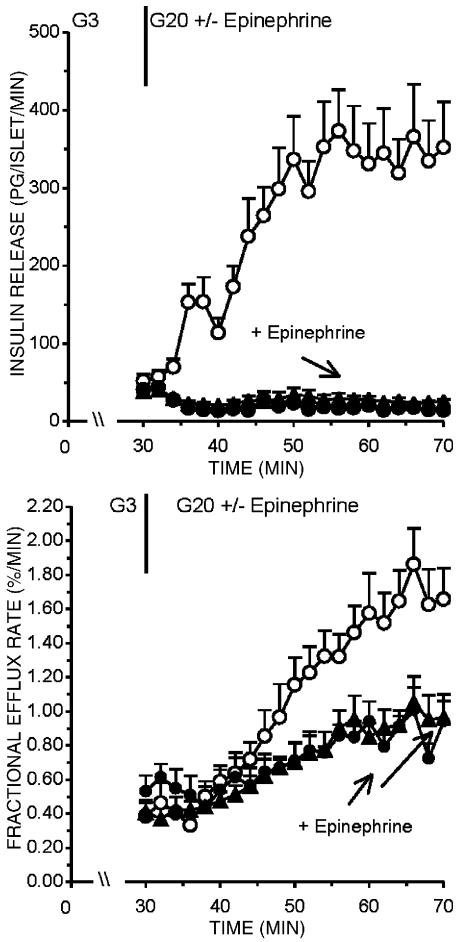

Fig. 4. Influence of epinephrine on secretion and PLC activation. Three groups of islets were studied. All groups were incubated for 3 hours in KRB medium supplemented with 5 mmol/L glucose and trace amounts of [^{3}H]inositol to label their phosphoinositide pools. After this, the islets were perifused for 30 minutes with 3 mmol/L glucose before stimulation with 20 mmol/L glucose alone (open circles) or 20 mmol/L glucose plus 0.1 (closed circles) to 1 μmol/L epinephrine (closed triangles). Insulin secretion rates (top) and fractional efflux rates of [^{3}H]inositol (bottom) of at least 4 separate experiments are shown. To prevent reincorporation of label back into phosphoinositide pools, 1 mmol/L inositol was included during the stimulatory period with 20 mmol/L glucose.

In the final experiments with epinephrine, we explored the potential impact of epinephrine on glucose-induced desensitization. Islets were incubated for 3 hours with 5 mmol/L glucose (controls), 10 mmol/L glucose (desensitized islets), or 10 mmol/L glucose plus 100 nmol/L epinephrine. This level of the catecholamine completely abolishes 10 mmol/L glucose–induced insulin secretion as established in Fig. 2. However, despite its profound inhibitory impact on the exocytosis of insulin, 100 nmol/L epinephrine did not spare or rescue the beta cell from 10 mmol/L glucose–induced desensitization (Fig. 5).

3.2. Studies with PGE_2

The final experiments examined the potential effects of PGE_2 on glucose-induced insulin secretion. Previous conflicting studies concerning the effects of this compound

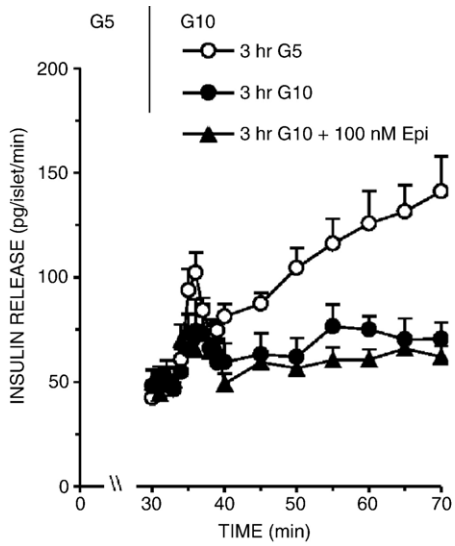

Fig. 5. Epinephrine fails to prevent 10 mmol/L glucose–induced desensitization. Three groups of islets were studied. The first group (open circles) was incubated for 3 hours in a KRB medium supplemented with 5 mmol/L glucose before being perifused. The second group (closed circles) was incubated for 3 hours in a KRB medium supplemented with 10 mmol/L glucose. The final group was incubated for 3 hours with 10 mmol/L glucose plus 100 nmol/L epinephrine (closed triangles), a level of the catecholamine that completely abolishes glucose-induced insulin secretion. All groups were subsequently perifused with 3 mmol/L glucose for 30 minutes and then stimulated with 10 mmol/L glucose for 40 minutes. At least 4 experiments were conducted under each condition.

on glucose-induced secretion were conducted using batch-incubated islets, batch-incubated pancreatic pieces, or static incubation of tumoral HIT cells [5,6,20,21]. These preparations yield no information on the kinetics of the secretory

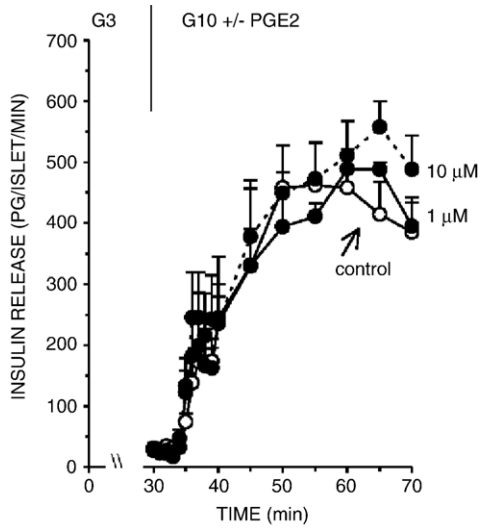

Fig. 6. Lack of effect of PGE_2 on glucose-induced insulin secretion from perifused islets. Three groups of islets were studied. The first group (open circles, same control data as in Fig. 2) was stimulated with 10 mmol/L glucose alone for 40 minutes. The second and third groups were stimulated with 10 mmol/L glucose plus 1 μmol/L (closed circles, solid line) or 10 μmol/L (closed circles, dashed line) PGE_2. At least 4 experiments were conducted under each condition.

response. The possibility exists that one phase is preferentially altered over the other and that the methodology used may contribute to this conflicting state of affairs. Despite reports demonstrating that PGE_2 inhibits glucose secretion from cultured neonatal pancreas pieces or HIT cells [5,6], but in agreement with 2 other studies using rat islets [20,21], we could not detect any adverse effect of PGE_2 on the kinetics or magnitude of 8 (results not shown) or 10 mmol/L glucose–induced secretion (Fig. 6). Even when used at levels more than 1000-fold greater than effective inhibitory concentrations of epinephrine on secretion, no adverse effect was observed.

4. Discussion

In the present series of experiments the effects of epinephrine and PGE_2 on insulin secretion from collagenase-isolated, perifused islets were determined. This preparation and approach was used for several reasons. First, the physiologic integrity of our preparation is retained and the magnitude of secretory responses observed compare favorably with the perfused pancreas preparation. For example, studies in rat have demonstrated that maximum responses to glucose stimulation with the latter preparation are about 20- to 40-fold greater than prestimulatory release rates [22-26]. Our 25- to 30-fold increase in response to 20 mmol/L glucose stimulation emphasizes that the factors controlling this robust secretory response are retained throughout the isolation procedure. Second, the dynamics of the secretory response and any preferential adverse impact of the inhibitors studied on either first- or second-phase responses can be easily ascertained. Finally, after isolation, our islets can be labeled with various tracers (in the present case, [^{3}H]inositol to label phosphoinositide pools), and the impact of glucose on the mobilization of label followed to determine PLC activation.

In the initial series of experiments, we first determined how epinephrine altered the secretory response to glucose stimulation. In response to 20 mmol/L glucose, a brisk secretory response was noted that, as mentioned above, was at least 25-fold greater than basal secretion rates. A concentration of 1 μmol/L epinephrine abolished this response, a finding made previously by Axen et al [13]. Considering that neither the glucose nor the epinephrine levels in vivo approach these values, additional studies were performed with lower concentrations of both compounds. Using 10 mmol/L glucose to stimulate secretion, we found that levels of epinephrine as low as 1 to 10 nmol/L inhibited secretion. An IC_{50} of approximately 5 nmol/L was calculated from the combined 10 mmol/L glucose data. Lower levels of the catecholamine (500 pmol/L) were also effective in reducing secretion evoked by 8 mmol/L glucose. This degree of physiologic α_2-adrenergic stimulation may be achieved in vivo [14] during stressful conditions.

Previous studies have established that chronic hyperglycemia desensitizes islets to subsequent glucose stimulation [1,15,17]. We have attributed the failure of desensitized islets to respond to glucose to events in the PLC signaling cascade [17,27]. Islets can be desensitized to a subsequent glucose stimulus by prior exposure to a number of compounds including glucose, carbachol, forskolin, glucosamine, or monomethylsuccinate. When compared with the responses of control islets, desensitized islets demonstrate markedly impaired PLC responses to glucose. Defective activation of this enzyme parallels defective insulin secretion. In the present studies, 2 additional issues related to PLC activation were explored. We determined the effect of epinephrine on glucose-induced PLC activation and whether the catecholamine protected the islets against glucose-induced desensitization.

Stimulation of perifused islets with 20 mmol/L glucose culminates in the activation of PLC, monitored in this and other studies [13,18] by an increase in [^{3}H]inositol efflux rates from [^{3}H]inositol-prelabeled islets. Parallel increments in secretion were also noted from these islets. Although 0.1 to 1 μmol/L epinephrine abolished secretion, it had a much less decisive inhibitory effect on PLC activation. It is difficult to compare our data to the previously published studies by Axen and coworkers [13] because comparable control studies were not included in this report. However, similar to the present findings, they also demonstrated that despite completely abolishing secretion, PLC activation was still demonstrable from epinephrine-treated islets. As previously suggested by Sharp [28], these and other results suggest that several mechanisms may contribute to the profound impact of epinephrine on the secretory process; a more proximal effect on PLC activation, and a more distal effect on the exocytotic apparatus.

Previous studies from our laboratory [1,17] have led us to conclude that excessive stimulation of PLC by glucose is responsible, at least in part, for the reduced sensitivity of these islets to a subsequent glucose stimulus. With the demonstration that epinephrine reduced but did not abolish the effects of 20 mmol/L glucose on PLC activation, we next determined whether the catecholamine altered the ability of glucose to induce desensitization. In these studies, islets were desensitized by a prior 3-hour exposure to 10 mmol/L glucose. In some studies, 100 nmol/L epinephrine, a level that completely abolishes 10 mmol/L glucose–induced secretion, was included together with 10 mmol/L glucose. When subsequently stimulated with 10 mmol/L glucose during a dynamic perifusion, no protective effect of epinephrine was noted. At first glance, these results would appear to question the concept that excessive stimulation of PLC by glucose is the immediate cause of desensitization. However, it should be remembered that whereas epinephrine completely abolished glucose-induced release, PLC was still activated, albeit to a lesser extent than control islets. It is possible that the residual activation of PLC, even at a reduced rate, is still sufficient to induce desensitization. Along this same line of reasoning, we have shown in other studies that sustained exposure of islets to levels of glucose

as low as 7 mmol/L can induce desensitization. In any event, future studies using different glucose and epinephrine levels should be directed at clarifying any potential protective effect of epinephrine on desensitization.

We were initially surprised by our failure to detect any inhibitory effect of PGE_2 on glucose-induced secretion, even when concentrations as high as 10 μmol/L, a level 10,000 times greater than the minimally effective inhibitory epinephrine concentration, were used. However, closer analysis of the approaches used in earlier studies gives some insight into the potential basis for these differences. The studies by Metz and coworkers [6] and Robertson and coworkers [29] were performed with cultured neonatal rat pancreas or HIT cells. Neither preparation shows the type of robust secretory response to glucose that characterizes the responses of islets used in these studies. For example, release rates in response to 300 mg/dL glucose increased only 2- to 3-fold from cultured neonatal pancreas [6]. A similar departure from the physiologic sensitivity of a normal beta cell also characterizes the glucose response of HIT cells [30]. It is difficult to extrapolate findings made with these preparations to the physiologic regulation of glucose-induced release when the responses of these preparations deviate in such a substantial fashion from normal beta cells. Finally, it should be noted that 2 other studies [20,21] also failed to document any adverse effect of PGE_2 on glucose-induced secretion, findings confirmed herein as well. We are left to conclude that unlike the situation observed in less responsive preparations, PGE_2 has no significant inhibitory role in the short-term regulation of glucose-induced insulin secretion from a preparation that retains the appropriate physiologic sensitivity to the hexose.

In summary, epinephrine at concentrations observed in vivo was a most effective inhibitor of insulin secretion. The catecholamine also partially reduced the stimulatory effect of glucose on PLC activation as well, but it did not spare the islet the impact of sustained hyperglycemia. In contrast to epinephrine, PGE_2 was impotent with regard to the inhibition of glucose-induced secretion and thus would appear to play little, if any, regulatory role in physiologic regulation of secretion. Our observations made with both epinephrine and PGE_2 also support the concept put forward by Newman and Brodows [14] that the in vivo effects of PGE_2 infusion may be indirect, mediated by PGE_2-induced catecholamine secretion. Finally, to more accurately characterize the profound inhibitory effects of epinephrine on the beta cell, the biochemical characterization of islets from catecholamine-deficient mice [31], islets that exhibit hyperresponsiveness to glucose and hyperinsulinemia in vivo, would appear most desirable.

Acknowledgment

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