Homologous desensitization of pancreatic beta cells to glucose response by polyunsaturated fatty acids

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We have shown recently that a mixture of 10 mM linoleic acid (18:2,ω6) and 5 mM linolenic acid (18:3,ω3) enhanced insulin secretion by microdissected islets. In the present study, our aim was to examine the effect of a prestimulus with this mixture of 10 mM linoleic acid and 5 mM linolenic acid on glucose and arginine-stimulated insulin response, by perifused isolated islets.

Insulin secretion by untreated (control) islets estimated as integrated area under the curve (AUC/20 mins) above basal during three pulses of 27.7 mM glucose separated by 20 min periods of basal (washout) perifusions were 2950 ± 585 pg, 5185 ± 1258 pg, and 2410 ± 921 pg, a greater response (P < 0.03) being obtained during the second glucose challenge. The insulin response of the islets to the glucose challenge was blunted profoundly if the islets were first exposed to a 20 min period of perifusion with the fatty acid mixture followed by a 20 min washout. Thus, the insulin AUC/20 min decreased from a control value of 2410 ± 921 pg to 337 ± 201 pg (P < 0.03) after prestimulus with the fatty acids. In contrast, fatty acid exposed islets were still responsive to 10 mM arginine. These data suggest that (1) a mixture of 10 mM linoleic acid and 5 mM linolenic acid can desensitize isolated perifused islets to glucose but not arginine; and (2) glucose intolerance associated with lipemia may result, at least in part, from a direct effect on the islets of Langerhans.

Keywords: desensitized islets; fatty acids; insulin secretion

Introduction

Presently, there is emphasis on the provision of essential fatty acids as a result of their possible efficacy, in patients receiving total parenteral nutrition[1,2] and patients with cystic fibrosis.[3-5] In addition, it has been shown recently that polyunsaturated fatty acids enhance insulin secretion in vivo[6] and in vitro.[7] Since adverse metabolic effects, including glucose intolerance associated with elevated plasma lipids, have been reported,[8-12] one question arising from these observations has been if the exposure of the pancreatic islets of Langerhans to a fat challenge could influence the islet response to glucose stimulation. From numerous recent reports, now it is known that the exposure of pancreatic islets to a chronic stimulation by glucose results in a desensitization of the islet to further glucose stimulation of insulin secretion.[13-19] This desensitization phenomenon has been described recently as a new phase of insulin secretion.[20] The possibility of such a desensitization effect by fatty acids has not been shown. In the present study, we have examined, in acute experiments, the effect of a prestimulus of polyunsaturated fatty acids on glucose and arginine-stimulated insulin output by isolated perifused pancreatic islets.

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Materials and methods

Isolation of islets

In each experiment, three adult CDI female mice (Charles River Laboratories) fasting for at least four hours but allowed free access to drinking water were used. The animals were sacrificed by stunning a blow to their heads followed by decapitation. The pancreas from each animal was taken with a flat-bottomed plastic dish into which a mixture of wax and Norit A charcoal (American Drug and Chemical Company, Inc., New York) had been melted to create a smooth surface. Two large islets were then isolated from the tail region of the pancreas by the technique of microdissection[21] using an operating microscope (Carl Zeiss 30184, Germany).

Perifusion

The six islets thus isolated were pooled into a plastic flow-through perifusion chamber of a volume of 0.5mL (Millipore Corporation) and preperifused at a constant rate of 1 ml/min with a modified Krebs-Ringer bicarbonate (KRB) buffer containing 5.5 mM glucose (basal) for 1 hr at 37° C. The KRB was maintained at pH 7.4 by continuous gassing with a mixture of 95%/5%, O_2/CO_2. This buffer comprised 120 mm NaCL, 5 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM $MgCl_2$, and 25 mM $NaHCO_3$, and in addition contained 100 KIU/ml Trasylol (Sigma Chemical Co.) and 2% bovine albumin (Armour Pharmaceuticals, Kankakee, IL). This albumin was free of fatty acids as well as insulin-like activity. The 2% albumin concentration was sufficient to bring into solution the concentrations of the fatty acids (Sigma Chemical Co.) used in this study approximating to an albumin fatty acid ratio of 1:50. After the preperifusion, basal effluent samples were collected before the perifusion was continued in 20 min cycles with the addition of a nutrient stimulus alternating with a basal (washout) perifusion. Solutions were changed using a stopcock. Effluent perifusate was collected on ice at 2 min intervals and stored frozen until radioimmunoassay for insulin.[22]

Data analysis

Insulin output was analyzed as area under the curve[7] during a 20 minute period of peak response to a nutrient present in the perifusate. Duplicates of RIA data were assessed independently and statistical evaluation was done by the Wilcoxon Rank Sum test.

Results

Effect of 27.7 mM glucose on insulin secretion (Figure 1)

In control experiments, islets were perifused with three 20 min pulses of 27.7 mM glucose which is the maximally effective concentration for stimulating insulin release,[23] each preceded by a 20 min washout (basal) perifusion. Insulin AUC/20 min were increased above basal as shown in *Figure 1*, a significantly greater insulin response being obtained during the second glucose challenge ($P < 0.03$).

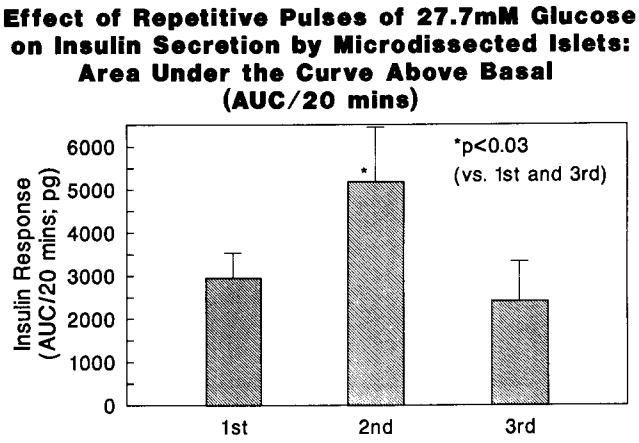

Figure 1 Effect of 27.7 mM glucose on insulin secretion. Islets were perifused with three 20 min pulses of 27.7 mM glucose separated by 20 min periods of basal (5.5 mM) glucose perifusion. Effluent samples were assayed for insulin and the data analyzed as areas under the curve over 20 min of peak insulin response (AUC/20 min) from three separate experiments.

Effect of fatty acid prestimulus on islet response to glucose (Figures 2–5)

The insulin response of the islets to a 27.7 mM glucose challenge was blunted profoundly if the glucose perifusion was preceded by a mixture of 10 mM linoleic acid (18:2,ω6) and 5 mM linolenic acid (18:3,ω3) as illustrated in *Figure 2*. However, the same islets were still responsive to 10 mM arginine stimulation (*Figure 2*) or to a second challenge with the fatty acid mixture (data

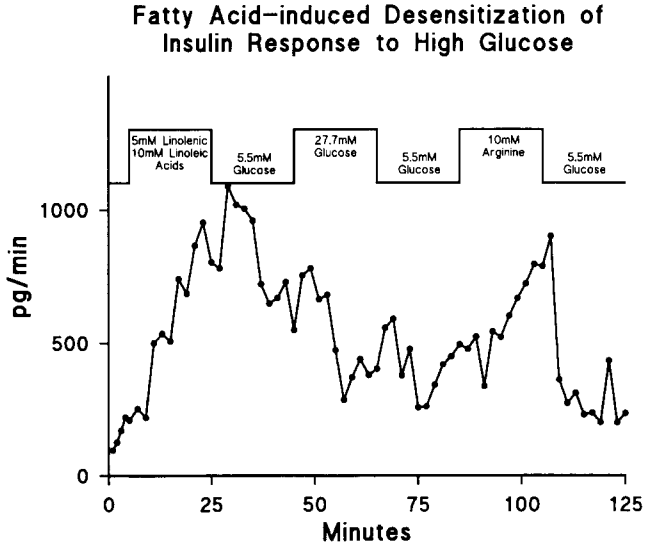

Figure 2 Effect of fatty acid prestimulus on islet response to glucose. Islets were challenged with 27.7 mM glucose pulse after a washout following a perifusion with a mixture of 10 mM linoleic and 5 mM linolenic acids. The response of the same islets to arginine perifusion was then performed as a control. This figure represents one of six separate experiments.

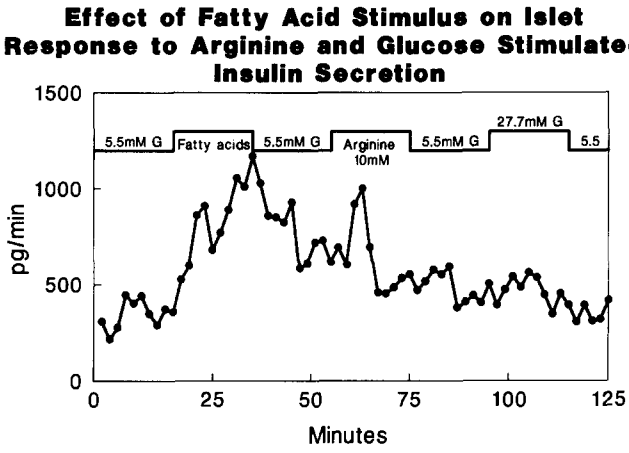

Figure 3 Effect of fatty acid prestimulus on islet response to arginine. This experiment was performed as described in *Figure 3*, except that the arginine perifusion was performed prior to a glucose challenge.

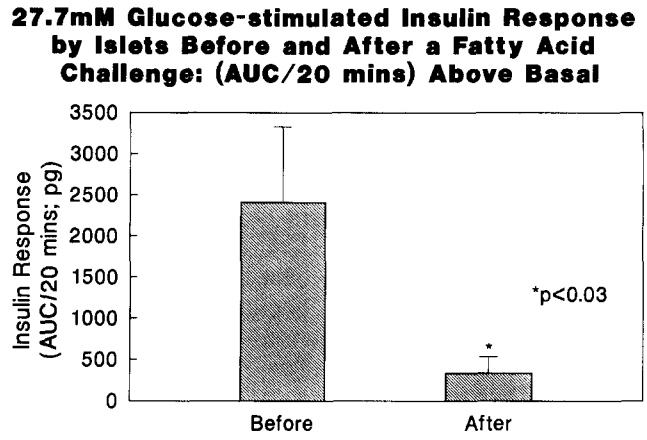

Figure 5 AUC/20 min above basal of insulin response to 27.7 mM glucose by islets before and after fatty acid challenge. The peak of insulin response to glucose over 20 min before (control) and after islets were exposed to fatty acids was evaluated in six separate experiments.

not shown). As shown in *Figure 3*, interchanging the positions of arginine and glucose in the perifusion profile did not alter the pattern of their insulin secretory effect after the islets had been exposed to the fatty acid mixture. Thus, while the islets remained responsive to arginine, their desensitization to 27.7 mM glucose was still prominent one hour after the withdrawal of the fatty acids. As pointed out in our preceding report,[7] insulin secretion after the 20 min washout (basal perifusion), following withdrawal of the fatty acids, remained significantly elevated above basal (*Figures 2* and *3*). However, this did not eliminate the responsiveness of the islets to 10 mM arginine stimulation, despite the fact that this arginine concentration may not be the maximum dose as shown in *Figure 4* obtained from dose-response experiments. It should be noted that the stimulatory effect of arginine, even on untreated (control) islets, appears to be transient as insulin secretion promptly returns to basal level despite the continued presence of the secretagogue (*Figure 4*). Also in *Figure 3*, insulin secretion during the washout period, following arginine withdrawal, had returned to basal level before 27.7 mM glucose challenge which still failed to stimulate insulin output. The failure of a pulse of 27.7 mM glucose to stimulate insulin secretion after a washout following fatty acid withdrawal (*Figure 2*) may therefore not be due to the sustained elevated insulin release. *Figure 5* is an assessment of the insulin response to 27.7 mM glucose challenge before and after a fatty acid prestimulus. Insulin output (AUC/20 min) fell from 2410 ± 921 pg before to 337 ± 201 pg after ($P < 0.03$).

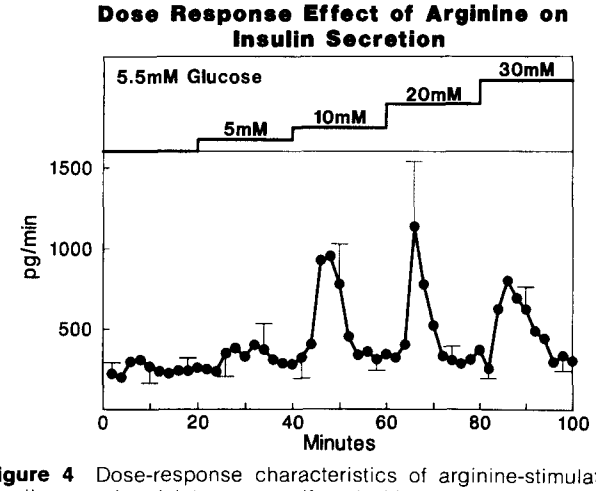

Figure 4 Dose-response characteristics of arginine-stimulated insulin secretion. Islets were perifused with increasing concentrations of arginine for 20 min at each dose, on a background of 5.5 mM glucose. Mean ± SE, $n = 5$ experiments.

Effect of palmoxirate on the fatty acid induced desensitization (Figure 6)

We and others have shown recently that the inhibition of fatty acid oxidation by palmoxirate, a specific carnitine acyltransferase inhibitor, blocked the stimulatory effect of these fatty acids on insulin.[7,24] On the basis of these observations, we tested the effect of palmoxirate on the desensitization of islets to glucose. As shown in *Figure 6*, raising the glucose concentration from basal to 27.7 mM caused a slow response in the stimulation of insulin secretion which was also slow in returning towards basal after reducing glucose to 5.5 mM. When the fatty acid mixture was added to the 5.5 mM glucose perifusion in the presence of palmoxirate, there was prompt reduction of insulin secretion to basal levels. Following the inhibition of fatty acid stimulated insulin secretion by palmoxirate, the characteristic postinhibitory "rebound off response" in basal insulin release seen in other studies[7,25] was observed. Increasing the glucose concentration to 27.7 mM from a basal 5.5 mM glucose caused a further stimulation of the already elevated insulin secretion (*Figure 6*).

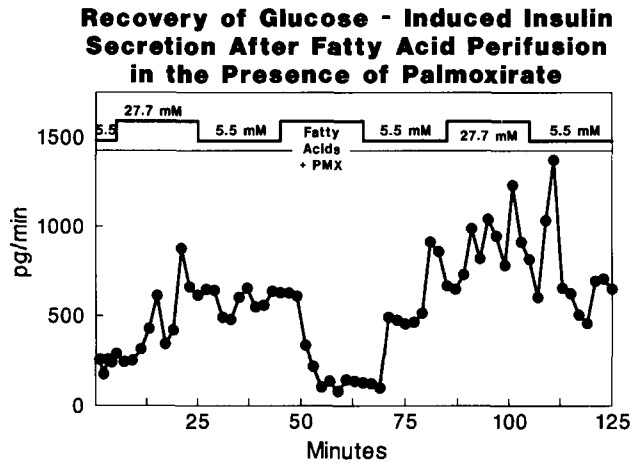

Figure 6 Effect of inhibition of fatty acid oxidation on desensitization. Two pulses of 27.7 mM glucose stimulus were separated by a pulse of the fatty acid mixture in the presence of 2 mM palmoxirate. Each of these pulses was preceded by a 20 min washout with 5.5 mM glucose. This figure represents one of four different experiments.

Discussion

Although numerous studies, as already mentioned, have shown that the exposure of the pancreatic islets of Langerhans to a chronic glucose stimulation lasting 24–96 hours, in vivo and in vitro, results in the desensitization of the islet insulin response to a subsequent glucose challenge, currently, there is a scarcity of data to show that a polyunsaturated fatty acid exposure of islets can directly desensitize their insulin response to a glucose effect. Our data from the present in vitro study clearly shows that a prestimulus with a mixture of linoleic and linolenic acids at high concentrations can render isolated perifused pancreatic islets unresponsive to a subsequent glucose challenge in acute experiments. The concentrations of the fatty acids used in this experiment are similar to those that have been measured in mixed meals,[6] and we have since shown in another study that these concentrations individually elicit maximal effects on insulin secretion in isolated islets.[26] Although these concentrations represent supraphysiologic levels of these fatty acids, when we checked the residual insulin contents of these fatty acid treated islets using an extraction procedure,[27] it was found that the total amount of insulin secreted during these experiments varied between 10–16% of the overall islet insulin concentrations. This suggests that it is unlikely that the fatty acid effect which we have observed could be due to a detergent action, since such a toxicity would cause the islet cells to burst and release most of their hormone contents. Besides, our experiments using palmoxirate have shown that the desensitization effect of fatty acids to glucose stimulus could be blocked by the addition of this pharmacologic agent to the fatty acid perifusate.

The reason for our observation in control experiments that a second glucose pulse gave rise to a higher insulin response than the first and third pulses is not clear, but may be due to a priming effect of the first glucose pulse.[28,29] In consideration of the possible influence of this phenomenon on our results, the position of the nutrient secretagogues in perifusing the islets was randomly switched during this study, and, as evident in *Figures 2* and *3*, this does not alter the pattern of insulin secretory response to arginine and glucose after islet pre-exposure to the fatty acids. It can not be inferred from the present study whether the desensitization to glucose stimulus is caused specifically by the polyunsaturated fatty acids we have used or if saturated fatty acids would also induce this desensitization phenomenon. However, given that long-chain saturated fatty acids have only a limited ability to cross the inner mitochondria membrane as CoA esters, we speculate that saturated fatty acids may not produce a desensitization effect as pronounced as that caused by polyunsaturated fatty acids. Indeed, a recent study indicates that long chain saturated fatty acids such as palmitate are not stimulators of insulin release but may amplify the insulin secretory response of islets to glucose.[30] Preliminary results (data not shown) in our laboratory, in which palmitate at high concentrations (10 mM) failed to stimulate insulin output support these observations. It is to be noted that there is a delay in insulin response to glucose as well as a low 3–4-fold glucose-induced insulin stimulation over basal in this and other studies using untreated (control) mouse islets.[31-33] The reason for this is unclear but may be related to the fact that glucose induced insulin secretion observed in these studies is monophasic although pulsatile, but lacking the well-described first phase insulin release which characteristically is more pronounced than the second phase.[34] Recently, an interesting observation has been made that the glycolytic pathway in the beta cell cytoplasm appears to be responsible for the glucose-induced first phase in insulin secretion, while the Krebs cycle taking place in the mitochondria mediates the second phase insulin release[35] which, of course, is known to be a slower process. It would therefore appear that the slow monophasic insulin response to glucose by mouse beta cells that we and others have observed could be due to a physiologic delay in the generation of Krebs cycle intermediate(s) involved in the insulin secretory signal.[36]

The mechanism for the fatty acid induced desensitization of islets to glucose sensitivity is not clear. However, one possibility involves the role of ATP. The hypothesis is that the metabolism of nutrients and concomitant generation of ATP would lead to the closure of the ATP-sensitive K^+ channels in the beta cell plasma membrane, and would initiate the cascade of events that triggers insulin secretion.[37] Thus, as shown in *Figure 7*, the β-oxidation of fatty acids in the mitochondria would generate abundant ATP. Since the phosphofructokinase and pyruvate kinase enzymes of the glycolytic pathway are inhibitable by increased ATP levels,[38] the effect of an overwhelming production of ATP by fatty acid oxidation would be to shut down glucose metabolism and consequently reduce insulin response to glucose. However, it remains to be

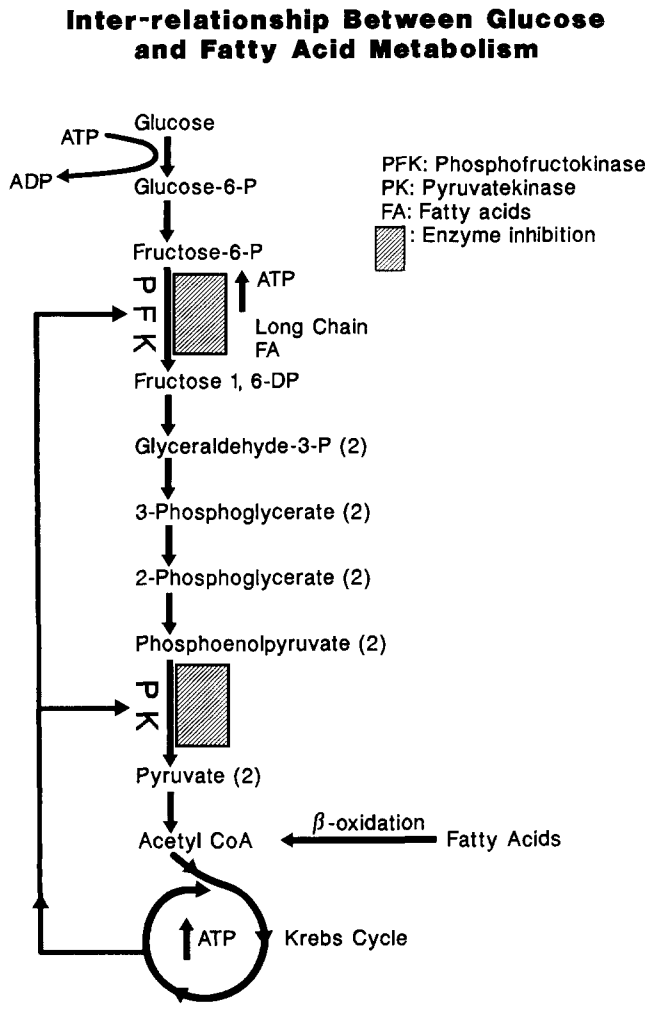

Figure 7 Inter-relationship between glucose and fatty acids metabolism. The β-oxidation of fatty acids in the beta cell mitochondria would generate abundant ATP. SInce the PFK and PK enzymes of the glycolytic pathway are inhibitable by increased ATP levels, the effect of an overwhelming production of ATP by fatty acid oxidation would be to shut down glucose metabolism and consequently reduce insulin secretion.

examined if the fatty acid induced desensitization involves a defect in the phosphoinositide hydrolysis in the islets, as has been shown with a chronic hyperglycemia model.[39] Recent studies have also implicated arachidonic acid and/or its metabolites in nutrient-stimulated insulin release[40-42] and, furthermore, it was shown that hydroxyperoxy-icosanoids generated in the metabolism of arachidonic acid inhibited glucose-induced insulin release by a negative feedback mechanism.[40,42]

Although the hypothesis of an ATP-mediated effect may be consistent with a recent observation[43] that chronic exposure to high glucose causes an impairment of K^+ channel function in perifused rat pancreatic islets, other metabolic feedback phenomenon or recruitment of multiple heterogenous secretory compartments[44] may explain the homologous desensitization of islets to glucose response induced by these polyunsaturated fatty acids. This fatty acid induced desensitization of the beta cell to glucose but not arginine effect suggests a fundamental difference between the mechanisms by which glucose and arginine stimulate insulin release. Presently, there is no agreement on the mechanism of arginine-induced insulin secretion, but current hypotheses include depolarization of the plasma membrane with subsequent gating of voltage-sensitive Ca^{2+} channels and/or to some other biophysical effect,[45] insulinotropic action of polyamines endogenously formed from arginine.[46] Whatever the mechanism involved, it is obvious that such a mechanism does not involve a metabolite that can cause the desensitization of islets to glucose stimulus. Indeed, it is of interest that an earlier study had shown that the stimulation of insulin and glucagon release by arginine does not involve the metabolism of this amino acid.[47]

In this report, we have shown that a mixture of high concentrations of linoleic and linolenic acids can desensitize isolated perifused murine pancreatic islets to glucose response. These data suggest the possibility that lipemia may contribute to glucose intolerance by a direct effect on the islets of Langerhans, consistent with recent observations of linear relationship between glucose area under the curve and elevated free fatty acid levels.[48]

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