Addition of L-glutamine to a linoleic acid perifusate prevents the fatty acid-induced desensitization of pancreatic islet response to glucose

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Previous studies showed that exposure of pancreatic islets to polyunsaturated fatty acids (PUFA) can render the beta cells unresponsive to glucose, thus suggesting the possibility that prolonged obligatory use of lipid preparations containing high concentrations of essential fatty acids during total parenteral nutrition may adversely affect glucose tolerance. In the present study we examined the effect of pretreatment of isolated murine islets with 10 mmol/L linoleate (18:2, ω6) alone or in the presence of 20 mmol/L-glutamine on the response of both alpha and beta cells to 27.7 mmol/L glucose perifusion at 37° C.

The incremental areas under the curve/20 mins (AUC/20 mins) for insulin output stimulated by 27.7 mmol/L glucose were 1552.8 ± 276.3 pg and 220.4 ± 163.9 pg ($P < 0.001$), ($n = 6$), respectively, before and after treatment with linoleic acid alone. In experiments in which the islets were treated with linoleate in the presence of L-glutamine there was no difference in the incremental insulin AUC/20 mins, in response to 27.7 mmol/L glucose before and after the fatty acid treatment (2051.8 ± 420.5 pg versus 2159.2 ± 317.6 pg, respectively, $n = 6$). Glucose-induced suppression of glucagon secretion, which was lost after perifusion of islets with the fatty acid alone, was observed when glutamine was added to the linoleate perifusate.

In conclusion, the addition of L-glutamine to linoleic acid perifusion of isolated islets completely blocked the PUFA-induced desensitization of both pancreatic alpha and beta cells to glucose effect.

Keywords: glutamine; insulin secretion; polyunsaturated fatty acid; glucagon release; glucose regulation

Introduction

Earlier studies on the nutritional benefits of providing linoleic acid (18:2,ω6) in the diet resulted in the identification of this nutrient as an essential fatty acid,[1,2] and presently the focus has been on the reported efficacy of essential fatty acids in a variety of conditions including cystic fibrosis[3,4] and in total parenteral nutrition, (TPN).[5–10] Recent experiments designed to assess the metabolic implications of the provision of essential fatty acids during TPN have shown that in animal models these fatty acids have a stimulatory effect on insulin release, which is accompanied by the desensitization of pancreatic beta cell response to glucose.[11,12] In these studies it was suggested that the mechanism of this polyunsaturated fatty acid (PUFA)-induced desensitization of the beta cell was linked to the fatty acid oxidation. Whether the PUFA-induced desensitization of beta cells also affects the pancreatic alpha cell response to glucose is not known.

The amino acid, L-glutamine, is among nutrients currently being evaluated for inclusion in TPN regimens,

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and in a variety of experiments on animals and humans it has been shown that the provision of glutamine might be efficacious, particularly in postoperative patients receiving TPN.[13-17] It is known that glutamine is one of the most abundant amino acids in the body and is the source of nitrogen for many biosynthetic pathways.[16,17] In addition, it has been well documented that glutamine is also an important energy-yielding substrate for gastrointestinal tract,[18] exocrine and endocrine pancreas,[19,20] and other rapidly growing cells.[21,22] Other studies have also shown that glutamine plays an important role in the metabolic processes associated with recovery in the physiologic alterations that accompany critical illness or injury.[23,24] Of interest is the demonstration that glutamine has an inhibitory effect on the oxidation of glucose pyruvate and fatty acids in pancreatic islets.[20,25,26]

The aims of the present study therefore were to examine whether the PUFA-induced desensitization of the beta cell also affects the pancreatic alpha cell response to glucose and to explore the possible use of glutamine as an inhibitor to fatty acid oxidation to counteract the desensitization.

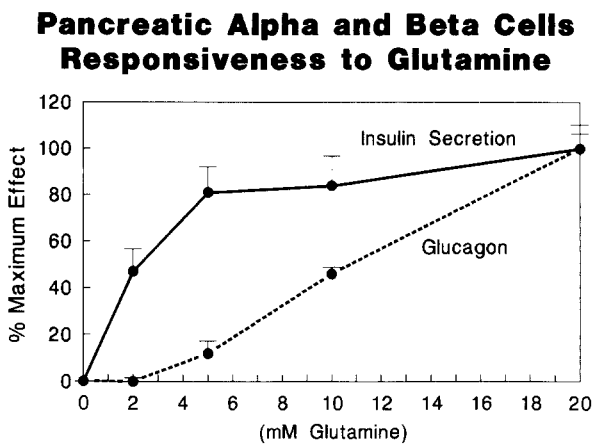

Figure 1 Dose response characteristics for the effect of glutamine on insulin (■) and glucagon (●) secretion. The maximal effect of glutamine on pancreatic hormone release (i.e., suppression of insulin and stimulation of glucagon output) was taken as 100% at a given concentration. The effects obtained at other concentrations were then calculated as percentage of the maximal effect. Data represent mean ± SEM, $n = 6$, and * denotes concentrations of L-glutamine at which statistically significant effects were observed with at least $P < 0.05$.

Methods and materials

Isolation of islets

In each experiment, three adult CD1 female mice (Charles River Laboratories, Raleigh, NC, USA), fasted for at least 4 hours but allowed free access to drinking water, were sacrificed by a blow to their heads followed by decapitation. The pancreas from each animal was taken into a dissection dish and two large islets were then isolated from the tail region of the organ by the technique of microdissection.[27]

Perifusion of islets

The six isolated islets were pooled into a plastic flow-through perifusion minichamber and preperifused at a constant rate of 1 ml/min with a modified Krebs-Ringer bicarbonate (KRB) buffer containing 5.5 mmol/L 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 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.1 mmol/L $MgCl_2$, 25 mmol/L $NaHCO_3$, and in addition contained 100 KIU/mL trasylol (Sigma Chemical Co., St. Louis, MO USA), and 2% bovine albumin (Armour Pharmaceuticals, Kankakee, IL USA) that was free of fatty acids and insulin-like activity. The albumin concentration was sufficient to bring as much as 10 mmol/L linoleate (Sigma Chemical Co.) into solution as described in a recent report.[28] After preperifusion basal effluent samples were collected on ice before the perifusion was continued in 20 min cycles in one of two protocols that essentially yielded the same results. In the first protocol, the glucose concentration was raised from a basal 5.5 mmol/L to 27.7 mmol/L, and this glucose perifusate was immediately followed by 10 mmol/L linoleate perifusion on a background of 5.5 mmol/L glucose in the absence or presence of freshly prepared 20 mmol/L L-glutamine (Sigma Chemical Co.). A washout period of basal glucose was then performed before the islets were retested with 27.7 mmol/L glucose. In the second protocol, after the first stimulation of islets with 27.7 mmol/L glucose a basal glucose washout was performed before the islets were perifused with the fatty acid with or without glutamine. The islets were then immediately retested with 27.7 mmol/L glucose. Solutions were changed using a stopcock, and effluent perifusate samples taken at 2 min intervals were stored frozen until radioimmunoassay for insulin[29] and glucagon.[30] The linoleate concentration used in this study was derived from an earlier report,[28] which suggested that this was maximally effective, while the glutamine concentration chosen was higher than was maximally effective in untreated (control) islets because of the high concentration of the linoleate to which the islets in the present study were exposed, and also because of the differential responsiveness of alpha and beta cells to the same concentrations of glutamine.[31]

Data analysis

Insulin output was assessed as integrated area under the curve (AUC/20min) above basal, while glucagon output has been presented as secretory responses in pg/min to the different nutrients because of the suppressive effect of high glucose on glucagon release. All values were expressed as mean ± standard error of the mean (SEM). Statistical evaluation requiring multiple comparisons was performed using a one-way analysis of variance (ANOVA) computer program, and depending on the outcome of ANOVA the Bonferroni method was used to assess the significance of differences between groups. A Student t test was used to compare the difference between the means of two groups. For all tests a value of $P < 0.05$ was considered significant.

Results

Dose response characteristics for the effects of L-glutamine on insulin and glucagon release by untreated islets (Figure 1)

In a preliminary report we showed that glutamine simultaneously suppressed insulin release while stimulating glucagon secretion.[31] However, the pancreatic beta cells appear to be more sensitive to glutamine than the alpha cells. Thus, as shown in *Figure 1*, whereas the effect

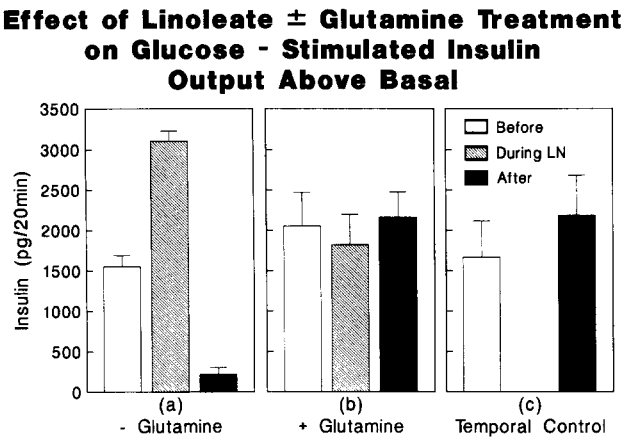

Figure 2 Effect of linoleate prestimulus on insulin response to 27.7 mmol/L glucose. Islet insulin output in response to 27.7 mmol/L glucose perifusion before (□) and after (■) linoleate treatment, as well as insulin secretion during (■) the period of fatty acid perifusion in the absence (2a) or presence (2b) of glutamine was assessed as insulin area under the curve (AUC/20mins) above basal. A temporal control was performed in basal glucose perifusate (figure 2c) over the duration of time during which the two other groups of islets were perifused with linoleate ± glutamine. Data represent mean ± SEM, $n = 6$.

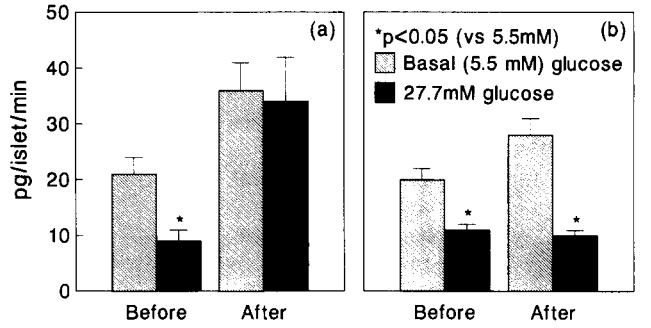

Figure 3 Effect of linoleate ± L-glutamine on 27.7 mmol/L glucose-induced suppression of glucagon secretion. Effluent perifusate samples collected at 2-min intervals during the experiments described in *Figure 2a* were assayed for glucagon content. This figure illustrates mean ± representative SEM of data from six separate experiments for each of (a) performed in the absence and (b) in the presence of L-glutamine.

of this amino acid on glucagon secretion continued to increase in a linear fashion over the range of concentrations tested, the same concentrations of glutamine were able to produce a saturating effect on insulin output with an apparent K_m of about 2.5 mmol/L.

Effect of linoleate prestimulus without or with glutamine on glucose-stimulated insulin output (Figures 2a and 2b)

Incremental insulin output from islets stimulated with 27.7 mmol/L glucose before and after linoleate treatment in the absence of glutamine is shown in *Figure 2a*. The insulin AUC/20min above basal were 1552.8 ± 276.3 pg and 220.4 ± 163.9 pg ($P < 0.001$, $n = 6$) before and after linoleate prestimulus, respectively. It can also be seen that insulin release was also significantly ($P < 0.05$) enhanced above basal during the fatty acid perifusion *(Figure 2a)*. When these experiments were repeated with the addition of glutamine to the linoleate perifusate, there was no difference in the insulin response (2051.8 ± 420.5 pg versus 2159.2 ± 317.6 pg, $n = 6$, respectively) to 27.7 mmol/L glucose stimulation of islets before and after linoleate exposure *(Figure 2b)*. The addition of glutamine to the linoleate perifusate *(Figure 2b)* was able to block completely the loss of insulin response seen in control experiments *(Figure 2a)*, but only caused a reduction of the fatty acid stimulation of insulin output by the islets. In parallel experiments set up as temporal controls during these studies 27.7 mmol/L glucose-stimulated insulin secretion was present before and after basal (5.5 mmol/L) glucose perifusion *(Figure 2c)* over the time period during which the other groups of islets were treated with linoleate in the absence *(Figure 2a)* or presence *(Figure 2b)* of L-glutamine.

Effect of linoleate prestimulus without or with glutamine on glucose-induced suppression of glucagon secretion (Figure 3)

Figure 3 shows that the normal suppressive effect of high glucose on glucagon secretion observed in islets exposed to 27.7 mmol/L glucose before linoleate treatment in the absence of glutamine was not seen after the islets had been treated with the fatty acid alone *(Figure 3a)*. However, when the linoleate perifusion was performed in the presence of glutamine, the suppressive effect of 27.7 mmol/L glucose on glucagon secretion was present before and after the fatty acid treatment *(Figure 3b)*.

Discussion

In the present study we have shown that a prestimulus of isolated perifused islets with the essential fatty acid linoleate alone can desensitize both pancreatic alpha and beta cells to glucose effect. In addition, our data show that this desensitization to glucose induced by linoleate can be completely blocked if L-glutamine is added to the fatty acid perifusate. Because previous studies suggested that the mechanism of PUFA-induced desensitization of pancreatic beta cells to glucose effect may be linked to fatty acid oxidation,[11,12] the blockade of this phenomenon by glutamine can be attributed to the inhibitory effect of this amino acid on fatty acid beta-oxidation,[20] although it could also be argued that the protective effect of glutamine results from a preferential utilization of the amino acid over that of the fatty acid. It is noteworthy that, although the desensitization was completely blocked by 20 mmol/L glutamine, the linoleate-induced stimulation of insulin release was only partially blocked. This observation supports our hypothesis that this fatty acid-stimulated insulin release occurs by multiple mechanisms, which includes fatty acid oxidation.[28] It is therefore possible that glutamine

blocks the pancreatic beta cell desensitization by inhibiting the stimulatory effect of linoleate that is due to the fatty acid oxidation. An alternative interpretation may be that glutamine blocks the release of a pool of insulin by linoleate, thus preserving it for subsequent release in response to glucose. It is to be noted that in a previous work we observed a stimulatory effect of L-arginine on insulin release,[12] in contrast to the inhibitory effect of L-glutamine seen in an earlier study.[31]

The antioxidant role of glutamine is generated via the glutaminase-mediated deamidation of this amino acid to glutamate and the subsequent incorporation of the latter into glutathione, a tripeptide consisting of glutamate, cysteine, and glycine. Indeed, we have recently shown that the substitution of L-glutamine with 3 mmol/L glutathione in the fatty acid perifusate also causes a blockade of the linoleate-induced desensitization to glucose.[32] These observations are consistent with a recent report that glutathione infusion enhances insulin secretion in elderly subjects with impaired glucose tolerance.[33] Glutathione is an abundant endogenous nucleophile whose depletion in an in vitro model such as ours may be rapid and consequently produce adverse effects on cellular processes, including detoxification of reactive electrophiles, amino acid transport, protein and DNA synthesis, protection of protein thiol groups essential for maintaining cellular integrity against oxidation, and the restoration of other free radical scavengers and antioxidants such as vitamins E and C to their reduced states.[34,35]

It is of interest that the apparent K_m for the bioeffect of glutamine on the mouse pancreatic beta cell in the present study was found to be about 2.5 mmol/L which is consistent with the K_m reported for the activity of glutaminase in the rat small intestine and liver mitochondria.[36,37] The similarity in the K_m for the bioactivity of L-glutamine in the pancreatic beta cell and other cell types, in contrast to pancreatic alpha cell, suggests that the primary islet cell of glutamine activity may be the beta cell, and that the stimulatory effect of high concentrations of this amino acid on glucagon secretion occurs as a counter-regulatory response to a potent suppressive effect on insulin secretion.[31] Although the concentrations of linoleate and L-glutamine used in these experiments are supraphysiologic, it seems quite appropriate in relating these observations to the TPN setting in which patients are infused with pharmacologic concentrations of nutrients.

It is to be noted that clinically available lipid emulsions are mainly composed of essential fatty acids, which are very rapidly oxidized in the presence of oxygen with the formation of peroxides and toxic free radicals whose deleterious effects are regulated by antioxidants such as vitamin E.[38] However, we are presently unaware of any study in which this PUFA-induced desensitization of the endocrine pancreas to glucose sensitivity has been reported to occur clinically, presumably owing to the abundance of antioxidant defense mechanisms in vivo. However, it has been shown in an experimental rat in vivo model that the PUFA-induced desensitization is dependent on the length of time of exposure of the pancreas to the fatty acids.[11] This phenomenon therefore remains a potential clinical risk in critically ill individuals on prolonged lipid-enriched TPN. In these individuals the baseline antioxidant defense mechanisms may be depleted prior to the TPN. It may be of benefit to such patients if their TPN regimen includes an antioxidant factor such as glutamine, which has also been extensively shown to play other efficacious role in both experimental and clinical TPN.[13–17,39,40] It has now been shown that glutamine-enriched parenteral solutions are safe for clinical use.[16]

In conclusion, the present study provides additional support for an efficacious role for glutamine in lipid-enriched total parenteral nutrition.

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