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Pancreatic islet insulin secretion and metabolism in adult rats malnourished during neonatal life

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Pancreatic islets were isolated from rats that had been nursed by dams fed with a control or an 8·7 % protein diet during the first 12 d of the lactation period. Glucose-induced insulin secretion from islets in the 8·7 % protein group was reduced 50 %. The islet insulin and DNA content were similar, whereas the pancreatic insulin content was reduced by 30 % in the rats fed 8·7 % protein. In order to elucidate the mechanism responsible for the attenuation of insulin secretion, measurements were performed of the activity of several islet enzymes that had previously been supposed to be involved in the coupling of glucose stimulation to insulin secretion. Islet glucose oxidation was unaffected, but glucose-stimulated hydrolysis of phosphatidylinositol was reduced by one-third in the islets of rats fed 8·7 % protein. The activity of mitochondrial glycerophosphate dehydrogenase was similar in islets of rats fed the 8·7 % protein diet and those fed the control diet. The activity of Ca-independent phospholipase A_2 was increased fourfold in the islets of rats fed 8·7 % protein. It is concluded that impairment of glucose-induced insulin secretion in rats fed a low-protein diet may be caused by attenuation of islet phosphatidylinositol hydrolysis, and it is tentatively suggested that the increased activity of Ca-independent phospholipase A_2 in islets of rats fed a low-protein diet may participate in the stimulation of apoptosis.

Protein malnutrition: Pancreatic islet metabolism: Insulin secretion: Phosphoinositides: Phospholipase A_2: Glycerophosphate dehydrogenase

Malnutrition-related glucose intolerance is commonly seen in developing countries (WHO, 1985) and it has been suggested that this may be due to malnutrition-induced changes in the pancreatic β-cells (Rao, 1988). Epidemiological studies have also pointed to a connection between low birth weights, presumably caused by protein or energy deficiency during intrauterine growth, and development of type-2 diabetes late in life (Hales *et al.* 1991). Animal studies aiming at testing this hypothesis have revealed an impaired insulin secretion from islets isolated from fully grown animals that had received a protein-deficient diet *in utero* and/or during early life (Swenne *et al.* 1988; Moura *et al.* 1996; Reis *et al.* 1997; Latorraca *et al.* 1998*b*; Wilson & Hughes, 1998), whereas glucoregulatory control in these animals seemed to be maintained unless the fully grown animals were further challenged by a high-fat diet (reviewed by Holness, 1999).

Studies of the mechanism of impaired insulin secretion are important, partly because they may enable the affected individuals to receive more efficient treatment, and partly because they may help to elucidate the pathogenesis of malnutrition-induced type-2 diabetes.

Accordingly, different protein-restriction regimes have been developed in animal models to investigate the metabolic derangements characterizing the glucose-intolerant state. These animal models differ both in the choice of when and for how long they are subjected to the protein-deficient diet and in their age at the time of the metabolic investigation. In many cases a protein-deficient diet is fed to the dams during pregnancy and/or lactation and the pups are studied in the fetal state (Dahri *et al.* 1991), after weaning (Latorraca *et al.* 1998*a*), or at approximately 12 weeks of age. In the last case, rats received either a normal diet (Desai, 1997; Latorraca *et al.* 1998*b*; Wilson & Hughes, 1998) or a protein-deficient diet (Sener *et al.* 1996) in the period from weaning until they were killed. In other cases the protein-deficient diet was given to the pups in a short (Swenne *et al.* 1987) or longer (Carneiro *et al.* 1995) period after weaning, and the animals were studied as adults.

The present study was performed in a rat model where the

Abbreviations: EGTA, ethylene glycol-bis (β-aminoethyl ether) N,N,N′, N′-tetra-acetic acid; IP, inositol monophosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate.

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dams were fed an 8·7 % protein diet *ad libitum* during the first 2 weeks of the lactation period and where the offspring were studied at the age of 80 d. Reduction of food protein content has previously been shown to cause deficient maternal food intake and milk yield (Sampson *et al.* 1986). The composition of the milk is somewhat changed compared with that of controls. The protein concentration is 10–15 % lower and the lipid concentration approximately 15 % higher (Pine *et al.* 1994). The increase in lipid content is, however, not able to compensate for the pups' energy loss due to a heavily reduced milk intake, and thus an ensuing decrease in weight gain of the litters is observed (Barbosa *et al.* 1999). This animal model therefore aims at reflecting protein–energy malnutrition-induced type-2 diabetes.

The aim of the present study was to characterize the metabolic changes in the pancreatic islets that are associated with the onset of malnutrition-induced diabetes. Especially we wanted to see whether the malnutrition-induced changes in insulin secretion could be explained by changes in known stimulus–secretion coupling systems in the islets. Among these we chose to measure glucose oxidation, mitochondrial glycerophosphate dehydrogenase, phosphoinositide metabolism and phospholipase A_2. It has long been known that glucose-induced insulin secretion is dependent on islet glucose metabolism through glycolysis or through the glycerophosphate shuttle, which results in an ATP-dependent closure of the K^+_{ATP} channels and further in depolarization and opening of the voltage dependent Ca^{2+} channels. The resulting influx of Ca^{2+} then triggers insulin secretion (Newgard & McGarry, 1995). However, glucose also regulates insulin secretion by other means that are not yet fully understood. Consequential to glucose metabolism in islets is a breakdown of islet polyphosphoinositides, with the formation of diacylglycerol and the inositol phosphates inositol monophosphate (IP), inositol 1,4-bisphosphate (IP_2) and inositol 1,4,5-trisphosphate (IP_3) (Fig. 1). IP_3 induces the release of Ca^{2+} from the endoplasmic reticulum and diacylglycerol may stimulate protein kinase C. Both of these events may result in stimulation of insulin secretion (Montague *et al.* 1985). This pathway may also be initiated by activation of muscarinic receptors and is supposed to be responsible for activation of insulin secretion by muscarinic cholinergic agents (Laychock, 1983). Islet cytosolic calcium-independent phospholipase A_2 may also be part of the K^+_{ATP} channel-independent pathway (Gross *et al.* 1993). Phospholipase A_2 catalyses the removal of arachidonic acid from the 2-position in phospholipids and the product arachidonic acid may act as a coupling factor in regulation of glucose-induced insulin secretion (Wolf *et al.* 1986; Metz *et al.* 1987).

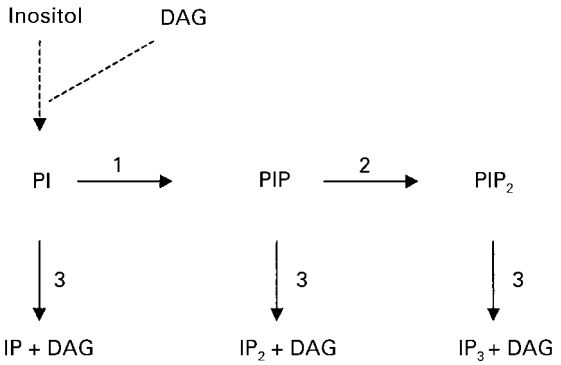

Fig. 1. Phosphoinositide metabolism. The numbered reactions are catalysed by (1) phosphatidylinositol 4-kinase, (2) phosphatidylinositol 4-phosphate 5-kinase and (3) phospholipase C. DAG, diacylglycerol; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate, IP, inositolmonophosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate.

Only a few of the previous investigations in protein-malnourished rats include studies of pancreatic islet metabolism and even fewer concentrate on the rat models that most directly reflect the impact of early protein–energy malnutrition on adult islet metabolism, i.e. the situation where the pups are malnourished only during fetal life or during lactation. In an early study Barbosa *et al.* (1993) demonstrated an abolition of the so-called phosphate flush in the islets, and Wilson & Hughes (1998) found unaltered islet glucose metabolism and presented evidence to suggest that the defect in insulin release may be due to defects in both the K^+_{ATP}-dependent and the K^+_{ATP}-independent secretory pathways. If the protein malnutrition is continued after weaning, further malfunctions may be seen. In the study by Carneiro *et al.* (1995) it was shown that Ca^{2+} handling in islets was affected, and Sener *et al.* (1996) found a reduction in the activity of the glycerol phosphate shuttle, confirming their previous finding of impaired activity of mitochondrial glycerophosphate dehydrogenase (Rasschaert *et al.* 1995); they also found a decrease in leucine transamination, whereas islet glucose oxidation was still unaltered.

The main finding of the present study was that attenuation of glucose-induced insulin secretion was associated with attenuation of glucose-induced phosphatidylinositol hydrolysis, whereas glucose oxidation and the activity of mitochondrial glycerol phosphate dehydrogenase was unaffected, and calcium-independent phospholipase A_2 increased.

Materials and methods

Materials

Crude bacterial collagenase as well as enzymes and co-enzymes used in the enzymic assays were obtained from Boehringer, Mannheim, Germany. Human serum albumin was from Behringswerke AG, Marburg, Germany. Bovine albumin and lipids were from Sigma Chemical Company, St Louis, MO, USA. [^{125}I]Insulin and guinea-pig anti-insulin serum were from Novo Nordisk A/S, Bagsværd, Denmark. [^{3}H-methyl]-diphosphatidylcholine, [U-^{14}C]-glucose and myo-[^{3}H]-inositol were from Amersham International, Amersham, Bucks, UK. The 8·7 % protein diet (Altromin C 1003) was from Chr. Petersen A/S, Ringsted, Denmark. The control diet was from Brogården, Gentofte, Denmark.

Animals

Pregnant Wistar rats were allowed free access to a

conventional control diet until delivery. During lactation the rats were divided into a control group receiving a conventional rat diet (22·5 % protein) *ad libitum* and a group receiving an 8·7 % protein diet *ad libitum* during the first 2 weeks of the lactation period, and thereafter the same diet as the control group during the rest of the lactation period. The number of pups in each group was adjusted to six. Since gender differences in insulin levels and glucose tolerance have been observed in some feeding experiments (Hales *et al.* 1996; Desai *et al.* 1997), it was decided to use only male offspring in the experiments. Accordingly, the female offspring were discarded after weaning, whereas the male offspring were weaned onto the control diet and were fed on this diet until the age of 81 d, when the experiments described below were carried out. The Danish ethical committee for animal experiments approved the described feeding schedule.

Islet isolation

Islets were isolated by the ductal tissue collagenase digestion method described by Van Suylichen *et al.* (1992).

Total islet and pancreatic insulin content

Total pancreatic insulin content was determined after extraction of the pancreas in acetic acid (Bonnevie-Nielsen, 1982), and islet insulin content was determined after extraction in acidified ethanol (Trimble & Renold, 1981).

Insulin release

Insulin release was measured both in batch-type incubations and in a perifusion system. In the batch-type incubations, five islets were incubated for 120 min in 600 μl Krebs buffer containing 0·2 % human serum albumin and 3·3 or 20 mM-glucose. The supernatants were used for determination of insulin. In the perifusion experiments, one islet was placed in each of six perifusion chambers (200 μl) filled with Bio-Gel P-2 (Bio-Rad Laboratories, Herts., UK). The islets were perifused with D-glucose (10 mmol/l; 350 μl/min) supplied by a pulsation-free syringe pump (WPI SP220i; World Precision Instruments, Sarasota, FL, USA). The first 30 min were without sampling and then for the next 35 min samples for insulin determination were taken every 0·5 min. The insulin was determined by radioimmunoassay.

Glucose oxidation

Glucose oxidation was determined as production of $^{14}CO_2$ from [U-^{14}C]-glucose (Thams *et al.* 1986).

Phosphoinositide metabolism

Islets were labelled for 24 h in TCM 199 (Medium 199; Life Technologies, Taastrup, Denmark) culture medium (1·26 mM Ca^{2+}, 5·5 mM glucose) supplemented with 40 μCi/ml [^{3}H]inositol. Islets were then washed once with TCM 199 medium containing 10 mM LiCl followed by two washes with Krebs–Ringer–Hepes medium supplemented with 10 mM LiCl. For determination of [^{3}H]inositol phosphate production, twenty islets were preincubated for 45 min at 37°C in 0·25 ml Krebs–Ringer–Hepes medium containing 3·3 mM-glucose and supplemented with 10 mM-LiCl before addition of 0·25 ml 3·3 or 30·1 mM-glucose or 200 μM-carbachol. Thus, both receptor-independent and muscarinic activation of the phosphoinositide turnover were studied. After incubation for 30 min, the reaction was stopped by addition of 0·5 ml ice-cold $CHCl_3$–methanol–HCl (200:100:1, v/v) and 0·1 ml 100 mM-EDTA. Further processing of the samples was performed as described previously (Thams *et al.* 1995).

Glycerophosphate dehydrogenase

Mitochondrial glycerophosphate dehydrogenase activity was measured essentially as described by MacDonald *et al.* (1996). Islet homogenate was prepared in a solution of 230 mM mannitol, 70 mM sucrose and 5 mM-Hepes pH 7·5 (two islets/μl). The reaction was carried out for 30 min at 37°C in 200 μl 50 mM Bicine buffer, pH 8·0, containing 1 mM-KCN, 4 mM-iodonitrotetrazolium violet, 50 mM-D,L-glycerol 3-phosphate and homogenate from forty islets. The reaction was stopped by addition of 1 ml ethyl acetate, and the ethyl acetate layer was separated from the aqueous layer by centrifugation for 3 min. The absorbance of the reduced iodonitrotetrazolium violet in the extract was measured at

Table 1. Body weight and characteristics of pancreatic islets isolated from 81-d-old rats fed a control or a protein-deficient diet

(Mean values with their standard errors)

	Control		8·7 % Protein diet		
	Mean	SE	Mean	SE	*n*
Body weight at day 14 (g)	31·3	0·6	27·0*	0·6	6
Body weight at day 81 (g)	359	5	336*	8	10
Pancreas weight (g)	1·37	0·06	1·19*	0·06	10
Pancreas weight/body weight (%)	0·36	0·05	0·38	0·05	10
DNA/islet (ng)	36·8	2·7	32·3	2·6	11
Insulin/pancreas (μg/g)	194	21	136*	8	10
Insulin/islet (ng)	55·9	6·8	48·8	7·1	8

Mean values were significantly different between groups: *$P<0·05$.

For experimental procedures, see p. 149.

490 nm. Blanks contained no substrate. Addition of NAD^+ to the assay mixture did not result in any further increase in absorbance, showing that KCN effectively prevented co-measurement of cytosolic glycerophosphate dehydrogenase.

Phospholipase A_2

Phospholipase A_2 catalyses the hydrolysis of phosphatidylcholine to lysophosphatidylcholine and fatty acid. However, since the enzyme also has lysophospholipase activity, lysophosphatidylcholine may be further hydrolysed to glycerophosphatidylcholine and fatty acid. Accordingly, the activity of phospholipase A_2 was measured as formation of ^{3}H-lysophosphatidylcholine and ^{3}H-glycerophosphatidylcholine from [^{3}H-choline]-phosphatidylcholine. An islet homogenate was prepared in 50 mM Tris buffer, pH 7·0, containing 1 mM ethylene glycol-bis (β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) and centrifuged at 100 000 ***g*** for 1 h. The supernatant was used for assay of cytosolic Ca-independent phospholipase A_2, as described previously (Capito *et al.* 1999).

DNA

DNA was measured by a fluorimetric method (Kissane & Robins, 1958).

Statistics

The results are given as means with their standard errors. Statistical comparison of the data was made by Student's unpaired *t* test or by the ANOVA followed by the Newman–Keuls test for multiple comparisons. Non-significant data: $P>0{\cdot}05$.

Results

Pancreas and body weight

The growth of the pups in the 8·7 % protein group was somewhat retarded compared to that of the control group (Table 1). At day 14, the last day with the low-protein diet, the mean body weight of the pups was 15 % lower than that of the control group. By the day of the metabolic experiments (day 81) the body weights of the animals in the 8·7 % protein group were still somewhat lower than those of the controls. The weight of the pancreas was reduced proportionally in the 8·7 % protein group, thus making up 0·4 % of the body weight in both groups. Judged from the DNA measurements, the number of cells per islet did not differ between the groups.

Insulin content of pancreas and islets

The insulin content of the pancreas was 30 % lower in the group fed 8·7 % protein than in the control group (Table 1). However, the insulin content per islet was similar in the two groups, suggesting that the pancreas in the group fed 8·7 % protein contained fewer β-cells.

Insulin release

Insulin release was measured both in batch-type incubations and in perifusion experiments, where the dynamics of the secretion were studied. In the batch-type experiments the insulin secretion from the islets of rats fed 8·7 % protein was considerably lower than from the control islets, both at 3·3 mM-glucose and at 20 mM glucose, although the difference at 3·3 mM-glucose did not reach statistical significance. At 3·3 mM-glucose the secretory rate was 0·29 (SE 0·09) pmol/h per islet and 0·84 (SE 0·32) pmol/h per

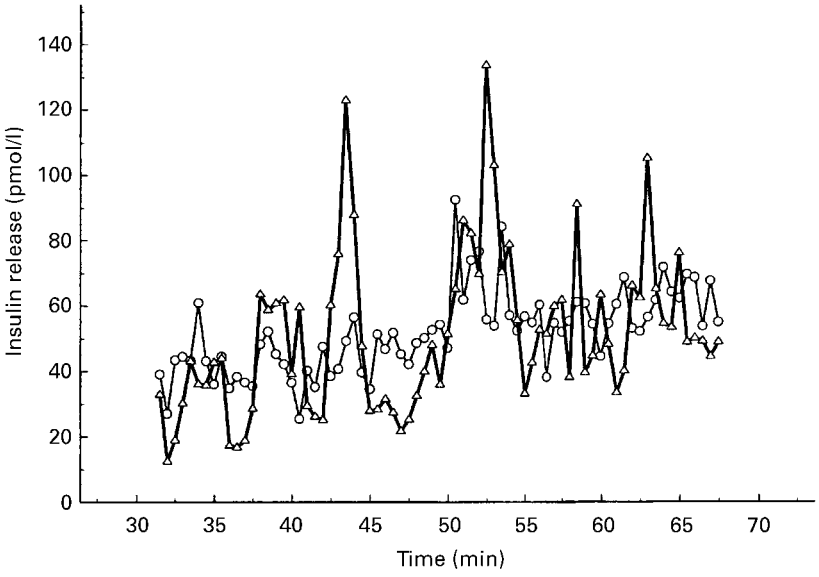

Fig. 2. Representative single islet perifusion of an islet isolated from a normal rat (△) or a rat fed an 8·7 % protein diet (○). Perifusion was carried out for 65 min with 10 mmol/l D-glucose. Sampling of insulin was performed every half-min for the last 35 min.

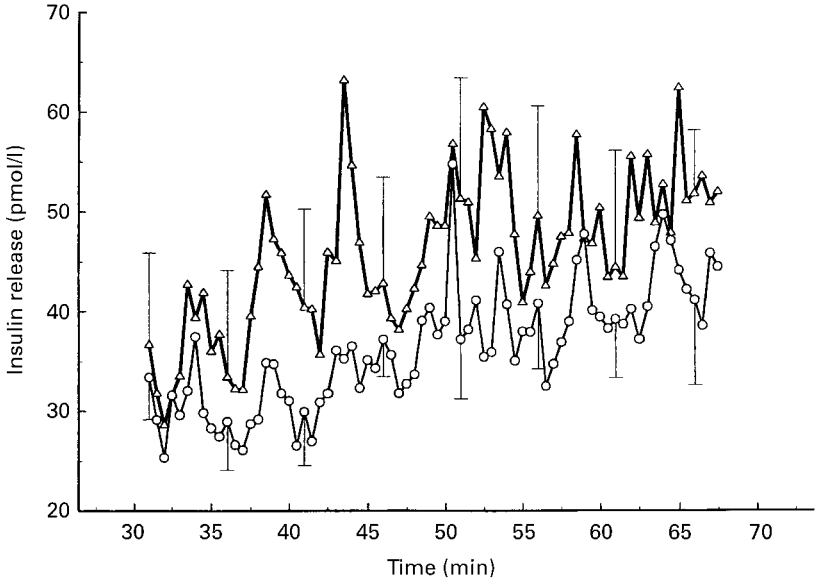

Fig. 3. Means of single islet perifusions of islets isolated from normal rats (△) (*n* 5) or rats fed an 8·7 % protein diet (○) (*n* 6). Perifusion was carried out for 65 min with 10 mmol/l D-glucose. Sampling of insulin was performed every half-min for the last 35 min. At 5 min intervals, points are means with their standard errors represented by vertical bars.

islet in the rats fed 8·7 % protein and the control rats respectively (*n* 8, NS). At 20 mM-glucose the secretory rate averaged 2·57(SE 0·19) pmol/h per islet and 4·72 (SE 0·53) pmol/h per islet respectively (*n* 6; $P<0·05$).

The dynamic insulin release pattern in response to 10 mmol/l D-glucose was oscillatory using either normal rat islets or islets from 8·7 % protein-fed rats in the perifusion set-up. Frequency (approximately one cycle every 4–5 min) appeared not to be changed by the low-protein diet (Figs 2 and 3), whereas the amplitude in the insulin response to D-glucose was smaller in islets isolated from the rats fed 8·7 % protein compared to islets isolated from control rats (Figs. 2 and 3). The oscillatory pattern seen in Fig. 2, which represents the mean of five to six single islet perifusions, indicates synchronism in the release of insulin from the single islets, isolated either from normal rats or those fed 8·7 % protein.

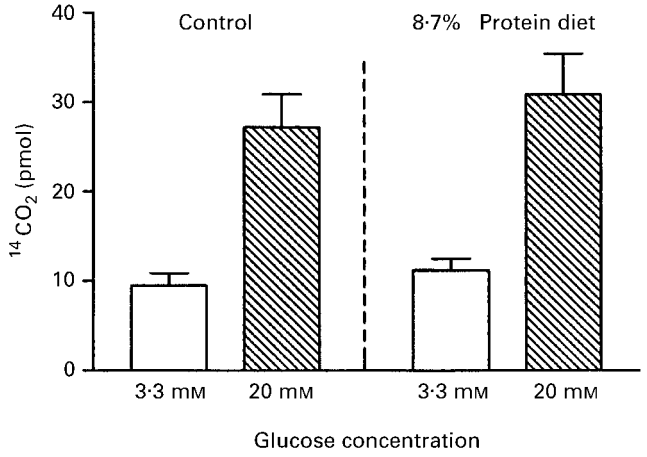

Fig. 4. Glucose oxidation in 2 h in islets from control rats, and those fed an 8·7 % protein diet. The glucose oxidation was measured as formation of $^{14}CO_2$ from U-^{14}C-glucose. The data from 30 batches of islets are means with their standard errors represented by vertical bars.

Glucose oxidation

The attenuation of insulin secretion in islets from rats fed 8·7 % protein was not caused by a reduction of glucose oxidation in the islets. The glucose oxidation measured as formation of $^{14}CO_2$ from [U-^{14}C]-glucose was increased threefold ($P<0·05$) when the glucose concentration was raised from 3·3 to 20 mM-glucose (Fig. 4). However, similar basal and stimulated values were obtained in the islets of control rats and those fed 8·7 % protein.

Mitochondrial glycerophosphate dehydrogenase

In attempting to explain the reduction in glucose-induced insulin secretion in the islets of rats fed 8·7 % protein, the activity of putative regulatory enzymes downstream of glucose oxidation was measured.

The activity of mitochondrial glycerophosphate dehydrogenase was measured in islet homogenates and was found to be identical in the two groups of islets, with a mean activity of 121 (SE 11) and 115 (SE 11) fmol/ng DNA/min in control rats and those fed 8·7 % protein respectively (*n* 6).

Calcium-independent phospholipase A_2

The presence of cytosolic Ca-independent phospholipase A_2 in islets has been demonstrated previously (Gross *et al.* 1993) and it has been suggested that arachidonic acid, which

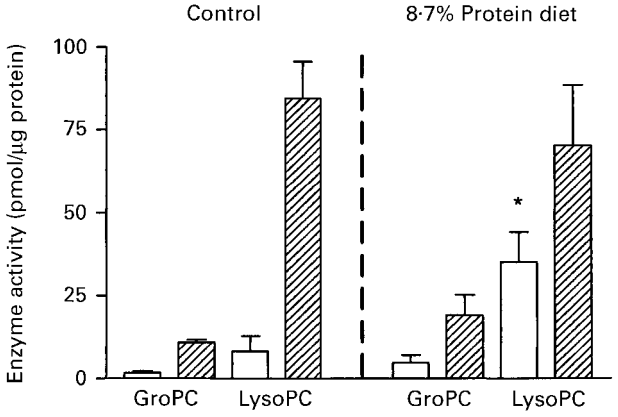

Fig. 5. Activity of cytosolic Ca-independent phospholipase A_2.The enzyme activity of control islets and those from rats fed an 8·7 % protein diet was measured over 2 h in a cytosolic fraction in the presence of 1 mM-ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetra-acetic acid (EGTA) (□) bars) or in the presence of 1 mM-EGTA and 5 μM-phosphatidylinositol biphosphate (▨). GroPC, glycerophosphatidylcholine; LysoPC, lysophosphatidylcholine. The data from seven to nine experiments are means with their standard errors represented by vertical bars. Significantly different from control diet: *$P<0·05$.

is liberated in the reaction, may be a putative coupling factor in islet β-cells. Since the enzyme also has lysophospholipase activity, the production of lysophosphatidylcholine as well as glycerophosphatidylcholine was measured in the present experiments. The enzyme was measured both at basal conditions, i.e. in the presence of EGTA, and in the presence of 5 μM-phosphatidylinositol bisphosphate, which we have previously shown is an efficient activator of the enzyme (Capito & Thams, 2000) (Fig. 5). The basal lysophosphatidylcholine production was increased fourfold in the islets of rats fed 8·7 % protein, whereas the phosphatidylinositol bisphosphate-stimulated activity was unchanged.

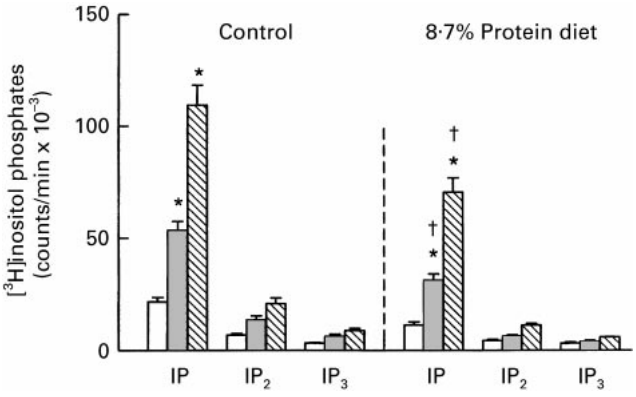

Fig. 6. Inositol phosphate production in 30 min in 20 islets from control rats and from rats fed an 8·7 % protein diet. Release of inositol monophosphate (IP), inositol 1,4-bisphosphate (IP_2) and inositol 1,4,5-trisphosphate (IP_3) in response to 3·3 mM-glucose (□), 16·7 mM-glucose (▧) and 100 μM carbachol (■) was determined in islets that had been pre-labelled for 24 h with ^{3}H-inositol. The data from six to nine experiments are means with their standard errors represented by vertical bars. Statistical evaluation of the data was performed by ANOVA followed by the Newman–Keuls test for multiple comparisons. Significantly different from 3·3 mM-glucose: *$P<0·05$; significantly different from control islets: †$P<0·05$.

Phosphoinositide metabolism

The islet phosphoinositides were labelled endogenously with ^{3}H-inositol, whereafter the islets were stimulated with glucose or carbachol. The labelling level obtained in the islets of control rats and those fed 8·7 % protein was not statistically different (data not shown). In the presence of 16·7 mM-glucose and 100 μM-carbachol the IP production was significantly stimulated in both control islets and those from rats fed 8·7 % protein (Fig. 6). The basal IP production seen in the presence of 3·3 mM-glucose was similar in the control islets and those from rats fed 8·7 % protein. However, the IP production rate in the islets from rats fed the low-protein diet in the presence of 16·7 mM-glucose or 100 μM-carbachol averaged only 65 % of that seen in the control islets.

Discussion

The present study was designed to investigate whether defects in proposed stimulus–secretion coupling systems in the pancreatic islets were associated with the attenuation of glucose-induced insulin secretion that is observed in adult rats that have been protein-malnourished during early life.

In experimental models where the animal is subjected to perinatal protein malnutrition, the development of the endocrine pancreas is severely compromised and a reduction of islet size, islet vascularization and pancreatic insulin content is seen at the time of birth (Dahri *et al.* 1991; Petrik *et al.* 1999). However, the amount of insulin per islet is unaffected (Cherif *et al.* 1998). The reduction in β-cell mass and pancreatic insulin content persists until adulthood (Bertin *et al.* 1999), signalling a change in the balance between β-cell replication and apoptosis (Garafano *et al.* 1999; Petrik *et al.* 1999).

In the present experiments the protein-restricted diet was fed to the dams during the first 14 d of the lactation period. This relatively short period of moderate protein malnutrition introduced disturbances in pancreas development, since the pancreatic insulin content in the adult rats was only two-thirds that found in the control rats.

As expected, the glucose-induced insulin secretion from the rats fed 8·7 % protein was impaired. In batch-type incubations the secretory rate in the presence of 20 mM-glucose was only 50 % of that in the control islets. This was not due to lower insulin content or a diminished number of cells in the islets, since these parameters were not affected by the protein malnutrition. Analogous data on insulin content have been found in similar animal models (Rasschaert *et al.* 1995; Wilson & Hughes, 1998). The dynamics of the secretory response, on the other hand, were not affected by the protein restriction. The oscillatory frequency of insulin secretion determined in the perifusion set-up was similar in islets of control rats and those fed 8·7 % protein.

Feeding-induced impairment of glucose-induced insulin secretion is in many cases positively correlated with

reductions in the islet glucose oxidation rate (Hedeskov & Capito, 1974; Capito *et al.* 1992). However, in agreement with previous data from islets isolated from protein malnourished animals (Sener *et al.* 1996; Wilson & Hughes, 1998), the islet CO_2 production from glucose was not affected by the protein-restricted diet in the present experiments.

Disturbances in phosphoinositide turnover have previously been found to be associated with the diabetic state in islets from neonatally streptozotocin-injected rats (Morin *et al.* 1996). We therefore tested the possibility that this pathway may be affected in the islets of rats fed 8·7 % protein. As we have previously found in mouse islets (Thams *et al.* 1995), glucose primarily stimulated the breakdown of phosphatidylinositol in control islets, while the stimulation of IP_2 and IP_3 formation did not reach statistical significance. The same pattern was observed in the presence of carbachol. In the islets of rats fed 8·7 % protein, however, a significant impairment of phosphatidylinositol hydrolysis was observed. Although the basal IP production in the presence of 3·3 mM-glucose was similar in control islets and those from protein-malnourished rats, both the glucose- and carbachol-stimulated phosphatidylinositol hydrolysis was reduced by one-third. Since glucose-induced hydrolysis of islet polyphosphoinositides is believed to occur secondarily to glucose-induced Ca^{2+} influx (Prentki & Matschinsky, 1987), the observed attenuation of polyphosphoinositide metabolism might be caused by impairment of the Ca^{2+} handling in the islets. This would be in agreement with the findings of Carneiro *et al.* (1995) in islets from long-term protein-malnourished rats. On the other hand, since carbachol-induced phosphatidylinositol breakdown was also reduced in the islets of rats fed 8·7 % protein, and since carbachol is known to stimulate phospholipase C in a receptor-mediated fashion, it is more likely that the enzyme itself is affected by the protein malnutrition.

Signals from glucose metabolism may also be transmitted via the glycerol phosphate shuttle, and in some diabetic animal models the activity of mitochondrial glycerophosphate dehydrogenase is reduced (Sener *et al.* 1993; Fabregat *et al.* 1996; MacDonald *et al.* 1996). In islets from adult rats that had been fed with a protein-deficient diet from conception to adulthood, a similar reduction in the activity of the glycerol phosphate shuttle and of the mitochondrial glycerolphosphate dehydrogenase was found (Rasschaert *et al.* 1995; Sener *et al.* 1996). However, if the administration of the low-protein diet was restricted to the gestational period, no change in the activity of mitochondrial glycerophosphate dehydrogenase was observed (Rasschaert *et al.* 1995), which is in agreement with the findings in the present experiments, where we have used a similar moderate protein-restriction regime.

It is generally agreed that glucose stimulates insulin secretion by both a K^+_{ATP} channel-dependent and a K^+_{ATP} channel-independent pathway. Based on experiments in islets from adult rats where a 5 % protein diet was given to the dams during the gestational and lactation period, Wilson & Hughes (1998) suggested that both of these glucose-regulated pathways were affected. The exact nature of the K^+_{ATP} channel-independent pathway has not yet been elucidated, but it is believed that a rise in islet ATP:ADP is implicated in stimulation of both pathways (Sato & Henquin, 1998). Islet cytosolic Ca-independent phospholipase A_2 may be a possible target for ATP in the K^+_{ATP} channel-independent pathway (Gross *et al.* 1993). Since a reduction in cytosolic Ca-independent phospholipase A_2 might thus theoretically explain the impairment of glucose-induced insulin secretion in the islets of rats fed 8·7 % protein, we measured the activity of cytosolic Ca-independent phospholipase A_2 in cytosolic fractions of the islets of these rats. In contrast to what could be expected from the theoretical considerations earlier, we found that the activity of this enzyme was increased in the islets. It has recently been shown in cardiac myocytes (Isenović & LaPointe, 2000) that lysophosphatidylcholine produced by Ca-independent phospholipase A_2 stimulated the synthesis of inducible nitric oxide synthase, which again may participate in stimulation of islet apoptosis (Sjöholm, 1998). Although this hypothesis warrants closer investigation, the possibility exists that the observed increase in the activity of islet Ca-independent phospholipase A_2 may be functionally coupled with the observed increase in apoptosis in protein-malnourished rats (Garafano *et al.* 1999; Petrik *et al.* 1999).

In conclusion, the present data have demonstrated that the insulin secretory defects seen in adulthood in rats that had been protein malnourished for a short period early in life were correlated with, and may have been caused by, changes in islet metabolism. Particularly, we have found that the glucose-induced hydrolysis of polyphosphoinositides was impaired by the protein malnutrition. Furthermore, our data point to the possibility that increased activity of islet cytosolic Ca-independent phospholipase A_2 may participate in the accelerated apoptosis observed in islets from protein-malnourished rats.

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