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EFFECT OF GLUCOSE ON PROTEIN PHOSPHORYLATION IN RAT PANCREATIC ISLETS

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Summary

Isolated rat pancreatic islets, incubated in the presence of extracellular ^{32}Pi to steady state ^{32}P incorporation into cellular phosphopeptides, were exposed to glucose for 10 min. Glucose (16.7 mM) significantly stimulated the phosphorylation of six phosphoproteins with molecular weights of 15,000, 35,000, 49,000, 64,000, 93,000 and 138,000. Mannoheptulose (16.7 mM) markedly inhibited glucose-stimulated phosphorylation of these six phosphoproteins. This protein phosphorylation might be important in mediating glucose-stimulated insulin release.

Introduction

Insulin secretion is stimulated by many substances of which glucose is the major stimulator. The mechanism of the stimulation is not known. Whether this is the glucose molecule itself acting on a membrane receptor or the result of intracellular glucose metabolism is still unresolved (1-2).

Phosphorylation of proteins has been shown to be an important regulatory mechanism in metabolic pathways (3-5). If these protein phosphorylations do reflect an intermediary step of general importance in substances actions, such a phenomenon might be demonstrable in pancreatic islets in response to glucose. For this reason, we investigated the effect of glucose on the phosphorylation of intact rat pancreatic islets.

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Materials and Methods

Islets were isolated from the pancreas of male Wistar rats (250-300g) fed ad libitum according to the method of Lacy & Kostianovsky (6) with a slight modification (7). $KH_2{}^{32}PO_4$ was added to a phosphate-free Krebs-Ringer bicarbonate solution, yielding an inorganic phosphate of approximately 0.4 mM and a radiochemical concentration of approximately 200 µCi/ml.

About 40 islets were incubated in 1 ml of the above medium with 5 mM glucose for 120 min at 37°C in an atmosphere of 95% O_2-5% CO_2 with gentle shaking. Glucose was added to a final concentration of 5 mM or 16.7 mM and the incubation was continued for an additional 10 min. Mannoheptulose was also added to the medium as required. At the end of the incubation, the medium was rapidly removed from the islets. The labeled islets were washed once with phosphate-free Krebs-Ringer bicarbonate solution. The islets were dissolved in 0.2 ml of 20 mM Tris-acetate buffer (pH 7.4), containing 10 mM sodium acetate, 1 mM EDTA, 3% SDS and 2.5% mercaptoethanol, according to the method of Rubin (8) and were incubated at 37°C for 15 min. Then, the mixture was boiled for 3 min. After the treatment of the mixture with 90% acetone at -20°C for 15 hours, the sediment was again dissolved in 50 µl of the avobe Tris-acetate buffer and boiled for 1 min. SDS-polyacrylamide gel electrophoresis was performed by the method of Fairbanks et al. (9) with a slight modification (10). Samples containing 40-60 µg islet protein, tracking dye (bromophenol blue) and 10% sucrose were subjected to electrophoresis in 0.1% SDS/ 5.6% polyacrylamide gel. The gels were fixed, stained with Coomassie blue and destained. The gels were scanned at 620 nm with a spectrophotometer. Molecular weights were determined by calibration of the gels with polypeptides of known molecular weights (cytochrome c, ovalbumin, bovine serum albumin and RNA-polymerase). The radioactivity incorporated into protein was determined by slicing the gels into 2 mm segments, placing the segments in vials with 5 ml H_2O, and determining the Cerenkov radiation in a liquid scintilation counter according to the method of Rubin & Rosen (11). The amount of insulin in the medium were determined by a double antibody radioimmunoassay of Morgan & Lazarow (12). Protein was measured by the method of Lowry et al. (13).

$KH_2{}^{32}PO_4$ (0.5 Ci/ mmol) were obtained from New England Nuclear Corp. Collagenase (class 4) was purchased from Worthington Biochemical Corp. Cytochrome c was purchased from Sigma Chemical Comp. RNA-polymerase (from E. coli) was obtained from Boehringer Mannheim Comp. All other reagents were of analytical grade.

Results and Discussion

As shown in Figure 1, between molecular weights of 12,000 and 250,000 the rat pancreatic islets showed a consistent pattern of approximately 30 bands of polypeptides.

The incorporation of ^{32}P into selected peptides increased steadily to the preincubation time of 120 min and was essentially unchanged between the 2nd and 3rd hour (Fig. 2). This pattern is seen with all other phosphopeptides in the islets. In this experiment the amount of insulin in the medium was 26 µu/islet/30 min, 73 µu/islet/60 min, 90 µu/islet/90 min, 123 µu/islet/120 min and 192 µu/islet/180 min, respectively. The incorporated

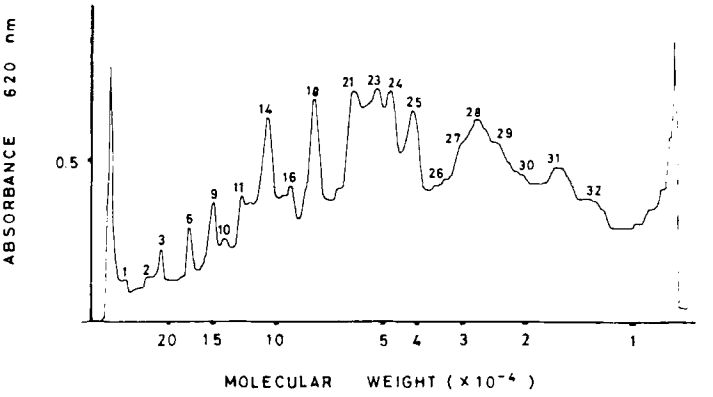

Figure 1: SDS-polyacrylamide gel scan of rat pancreatic islets.

Gel contained 40 µg of protein, and other details were the same as described in Materials and Methods.

^{32}P was alkali-labile, but was stable to acid or hydroxylamine treatment (data not shown). All subsequent experiments were performed with a pre-incubation time of 120 min.

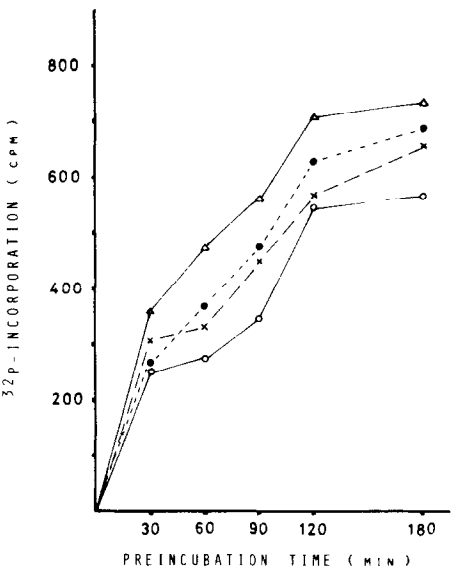

Figure 2: ^{32}P incorporation into selected phosphopeptides in rat pancreatic islets.

Rat pancreatic islets were incubated with $KH_2{}^{32}PO_4$ for 30, 60, 90, 120 and 180 min. Other details were the same as described in Materials and Methods. Data are from a representative experiment.
○ , mol wt 207,000; △ , mol wt 127,000; × , mol wt 35,000; ● , mol wt 15,000.

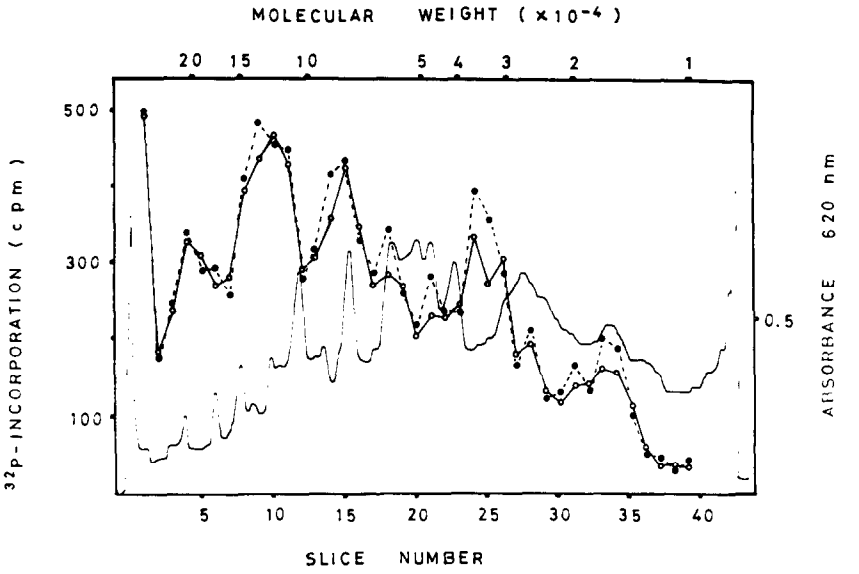

Figure 3: Effect of glucose on peptide phosphorylation in rat pancreatic islets.

Rat pancreatic islets were incubated with $KH_2{}^{32}PO_4$ for 120 min and glucose was added to a final concentration of 5 mM or 16.7 mM and the incubation was continued for an additional 10 min. Other details were the same as described in Materials and Methods. The solid line shows the gel scan at 620 nm. Data are from a representative experiment.
○ , 5 mM glucose; ● , 16.7 mM glucose.

Figure 3 illustrates the pattern of phosphorylation of control islets with 5 mM glucose and the effect of incubation with 16.7 mM glucose for 10 min. Rat pancreatic islets revealed approximately 15 phosphopeptides. Glucose stimulated significantly phosphorylation of six proteins with molecular weights of 15,000, 35,000, 49,000, 64,000, 93,000 and 138,000 (Table 1). In these experiments glucose (16.7 mM) stimulated insulin release by 73 ± 12% (n = 6). Mannoheptulose inhibited significantly the glucose-stimulated phosphorylation of these six proteins (Table 1 and Fig. 4). In these experiments mannoheptulose also markedly inhibited the glucose-stimulated insulin release (data not shown). In comparison with the control (5 mM glucose) 16.7 mM mannoheptulose plus 5 mM glucose did not show significant inhibition both in phosphorylation of protein and in insulin release (data not shown).

Table 1: Effects of glucose on peptide phosphorylation and mannoheptulose on glucose-stimulated phosphorylation in rat pancreatic islets.

Peptide molecular Weight X 10^{-3}	% increase in phosphorylation ($p < 0.05 - < 0.001$)	
	16.7 mM glucose (n = 6)	16.7 mM glucose plus 16.7 mM mannoheptulose (n = 3)
207	n.s.	n.s.
150	n.s.	n.s.
138	9 ± 2	-12 ± 4
127	n.s.	n.s.
107	n.s.	n.s.
93	16 ± 3	-18 ± 3
82	n.s.	n.s.
64	20 ± 3	-23 ± 4
53	n.s.	n.s.
49	20 ± 2	-16 ± 3
43	n.s.	n.s.
35	18 ± 3	-23 ± 3
32	n.s.	n.s.
27	n.s.	n.s.
21	n.s.	n.s.
15	22 ± 2	-14 ± 2

Rat pancreatic islets were incubated with $KH_2{}^{32}PO_4$ for 120 min and 5 mM or 16.7 mM glucose was added to the medium, or 16.7 mM glucose or 16.7 mM glucose plus 16.7 mM mannoheptulose was added to the medium and the incubation was continued for additional 10 min. The effect of glucose (16.7 mM) was compared with control (5 mM glucose), while the effect of glucose (16.7 mM) plus mannoheptulose (16.7 mM) was done with glucose (16.7 mM) alone. The number of individual experiments yielding such paired comparisons is given in parentheses. Values are given as means ± S.E.

n.s., not significant.

An explanation for these results of phosphorylation of rat pancreatic islets is not apparent. It has been reported that glucose (16.7 mM) induced a slight but significant increase in cAMP concentration in rat pancreatic islets incubated for 10 min (7). And so these enhanced phosphorylation caused by glucose may be partly mediated by cAMP-dependent protein kinase in rat pancreatic islets. It has been already reported that pancreatic islets have the cAMP-dependent protein kinase (14-15).

Mannoheptulose competitively inhibits glucose phosphorylation in pancreatic islets, and has a potent inhibitory action on glucose-induced insulin

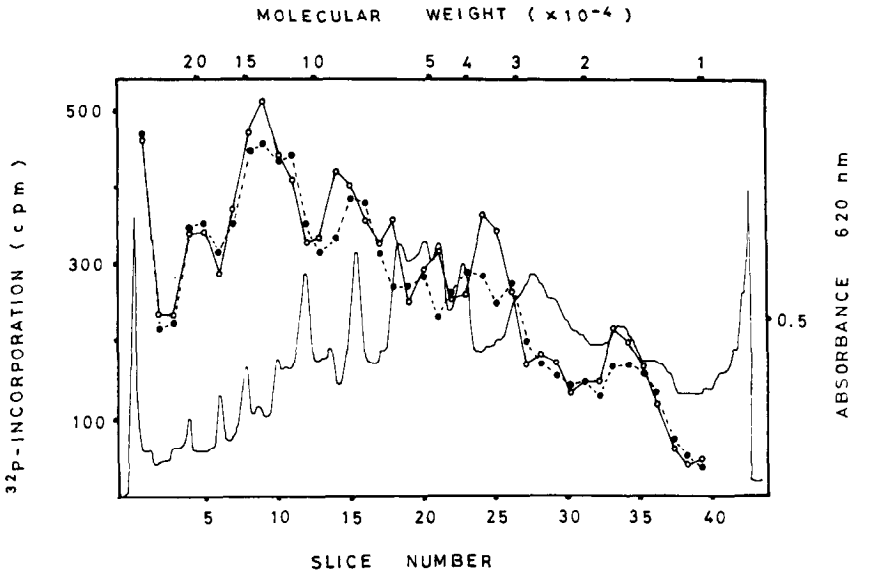

Figure 4: Effect of Mannoheptulose on glucose-stimulated phosphorylation in rat pancreatic islets.

Rat pancreatic islets were incubated with $KH_2{}^{32}PO_4$ for 120 min and glucose (16.7 mM) or glucose (16.7 mM) plus mannoheptulose (16.7 mM) was added to the medium and the incubation was continued for an additional 10 min. Other details were the same as described in Materials and Methods. The solid line shows the gel scan at 620 nm. Data are from a representative experiment.
o , 16.7 mM glucose; • , 16.7 mM glucose plus 16.7 mM mannoheptulose.

release (16-18). It has been reported that mannoheptulose inhibited glucose-induced increase in cAMP concentration in the rat pancreatic ielets (19). The present experiments showed that mannoheptulose also inhibited glucose-stimulated phosphorylation of protein in the islets. The mechanism of this inhibition by mannoheptulose is not known. If the existence of glucoreceptor is able to be supposed on or in the islets, mannoheptulose might competitively bind with glucoreceptor and block the effect of glucose.

In summary, we have demonstrated that glucose stimulated the phosphorelation of six proteins in intact rat pancreatic islets, and this was inhibited significantly by mannoheptulose. This glucose-stimulated phosphorylation of proteins might be important in mediating the glucose-stimulated insulin release. The phosphorylation appears to be mediated by cAMP-

dependent protein kinase, but it is impossible to deny that the phosphorylation may be mediated by cAMP-independent protein kinases.

Further studies will be necessary to characterize the specificity of the phosphorylation of these proteins, to identify their subcellular distribution, and to identify their role in the response to glucose.

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