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Incorporation of (U-^{14}C)-Glucose into Glycogen in Normal Rat Pancreatic Islets

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The incorporation of glucose into glycogen was determined in pancreatic islets isolated from normal rats and incubated with glucose (5 or 20 mM) and compounds known to affect glycogen metabolism in other tissues. Incubation of pancreatic islets with glucose (20 mM) induced a marked increase in radioactive glycogen. Exposure to epinephrine in the presence of glucose (20 mM) slightly increased incorporation of glucose into glycogen. In contrast the incorporation of glucose into glycogen was not affected when isolated islets were exposed to glucagon or insulin, whereas anti-insulin serum in the incubation medium decreased radioactive glycogen formation.

Key words: Pancreatics islets, Glycogen, Epinephrine.

It has been known for a long time that deposition of glycogen is a major cause of the vacuolated appearance of the B cells seen in the early phase after subtotal pancreatectomy (5, 13-16, 20, 23, 24). Subsequent studies have shown that glycogen is a normal constituent of the pancreatic islets (7, 18) which are enzymatically equipped for the formation and degradation of glycogen (4). The significance of glycogen in pancreatic B cells is, however, unsettled. The present investigation was carried out to study mechanisms involved in the incorporation of glucose into glycogen in pancreatic cells.

Materials and Methods

Reagents. Collagenase type IV was from Worthington Biochemical Co.

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(Freehold, New Jersey); crystalline bovine albumin, epinephrine and D-mannoheptulose were from Sigma; D-(+)-glucose was from Merck; (U-^{14}C)-glucose (specific activity 291 mC/μmol) was from the Radiochemical Centre; glucagon and insulin were gifts from NOVO Industry (Novofarma, Madrid); anti-insulin serum was from Miles Laboratories Ltd. (Slough). All reagents were of analytical grade.

Incubation medium. Pancreatic islets were obtained from fed male Wistar rats (200-250 g) as previously described (12). The incubation medium was Krebs-Ringer bicarbonate buffer (22) pH 7.4 containing 1 mg/ml crystalline bovine albumin. To this, (U-^{14}C)-glucose and when necessary unlabelled glucose was added. The substance under study was added at the concentration described in Results to one portion of the incubation medium and the remainder served as control incubation medium.

Experimental conditions. Islets in batches of 40 were preincubated in a metabolic shaker for 30 min in 0.5 ml of medium containing glucose 3.2 mM. At the end of the preincubation period, the medium was removed and the islets incubated for 30, 60, 90 or 120 min in 0.5 ml of buffer containing (U-^{14}C)-glucose (final specific activity 0.2 μC/μmol) and as required the substance under study. At least six series of experiments were performed at each experimental condition.

Glycogen determination. At the end of each incubation period, the medium was carefully removed and the islets were homogenized (20 strokes, 1,500 rpm) in 0.03N HCl (125 μl) and were prepared as described by PASSONNEAU and LAUDERDALE (19). Aliquots (100 μl) of the homogenate were spread evenly on pieces of filter paper (Whatman 31 ET chromatography paper 2 × 2 cm). The filter paper, which completely absorbed the sample within 1-2 s, was dropped immediately into a beaker containing a minimum of 7 ml cold 66 % (v/v) ethanol for each paper and stirred with a teflon-covered stirring rod. The rod was kept from physical contact with the papers by a stainless-steel screen. The papers were subsequently washed three times for 40 min in 66 % ethanol. They were then briefly rinsed (5 min) with acetone and dried under a heat lamp, until the odor of acetone was no longer present. Filter papers were placed in glass scintillation vials containing 10 ml of 0.6 % (w/v) 2,5-diphenyloxazole (PPO) and 0.02 % (w/v) 1,4-bis-[5-phenyl-2-oxazolyl])-benzene (POPOP) in toluene and then were counted.

Results

Effect of glucose. Figure 1 shows the incorporation of glucose ^{14}C into glyco-

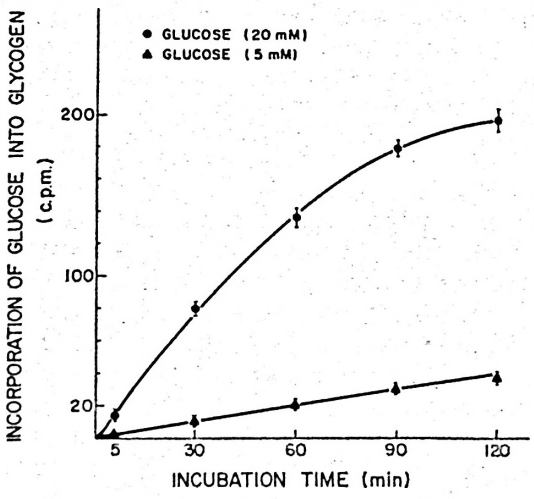

Fig. 1. *Incorporation of (U-^{14}C)-glucose into glycogen by isolated rat islets incubated for 30, 60, 90 and 120 minutes in different concentration of glucose.* Results are means ± S.E.M. of 6-8 experiments.

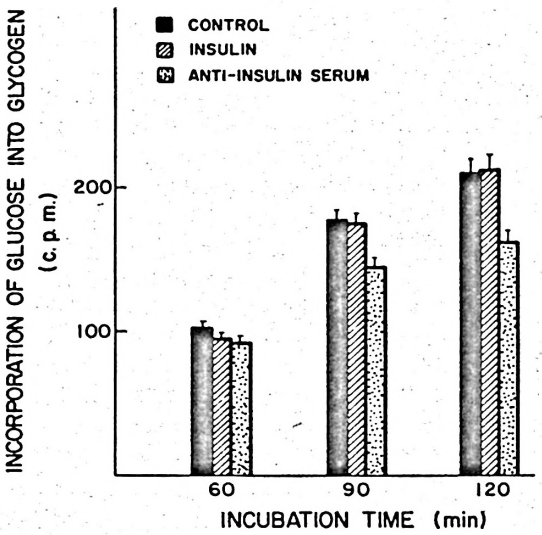

Fig. 2. *Effects on the incorporation of (U-^{14}C)-glucose into glycogen of exposing isolated rat islets to exogenous insulin (1.25 mU/ml) and to an insulin-free medium by adding anti-insulin serum, in the presence of glucose 20 mM.*
Results are means ± of 6-8 experiments.

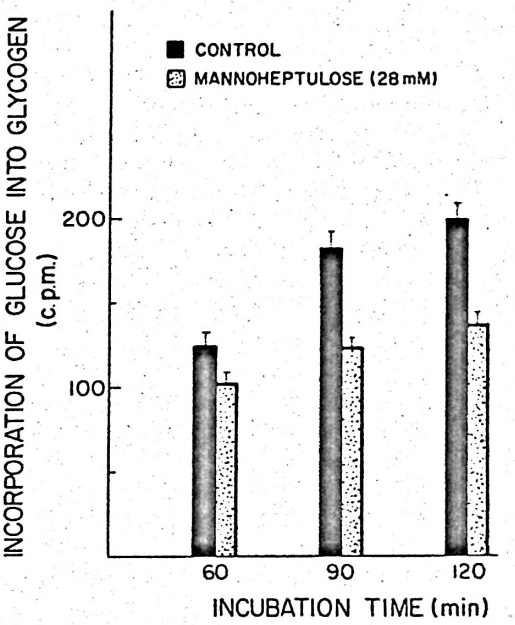

Fig. 3. *Effect of D-mannoheptulose on the incorporation (U-^{14}C)-glucose into glycogen by isolated rat islets incubated in the presence of glucose 20 mM.*
Results are means ± S.E.M. of 6-8 experiments.

gen in isolated islets exposed to glucose. At a high glucose concentration (20 mM), there was a nearly linear increase in radioactive glycogen after a short time lag. At the low glucose concentration (5 mM), there was also an increase in radioactive glycogen similar in pattern to that seen for high glucose concentration, but with much lower incorporation of glucose ^{14}C into glycogen.

Effect of glucagon and epinephrine. Table I shows the effects of glucagon (15 μg/ml) in the presence of glucose 20 mM on glucose incorporation into glycogen in isolated islets. Glucagon had no significant effect on glucose incorporation, whereas epinephrine (2 μg/ml) increased incorporation slightly during the first 90 min of incubation and markedly at 120 min.

Table I. *Effect of Glucagon and Epinephrine upon the incorporation of (U-^{14}C)-glucose into glycogen by isolated islets of Langerhans incubated in the presence of glucose 20 mM (c.p.m.).*
Results are means ± S.E.M. of 6-8 experiments.

	INCUBATION TIME (MIN.)			
	30	60	90	120
Glucagon	77 ± 8	114 ± 10	165 ± 15	180 ± 18
Control	85 ± 8	110 ± 7	174 ± 17	190 ± 19
Epinephrine	96 ± 5	138 ± 8	194 ± 16	257 ± 23
Control	80 ± 7	115 ± 7	164 ± 13	196 ± 18

Effect of insulin. Bovine insulin added to the incubation medium at the concentration of 1.25 mU/ml did not affect glucose incorporation into glycogen in isolated rat islets (fig. 2). When the effect of endogenous insulin secreted by B cells, in response to glucose 20 mM, was neutralised by adding anti-insulin serum to the incubation medium, the incorporation of glucose into glycogen decreased in respect to the control islets.

Effect of D-mannoheptulose. Islets were preincubated in D-mannoheptulose (14 mM) for 30 min before changing the incubation medium and were then incubated with either glucose or glucose plus D-mannoheptulose (28 mM). (fig. 3) D-Mannoheptulose reduced the incorporation of glucose into glycogen by approximately 30 %.

Discussion

The aim of this work was to determine whether isolated rat pancreatic islets incorporate glucose into glycogen and to investigate the effects of substances known to influence glycogen metabolism in other tissues. The method used was based on the fact that radioactive glucosyl moieties incorporated into polysaccharide are precipitated by aqueous ethanol on filter paper and can be separated from small molecular weight material such as glucose which is soluble in the aqueous phase. In addition, it has the advantages of being rapid, of having readily available substrates, and of requiring only small amounts of material.

The present findings show that at high glucose concentration, pancreatic islets are capable of converting glucose into glycogen, at least in rats, and that extracellular glucose concentration plays an important role in the regulation of glucose incorporation into glycogen in pancreatic islets. Thus, B-cells were able to accumulate and maintain large glycogen stores in the presence of glucose at a concentration of 20 mM, whereas glycogen stores were markedly diminished at low glucose levels. These findings indicate a close relation between glycogen stores of B cells and glucose concentration to which they are exposed, in agreement with previous reports (1.7). It is noteworthy that the pattern of glucose incorporation in B cells is very similar to that seen in other tissues (17, 21).

Glucagon failed to stimulate glycogenolysis in the pancreatic islets (7, 10). It is apparent from the present data that glucagon tested at a glucose concentration of 20 mM had no effect upon the incorporation of glucose into glycogen. The lack of effect of glucagon on incorporation of glucose into glycogen was probably unrelated to effects of increased insulin secretion, since exogenous insulin added to the incubation medium failed to stimulate glucose incorporation in respect to the control islets. Nevertheless, when insulin secreted by the islets in response to high glucose concentration was suppressed of the incubation medium by adding anti-insulin serum, the incorporation of glucose into glycogen slightly decreased. Therefore, the notion that insulin might play a role in glycogen metabolism in B cells cannot be ruled out at present.

Epinephrine increased the incorporation of glucose into glycogen in the present study. It is therefore noteworthy that epinephrine inhibits the synthesis of cyclic-3', 5'-AMP in pancreatic islets (11) and interferes with glucose metabolism below fructose-1,6-diphosphate, resulting in accumulation of early glycolytic intermediates including glucose-6-phosphate (9). D-Mannoheptulose, which also inhibits the synthesis of cyclic-3', 5'-AMP (6), but suppresses the increase in glucose-6-phosphate in islets (2), diminished the incorporation of glucose into glycogen in isolated rat islets in the

present study. Taken together, the findings may support the notion that glucose-6-phosphate might be important in the control of glycogen level in B cells.

Acknowledgements

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Resumen

Se estudia la incorporación de glucosa a glucógeno en islotes aislados de ratas normales. Para ello se ha investigado el efecto que sobre esta incorporación ejercían sustancias que se sabe influyen en el metabolismo del glucógeno en otros tejidos.

Los resultados obtenidos indican que, en presencia de altas concentraciones de glucosa en el medio extracelular, los islotes pancreáticos incorporan glucosa a glucógeno, existiendo una estrecha relación entre el glucógeno almacenado en las células B y la concentración de glucosa a la que éstas hayan sido expuestas. La epinefrina, en presencia de altas concentraciones de glucosa (20 mM), aumenta ligeramente la incorporación de glucosa a glucógeno. La incorporación no se vió afectada cuando los islotes fueron expuestos, en presencia de glucosa 20 mM, a glucagón o insulina exógena. La supresión en el medio de incubación, de la insulina segregada por los islotes en respuesta a glucosa 20 mM mediante la adición al medio de cantidades suficientes de suero anti-insulina, disminuye la formación de glucógeno radiactivo, por lo que de los presentes resultados no se puede excluir que la insulina juegue algún papel en la regulación del metabolismo del glucógeno en los islotes pancreáticos.

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