Protection of the Pancreatic β -Cell Glucoreceptor Mechanism for Insulin Secretion during Culture in Chemically Defined Medium

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High concentrations of glucose have a protective effect on the glucoreceptor mechanism for insulin secretion during culture of pancreatic islets in chemically defined media. To study at what level glucose exerts this effect, insulin secretion from β -cell-rich mouse pancreatic islets was measured before and after culture for 1 week in the presence of different substances. Before culture, glucose and inosine were potent stimulators, mannose and fructose were less potent and xylitol had no effect on secretion. Culture in 3 mM-glucose resulted in a 10-fold decrease in the insulin response to glucose stimulation. A less marked decrease was noted after culture in 20 mM- or 30 mM-glucose. Inosine-stimulated secretion was much decreased after culture in high concentrations of glucose, whereas the responses to mannose or fructose were unchanged. After culture in 30 mM-mannose, glucose-stimulated secretion was similar to that observed after culture in high concentrations of glucose, whereas the response to mannose had much decreased. There were no secretory responses to glucose or fructose after culture in 30 mM-fructose, or to glucose or xylitol after culture in 30 mM-xylitol. Culture in 10 mM-inosine did not preserve any significant response to glucose or inosine. The insulin contents of islets and culture media were higher after culture in high concentrations of glucose, mannose or inosine than after culture in fructose, xylitol or low concentrations of glucose. It is suggested that glucose, and to some extent mannose, preserves the glucoreceptor mechanism for insulin secretion by influencing an early stage in glucose metabolism, presumably glucokinase activity.

Maintenance of glucose-stimulated insulin release during culture of pancreatic islets in a chemically defined medium requires the presence of high concentrations of glucose (Buitrago *et al.*, 1975; Frankel *et al.*, 1976; Gylfe, 1977). This effect of glucose seems to result from protection of the glucoreceptor mechanism initiating insulin release (Gylfe, 1977). There is a great deal of evidence supporting the idea that glucose is recognized as an insulin secretagogue by virtue of its metabolism (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft *et al.*, 1973; Hellman *et al.*, 1974; Capito & Hedekov, 1976; Malaisse *et al.*, 1976, 1977). A stimulatory signal compound is probably formed during glycolysis at or beyond the triose phosphate level (Hellman *et al.*, 1974; Capito & Hedekov, 1976; Sugden & Ashcroft, 1977). To identify the level at which glucose exerts its protective effect on β -cell function during culture, glucose-stimulated insulin release was recorded after culture of β -cell-rich pancreatic islets in the presence of high concentrations of substrates that enter the glycolytic pathway at different levels. I further studied whether long-term exposure of the β -cells to poor secreta-

gogues or non-secretagogues resulted in adaptation of the insulin-releasing mechanism to these compounds.

Experimental

Materials

Reagents of analytical grade and deionized water were used. Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], D-glucose, D-mannose, D-fructose, xylitol, inosine and bovine serum albumin (fraction V) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Tissue culture medium 199 (TCM 199; 10 times concentrated) (Salk *et al.*, 1954) was obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, and benzylpenicillin from A.B. Kabi, Stockholm, Sweden. ^{125} I-labelled insulin was from Farbwerke Hoechst A.-G., Frankfurt/Main, Germany, and crystalline mouse insulin was prepared by Novo A/S, Copenhagen, Denmark.

Media

The basal medium used for isolation, culture and incubation consisted of a modified TCM 199 medium

with Hanks salts (Salk *et al.*, 1954), lacking glucose and Phenol Red and buffered with 25 mM-Hepes. All media contained bovine serum albumin (1 mg/ml); the culture media were also supplemented with benzylpenicillin (50 µg/ml). The preparation and sterilization of the media have previously been described (Buitrago *et al.*, 1975).

Animals and isolation of islets

Adult mice, homozygous for the gene *ob*, were from a local colony (Hellman, 1965). Fresh pancreatic islets were isolated from animals starved overnight, by free-hand micro-dissection (Hellerström, 1964) at room temperature (22°C) in basal medium containing 3 mM-glucose.

Culture and incubation of islets

Isolated islets were transferred to sterile Petri dishes (Heger Plast A.B., Stallarholmen, Sweden; diameter 5 cm). Each dish contained two islets in 4 ml of medium TCM 199, modified as indicated in the Tables. The islets were then stored for 7 days at 37°C in humidified air. The amounts of insulin in the media were determined at the end of the culture period. After culture, the islets were rinsed and preliminarily incubated for 60 min in 1 ml of basal medium containing 1 mM-glucose. Insulin release was studied during a subsequent 60 min period of incubation of two islets in 300 µl of medium supplemented with a low or a high concentration of the substance to be tested, as

indicated in the Tables. Both preincubation and incubation were performed in closed vials at 37°C with air as gas phase. The same procedure was followed for preincubation and incubation of non-cultured islets.

Weighing of islets and assay of insulin

After incubation the islets were freeze-dried (−40°C; 0.1 Pa) and weighed on a quartz-fibre balance (Lowry, 1953). Islets used for determination of insulin content were then subjected to ultrasonic disintegration in 1 mM-HCl [Sonifier B-12 (Branson Sonic Power Co., Danbury, CT, U.S.A.) equipped with a micro-tip and run at a meter reading of 50 W]. Insulin in islet extracts and media was assayed radio-immunologically (Heding, 1969) by using crystalline mouse insulin as the reference.

Presentation of results

Insulin values were expressed per unit dry weight of islets. Unless otherwise indicated, statistical significance was estimated from the mean $\pm$ S.E.M. of differences between paired test and control incubation in a series of repeated experiments.

Results

Insulin-secretion data from fresh islets are presented in Table 1. Glucose (30 mM), mannose (30 mM), fructose (30 mM) and inosine (10 mM) all stimulated

Table 1. *Insulin release from fresh pancreatic islets*

After preliminary incubation of the islets for 60 min in the basal medium containing 1 mM-glucose, batch-type incubations were performed for 60 min in media supplemented as indicated. Results are given as mean values $\pm$ S.E.M. with the numbers of experiments in parentheses. The statistical significance was estimated from the differences between paired test and control incubations.

Supplement to incubation medium	Concn. (mM)	Insulin released (ng/h per µg dry wt. of islets)		P
		Primary data	Test minus control	
Glucose	1 (control)	0.31 $\pm$ 0.04 (24)		
	30	2.87 $\pm$ 0.33 (23)	2.58 $\pm$ 0.31 (23)	<0.001
Mannose	1 (control)	0.24 $\pm$ 0.04 (10)		
	30	0.79 $\pm$ 0.17 (10)	0.55 $\pm$ 0.20 (10)	<0.025
Fructose	1 (control)	0.19 $\pm$ 0.03 (10)		
	30	0.59 $\pm$ 0.10 (10)	0.40 $\pm$ 0.09 (10)	<0.005
Xylitol	1 (control)	0.43 $\pm$ 0.06 (10)		
	30	0.78 $\pm$ 0.28 (10)	0.34 $\pm$ 0.27 (10)	
Inosine	1 (control)	0.71 $\pm$ 0.10 (10)		
	10	2.38 $\pm$ 0.33 (10)	1.68 $\pm$ 0.33 (10)	<0.001

Table 2. *Insulin release from cultured pancreatic islets*

After culture of the islets for 7 days in the basal medium supplemented as indicated, the islets were rinsed and incubated preliminarily for 60 min in a medium containing 1 mM-glucose. Batch-type incubations were then performed for 60 min in media supplemented as indicated. Results are given as mean values $\pm$ S.E.M. with the numbers of experiments in parentheses. The statistical significance was estimated from the difference between paired test and control incubations.

Supplement to culture medium	Supplement to incubation medium	Concn. (mM)	Insulin released (ng/h per μ g dry wt. of islets)		P
			Primary data	Test minus control	
3 mM-Glucose	Glucose	1 (control)	0.05 $\pm$ 0.00 (10)		
		20	0.28 $\pm$ 0.07 (10)	0.23 $\pm$ 0.07 (10)	<0.01
20 mM-Glucose	Glucose	1 (control)	0.11 $\pm$ 0.03 (19)		
		20	0.66 $\pm$ 0.09 (19)	0.56 $\pm$ 0.07 (19)	<0.001
	Inosine	1 (control)	0.16 $\pm$ 0.04 (10)		
		10	0.41 $\pm$ 0.07 (10)	0.25 $\pm$ 0.06 (10)	<0.005
30 mM-Glucose	Glucose	1 (control)	0.08 $\pm$ 0.01 (27)		
		30	1.06 $\pm$ 0.07 (28)	0.96 $\pm$ 0.07 (27)	<0.001
	Mannose	1 (control)	0.08 $\pm$ 0.02 (9)		
		30	0.90 $\pm$ 0.15 (9)	0.82 $\pm$ 0.13 (9)	<0.001
	Fructose	1 (control)	0.12 $\pm$ 0.01 (11)		
		30	0.53 $\pm$ 0.10 (11)	0.42 $\pm$ 0.10 (11)	<0.005
	Xylitol	1 (control)	0.07 $\pm$ 0.01 (9)		
		30	0.08 $\pm$ 0.01 (9)	0.02 $\pm$ 0.01 (9)	
30 mM-Mannose	Glucose	1 (control)	0.06 $\pm$ 0.02 (9)		
		30	0.82 $\pm$ 0.21 (9)	0.75 $\pm$ 0.21 (9)	<0.01
	Mannose	1 (control)	0.05 $\pm$ 0.01 (9)		
		30	0.18 $\pm$ 0.03 (9)	0.13 $\pm$ 0.03 (9)	<0.005
30 mM-Fructose	Glucose	1 (control)	0.17 $\pm$ 0.04 (11)		
		30	0.12 $\pm$ 0.03 (11)	-0.05 $\pm$ 0.05 (11)	
	Fructose	1 (control)	0.09 $\pm$ 0.02 (10)		
		30	0.07 $\pm$ 0.01 (11)	-0.02 $\pm$ 0.02 (10)	
30 mM-Xylitol	Glucose	1 (control)	0.08 $\pm$ 0.01 (8)		
		30	0.09 $\pm$ 0.01 (9)	0.00 $\pm$ 0.01 (8)	
	Xylitol	1 (control)	0.08 $\pm$ 0.01 (9)		
		30	0.07 $\pm$ 0.01 (7)	-0.02 $\pm$ 0.01 (7)	

Table 2—continued

Supplement to culture medium	Supplement to incubation medium	Concn. (mM)	Insulin released (ng/h per μ g dry wt. of islets)		P
			Primary data	Test minus control	
10mM-Inosine	Glucose	1 (control)	0.10 $\pm$ 0.01 (20)		
		20	0.14 $\pm$ 0.02 (20)	0.05 $\pm$ 0.02 (20)	<0.05
	Inosine	1 (control)	0.10 $\pm$ 0.01 (10)		
		10	0.18 $\pm$ 0.04 (10)	0.07 $\pm$ 0.03 (10)	

Table 3. Insulin content of pancreatic islets and media after culture

Batches of two islets were cultured for 7 days in 4ml of the basal medium supplemented as indicated. Results are given as mean values $\pm$ S.E.M. with the numbers of experiments in parentheses. For assessment of the statistical significance between test and control groups Student's *t* test was applied. *P<0.025; **P<0.005; ***P<0.001.

Supplement to culture medium	Concn. (mM)	Insulin content (ng/ μ g dry wt. of islets)		
		Islets	Culture medium	Islets plus culture medium
Glucose	3 (control)	44.8 $\pm$ 3.7 (6)	33.2 $\pm$ 6.8 (10)	75.4 $\pm$ 9.6 (6)
	20	61.1 $\pm$ 2.0*** (15)	125.9 $\pm$ 10.5*** (20)	177.2 $\pm$ 13.6*** (15)
	30	61.6 $\pm$ 3.1* (25)	119.3 $\pm$ 7.1*** (27)	177.9 $\pm$ 7.3*** (24)
Mannose	30	60.0 $\pm$ 3.5* (9)	100.5 $\pm$ 8.1*** (9)	160.6 $\pm$ 10.9*** (9)
Fructose	30	53.4 $\pm$ 6.1 (6)	30.4 $\pm$ 2.2 (11)	83.7 $\pm$ 7.9 (6)
Xylitol	30	51.6 $\pm$ 4.2 (9)	14.5 $\pm$ 1.3* (9)	66.1 $\pm$ 4.6 (9)
Inosine	10	63.1 $\pm$ 2.4*** (16)	121.3 $\pm$ 14.4*** (20)	179.5 $\pm$ 18.3** (16)

insulin secretion compared with control islets incubated in 1 mM of the same compound. Glucose was a much more potent secretagogue than were mannose and fructose. Insulin release in the presence of 10mM-inosine did not differ significantly from that in 30mM-glucose. Even 1mM-inosine stimulated insulin secretion when compared with the effect of 1mM-glucose (*P*<0.001, Student's *t* test). Xylitol had no significant effect on insulin secretion when added in 1 or 30mM concentrations.

Insulin secretion data during incubation after culture in different media are shown in Table 2. Glucose-stimulated insulin secretion decreased during culture and was best maintained by culture in the presence of 20 or 30mM-glucose or 30mM-mannose. A small response was also observed when the islets had been cultured in 3mM-glucose or 10mM-inosine. Fructose (30mM) or xylitol (30mM) could not maintain glucose-stimulated insulin secretion during culture. Insulin secretion stimulated by mannose and

fructose after culture in 30mM-glucose did not differ significantly from responses of fresh islets. However, culture in 30mM-mannose decreased the subsequent response to mannose after culture, and no response to fructose was observed after culture in 30mM-fructose. The insulin response to 10mM-inosine was greatly decreased by culture in 20mM-glucose and even more decreased by culture in 10mM-inosine. Xylitol (30mM) did not stimulate insulin secretion after culture in either 30mM-glucose or 30mM-xylitol.

Table 3 shows the insulin content of islets and culture media. When the islet contents of insulin after culture were compared, similar amounts were found in islets cultured in 20 or 30mM-glucose, 30mM-mannose or 10mM-inosine. A significantly lower content was recorded for islets cultured in 3mM-glucose, and intermediary values after culture in 30mM-fructose or 30mM-xylitol. The culture media contained insulin in the range of 100–125 ng/ μ g dry wt. of islets after culture in 20 or 30mM-glucose,

30mM-mannose or 10mM-inosine, whereas less than one-third of these values were found after culture in 3mM-glucose, 30mM-fructose or 30mM-xylitol. When the total amounts of insulin in the islets and culture media are compared, similar and high recoveries were obtained after culture in 20 or 30mM-glucose, 30mM-mannose or 10mM-inosine. The recoveries were, however, no more than half as great after culture in 3mM-glucose, 30mM-fructose or 30mM-xylitol.

Discussion

The present data on insulin release from fresh islets conform with observations made by other authors (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft *et al.*, 1972, 1973; Capito & Hedekov, 1976). The secretagogues tested are all believed to trigger insulin secretion through the formation of a signal compound in a later stage of glycolysis (Hellman *et al.*, 1974; Capito & Hedekov, 1976; Sugden & Ashcroft, 1977). Previous studies have shown that the pancreatic β -cells require high concentrations of glucose during culture for maintaining glucose-stimulated insulin secretion (Buitrago *et al.*, 1975; Frankel *et al.*, 1976; Gylfe, 1977). The insignificant stimulatory effect of glucose observed after culture in low concentrations of glucose has been attributed to a defective glucoreceptor mechanism (Gylfe, 1977). This is in contrast with the decreased response after culture in high glucose concentrations, which is due to a diminished content of insulin in β -cells with an intact glucoreceptor (Gylfe, 1977).

The observation that the insulin secretion stimulated by mannose and fructose was fully maintained during culture in a high glucose concentration also indicates preservation of the glucoreceptor. In these experiments the islet insulin content was probably sufficient for obtaining a maximal response to mannose and fructose, but too low for a maximal response to the more potent secretagogue glucose. If the maintenance of metabolic pathways depends on the utilization of these pathways, the decreased response to inosine observed after culture in high concentrations of glucose might result from decreased inosine metabolism. Also mannose appeared to preserve glucose-stimulated insulin secretion during culture. The protective effect of mannose on the glucoreceptor was, however, less marked, the mannose stimulation of insulin secretion being much decreased.

Although fructose and inosine stimulated insulin secretion from fresh islets, these compounds could not preserve the glucoreceptor during culture. Fructose, like mannose, can enter the glycolytic pathway at the fructose 6-phosphate level, but animal hexokinases have a relatively low affinity for fructose (Walker, 1966; Colowick, 1973). The natural metabolism of fructose may therefore involve the formation of fructose 1-phosphate and either entry

into the glycolytic pathway at the fructose 1,6-bisphosphate level or breakdown to glyceraldehyde and dihydroxyacetone phosphate and thus entry at the same level as inosine. If so, the present lack of a protective effect of fructose and inosine may indicate the importance of the early stages of glucose metabolism for the protection of the glucoreceptor mechanism. However, the fructose concentration chosen was at least 10 times the K_m values characteristic for animal hexokinases (Walker, 1966), which should favour fructose entry at the fructose 6-phosphate level.

Apart from hexokinase, the β -cells have glucokinase, i.e. a high- K_m phosphorylating enzyme (Ashcroft & Randle, 1970). In different tissues this enzyme has K_m values for glucose in the 12–20mM range (Walker, 1966; Ashcroft & Randle, 1970; Colowick, 1973), for mannose about 50mM (Walker, 1966) and for fructose more than 800mM (Colowick, 1973). Induction of glucokinase formation has been suggested as an explanation for the acquired postnatal glucose sensitivity of glucose-insensitive β -cells of fetuses (Ågren *et al.*, 1975). Glucokinase induction occurs during culture in a high-glucose medium (Andersson *et al.*, 1974) and might well explain how glucose protects the glucoreceptor mechanism during culture. The different affinities of glucokinase for glucose, mannose and fructose correlate well with their potency in maintaining glucoreceptor function and may therefore also reflect their ability to maintain the formation of this enzyme.

The insulin response to mannose, fructose and inosine was much decreased or even absent if the islets had been cultured in the presence of the particular compound, an effect that can hardly be explained by a decreased islet content of insulin. Xylitol, which had no effect on insulin secretion in fresh islets, lacked stimulatory effect also after culture with 30mM-xylitol. Thus, in terms of stimulatory potency there was no sign of β -cell adaptation to these substances during culture.

The recoveries of insulin from islets and culture media were high and similar after culture in 20 or 30mM-glucose, 30mM-mannose or 10mM-inosine, but low after culture in 3mM-glucose, 30mM-fructose or xylitol. Since high concentrations of glucose, mannose (Pipeleers *et al.*, 1973) and inosine (Andersson & Hellerström, 1977) stimulate insulin biosynthesis, whereas fructose and xylitol do not (Pipeleers *et al.*, 1973), there seems to be a correlation between stimulation of biosynthesis and recovery of insulin after culture. However, there is a clear dissociation between the stimulatory effect of inosine on insulin biosynthesis and its poor ability to maintain insulin secretion during culture. A similar dissociation has been observed previously for leucine (Gylfe, 1977). These data and the different dose-response relationships for glucose stimulation of

insulin release and biosynthesis (Pipeleers *et al.*, 1973) support the suggestion that the two processes are triggered by two distinct signal compounds (Ashcroft, 1976).

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