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Lipid Metabolism of Isolated Mouse Pancreatic Islets Maintained in Culture at Different Glucose Concentrations

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ABSTRACT

The lipid metabolism of isolated mouse pancreatic islets, which had been cultured for one week at 3.3 or 16.7 mM glucose, was examined in short-term experiments performed at the end of the culture period. It was found that: a) high-glucose cultured islets oxidized palmitate at a higher rate: and b) both groups of cultured islets increased the rate of incorporation of (U-^{14}C)-glucose into triacylglycerols and phospholipids in response to an acute glucose challenge. This latter effect was, however, more pronounced after culture at a high glucose concentration. The present data suggest that long-term exposure to high concentrations of glucose leads to a general increase of islet oxidative metabolism. Moreover, high glucose culture seems to induce an increased conversion of carbohydrates into triacylglycerols and membrane phospholipids.

INTRODUCTION

In maturity-onset diabetes, the pancreatic islets are exposed to elevated blood glucose concentrations for considerable periods of time. The cause of the progressive decline in insulin secretory capacity is, so far, entirely unknown. Therefore, it is important to evaluate various aspects of the B-cell metabolism under prolonged hyperglycemic conditions. The use of isolated islet culture techniques makes it possible to perform long-term studies of pancreatic islet metabolism at both different nutritional states and functional loads on the insulin production (1,4,5,6,7,8).

Recent studies in isolated pancreatic islets have indicated that long-chain fatty acids may have an important role in the energy metabolism of the B-cells (9). Moreover, it was demonstrated that the incorporation of labelled glucose and palmitic acid into triacylglycerols and, to a greater extent, into the phospholipid fraction, was dependent upon the extracellular concentration of glucose in acute in vitro experiments (10). It was the aim of the present study to examine the regulation of long-chain fatty acid oxidation and the biosynthesis of triacylglycerols and phospholipids in mouse pancreatic islets maintained

in tissue culture for one week at different glucose concentrations.

MATERIALS AND METHODS

Chemicals: Palmitic acid, tripalmitoylglycerol and antimycin A were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Fatty acid-poor bovine albumin prepared according to Chen (12) was from Miles Laboratories, Kankakee, Ill., U.S.A. PPO (2,5-Diphenyloxazole), dimethyl-POPOP(1,4-bis(4-methyl-5-phenyloxazol-2-yl) benzene) and hyamine hydroxide (1 M solution in methanol) were from Packard Instruments Co., Downers Grove, Ill., U.S.A. 2',7'-dichlorofluorescein, all organic solvents and inorganic salts were of analytical grade quality and were purchased from E.G. Merck A.G., Darmstadt, West Germany. Egg yolk lipids (12 mg/ml) were kindly provided by Vitrum AB, Stockholm, Sweden. (U-^{14}C)Glucose (250 mCi/mmol) and (U-^{14}C)palmitate (256 mCi/mmol) were from the Radiochemical Centre, Amersham, Bucks, U.K. Tissue culture media ingredients were from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. Deionized and doubly distilled water were used in all experiments.

Tissue culture technique: Pancreatic islets were obtained from adult, male NMRI mice, starved overnight. Isolation was carried out under aseptic conditions using a modified collagenase digestion method (17). The islets were cultured in plastic Petri dishes (4) in tissue culture medium 199, supplemented with 10% (v/v) calf serum, penicillin (100 units/ml) and streptomycin (0.1 mg/ml). The concentrations of glucose was either 3.3 mM or 16.7 mM. After 7 days of culture the islets were harvested with the aid of a braking pipette, rinsed and immediately transferred in groups of 20 into media described below.

Short-term incubations: Rates of palmitate oxidation and incorporation of (U-^{14}C)glucose into lipids were estimated as previously described in detail (9, 10). The islets were incubated in glass vials (18) containing a bicarbonate-buffered medium pH 7.4 (16) with the additions indicated in the Tables. The buffer was equilibrated with O_2:CO_2 (95%:5%) before and during the incubations, which were carried out at +37°C in a shaking water bath.

Palmitate oxidation: 1 mM-Palmitic acid was converted into its sodium salt with 15 mM-NaOH and coupled to fatty acid-poor albumin. After incubation for 1 h in a volume of 250 /ul the metabolic activity was stopped with 0.01 /uM-antimycin A in 70% (v/v) ethanol and 0.4 M-Na_2HPO_4, pH 6.0, was added to liberate $^{14}CO_2$. Labelled carbon dioxide was trapped in Hyamine during a second 2 h incubation period at +37°C, which was followed by addition of 10 ml scintillation solution (POP, 5 g; dimetyl-POPOP, 0.05 g, toluene 1000 ml). Blank incuba-

tions performed without islets were treated in the same way and served as controls.

(U-^{14}C)Glucose incorporation into lipids: Groups of 20 islets were incubated for 3 h in 50 µl medium containing labelled glucose and the incorporation subsequently terminated by addition of non-labelled glucose. The islets were immediately rinsed twice and then transferred to 100 µl of a mixed salt solution (Folch et al., 1957), for disruption by sonication (Ultrasonic Disintegrator, 60 W, MSE, London, S.W.1, U.K.). Lipids were extracted from the islet sonicate in 2 ml of chloroform:methanol (2:1, v/v), to which was added 40 µl of a carrier lipid mixture (tripalmitoylglycerol, palmitic acid and an extract of egg yolk phospholipids). After four washings as described by Folch et al. (14), the lipid solvent extracts were evaporated to dryness under pure nitrogen. The residue was then redissolved in 40 µl of chloroform:methanol (2:1 v/v) and applied to precoated t.l.c. plates of Silica Gel G (E. Merck A.G., Darmstadt, W. Germany), previously activated at +100°C for 1 h. The solvent system for t.l.c. was n-hexane:diethyl ether:methanol:acetic acid (90:20:2:3 by volume) (15). The spots corresponding to tripalmitoylglycerol and the phospholipid mixture were identified after spraying the plates with 2',7'-dichlorofluorescein in ethanol (0.2 mg/ml) and the scrapings of the spots were mixed with 10 ml of a scintillation solution supplemented with 0.5 ml methanol (11).

Radioactivity measurement and expression of the results: The radioactivity of the different samples was estimated in a Packard liquid scintillation counter. Correction for quenching was achieved by the channels-ratio method. Rates of oxidation of palmitate and incorporation of glucose were calculated from the specific radioactivities of (U^{14}C)glucose and (U-^{14}C)palmitate in the incubation media. All results are expressed as mean values ± S.E.M. Differences between means were evaluated with Student's t-test.

RESULTS AND DISCUSSION

The rate of oxidation of 1 mM-(U-^{14}C)palmitate (Table 1), in the absence of glucose, of the cultured islets was comparable to that of freshly isolated islets, confirming our previous findings of good islet preservation following culture under similar conditions (2). Thus, by approximating a mean islet weight of 0.5 µg, the oxidation rate of low glucose cultured (3.3 mM) islets was 2.9 pmol/µg dry weight per h (Table 1). The corresponding rates of palmitate oxidation, at 0.7 mM-palmitate, in islets isolated from obese hyperglycemic mice or their lean litter mates was 3.1 and 1.8 pmol/µg dry weight per h, respectively (9).

Table 1. Oxidation of 1 mM-(U-^{14}C)palmitate by cultured islets.

Glucose concentration of the culture medium (mM)	Rate of palmitate-oxidation (pmol/20 islets per h) No glucose	16.7 mM glucose
3.3	29.2 ± 4.1 (12)	15.4 ± 2.7^{x} (16)
16.7	63.6 ± 4.9^{+} (14)	11.9 ± 2.6^{x} (19)

+ = $p < 0.001$ for the difference in rate of palmitate oxidation between islets cultured at 3.3 and 16.7 mM-glucose. x denotes $p < 0.001$ for the inhibitory effect of 16.7 mM-glucose on palmitate oxidation.

Culture for one week at a high glucose concentration (16.7 mM) was found to increase considerably the rate of palmitate oxidation (Table 1), c.f. islet glucose oxidation (1). Similarly, we recently observed that islets exposed in culture to L-leucine (8) or adenosine (3) for one week, displayed an increased rate of glucose oxidation at the end of the culture period. Together these data suggest that an abundant supply of exogenous nutrients may result in a general stimulation of islet oxidative metabolism, possibly exerted at the level of oxidative phosphorylation or the tricarboxylic acid cycle.

When the palmitate oxidation experiments were performed in the presence of 16.7 mM-glucose, an inhibition of the palmitate oxidation of low and high glucose cultured islets by 50% and 80% respectively, was observed (Table 1). A similar inhibitory effect of D-glucose was found in islets isolated from obob-mice (9). The decrease in $^{14}CO_2$-production from labelled palmitate under conditions of abundant exogenous glucose may be explained by a competition for available palmitate between oxidative metabolism and incorporation into lipids. Thus, it seems as if glucose is the preferred fuel for oxidation in the pancreatic islets during hyperglycemia. Long-chain fatty acids, on the other hand, are stored as tricylglycerols or utilized for the increased biosynthesis of phospholipids secondary to an increased membrane turnover following glucose stimulation of insulin secretion.

This latter suggestion is supported indirectly by the results summarized in Table 2. Both groups of cultured islets increased the rate of incorporation of (U-^{14}C)glucose into lipids in response to an elevation of the concentration of labelled glucose from 3.3 to 16.7 mM during the subsequent short-term incubation ($p < 0.01$). The glucose-stimulated increment of incorporation into total islet lipids amounted to 330 and 550% under the two different short-term experimental conditions for low- and high glucose cultured islets, respectively (Table 2). We have previously reported similar effects of glucose on non-cultured islets

isolated from obob-mice using either (U-^{14}C)glucose or (U-^{14}C)palmitate as precursors of lipids (10). Indeed, in obob-mice, 96% of the radioactivity originating from incorporated (U-^{14}C)glucose is recovered as glyceride-glycerol.

Table 2. Incorporation of (U-^{14}C)glucose into triacylglycerols and phospholipids of cultured islets.

Glucose concentration of the culture medium (mM)	Rate of (U-^{14}C)glucose incorporation (pmol/20 islets per h) Glucose (mM)			
	3.3	16.7	3.3	16.7
	triacylglycerols		phospholipids	
3.3	0.3 ± 0.1 (9)	0.7 ± 0.1 (10)	1.1 ± 0.3 (9)	3.9 ± 0.5 (10)
16.7	0.4 ± 0.1 (12)	1.3 ± 0.1^{x} (14)	1.1 ± 0.2 (12)	7.0 ± 0.6^{x} (14)

Number of experiments given within parentheses. x = $p < 0.001$ for the difference in rate of (U-^{14}C)glucose incorporation between islets cultured in 3.3 and 16.7 mM glucose.

Looking in more detail at the effects of the preceeding culture conditions on the subsequent incorporation of labelled glucose, it is apparent that there was no difference between the two groups of islets when the incorporation rates at 3.3 mM-(U-^{14}C)glucose are considered. Conversely, the ^{14}C-incorporation into both triacylglycerols and phospholipids at 16.7 mM-(U-^{14}C)glucose was twice as high in the high-glucose cultured islets as compared to those cultured in the low-glucose medium. Thus, a prolonged exposure to high glucose seems to induce both an increased capacity of the islets to convert carbohydrates into triacylglycerols and an increased turn-over of the phospholipids of cellular membranes. It is worthy of note, that culture in high glucose media does not invariably lead to an increased biosynthesis of lipids as has recently been shown for smooth muscle cells and vascular endothelium (19,13). It thus seems possible that the stimulatory effect of glucose is linked to its function as the primary modulator of insulin biosynthesis and release. If we consider that islets cultured at 3.3 mM glucose are refractory, as regards insulin release, to an acute glucose challenge (7), the present data suggest that the rates of insulin release and phospholipid biosynthesis may be controlled by glucose at different stages of the stimulus-secretion sequence.

Moreover, the present study shows, in agreement with previous studies on cultured mouse pancreatic islets (1,5,6,7), that profound changes of islet cell function are induced by exposure to different extracellular glucose concentra-

tions for prolonged time periods. We have, however, been unable to demonstrate any harmful effects of a high extracellular glucose concentration on different aspects of islet cell function. On the contrary, prolonged exposure of islets to high concentrations of glucose induces an adaptive response leading to: a) an increased turn-over rate of insulin (5); b) storage of energy in the form of glycogen (6) and neutral lipids; c) a high turn-over of membrane phospholipids; and d) a general stimulation of the oxidative metabolism of the pancreatic B-cells. The present in vitro study, thus lends no further support to the view that hyperglycemia per se, may be responsible for the progressive decline in insulin secretory capacity, commonly observed in diabetes mellitus.

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