The Metabolism of Lipids in Mouse Pancreatic Islets

THE OXIDATION OF FATTY ACIDS AND KETONE BODIES

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The rate of oxidation of ^{14}C -labelled fatty acids and of ketone bodies was measured in isolated pancreatic islets of obese-hyperglycaemic mice (*ob/ob*). The following main observations were made. 1. Octanoate, palmitate and oleate were all converted into CO_2 by the pancreatic islets. Octanoate was oxidized with the highest rate followed by palmitate and oleate. 2. The rate of oxidation of 0.7 mM-palmitate was 3.1 pmol/h per μg dry weight. This was decreased by 50% in the presence of 16.7 mM-glucose. The rate of palmitate oxidation was also inhibited by 2-bromostearate. The palmitate oxidation showed a concentration-dependent increase, which was most marked between 0.25 and 1.0 mM. 3. Octanoate (5 mM) had no effect on the rate of oxidation of 3.3 mM-glucose. 4. Acetoacetate (5 mM) and D-3-hydroxybutyrate (5 mM) were oxidized at rates of 5.9 and 5.4 pmol/h per μg dry weight respectively. These rates were less than 10% of those found in kidney-cortex slices. The magnitude of the oxidation rates found for fatty acids and for ketone bodies suggests that these substrates represent important metabolic fuels for the pancreatic B-cells.

The stimulatory effect of glucose on the release of insulin shows a close relationship to its rate of intracellular degradation in the pancreatic B-cell (Grodsky *et al.*, 1963; Ashcroft *et al.*, 1970; Hellman *et al.*, 1974). Amino acids represent another group of potent insulin secretagogues, which have also been shown to be metabolized by the B-cells, though no obvious correlation was found between the oxidation rate and the insulin-releasing capacity of various amino acids (Hellman *et al.*, 1971).

The role of fatty acids and of ketone bodies in the regulation of insulin secretion in non-ruminant mammals is not yet clearly defined. The ability of medium-chain and long-chain fatty acids as well as ketone bodies to stimulate the secretion of insulin shows a dependence on the glucose concentration (Malaisse & Malaisse-Lagae, 1968; Goberna *et al.*, 1974). The interspecies variations in the insulin-releasing potencies of these substrates are frequent (Coore & Randle, 1964; Schalch & Kipnis, 1965; Montague & Taylor, 1968; Crespin *et al.*, 1973). The mechanism of the insulin-releasing action of fatty acids and of ketone bodies is so far obscure, and little is known about the role of lipids as fuels for oxidative metabolism in the pancreatic B-cells. We have with quantitative methods demonstrated the presence of both 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) and D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) in the pancreatic islets (Hammar & Berne, 1970; Berne, 1975a). These enzymes are necessary for the degradation of fatty acids and of D-3-hydroxybutyrate respectively. Among fatty acids

octanoate was found to be oxidized in guinea-pig (Edwards *et al.*, 1972) and rat islets (Cole & Logothetopoulos, 1974). Hitherto, no attempts have been made to study the ability of the islets to oxidize ketone bodies.

The aim of the present work was therefore to study the rate of oxidation of fatty acids and of ketone bodies in the pancreatic islets. Fatty acids of different chain length and of different degree of saturation were used.

Experimental

Chemicals

Antimycin A, ethyl acetoacetate, palmitic acid, octanoic acid, oleic acid and sodium DL-3-hydroxybutyrate were all obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Hyamine hydroxide (1 M solution in methanol), 2,5-diphenyloxazole and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene were obtained from the Packard Instrument Co., Downers Grove, Ill., U.S.A. Fatty acid-poor bovine albumin, which was prepared as described by Chen (1967), was purchased from Miles Laboratories, Kankakee, Ill., U.S.A. 2-Bromostearate was obtained from Fluka A.G., Buchs, Switzerland. Toluene and all inorganic chemicals were of analytical grade and were obtained from E. Merck A.G., Darmstadt, Germany. The radioactive materials used were all purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Deionized double-distilled water was used in all experiments.

Animals

Obese-hyperglycaemic (gene symbol, *ob/ob*) and lean (gene symbols, *Ob/Ob*, *Ob/ob*) mice, 6–8-months old and of both sexes, were used. The animals belonged to the non-inbred stock which has been kept at the Department of Histology, University of Uppsala since 1959, and which originated from The Jackson Laboratory, Bar Harbor, Maine, U.S.A. They were reared on standard mouse pellets (Astra-Ewos, Södertälje, Sweden) and were starved overnight before the experiments.

Methods

Preparation of incubation media. All the incubation media used were based on a Gey & Gey (1936) bicarbonate buffer, pH 7.4, which was gassed for at least 10 min with $O_2 + CO_2$ (95:5) before use.

n-Octanoic acid was neutralized with an equimolar amount of 1.5M-NaOH before it was added to the buffer. The radioactive fatty acid was obtained as the sodium salt. In order to couple the long-chain fatty acid anions to albumin, palmitic acid and oleic acid with radioactive tracer added were converted into their sodium salts with 15mM-NaOH by gentle heating (60–80°C). The warm solutions of the saponified fatty acids were added to a Ca^{2+} -free buffer containing 10mg of fatty acid-poor albumin/ml. $CaCl_2$ was finally added. The fatty acid/albumin ratio was 4–7:1 (w/w).

Sodium DL-3-hydroxybutyrate was added directly to the buffer. Ethyl acetoacetate was hydrolysed with an equimolar amount of 2M-NaOH for 1 h at 40°C (Krebs & Eggleston, 1945). The acetoacetate was then freeze-dried to remove ethanol and water.

Before being added to the incubation media glucose, acetoacetate and sodium DL-3-hydroxybutyrate were treated with 0.01M-HCl for 2 h and then freeze-dried. This procedure markedly decreased the control values by removing 'carbonate-like' impurities which are otherwise present in the radioactive substrates (Merlevede *et al.*, 1963).

The radioactive substrates were used in the media with the following final specific radioactivities: D-[U- ^{14}C]glucose (1.4mCi/mmol), *n*-[1- ^{14}C]octanoate (0.7mCi/mmol), [U- ^{14}C]palmitate and [U- ^{14}C]oleate (each at 4.8mCi/mmol), [3- ^{14}C]acetoacetate and sodium DL-3-hydroxy[3- ^{14}C]butyrate (each at 2.5mCi/mmol).

Isolation of islets and incubation procedure. Islets were isolated either by micro-dissection (Hellerström, 1964) or by collagenase digestion (Moskalewski, 1965; Howell & Taylor, 1968). The former procedure was used in a series of experiments in which the oxidation rates of palmitate, oleate, acetoacetate and DL-3-hydroxybutyrate were measured. Since it was found in separate experiments that the two procedures

yielded equal rates of oxidation, collagenase isolation was used when a large number of islets were required.

The micro-dissection of the islets was performed in a Krebs–Ringer phosphate buffer (Umbreit *et al.*, 1964), pH 7.4, at 4°C; 15–20 islets were obtained from each animal. Collagenase isolation was carried out by using crude collagenase (code CLS; Worthington Biochemical Corp., Freehold, N.J., U.S.A.; 6mg/ml) in a Krebs–Ringer phosphate buffer, pH 7.4. Some 100–300 islets could be obtained from a single obese mouse with a maximum yield of about 1mg dry weight of islet tissue. Groups of three to five micro-dissected or seven to ten collagenase-isolated islets were transferred to 100 μ l of the incubation medium which was contained in a small centre well inside a scintillation vial (Keen *et al.*, 1963). The vials were thoroughly gassed with $O_2 + CO_2$ (95:5) before they were capped and then incubated for 1 h in a shaking water bath (100 strokes/min) at 37°C. After incubation, the islet metabolism was stopped by injecting 100 μ l of a solution of 0.01 μ M-antimycin A in 70% (v/v) ethanol into the centre well. Methanolic Hyamine (250 μ l) was then injected into the vial followed by an injection into the centre well of 100 μ l of 0.4M- Na_2HPO_4 adjusted to pH 6 to release the CO_2 into the medium (Edwards *et al.*, 1972). After another 2 h shaking in the water bath the centre wells were removed and 10ml of a toluene-based scintillation mixture [1 litre of toluene, 5g of 2,5-diphenyloxazole and 0.05g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] was added to the Hyamine. The radioactivity was then determined in a Packard liquid-scintillation counter, model 3380. Each sample was counted for 2 $\times$ 5 min. Necessary quench corrections were based on an external-standard channels-ratio method. Corrections for controls were made by incubating vessels with the complete medium but without islets. A sample of the incubation medium was added to 250 μ l of Hyamine and was used to calculate the specific radioactivity of the substrates.

Islet weights. The islets were recovered from the incubation media. They were rapidly rinsed twice in double-distilled water, and then frozen and freeze-dried. Weighing was performed on a quartz-fibre balance.

DNA measurements. Hinegardner's (1971) modification of the method for DNA determination developed by Kissane & Robins (1958) was used in some experiments. Calf thymus DNA was used as the standard and fluorescence was measured in a Farrand ratio fluorimeter (Farrand Optical Co., Mt. Vernon, N.Y., U.S.A.) in a volume of 150 μ l.

Calculation of the results. The results were calculated on the basis of the specific radioactivities in the incubation media. No corrections were made for the presence of intracellular pools of the fatty acids and of the ketone bodies or their metabolites. It is

known, however, that the steady-state concentrations of fatty acids and the concentrations of intermediates of the β -oxidation chain are very low in animal tissue (Garland *et al.*, 1965). On the other hand, lipolytic cleavage of intracellular triacylglycerol stores or of membrane phospholipids may provide a continuous supply of fatty acids to the β -oxidation chain.

The term 'experiment' refers to one batch of islets. Two to four batches were obtained from each animal. Unless otherwise stated, islets from at least six animals were used to calculate the mean values. Differences between means were tested by Student's *t* test.

Results

The production of CO₂ from palmitate, octanoate and ketone bodies was linear for at least 2 h. The time of incubation was therefore set to 1 h.

Fatty acid oxidation

Of the fatty acids octanoate (C_{8:0}) was oxidized most rapidly, followed by palmitate (C_{16:0}) and oleate (C_{18:1}). Octanoate (0.5mM) was oxidized at a rate of 80.3±3.7 (mean±s.e.m.) pmol/h per µg of DNA. Increasing the octanoate in the medium to 5mM resulted in an enhancement in the rate of oxidation to 137.4±9.1 pmol/h per µg of DNA. The rate of palmitate oxidation in the B-cell-rich islets from obese-hyperglycaemic mice was 3.1±0.2 pmol/h per µg dry wt. whereas oleate was oxidized at the slightly lower rate of 2.1±0.2 pmol/h per µg dry wt. In the lean mice the rate of palmitate oxidation was

significantly (*P*<0.01) decreased compared with the obese animals and reached 1.8±0.3 pmol/h per µg dry wt.

Palmitate was chosen as a representative of the physiologically important fatty acids and its rate of oxidation by the islets was investigated under a further series of experimental conditions. The oxidation rate showed a concentration-dependent increase up to at least 2mM (Fig. 1), with the steepest rise below 1mM. There was a 50% inhibition of this rate by 2-bromostearate, which is a potent inhibitor of long-chain fatty acid oxidation in heart muscle (Randle, 1969) (Table 1).

To investigate possible mutual interactions between carbohydrate and fatty acid metabolism, the rate of oxidation of glucose without and with octanoate present in the incubation medium was measured, as was the rate of palmitate oxidation at low and high glucose concentration. Octanoate did not affect the glucose-oxidation rate, but raising the glucose concentration from 3.3 to 16.7mM decreased the palmitate-oxidation rate by 50% (Table 2).

Ketone-body oxidation

Acetoacetate and DL-3-hydroxybutyrate were both oxidized to CO₂ by the pancreatic islets. The oxidation rates were of the same order but they were less than 10% of those found in slices of the kidney cortex from the same animals (Table 3).

In Table 4 the results given in detail above have been summarized and expressed as pg-atoms of C utilized for oxidation/h per µg dry wt. It is evident that the long-chain fatty acids present in the media at physiological concentrations provide about equal amounts of carbon for oxidation as 3.3mM-glucose, whereas the ketone bodies are less effective in this respect.

Fig. 1. Effect of the palmitate concentration on the oxidation of palmitate by pancreatic islets

Collagenase-isolated islets from two obese-hyperglycaemic mice were incubated for 1 h in the presence of palmitate complexed to fatty acid-poor bovine albumin (10mg/ml). The CO₂ output was measured and the oxidation rate calculated as described in the Experimental section. Each point represents quadruplicate determinations ±s.e.m.

Table 1. Effect of 2-bromostearate on the oxidation of [U-¹⁴C]palmitate in pancreatic islets from obese-hyperglycaemic mice

Collagenase-isolated islets from a single animal were preincubated for 20min with 0.25mM-2-bromostearate coupled to fatty acid-poor bovine albumin (10mg/ml) or with buffer containing only the albumin. After the pre-incubation period radioactive palmitate was added to a final concentration of 0.25mM and the incubation was continued for 1 h. The CO₂ output was assayed and the oxidation rate calculated as described in the Experimental section. Results are given as means±s.e.m. and the numbers of experiments are given in parentheses. * *P*<0.01 for the difference.

	Concentration of the substrate (mM)	Oxidation rate (pmol of substrate/h per µg of DNA)
Palmitate	0.25	21.9±3.0 (5)*
Palmitate+ 2-bromostearate	0.25	10.5±1.3 (5)

Discussion

The present investigation shows that both medium-chain and long-chain fatty acids and ketone bodies are oxidized by the pancreatic islet cells of the obese-hyperglycaemic mice. The oxidation rates found for these substrates were of such a magnitude that they can be assumed to represent an important part of the total islet energy metabolism. This was particularly evident when the energetic values of the various substrates were compared (Table 4).

In the obese-hyperglycaemic animals, B-cells compose more than 90% of the islets (Gepts *et al.*, 1960; Hellman, 1961), indicating that the present results are representative of this cell type. The B-cells of obese-hyperglycaemic mice have been shown to respond normally to various insulin secretagogues (cf. Lernmark, 1971; Hellman *et al.*, 1973). In addition, the glucose-oxidation rate of these cells is of the same order and has the same characteristic dependence on the glucose concentration as that of normal mice (Hellman *et al.*, 1974). Of further importance for this study are results indicating that there is no difference between serum unesterified fatty acid concentrations in obese-hyperglycaemic and lean mice neither in the fed state nor after a 24h period of starvation (Westman, 1968; Abraham *et al.*, 1971).

The significant enhancement of palmitate oxidation in the islets of the obese mice in comparison with the lean mice is difficult to explain in view of the similarities found in the adjustment of the concentration of serum unesterified fatty acids. The difference may, however, reflect a methodological rather than biological variation, since micro-dissection of the relatively small islets from lean mice may increase the proportion of damaged islet cells, which would be manifest as a lowered oxidation rate. Indirect support of this is provided by the observation that islets maintained in tissue culture for 6 days display an increased rate of palmitate oxidation as compared with freshly isolated islets (C. Berne & A. Andersson, unpublished work). In fact, electron-microscopic examination of cultured islets showed a much smaller

proportion of injured cells than in the freshly isolated islets (Andersson, 1974).

The general pattern of fatty acid oxidation by the B-cells was similar to that in other tissues in that the rate of oxidation showed an inverse relationship to the chain length of the fatty acid (Björntorp, 1968; Scheig, 1968; Fischel & Oette, 1974). It should be noted, however, that *in vivo* octanoate may be of minor importance, since this fatty acid constitutes only a small amount of the dietary fat in adult mammals. By contrast, the two long-chain fatty acids palmitate and oleate comprise more than 50% of the serum unesterified fatty acids in most mammalian species (Spector, 1971). Also the palmitate concentration used was in the normal physiological range of the obese mouse, which changes its serum unesterified fatty acid concentration from 0.29 to 0.89 mM when subjected to a 24h starvation period (Abraham *et al.*, 1971). The mechanism behind the faster oxidation of

Table 2. Effect of octanoate and glucose on the oxidation of [$U-^{14}C$]glucose and [$U-^{14}C$]palmitate

For the oxidation of [$U-^{14}C$]glucose, collagenase-isolated islets were incubated for 1.5h in 160 μ l of medium. Octanoate was added as the sodium salt. For the oxidation of [$U-^{14}C$]palmitate micro-dissected islets were incubated for 1h in the presence of palmitate complexed to fatty acid-poor bovine albumin (10mg/ml). The CO_2 output was assayed and the oxidation rate calculated as described in the Experimental section. Results are given as means $\pm$ S.E.M. and the numbers of experiments are given in parentheses. Islets from four mice were used in each experimental group. * Not significant. $\dagger P < 0.005$.

	Concn. of the substrates (mM)	Oxidation rate (pmol of substrate/ h per μ g dry wt.)
[$U-^{14}C$]Glucose	3.3	6.8 $\pm$ 0.6 (27)
[$U-^{14}C$]Glucose + octanoate	3.3 5.0	7.7 $\pm$ 0.6 (25)*
[$U-^{14}C$]Palmitate + glucose	1.0 3.3	3.1 $\pm$ 0.4 (11)
[$U-^{14}C$]Palmitate + glucose	1.0 16.7	1.5 $\pm$ 0.3 (14) $\dagger$

Table 3. Oxidation of ketone bodies in pancreatic islets and kidney cortex from obese-hyperglycaemic mice

Micro-dissected islets and slices of kidney cortex were incubated for 1h in the presence of DL-3-hydroxy[3- ^{14}C]butyrate and [3- ^{14}C]acetoacetate. The CO_2 output was measured and the oxidation rate calculated as described in the Experimental section. Results are given as means $\pm$ S.E.M. and the numbers of experiments are given in parentheses.

	Concentration of the substrate (mM)	Oxidation rate (pmol of substrate/h per μ g dry wt.)	
		Pancreatic islets	Kidney cortex
DL-3-Hydroxybutyrate	10	5.4 $\pm$ 0.5 (39)	54.8 $\pm$ 5.0 (13)
Acetoacetate	5.0	5.9 $\pm$ 1.6 (40)	86.9 $\pm$ 8.0 (16)

Table 4. Oxidation of glucose, long-chain fatty acids and ketone bodies by pancreatic islets from obese-hyperglycaemic mice

The oxidation rates presented above (see Tables 2 and 3 and the text) have been expressed as pg-atoms of C oxidized/h per μg dry wt. instead of the previously used pmol of substrate oxidized/h per μg dry wt. to permit a better comparison between different carbon sources. Results are given as the recalculated means $\pm$ S.E.M.

	Concentration of the substrate (mM)	Oxidation rate (pg-atoms of C/h per μg dry wt.)
Glucose	3.3	40.8 ± 3.6
Palmitate	0.7	49.6 ± 3.2
Oleate	0.7	37.8 ± 3.6
DL-3-Hydroxybutyrate	10.0	21.6 ± 2.0
Acetoacetate	5.0	23.6 ± 6.4

octanoate in comparison with the long-chain fatty acids awaits further study but may involve the different routes for disposal within the cells found for fatty acids of different chain length. It is thus known that octanoate is incorporated into lipids to a negligible extent by the rat liver (Scheig, 1968) and also by the pancreatic islets of obese mice (C. Berne, unpublished work), whereas long-chain fatty acids are readily incorporated into lipids by the same tissues (Berne, 1975b; Scheig, 1968). This would make relatively more of the medium-chain fatty acids available for oxidation. Moreover, in contrast with long-chain fatty acids, octanoate may enter the mitochondrial matrix without being transported by a carnitine-dependent pathway (Fritz, 1961).

The existence of a glucose-fatty acid cycle (Randle *et al.*, 1965) in the B-cell has been expected on the basis that glucose 6-phosphate accumulated in rat islets exposed to octanoate (Montague & Taylor, 1968). The results of subsequent studies did not support this idea, however, since octanoate failed to raise the islet concentration of citrate (Hellman & Idahl, 1972). The present results agree with the latter report in that glucose oxidation was not inhibited by octanoate. On the contrary, evidence was obtained showing that a high concentration of glucose actually depressed the oxidation of palmitate by about 50%. The mechanism underlying this inhibition can be explained by the finding that a high glucose concentration stimulates the rate of palmitate incorporation into islet lipids by twofold (Berne, 1975b). Competition for long-chain acyl-CoA between esterification and oxidative degradation has been demonstrated in liver and in Ehrlich ascites-tumour cells (Ontko, 1972; Spector & Steinberg, 1965). The magnitude of the inhibition of palmitate oxidation found presently can be fully accounted for by the increase in the rate of palmitate esterification when calculated on a molar

basis. This makes it less likely that the effect of glucose merely reflects a dilution of radioactive acetyl-CoA.

The measurements of the rates of ketone-body oxidation were made with concentrations of substrate which are attained *in vivo* only after a prolonged period of starvation. The oxidation rates, being less than 10% of those found in the kidney-cortex slices, were low compared with those of other oxidizable fuels in the islets. It should be pointed out, however, that in the kidney cortex the rate of ketone-body utilization is high and provides up to 80% of the respiratory fuel (Weidemann & Krebs, 1969). The oxidation rates found in the islets taken together with the low ketone-body concentrations normally found in blood *in vivo* make it likely that ketone bodies play a less significant role in total energy metabolism of the islets, at least in the fed state. Starvation may, however, increase the contribution of ketone bodies to the islet energy production.

Since measurement of the uptake of substrates into the pancreatic islets *in vivo* is hardly possible at present, the relative contributions of different sources of energy to the total islet metabolism can only be estimated approximately. One approach to this problem would be to compare the oxidation rates of different substrates measured at physiological concentrations in the same assay system *in vitro*. To facilitate such a comparison the molar oxidation rates were recalculated to eliminate differences caused by variations in the number of carbon atoms of the different substrates. In this way the energetic value of various carbon sources is better taken into account. This calculation actually underestimates the energetic value of fatty acids since they have a slightly higher ATP yield per carbon atom than glucose, which is attributable to the formation of two reducing equivalents per cycle of the β -oxidation chain. It is evident from this comparison that the non-carbohydrate energy sources may be quite important in islet metabolism (Table 4). During starvation or in the post-absorptive state, when the blood glucose concentration decreases and lipolysis leads to the production of fatty acids and the concomitant formation of ketone bodies from the liver, the islets are likely to show an increased dependence on the lipid-derived substrates. It should be pointed out, however, that evidence was obtained which showed that when glucose is available at high concentrations it is utilized for oxidation in preference to fatty acids (Table 2.)

As was the case with amino acids (Hellman *et al.*, 1971), a direct correlation between the rate of oxidation of fatty acids and of ketone bodies and their insulin-releasing capacity is hard to establish. Of the substrates investigated in the present study only octanoate was able to increase insulin output in isolated pancreatic islets from obese-hyperglycaemic mice (Berne, 1973). The fact that octanoate was the fatty acid with the highest oxidation rate in the

B-cells may, however, be of importance in this context. Octanoate is known to stimulate insulin release both in man (Linscheer *et al.*, 1967) and in rat islets *in vitro* (Sanbar & Martin, 1967). The responses to long-chain fatty acids and ketone bodies on the other hand show large species differences, but appear to need a permissive action of glucose to manifest itself as shown in the rat (Malaisse & Malaisse-Lagae, 1968; Goberna *et al.*, 1974). The mere observation that octanoate, long-chain fatty acids and ketone bodies are oxidized should be of importance when their actions on the B-cell are taken into consideration. It is possible that these substrates in the course of their degradation may, for example, bring about changes in metabolite concentrations which are relevant to their effects on insulin release.

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