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Starvation-induced changes of palmitate metabolism and insulin secretion in isolated rat islets stimulated by glucose

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The influence of 48 h starvation on glucose-induced changes of palmitate metabolism and insulin release in isolated rat islets was investigated. (1) Islet insulin response to 20 mM-glucose was abolished after 48 h starvation, and it was restored by 0.25 mM-2-bromostearate, an inhibitor of fatty acid oxidation. (2) The increase in glucose concentration from 3 to 20 mM was accompanied by a 50% decrease in the oxidation rate of 0.5 mM-[U-^{14}C]palmitate in control (fed) islets, and a concomitant increase (100%) in its incorporation into triacylglycerol and phospholipid fractions. (3) Starvation induced a higher basal (3 mM-glucose) rate of palmitate oxidation, which was resistant to inhibition by 20 mM-glucose. The latter also failed to increase palmitate incorporation into islet triacylglycerols and phospholipids. (4) 2-Bromostearate (0.25 mM) strongly inhibited the high oxidation rate of palmitate in islets of starved rats, and allowed a normal stimulation of its incorporation rate into islet lipids by 20 mM-glucose. (5) The results suggest that starvation restricts islet esterification of fatty acids by inducing a higher rate of their oxidative degradation that is insensitive to regulation by glucose.

Isolated islets of fed mice oxidize palmitate, and this process is inhibited by glucose (Berne, 1975*a*). Glucose also increases concomitantly the incorporation rate of palmitate into islet triacyglycerols and phospholipids (Berne, 1975*b*). According to the 'fuel hypothesis' of the mechanism of insulin release (Malaisse *et al.*, 1979), there is a close correlation between islet O_2 consumption and secretory rate, provided that the sparing action of some metabolizable secretagogues on the oxidation of endogenous substrates is taken into consideration (Hutton & Malaisse, 1980). Islet lipids are considered important contributors to the maintenance of a constant endogenous respiratory rate in the absence of exogenous substrates (Hellerström, 1967; Malaisse & Sener, 1981). In fact, some of the stimulators and potentiators of insulin release are known to decrease the oxidation rate of [U-^{14}C]palmitate in islets prelabelled with ^{14}C (Sener *et al.*, 1978; Malaisse *et al.*, 1980*a*,*b*, 1982).

It is well known that islets isolated from starved animals show a poor insulin response to glucose. This phenomenon has been ascribed either to a defective cyclic AMP production by glucose-stimulated islets (Howell *et al.*, 1973; Selawry *et al.*, 1973; Capito & Hedeskov, 1974; Hellman, 1976; Wolters *et al.*, 1977) or to an impaired islet glucose metabolism (Idahl, 1974; Hedeskov & Capito, 1974; Levy *et al.*, 1976; Burch *et al.*, 1981; Welsh & Andersson, 1982). These factors, however, do not allow a full explanation of the starvation-induced secretory defect (Bouman *et al.*, 1979; Welsh *et al.*, 1983).

We have reported (Tamarit-Rodríguez *et al.*, 1984) that 2-bromostearate, an inhibitor of fatty acid oxidation (Chase & Tubbs, 1972), restores a normal insulin repsonse to glucose in islets from starved animals and also potentiates palmitate-induced insulin release. Starvation is known to influence the metabolic fate of fatty acids in some tissues by increasing their oxidative degradation and decreasing their esterification rate (McGarry & Foster, 1980). Isolated islets seem to be greatly dependent on the exogenous supply of fatty acids for the synthesis of triacylglycerols and phospholipids (Berne, 1975*b*); hence a decrease of their esterification might lead to a diminished turnover rate of phospholipid metabolism. Therefore the general aim of the present work was to investigate the effects of starvation on the oxidation and esterification rates of palmitate in isolated rat islets, in correlation with the secretory activity.

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Experimental

Chemicals

$Na^{125}I$, D-[5-^{3}H]glucose, D-[U-^{14}C]glucose, [U-^{14}C]palmitic acid and $NaH^{14}CO_3$ were from The Radiochemical Centre, Amersham, Bucks., U.K. Stearic acid, palmitic acid and 2-bromostearic acid were from Serva, Heidelberg, Germany. Collagenase was from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Activated charcoal, Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], antimycin A, 2',7'-dichlorofluorescein, 1,2-dipalmitoylglycerol, tripalmitoylglycerol, L-α-phosphatidyl-choline,-ethanolamine and -L-serine were from Sigma, St. Louis, MO, U.S.A. Purified bovine serum albumin was from Behringwerke, Marburg, Germany, and fatty-acid-poor bovine serum albumin from Miles Laboratories, Kankakee, IL, U.S.A. Hyamine hydroxide was from Packard, Downers Grove, IL, U.S.A. 3,5-Diaminobenzoate dihydrochloride was from EGA-Chemie, Steinheim, Germany. Crystalline pig and rat insulins were from Novo Industri, Copenhagen, Denmark; Sephadex G-25 and G-75 were from Pharmacia Fine Chemicals, Uppsala, Sweden; calf thymus DNA was from Boehringer, Mannheim, Germany. All organic solvents and inorganic salts were of analytical grade, from Merck, Darmstadt, Germany.

Methods

Islets were isolated by collagenase (3mg/ml) digestion (Lacy & Kostianovsky, 1967) of individual pancreases from adult Wistar Albino rats (250g body wt.) in 5ml of Hanks balanced salt solution (Hanks & Wallace, 1949). Two batches of 40 islets were transferred to the chambers of a double perifusion system (1.5–2.0ml dead space) and simultaneously perifused at 1ml/min with Krebs–Ringer bicarbonate buffer, containing 115mM-NaCl, 4.7mM-KCl, 2.56mM-$CaCl_2$, 1.2mM-KH_2PO4, 1.2mM-$MgSO_4$, 24.0mM-$NaHCO_3$ and 1% bovine serum albumin, and equilibrated with O_2/CO_2 (19:1) to pH7.4. Samples of the perifusion effluent were taken every 1min, diluted with radioimmunoassay buffer and stored at −40°C until the radioimmunoassay of insulin was performed (Herbert *et al.*, 1965). [^{125}I]Iodoinsulin, prepared from cyrstalline pig insulin (Hunter & Greenwood, 1962), was purified by gel filtration in Sephadex G-25 and G-75. Crystalline rat insulin was used as a radioimmunoassay standard.

Palmitate oxidation by isolated islets during 2h incubation at 37°C was measured as the $^{14}CO_2$ production from 0.5mM-[U-^{14}C]palmitate, precisely as described by Berne (1975*a*). The incubation medium was Krebs–Ringer bicarbonate buffered with 10mM-Hepes (pH7.4), supplemented with 1% fatty-acid-poor bovine serum albumin and equilibrated with O_2/CO_2 (19:1). Fatty acids were neutralized with an equivalent amount of NaOH (100mM) by gentle heating (70–80°C), and a sample was transferred to the incubation medium (1:200 dilution). Labelled palmitic acid in toluene was dried under N_2 and redissolved by addition of incubation medium containing unlabelled palmitate, to give a final specific radioactivity of 10Ci/mol. The results are expressed as pmol of palmitate equivalents oxidized with the same specific radioactivity as that of the incubation medium measured in triplicates. They were corrected according to the recovery of $^{14}CO_2$ from 24mM-$NaH^{14}CO_3$ (0.63μCi/mmol), measured in separate experiments ($36.8 \pm 5\%$, $n = 10$). Antimycin A (0.01μM), used to stop metabolism, decreased palmitate oxidation to 0.005 ± 0.002pmol/h per islet ($n = 19$).

[U-^{14}C]Palmitate (0.5mM; 10Ci/mol) incorporation into islet lipids was studied by the method described by Berne (1975*b*). The same incubation medium as for oxidation studies was used. Groups of 20–25 islets were transferred to a centre well, containing 100μl of radioactive medium, and hanging inside a scintillation vial gassed with O_2/CO_2 (19:1). They were incubated at 37°C for 1, 2 or 3h with gentle shaking. Incubation was stopped by removing the islets, which were washed twice in non-radioactive medium, transferred to 125μl of a salt solution described by Folch *et al.* (1957) and sonicated in a MSE Ultrasonic Disintegrator for 15s at position 9. Two samples (10μl each) were taken for DNA measurements. The rest of the islet homogenate was immediately extracted with 2ml of chloroform/methanol (2:1, v/v). After addition of a solution (30μl) of carrier lipids in chloroform/methanol containing triacylglycerols, diacylglycerols, phospholipids (extracted from rat epididymal fat-pad and liver) and palmitic acid (1–3mg/ml of each), the extract was washed (Folch *et al.*, 1957) and the organic phase dried under N_2 and redissolved in 40μl of chloroform/methanol. A sample (30μl) was then applied to precoated plates of silica gel G (20cm × 20cm; Merck) previously activated for 1h at 110°C. The lipids were separated in a solvent system of n-hexane/diethyl ether/methanol/acetic acid (90:20:2:3, by vol.) (Garland & Randle, 1963) and detected under u.v. light after spraying the plates with 2',7'-dichlorofluorescein (0.2mg/ml of ethanol). The spots corresponding to triacylglycerol, fatty acids, diacylglycerol and phospholipid markers were scraped off into scintillation vials. After addition of 0.15ml of methanol and 5ml of scintillation mixture (5g of 2,5-diphenyloxazole/l of toluene), the radioactivity was measured in an Intertechnique liquid-scintillation counter (model SL

3000). The radioactivity of the incubation medium (5 μl triplicates) was also assayed after addition of 0.15 ml of methanol, 0.25 ml of Hyamine hydroxide and 5 ml of scintillation mixture, for the calculation of palmitate specific radioactivity. Percentage recovery of various amounts of labelled palmitate from t.l.c. plates was constant (98.03 $\pm$ 1.2%), and no contamination of other spots was detected.

Glucose utilization and oxidation were measured as the rates of ^3H_2O and $^{14}CO_2$ production from D-[5-^{3}H]glucose and D-[U-^{14}C]-glucose respectively (Ashcroft *et al.*, 1972; Tamarit-Rodríguez *et al.*, 1977). The incubation medium was in both cases Krebs–Ringer buffered with 20 mM-Hepes and no bicarbonate (pH 7.4), and supplemented with 0.5% bovine serum albumin. Groups of ten islets were transferred to tightly capped polyethylene tubes (400 μl; Beckman) with a side opening, containing either 20 μl (utilization) or 50 μl (oxidation) of radioactive incubation medium. They were suspended inside scintillation vials and incubated for 2 h at 37°C with gentle shaking. Metabolism was stopped by addition of 5 μl of 0.4 M-HCl (utilization) or 10 μl of 0.5 M-HCl (oxidation) to the tubes. The ^3H_2O produced (utilization) in the inner polyethylene tubes was allowed to equilibrate with 0.5 ml of ^1H_2O placed in the outer scintillation vial for 24 h at 37°C. In oxidation studies, the $^{14}CO_2$ produced was trapped in 0.5 ml of Hyamine hydroxide after 1 h of shaking at room temperature. ^{14}C (or ^{3}H) radioactivity in $^{14}CO_2$ (or ^3H_2O) and in triplicate samples (5 μl each) of incubation medium was analysed after addition of 10 ml (or 5 ml) of a toluene-based (or PCS; Amersham International) scintillation mixture. Percentage recovery of $^{14}CO_2$ from traces of $NaH^{14}CO_3$ was 98.2 $\pm$ 1.4%.

DNA was measured in duplicate samples (10 μl) of islet homogenates, by the method of Kissane & Robins (1958), modified by Vytasek (1982). Calf thymus DNA was used as the standard, and the fluorescence was measured in a Perkin–Elmer spectrophotofluorimeter (model MPF3) in a volume of 280 μl.

Results

Insulin secretion by perifused rat islets

As shown in Fig. 1 and Table 1, increasing the glucose concentration from 3 to 20 mM induced a 4-fold increase of insulin release in islets of fed rats, and this response was almost abolished by 48 h of starvation. 2-Bromostearate (0.25 mM) did not affect insulin release from control (fed) islets at 3 mM-, 6 mM- or 20 mM-glucose (Tables 1 and 2), but restored a normal secretory response to 20 mM-glucose in islets of starved rats (Fig. 1, Table 1).

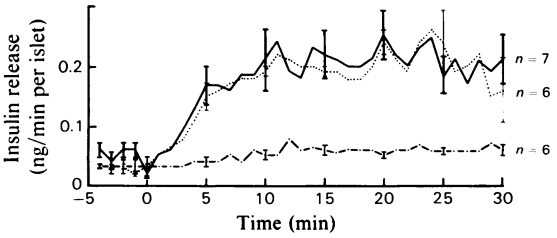

Fig. 1. *Insulin response to 20 mM-glucose by perifused rat islets, isolated from either fed or 48 h-starved rats, and the effect of 0.25 mM-2-bromostearate*

Details of analytical procedures are given in the Experimental section and the legend of Table 1. Mean secretion values (±S.E.M.) of islets isolated from either fed (——) or starved animals, treated (·····) with 0.25 mM-2-bromostearate or untreated (—·—), are shown at 5 min intervals.

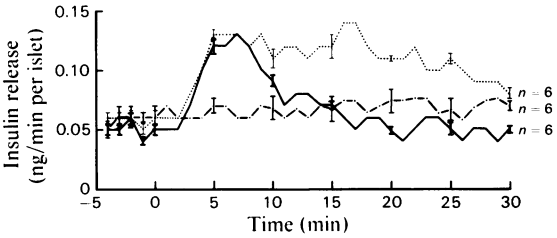

Fig. 2. *Insulin response to 0.5 mM-palmitate by perifused islets, isolated from fed rats, and the effect of 0.25 mM-2-bromostearate*

Methodological details are given in the Experimental section and legend of Table 2. Mean secretory values (±S.E.M.) of islets perifused with either 6 mM-glucose alone (—·—) or in combination with 0.5 mM-palmitate (——) or 0.5 mM-palmitate plus 0.25 mM-2-bromostearate (····) are shown at 5 min intervals.

Stearate (0.25 mM) was unable to prevent effects of starvation on insulin release (Table 1).

In the presence of 6 mM-, but not of 5 mM-, glucose, 0.5 mM-palmitate induced a modest and transient insulin response in fed islets that lasted for 10 min (Fig. 2, Table 2). Addition of 0.25 mM-2-bromostearate allowed a maintained islet secretory response to palmitate.

Palmitate oxidation by isolated islets

The islet production of $^{14}CO_2$ from 0.5 mM-[U-^{14}C]palmitate in control (fed) islets at 3 mM-glucose was significantly decreased by increasing the glucose concentration to 6 mM and 20 mM (Table 3). Islets from 48 h-starved rats exhibited a significantly higher (around 100%) rate of palmitate oxidation at 3 mM-glucose, which was unaffected by 20 mM-glucose. 2-Bromostearate (0.25 mM) decreased palmitate oxidation to very low values, irrespective of either glucose concentration (3, 6 or 20 mM) or the nutritional state of the animals.

Table 1. *Insulin response to 20 mM-glucose by perifused islets of fed and starved rats*

Two batches of 40 islets isolated from the same animal were simultaneously perifused at 3 mM-glucose (G3), with or without 0.25 mM-2-bromostearate (BrS) or 0.25 mM-stearate (S) (−30 to 0 min). At zero time they were stimulated by 20 mM-glucose (G20) in the absence or presence of either BrS or S (0 to 30 min). Values denote means ± S.E.M. of the total amount of insulin released during the stimulation period (0 to 30 min), and they were statistically compared by the non-paired Student's *t* test. Numbers of animals used in each experiment condition are shown in parentheses. *N.S. (not statistically significant) compared with line 1. ** $P<0.001$ compared with line 1.

Nutritional state	Additives: −30 to 0 min	Additives: 0 to 30 min	Insulin release (ng/30 min per islet)	*P*
1. Fed	G3	G20	5.57 ± 0.40 (7)	Control
2. Fed	G3 + BrS	G20 + Brs	5.57 ± 0.45 (6)	N.S.
3. Starved	G3	G20	1.97 ± 0.20 (8)	Control
4. Starved	G3 + BrS	G20 + BrS	5.40 ± 0.25 (8)*	<0.001
5. Starved	G3	G20	2.12 ± 0.41 (6)	Control
6. Starved	G3 + BrS	G20	5.42 ± 0.30 (6)*	<0.001
7. Starved	G3	G20	1.86 ± 0.24 (5)	Control
8. Starved	G3 + S	G20 + S	2.64 ± 0.26 (5)**	<0.05

Table 2. *Insulin response to 0.25 mM-palmitate (P) by perifused islets of fed rats*

Two batches of 40 islets isolated from the same animal were simultaneously perifused at 3 mM-glucose (−30 to 0 min) and then the medium was switched to one containing 5 mM- or 6 mM-glucose (G5 and G6 respectively) and other additives (0 to 30 min). Values denote means ± S.E.M. of the total amount of insulin released during min 0–10 and 11–30. They were statistically compared by the non-paired Student's *t* test. The same number of animals (six) was used in each experimental condition. Abbreviations: S, 0.5 mM-stearate; BrS, 0.25 mM-2-bromostearate; N.S., not statistically significant.

Additives	Insulin release (ng/islet): 0 to 10 min	*P*	11 to 30 min	*P*
1. G5	0.63 ± 0.10	Control	1.05 ± 0.24	Control
2. G5 + P	0.70 ± 0.06	N.S.	1.18 ± 0.07	N.S.
3. G6	0.67 ± 0.05	Control	1.35 ± 0.11	Control
4. G6 + P	1.05 ± 0.04	0.01	1.32 ± 0.08	N.S.
5. G6	0.66 ± 0.10	Control	1.36 ± 0.13	Control
6. G6 + BrS	0.70 ± 0.10	N.S.	1.38 ± 0.10	N.S.
7. G6 + P	1.03 ± 0.04	Control	1.36 ± 0.12	Control
8. G6 + P + BrS	1.06 ± 0.03	N.S.	2.30 ± 0.05	<0.001

Table 3. *Palmitate oxidation by isolated islets*

Two or three batches of islets from either fed (control) or starved rats were incubated at 37°C for 2 h in the presence of 0.5 mM-[U-^{14}C]palmitate and other additives, as indicated. Values denote means ± S.E.M. and were statistically compared by a non-paired Student's *t* test. Numbers of animals used in each experimental condition are shown in parentheses. (G3, G6 and G20, 3 mM-, 6 mM- and 20 mM-glucose; BrS, 0.25 mM-2-bromostearate). *N.S. (not significant) compared with line 5; ** $P<0.01$ compared with line 3.

Nutritional state	Additives	Palmitate oxidation (pmol/h per islet)	*P*
1. Fed	G3	0.95 ± 0.08 (22)	Control
2. Fed	G3 + BrS	0.14 ± 0.05 (6)	<0.001
3. Fed	G20	0.54 ± 0.03 (10)	<0.001
4. Fed	G20 + BrS	0.19 ± 0.05 (7)	<0.001
5. Starved	G3	1.75 ± 0.09 (12)	<0.001
6. Starved	G3 + BrS	0.20 ± 0.05 (11)	<0.001
7. Starved	G20	1.66 ± 0.16 (11)*	<0.001
8. Starved	G20 + BrS	0.18 ± 0.03 (9)	<0.001
9. Fed	G3	0.94 ± 0.05 (5)	Control
10. Fed	G3 + BrS	0.16 ± 0.07 (5)	<0.001
11. Fed	G6	0.69 ± 0.11 (5)**	<0.001
12. Fed	G6 + BrS	0.18 ± 0.05 (5)	<0.001

Palmitate incorporation into islet triacylglycerol and phospholipid fractions

The incorporation of 0.5mM-[U-^{14}C]palmitate into triacylglycerols of fed-rat islets reached an apparent isotopic equilibrium after 1h at either 3mM- or 20mM-glucose (Table 4). The ^{14}C content of triacylglycerols at equilibrium was significantly higher (more than 2-fold) in the presence of 20mM- than of 3mM-glucose. Radiolabelling of the islet phospholipid fraction tended towards a steady state after 2h of incubation at 3mM-glucose, whereas it proceeded linearly for 3h at 20mM-glucose (Table 4). The latter induced a significantly greater rate of islet phospholipid labelling than did 3mM-glucose at all times studied. In the following experiments we used an incubation time of 2h.

Table 5 shows that 2-bromostearate (0.25mM) did not modify the incorporation rate of [U-^{14}C]-palmitate into triacylglycerols and phospholipids of control (fed) islets at 20mM-glucose, but increased it significantly at either 3mM or 6mM. The increase was significantly greater at 6mM- than at 3mM-glucose. Addition of glycerol at various concentrations (1, 5 and 10mM) did not modify [^{14}C]-palmitate incorporation into islet lipids even in the presence of 2-bromostearate (Table 5).

Isolated islets from 48h-starved rats exhibited a similar rate of [^{14}C]palmitate incorporation into triacylglycerols and phospholipids to control

Table 4. *Time-dependence of palmitate incorporation into islet triacylglycerol and phospholipid fractions*
Two or three batches of isolated islets were incubated at 37°C with 0.5mM-[U-^{14}C]palmitate at either 3mM- or 20mM-glucose for the times indicated below. Details of the analytical procedure are given in the Experimental section. Values denote means ± S.E.M.; numbers of animals used in each experiment are shown in parentheses. All values recorded at 20mM-glucose were significantly higher ($P<0.001$) than the corresponding ones at 3mM.

Glucose (mM)	Time (h)	Phospholipids (pmol/μg of DNA)	P	Triacylglycerols (pmol/μg of DNA)	P
3	1	13.51 ± 0.50 (6)	Control	12.87 ± 0.18 (6)	Control
3	2	30.00 ± 1.40 (11)	<0.001	29.20 ± 3.60 (14)	<0.001
3	3	40.51 ± 1.20 (6)	<0.001	29.81 ± 2.80 (6)	<0.001
20	1	33.19 ± 1.06 (12)	Control	33.39 ± 1.40 (10)	Control
20	2	70.86 ± 2.64 (7)	<0.001	72.36 ± 3.60 (7)	<0.001
20	3	146.56 ± 10.70 (6)	<0.001	71.90 ± 4.16 (7)	<0.001

Table 5. *Palmitate incorporation into islet triacylglycerol and phospholipid fractions: effect of glucose and glycerol concentrations, starvation and 2-bromostearate*
Two or three batches of islets were incubated at 37°C with 0.5mM-[U-^{14}C]palmitate and other additives for 2h, and the radioactivity in triacylglycerols and phospholipid was assayed as described in the Experimental section. Values denote means ± S.E.M.; numbers of animals used in each experimental condition are given in parentheses. Statistical comparisons were performed by the non-paired Student's t test: *$P<0.001$ compared with line 5; **N.S. (not statistically significant) compared with line 5; †$P<0.05$ compared with line 2. Abbreviations: G3, G6 and G20, 3mM-, 6mM- and 20mM-glucose respectively; Gol.1, Gol.5 and Gol.10, 1mM-, 5mM- and 10mM-glycerol; BrS, 0.25mM-2-bromostearate.

Nutritional state	Additives	Phospholipids (pmol/h per μg of DNA)	P	Triacylglycerols (pmol/h per μg of DNA)	
1. Fed	G3	17.48 ± 0.68 (11)	Control	14.57 ± 1.79 (14)	Control
2. Fed	G3 + BrS	20.48 ± 0.53 (5)	<0.005	19.62 ± 1.17 (5)	<0.001
3. Fed	G6	15.81 ± 2.08 (6)	N.S.	14.99 ± 1.2 (6)	N.S.
4. Fed	G6 + BrS	23.54 ± 2.6 (6)†	<0.05	23.24 ± 3.3 (7)†	<0.001
5. Fed	G20	35.40 ± 1.34 (7)	<0.001	36.18 ± 1.81 (7)	<0.001
6. Fed	G20 + BrS	36.00 ± 1.72 (6)	<0.001	36.95 ± 2.60 (6)	<0.001
7. Starved	G3	18.01 ± 0.86 (5)	N.S.	13.88 ± 1.86 (5)	N.S.
8. Starved	G3 + BrS	17.96 ± 0.37 (5)	N.S.	12.99 ± 1.49 (5)	N.S.
9. Starved	G20	17.10 ± 2.10 (9)*	N.S.	13.31 ± 1.15 (9)*	N.S.
10. Starved	G20 + BrS	40.71 ± 2.50 (6)**	<0.001	36.93 ± 1.3 (6)**	<0.001
11. Fed	G3	11.74 ± 1.33 (5)	Control	13.92 ± 1.70 (5)	Control
12. Fed	G3 + Gol.1	12.74 ± 2.90 (5)	N.S.	13.71 ± 1.80 (5)	N.S.
13. Fed	G3 + Gol.5	12.23 ± 0.50 (4)	N.S.	13.78 ± 1.10 (5)	N.S.
14. Fed	G3 + Gol.5 + BrS	13.37 ± 0.14 (5)	N.S.	13.64 ± 0.39 (4)	N.S.
15. Fed	G3 + Gol.10	13.00 ± 2.80 (5)	N.S.	13.39 ± 2.80 (5)	N.S.
16. Fed	G3 + Gol.10 + BrS	12.63 ± 2.70 (5)	N.S.	14.02 ± 1.90 (5)	N.S.

Table 6. *Islet glucose metabolism*

Two or three batches each of ten islets were incubated at 37°C for 2h in medium containing either 3mM- or 20mM-[5-^{3}H]glucose (16.6 or 2.5Ci/mol respectively) or 3mM- or 20mM-D-[U-^{14}C]glucose (11.6 or 2.5Ci/mol respectively). Glucose utilization and oxidation rates were measured as the amounts of ^3H_2O and $^{14}CO_2$ produced from labelled glucose respectively. Values denote means ± S.E.M., and they were statistically compared by the non-paired Student's *t* test; the numbers of animals used in each experimental condition are given in parentheses. Abbreviations: G3 and G20, 3mM- and 20mM-glucose; BrS, 0.25mM-2-bromostearate. *N.S. (not statistically significant) compared with line 2; **N.S. compared with line 7.

Nutritional state	Additives	Glucose utilization (pmol/h per islet)	*P*
1. Fed	G3	14.17 ± 0.91 (16)	Control
2. Fed	G20	83.03 ± 2.28 (7)	<0.001
3. Fed	G20 + BrS	85.13 ± 2.72 (13)*	<0.001
4. Starved	G20	82.40 ± 1.41 (17)*	<0.001
5. Starved	G20 + BrS	81.20 ± 1.29 (15)*	<0.001
		Glucose oxidation (pmol/h per islet)	
6. Fed	G3	2.90 ± 0.12 (23)	Control
7. Fed	G20	15.71 ± 0.63 (11)	<0.001
8. Starved	G20	14.89 ± 0.77 (12)**	<0.001
9. Starved	G20 + BrS	15.41 ± 0.63 (9)**	<0.001

(fed) islets at 3mM-glucose, but it was not increased by 20mM-glucose (Table 5). 2-Bromostearate (0.25mM) did not modify the labelling rate of triacylglycerols and phospholipids at 3mM-glucose in those islets, but allowed a normal stimulation by 20mM-glucose.

Glucose metabolism of isolated islets

Raising the glucose concentration from 3 to 20mM increased at least 5-fold sugar-utilization and -oxidation rates in islets isolated from fed rats (Table 6). Neither 48h starvation nor 2-bromostearate significantly modified these metabolic parameters. Glucose-utilization rates have been found to be consistently depressed by starvation in previous islet studies (see the introduction). The discrepancy of our results may be due to the use of a different incubation medium, namely Krebs–Ringer exclusively buffered with 20mM-Hepes and no bicarbonate. However, we emphasize that 2-bromostearate did not exert any non-specific inhibitory effect on glucose metabolism.

Discussion

The experimental data on insulin release obtained here with a double perifusion system confirm our previous results (Tamarit-Rodríguez *et al.*, 1984), demonstrating the ability of 2-bromostearate to restore a normal insulin response to 20mM-glucose in islets from 48h-starved rats. Confirming also previous reports from our and other laboratories (Goberna *et al.*, 1974; Tamarit-Rodríguez *et al.*, 1984), palmitate induced a transient stimulation of insulin secretion at 6mM-glucose, but not at a lower concentration, that was changed into a sustained release by 2-bromostearate.

A change of glucose concentration from 3 to 20mM induces important changes of [U-^{14}C]-palmitate metabolism in isolated islets. Whereas the oxidation of palmitate ($^{14}CO_2$ production) was decreased by almost 50%, its incorporation into triacylglycerols and phospholipids was increased by more than 2-fold. As labelling of islet triacylglycerols reaches isotopic equilibrium after 1h at either 3mM- or 20mM-glucose, the higher islet radioisotope content obtained at the latter concentration may reflect a net increase of islet triacylglycerol stores. Islet phospholipid labelling at 20mM-glucose did not reach a steady state after 3h incubation, but was greater than that obtained at 3mM-glucose at all times studied, indicating a tendency towards a higher radioisotope content at equilibrium. These results are compatible with an increased rate of phospholipid and triacylglycerol synthesis and content in islets stimulated by 20mM-glucose. Evidence of an enhancing effect of glucose on the synthesis rate of some islet phospholipid classes has been reported (Laychock, 1983).

Islets from 48h-starved rats exhibited a higher rate of palmitate oxidation than did control (fed) islets at 3mM-glucose, which was not significantly decreased by 20mM-glucose. The latter also failed to increase the incorporation of [^{14}C]palmitate into triacylglycerol and phospholipid fractions. Inhibition of palmitate oxidation by 2-bromostearate resulted in a restoration of the glucose effect on palmitate incorporation into islet phospholipids

and triacylglycerols. This suggests that starvation blocks the ability of glucose to decrease fatty acid oxidation and to increase their esterification in islet cells. Similar changes of lipid metabolism occurring in livers of starved rats are also reversed by inhibitors of fatty acid oxidation (McGarry *et al.*, 1973; Ide & Ontko, 1981). This demonstrates that a direct inhibition of the latter process diverts exogenous fatty acids to the esterification pathway.

A physiological mechanism responsible for cellular regulation of fatty acid oxidation is now ascribed to hormone-induced modifications of acetyl-CoA carboxylase activity (Witters, 1981), which result in variations of malonyl-CoA concentrations, a specific inhibitor of carnitine acyltransferase (McGarry & Foster, 1980; Mills *et al.*, 1983). The starvation-induced increase in oxidation and decrease in esterification of fatty acids have been attributed to inhibition of acetyl-CoA carboxylase (Zammit & Corstorphine, 1982) and to a decreased sensitivity of carnitine acyltransferase to inhibition by malonyl-CoA (McGarry & Foster, 1981; Saggerson & Carpenter, 1982; Robinson & Zammit, 1982). It is unknown how the aforementioned enzymes are regulated in islet cells, but they are able to synthesize fatty acids from glucose (Berne, 1975*a*). Although of little quantitative importance, this process seems to be very dependent on glucose concentration, and might supply adequate malonyl-CoA concentrations for regulating the relative contributions of oxidation and esterification to the overall metabolism of exogenous fatty acids in islet cells. This idea is supported by the finding that starvation significantly decreases incorporation of labelled glucose into several phospholipid classes of mouse islets (Hallberg, 1983).

2-Bromostearate was not able to increase palmitate esterification in control (fed) islets at 3mM-glucose to that obtained at 20mM. This suggests that some other factors besides inhibition of fatty acid oxidation participate in glucose regulation of islet palmitate esterification. Glycerol 3-phosphate concentrations may under certain conditions restrict palmitate esterification in isolated hepatocytes (Lund *et al.*, 1980; Declercq *et al.*, 1982). Its production from glucose might also contribute to make islet fatty acid esterification dependent on sugar concentration. Consistent with this idea, the incorporation of [^{14}C]palmitate into islet phospholipids and triacylglycerols in the presence of 2-bromostearate was significantly higher at 6mM- than at 3mM-glucose. On the other hand, attempts to increase the incorporation of palmitate into islet lipids at 3mM-glucose by addition of glycerol were fruitless. It cannot be discarded, however, that islet cells may have a low glycerol kinase activity, as occurs in adipocytes (Newsholme & Start, 1973). Glycerol was also unable to stimulate insulin release in the presence of 3mM-glucose, with or without 0.25mM-2-bromostearate (J. Tamarit-Rodríguez & E. Vara, unpublished work).

In conclusion, glucose regulates the relative contributions of the oxidative and esterification pathways to the overall rate of exogenous fatty acid metabolism in the islets by favouring the latter. Starvation blocks this regulatory role of glucose as well as its stimulatory effect on insulin secretion. Both abilities of glucose are restored in islets of starved rats by 2-bromostearate, a fatty-acid-oxidation inhibitor.

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