Palmitate dependence of insulin secretion, “de novo” phospholipid synthesis and $^{45}\text{Ca}^{2+}$ -turnover in glucose stimulated rat islets

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Summary. Palmitate ability to modify D-[U- ^{14}C]glucose incorporation into different lipids (“de novo” synthesis), as well as sugar-stimulation of insulin release and $^{45}\text{Ca}^{2+}$ -fluxes, was investigated in islets of fed and 48-h starved rats. The fatty-acid induced dose-dependent, correlative increments of insulin secretion, $^{45}\text{Ca}^{2+}$ -influx and the “de novo” synthesis of each phospholipid fraction analysed at 20 mmol/l (but not 3 mmol/l) glucose. Omission of calcium reduced drastically ($p < 0.001$) insulin release and the “de novo” synthesis of neutral glycerolipids, leaving unaltered that of acidic phospholipids (phosphatidate and phosphoinositides). The increased synthesis of the latter is therefore not the consequence of stimulated secretion. It could initiate or contribute to maintain an increased turnover of islet phosphoinositides, thus generating some mediators of the calcium signalling system (inositol phosphates). Starvation

led to a drastic reduction ($p < 0.001$) of both insulin secretion, “de novo” synthesis of each lipid fraction, and $^{45}\text{Ca}^{2+}$ -influx in response to glucose and palmitate. The presence of a fatty-acid oxidation inhibitor (2-bromostearate or 2-tetradecylglycidate) prevented the effect of starvation on $^{45}\text{Ca}^{2+}$ -influx, as it has been shown to do on insulin secretion and palmitate incorporation into islet lipids. It is finally suggested that palmitate might amplify the insulin secretory response of islets to glucose, through the stimulation of the “de novo” synthesis of phosphoinositides and the subsequent generation of inositol phosphates, which would contribute to accelerated calcium turnover.

Key words: Islets of Langerhans, insulin release, “de novo” phospholipid synthesis, $^{45}\text{Ca}^{2+}$ -turnover.

Glucose is thought to stimulate insulin secretion by increasing the concentration of cytoplasmic calcium in B cells [1–3]. As emphasized in the past [4], this may be accomplished by either a net increase of cellular calcium uptake and/or a net release of the cation from intracellular stores. Islet phospholipid metabolism has been implicated in the mechanism of glucose-induced calcium release from intracellular stores through the generation of inositol 1, 4,5-trisphosphate (IP_3) mediated by the hydrolysis of phosphatidylinositol 4,5-bisphosphate [4, 5]. The possibility has also been raised that inositol 1,3,4,5-tetrakisphosphate (IP_4), a phosphorylated metabolite of IP_3 , might act at the plasma membrane facilitating calcium entry [6].

As already emphasized by others [7, 8], it is difficult to reconcile glucose activation of a phospholipase C-specific hydrolysis of polyphosphoinositides in B cells with the lack of firm evidence for the presence of a specific glucoreceptor. However, glucose does stimulate the “de novo” synthesis of islet phospholipids [9–12] and in this way it could also conceivably provide the B cells with some of the postulated messengers (IP_3 , IP_4 , diacylglycerol) of the calcium signalling system [13]. Palmitate is able to potentiate dose-de-

pendently (0 to 1 mmol/l) glucose-stimulation of “de novo” phospholipid synthesis and insulin release, both effects being suppressed on rats starved for 48 h [14]. Starved islets are also characterised by high oxidation rates of exogenous palmitate that are insensitive to inhibition by glucose, leading to low rates of fatty-acid incorporation into lipids [15]. This situation, and the secretory defect, are reversed by 2-bromostearate, a known fatty-acid oxidation inhibitor [15]. In the present work, we have investigated whether palmitate potentiation of glucose-induced insulin secretion is accompanied by an increased “de novo” synthesis of any specific phospholipid and/or an enhancement of islet $^{45}\text{Ca}^{2+}$ -uptake and how they are affected by starvation.

Materials and methods

Chemicals

D-[U- ^{14}C]glucose and Na^{125}I were from The Radiochemical Centre (Amersham, Bucks, UK). Activated charcoal, Hepes[4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 2', 7'-dichlorofluorescein and standard lipids were from Sigma Chemical Co (St. Louis, Mo,

Fig. 1. Time-dependent incorporation of 3 mmol/l (—•—) or 20 mmol/l (—) D-[U-¹⁴C]glucose (110 and 33 Ci/mol, respectively) into islet lipids at 1 mmol/l palmitate. The different symbols denote means $\pm$ SEM of 3 (60 min) or 5 (5, 15, 120 min) experiments. DG (*) and TG (■) are di- and tri-acylglycerols, respectively; PC (●) and PE (▲), phosphatidyl-choline and -ethanolamine; PI (★) and PA (△), phosphatidylinositol and phosphatidic acid; PPI (☆), polyphosphoinositides

Fig. 2. Effect of palmitate (Palm.) concentration (■, 0.25 mmol/l, $n=3$; □, 1 mmol/l, $n=7$ or 9), starvation (▨, $n=3$) and Ca^{2+} -omission (■, $n=5$ or 7) on 3 mmol/l (set of columns to the left in each panel) or 20 mmol/l (set of columns to the right) D-[U-¹⁴C] glucose (56 or 17 Ci/mol) incorporation into di- (DG) and tri-acylglycerols (TG), phosphatidyl-choline (PC) and -ethanolamine (PE). * $p < 0.01$ and ** $p < 0.001$ compared with the respective controls (open columns)

USA). Fatty-acid-poor bovine serum albumin was from Miles Laboratories (Kankakee, Ill, USA). Versilube F-50 was from Klöckner (Duisburg, FRG). 3,5-Diaminobenzoate dihydrochloride and Tris(hydroxymethyl)aminomethane (TRIS) were from Aldrich-Chemie (Steinheim, FRG). Crystalline pig and rat insulins were from Novo Industri (Copenhagen, Denmark); Sephadex G-25 and G-75 were from Pharmacia Fine Chemicals (Uppsala, Sweden), Collagenase and calf thymus DNA were from Boehringer (Mannheim, FRG). Palmitic and 2-bromostearic acids were from Serva (Heidelberg, FRG). 2-Tetradecylglycidic acid (McN-3802) was kindly supplied by McNeil Pharmaceutical (Spring House, Penn, USA). All organic solvents and inorganic salts were of analytical grade, from Merck (Darmstadt, FRG).

Methods

Islets were isolated by collagenase digestion [16] of the pancreas from adult Wistar Albino rats (males weighing 250 g). Insulin secretion was studied in batch-type incubations (3 islets/300 μ l) in Krebs Ringer bicarbonate (KRB), buffered with 10 mmol/l Hepes and equilibrated with O_2/CO_2 (19:1) to pH 7.4. Albumin (free of fatty acids) concentration was varied according to the amount of neutralised palmitic (0.5, 1.0 and 2.0% (w/v) albumin for 0.25, 0.50 and

1.0 mmol/l palmitate, respectively), 2-bromostearic (0.5% for 0.25 mmol/l 2-bromostearate) and 2 tetradecylglycidic acid (0% for 10 μ mol/l 2-tetradecylglycidate) added to the medium. [¹²⁵I] Iodoinsulin was prepared from crystalline pig insulin [17] and purified by gel filtration in Sephadex G-25 and G-75. Crystalline rat insulin was used as a standard of radioimmunoassay [18].

The "de novo" synthesis of islet lipids was measured by the incorporation of D-[U-¹⁴C]glucose. In the time-kinetic study (Fig. 1), the islets were preincubated for 30 min at 37°C in 25 μ l of the medium described above, and the incubation continued for different time-lengths after addition of another 25 μ l of the same medium, supplemented with the isotope. At the end of incubation, the tubes containing the medium and islets were rapidly frozen by plunging them in acetone chilled with dry ice. After addition of 50 μ l salt solution [19] containing 50 mmol/l glucose and 0.7 ml of acid methanol (containing 7 μ l conc. HCl) to the tubes, the content was sonicated in a MSE Ultrasonic Disintegrator. The lipids were then extracted with 1.3 ml of chloroform and 0.4 ml of salt solution for 1 h at room temperature. The organic phase was then washed 3 times with 1.0 ml of the aqueous phase of a system composed of chloroform/methanol/salt solution/concentrated HCl (266:133:100:1, v/v), after addition of 30 μ l of carrier lipids in chloroform/methanol/concentrated HCl (200:100:1, v/v). The carrier lipids were obtained from rat livers that were extracted with acidified chloroform/methanol (2/1, v/v). In other experiments (Fig. 2 and 3), the

Fig. 3. Effect of palmitate (Palm.) concentration (▨, 0.25 mmol/L, $n=3$; □, 1 mmol/L, $n=7$ or 9), starvation (▩, $n=3$) and Ca^{2+} -omission (■, $n=5$ or 7) on 3 mmol/L (set of columns to the left in each panel) or 20 mmol/L (set of columns to the right) D-[U- ^{14}C] glucose (56 or 17 Ci/mol) incorporation into polyphosphoinositides (PPI), phosphatidylinositol (PI) and phosphatidic acid (PA). + $p < 0.05$, ++ $p < 0.02$, * $p < 0.01$ and ** $p < 0.001$ compared with the respective controls (open columns)

islets were incubated with D-[U- ^{14}C]glucose for 120 min at 37°C. Incubation was stopped by removing the islets, which were washed twice in a non-radioactive medium, transferred to 125 μl of salt solution and sonicated. Two samples (10 μl each) were taken for DNA measurements. The rest of the islet homogenate was extracted with 2 ml of chloroform/methanol/concentrated HCL and washed as described above. The organic phase was dried under N_2 and redissolved in 40 μl of chloroform/methanol (2:1, v/v). A sample (30 μl) was then applied to precoated plates of silica gel 60 (20 cm $\times$ 20 cm; Merck) previously activated for 1 h at 110°C. The lipids were separated by unidimensional chromatography in two solvent systems that were subsequently applied: 1) n-hexane/diethyl ether/methanol/acetic acid (90:20:2:3, v/v), 2) chloroform/methanol/acetic acid/ H_2O (100:60:16:5, v/v). After spraying the plates with 2', 7'-dichlorofluorescein (0.2 mg/ml of ethanol), the following spots were detected under ultraviolet light and identified with adequate markers: polyphosphoinositides (PPI) (origin), lysophosphatidylcholine, phosphatidylcholine (PC), -serine and -inositol (PI), -ethanolamine (PE), phosphatidic acid (PA), fatty acids, di- (DG) and tri-acylglycerols (TG). Each spot was scraped off into a scintillation vial and its radioactivity measured [15]. The results are expressed in

pmol of labelled glucose, with the same specific radioactivity as that of the incubation medium, incorporated into each lipid fraction.

DNA was measured in duplicate samples (10 μl) of islet homogenates, by the method of Kissane & Robins [20], modified by Vytasek [21], using calf thymus DNA as standard. The mean $\pm$ SEM of the DNA content of rat islets was calculated as $0.07 \pm 0.004 \mu\text{g/islet}$ ($n=50$).

The uptake of $^{45}\text{Ca}^{2+}$ into isolated islets was measured with the lanthanum-wash procedure, allowing a better discrimination between extracellular and intracellular calcium [22]. Two to three batches each of ten islets were preincubated (30 min at 37°C) in 50 μl of the same incubation medium (2.56 mmol/L CaCl_2) used in secretion studies, layered on the top of a tube containing 50 μl of modified KRB (chloride as the only anion, buffered with 5 mmol/L TRIS to pH 7.4 and containing 2 mmol/L LaCl_3 and 6 mol/L urea) at the bottom with an intermediate layer of silicon-oil (130 μl Versilube F-50). Incubation was started by adding 50 μl of prewarmed incubation medium containing $^{45}\text{CaCl}_2$ (16 Ci/mol) and it was stopped after variable times (5–120 min) by centrifugation of islets through the oil-layer. The conic tip of the tube containing the islets was cut off and incubated further for 60 min at 37°C in 10 ml of a buffer of similar composition to that of the bottom layer but lacking urea. The tip was finally introduced into a scintillation vial and its radioactivity measured. Tubes without islets were systematically run in parallel to check for any possible contamination. Lanthanum-washing of extracellular radioactivity was apparently complete after 45 min and the remaining islet content of $^{45}\text{Ca}^{2+}$ stayed constant even after several hours of incubation with LaCl_3 .

The efflux of $^{45}\text{Ca}^{2+}$ from islets preloaded with the isotope for 120 min was followed by measuring the radioactivity remaining in the tissue after variable incubation times in a non-radioactive medium. To eliminate extracellular radioactivity, islets were centrifuged through an oil-layer immediately after their incubation in the non-radioactive medium and washed with LaCl_3 for 60 min, as described above in the method for the study of $^{45}\text{Ca}^{2+}$ -uptake.

Statistical analysis

The values in all Figures and Tables represent means $\pm$ SEM, and the figures in brackets indicate the number of animals used in each experimental condition. Statistical comparisons between pairs of means were made with Student's t-test for non-paired values. A two-tail p value lower than 0.05 between means was accepted as statistically significant.

Results

Insulin secretion by isolated islets (Table 1)

Glucose (20 mmol/L) increased approximately 10-fold the secretion of insulin at each palmitate concentration. The amount of insulin released was significantly higher (almost two-fold) at 1 mmol/L than 0.25 mmol/L palmitate ($p < 0.01$). Calcium omission did not significantly modify islet insulin secretion at 3 mmol/L glucose, but it reduced drastically the hormone response to 20 mmol/L glucose in the presence of palmitate ($p < 0.001$). Islets from 48-h starved rats showed considerably lower secretory rates than control (fed) islets at each glucose or palmitate concentration. They responded weakly but significantly to 20 mmol/L glucose although no difference between 0.25 mmol/L and 1.0 mmol/L palmitate could be appreciated.

Table 1. Effect of palmitate concentration, starvation and Ca^{2+} -omission on insulin secretion

Glucose (mmol/l)	Palmitate (mmol/l)	Ca Cl_2 (mmol/l)	Insulin release (ng/h $\times$ islet)				
			Fed (A)	<i>p</i>	Starved (B)	<i>p</i>	(A-B)
3	0.25	2.56	1.20 $\pm$ 0.05 (3)	Control	0.58 $\pm$ 0.01 (3)	Control	<i>p</i> < 0.001
3	0.25	0	0.89 $\pm$ 0.09 (6)	NS	–	–	–
3	1.0	2.56	2.60 $\pm$ 0.20 (3)	< 0.01	0.59 $\pm$ 0.12 (3)	NS	<i>p</i> < 0.01
3	1.0	0	2.00 $\pm$ 0.20 (5)	< 0.05	–	–	–
20	0.25	2.56	14.2 $\pm$ 1.00 (3)	Control	4.01 $\pm$ 0.52 (3)	Control	<i>p</i> < 0.001
20	0.25	0	4.05 $\pm$ 0.40 (5)	< 0.001	–	–	–
20	1.0	2.56	22.5 $\pm$ 0.08 (3)	< 0.01	4.58 $\pm$ 0.30 (3)	NS	<i>p</i> < 0.001
20	1.0	0	5.9 $\pm$ 0.50 (7)	< 0.001	–	–	–

Five batches each of three islets were incubated at 37°C for 120 min and the insulin released was radioimmunologically measured in the incubation medium. NS, non significant

Fig. 4. Time-dependent study of $^{45}\text{Ca}^{2+}$ -uptake by isolated islets at 3 mmol/l (open symbols) and 20 mmol/l (closed symbols) glucose, in the presence of different palmitate concentrations (○ and ●, 0 mmol/l; □ and ■, 0.25 mmol/l; △ and ▲, 1 mmol/l). **p* < 0.025, +*p* < 0.02, ++*p* < 0.005 and ****p* < 0.001 compared with the corresponding time values obtained in the absence of palmitate

“De novo” synthesis of islet lipids

The labelling of the different islet phospholipids with 3 mmol/l D-[U- ^{14}C]-glucose was very low and it increased steadily with time in the case of phosphatidylcholine, phosphatidylethanolamine, triacylglycerols and diacylglycerols (Fig. 1). After 5 min of incubation with 20 mmol/l D-[U- ^{14}C]-glucose, the content of isotope was already significantly increased (compared with the corresponding value at 120 min in 3 mmol/l labelled glucose) in polyphosphoinositides (4-fold, *p* < 0.001), phosphatidylinositol (2-fold, *p* < 0.01) and phosphatidate (6-fold, *p* < 0.001). The incorporation of labelled glucose increased progressively with time in all the lipid fractions. Isotopic equilibrium was apparently reached in phosphatidate, polyphosphoinositides and diacylglycerol between min 5 and 15. The other lipid fractions showed a constant rate of labelling

(phosphatidylcholine) or a tendency to reach isotopic equilibrium between min 60 and 120 (triacylglycerols, phosphatidylethanolamine, phosphatidylinositol).

As shown in Figures 2 and 3 the amount of 20 mmol/l D-[U- ^{14}C]-glucose incorporated into every islet lipid fraction analysed, was augmented approximately 2-fold by increasing the concentration of palmitate from 0.25 to 1.0 mmol/l. At the highest concentration, islets from 48-h starved rats incorporated significantly less labelled glucose in all the lipid fractions. Omission of calcium from the incubation medium reduced labelled glucose incorporation into diacylglycerol, triacylglycerol, phosphatidylcholine and phosphatidylethanolamine (Fig. 2) but not in the other fractions (Fig. 3). Although the reduction of 20 mmol/l D-[U- ^{14}C]-glucose incorporation into diacylglycerol did not reach statistical significance at 1 mmol/l palmitate, calcium omission induced a statistically significant decrease at 0.25 mmol/l palmitate of either 3 mmol/l (0.29 $\pm$ 0.05, *n* = 5 vs 1.36 $\pm$ 0.41, *n* = 3; *p* < 0.01) or 20 mmol/l (1.07 $\pm$ 0.23, *n* = 5 vs 2.29 $\pm$ 0.16, *n* = 3; *p* < 0.01) labelled glucose incorporation. The effect of calcium omission could be reproduced with 1 $\mu\text{mol/l}$ trifluoperazine in diacylglycerols (2.15 $\pm$ 0.3, *n* = 5 vs 3.01 $\pm$ 0.24, *n* = 9; *p* < 0.05), as well as in the other lipid fractions and insulin release (unpublished observations), at 20 mmol/l glucose and 1 mmol/l palmitate.

$^{45}\text{Ca}^{2+}$ -uptake into a lanthanum-nondisplaceable pool of islets

The time-dependent uptake of $^{45}\text{Ca}^{2+}$ into this pool was comparatively studied at two different glucose concentrations (3 and 20 mmol/l) and in the absence or presence of palmitate (0.25 and 1 mmol/l), as it is shown in Figure 4. Twenty mmol/l glucose induced a faster initial rate of uptake that was constant between 5 and 15 min and it led to a higher islet isotope content than 3 mmol/l glucose after 120 min of incubation. The addition of palmitate (either 0.25 mmol/l and 1.0 mmol/l) caused a significantly higher islet uptake of $^{45}\text{Ca}^{2+}$ than 20 mmol/l glucose alone after 5, 10

and 15 min of incubation with the isotope, but not after 60 or 120 min. This enhancing effect was significantly greater at 1 mmol/l than 0.25 mmol/l palmitate (5 min, $p < 0.005$, 10 and 15 min, $p < 0.001$). Palmitate (1 mmol/l) also increased significantly the loss of $^{45}\text{Ca}^{2+}$ from islets preloaded with the isotope for 120 min at 20 mmol/l glucose and then washed in nonradioactive medium ($p < 0.01$ after 5 min, or $p < 0.02$ after 10 and 20 min of washing) (Fig. 5).

A similar time-dependent study of $^{45}\text{Ca}^{2+}$ -uptake

Fig. 5. Effect of 1 mmol/l palmitate (closed symbols, ●) on $^{45}\text{Ca}^{2+}$ -efflux at 20 mmol/l glucose. It was measured as the amount of $^{45}\text{Ca}^{2+}$ (mean $\pm$ SEM) remaining in the islets after variable times of washing in a non-radioactive medium with the same composition of that used during preloading (20 mmol/l glucose $\pm$ 1 mmol/l palmitate). * $p < 0.01$ and ** $p < 0.02$ compared with the corresponding time-value in the absence of 1 mmol/l palmitate

was performed in starved islets which is summarised in Table 2. Starvation reduced both the short- (15 min) and long-term (120 min) $^{45}\text{Ca}^{2+}$ -uptake at 20 mmol/l glucose, both in the absence or presence of 1 mmol/l palmitate. This decrease was completely blocked by the fatty-acid oxidation inhibitors 2-bromostearate (0.25 mmol/l) and 2-tetradecylglycidate (10 $\mu\text{mol/l}$) that, on the other hand, did not exert any significant effect on $^{45}\text{Ca}^{2+}$ -uptake by fed islets.

Discussion

The results of the present work confirm that palmitate potentiates dose-dependently glucose-induced insulin secretion by isolated rat islets, and that this effect is prevented by 48 h starvation [14]. They also support previous studies demonstrating a stimulation of islet "de novo" phospholipid synthesis by glucose [9–12] and its dependence on the concentration of exogenous fatty-acids and their metabolic fate [14]. Moreover, the time-dependent study of islet lipid labelling with D-[U- ^{14}C]glucose performed here shows that, with the exception of phosphatidylcholine, all lipid fractions analysed tend towards isotopic equilibrium after 120 min of incubation. The increase of isotope content observed in each islet lipid fraction after this incubation time is then indicative of a net increase of the corresponding "de novo" synthesis. In the presence of 20 mmol/l glucose, palmitate induces generalised increments of the "de novo" synthesis of each islet lipid in a dose-dependent manner. They are all greatly and indiscriminately reduced in islets from 48-h starved animals [23] that are characterised by a predominance of oxidation versus esterification of fatty acids [15].

The omission of calcium reduced the insulin secretory response to glucose and palmitate, and it in-

Table 2. Effect of 0.25 mmol/l 2-bromostearate (BrS) and 10 $\mu\text{mol/l}$ 2-tetradecylglycidate (TDGA) on the uptake of $^{45}\text{Ca}^{2+}$ by islets of fed and 48 h starved rats, in the absence or presence of 1 mmol/l palmitate (P)

15 min $^{45}\text{Ca}^{2+}$ -uptake (pmol/islet)						
Additives	3 mmol/l Glucose			20 mmol/l Glucose		
	Fed (A)	Starved (B)	(A-B)	Fed (A)	Starved (B)	(A-B)
–	1.11 $\pm$ 0.08 (5)	1.23 $\pm$ 0.35 (4)	NS	4.86 $\pm$ 0.19 (6)	2.36 $\pm$ 0.10 (4)	$p < 0.001$
BrS	1.84 $\pm$ 0.54 (3)	1.46 $\pm$ 0.30 (4)	NS	3.78 $\pm$ 0.42 (5)	4.38 $\pm$ 0.63 (5) ^a	NS
TDGA	1.53 $\pm$ 0.52 (3)	1.70 $\pm$ 0.19 (5)	NS	4.16 $\pm$ 0.07 (4)	4.30 $\pm$ 0.21 (5) ^b	NS
P	1.43 $\pm$ 0.27 (4)	1.26 $\pm$ 0.09 (4)	NS	10.14 $\pm$ 0.86 (5)	3.61 $\pm$ 0.09 (4)	$p < 0.001$
P + BrS	1.55 $\pm$ 0.39 (5)	1.79 $\pm$ 0.33 (6)	NS	8.25 $\pm$ 0.57 (5)	8.20 $\pm$ 0.70 (6) ^b	NS
P + TDGA	1.83 $\pm$ 0.18 (3)	1.81 $\pm$ 0.34 (5)	NS	9.10 $\pm$ 0.22 (4)	9.39 $\pm$ 0.92 (5) ^b	NS
120 min $^{45}\text{Ca}^{2+}$ -uptake (pmol/islet)						
–	3.40 $\pm$ 0.13 (7)	1.14 $\pm$ 0.11 (4)	$p < 0.001$	13.04 $\pm$ 0.67 (7)	5.28 $\pm$ 0.25 (4)	$p < 0.001$
BrS	3.78 $\pm$ 0.19 (3)	4.48 $\pm$ 1.10 (4) ^a	NS	13.44 $\pm$ 1.02 (3)	14.42 $\pm$ 0.98 (5) ^b	NS
TDGA	4.21 $\pm$ 0.35 (3)	3.64 $\pm$ 0.52 (5) ^c	NS	14.94 $\pm$ 1.74 (3)	13.38 $\pm$ 0.36 (5) ^b	NS
P	3.88 $\pm$ 0.17 (4)	1.71 $\pm$ 0.14 (6)	$p < 0.001$	13.59 $\pm$ 1.04 (8)	6.44 $\pm$ 0.32 (6)	$p < 0.001$
P + BrS	3.59 $\pm$ 0.52 (3)	2.28 $\pm$ 0.24 (5) ^d	$p < 0.05$	13.08 $\pm$ 0.22 (4)	11.96 $\pm$ 0.55 (5) ^b	NS
P + TDGA	3.90 $\pm$ 0.55 (3)	3.13 $\pm$ 0.39 (4) ^c	NS	14.22 $\pm$ 1.42 (3)	13.58 $\pm$ 0.45 (5) ^b	NS

NS, non significant. ^a $p < 0.05$, ^b $p < 0.001$, ^c $p < 0.01$ and ^d NS compared with the corresponding value in the absence of BrS and TDGA

hibited the "de novo" synthesis of triacylglycerols and neutral phospholipids, whereas that of phosphatidic acid and phosphoinositides remained unaffected. The calcium-independence of the latter pathway points out that islet synthesis of acidic phospholipids is not the consequence of a stimulated secretion. An increased flux through this pathway could initiate or help to maintain the stimulation of islet phosphoinositide turnover by glucose [12], supplying the known messengers (inositol phosphates, diacylglycerol, phosphatidic acid) of the calcium signalling system [13]. This possibility is strengthened by increasing experimental evidence in favour of the calcium-dependence of glucose-stimulated hydrolysis of islet polyphosphoinositides [24, 25].

The "de novo" synthesis of neutral glycerolipids is actually thought to be regulated at the level of phosphatidate phosphohydrolase [26], which catalyzes phosphatidate conversion to diacylglycerol. Islet cells have such an enzyme activity which is translocated to plasma membranes when it is stimulated by glucose [27]. Its translocation and/or activity is known to be stimulated in hepatocytes by fatty-acids, and decreased by calcium omission or reduction (from 2.5 to 0.1 mmol/l) [28–30]. In this regard, the ability of calcium omission to decrease not only diacylglycerol labelling by 20 mmol/l D-[U-¹⁴C]glucose (at least at 0.25 mmol/l palmitate) but also that of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine is also compatible with the calcium-dependence of islet phosphatidate phosphohydrolase. The stimulation by glucose and palmitate of a calcium-dependent hydrolysis of phosphatidate might help to increase the concentration of diacylglycerol in islets. This interpretation is also supported by the reported calcium-dependence of ³H-glycerol labelling of both diacylglycerol and triacylglycerol in glucose-stimulated islets [31], although no information was given concerning the extent of isotopic equilibration. An opposite conclusion (calcium-dependent inhibition of phosphatidate phosphohydrolase) was drawn in a study showing that the calcium-ionophore A-23187 increases the labelling (60 min) of islet phosphatidate by ³H-glycerol, and decreases that of phosphatidylcholine, without significantly affecting other lipids at 2.8 mmol/l glucose [32]. In our opinion, the lack of a time-dependent investigation of ³H-glycerol incorporation (isotopic equilibration) hinders the interpretation that the increased phosphatidate labelling by A-23187 is due to a net increase in mass.

With regard to islet ⁴⁵Ca²⁺-fluxes, glucose behaved according to what has been previously reported [33]: it increased the influx (initial ⁴⁵Ca²⁺-uptake) of calcium into a lanthanum-nondisplaceable pool [22] that also resulted in enlargement. Palmitate dose-dependently stimulated glucose-induced influx of calcium into this pool, without affecting its size, and it also increased the outflow rate. This suggests that palmitate is able to ac-

celerate the turnover rate of an intracellular pool of islet calcium that is glucose-dependent. The accelerating effect of palmitate is apparently related to its metabolism, since it is greatly reduced by starvation and restored by fatty-acid oxidation inhibitors (2-bromostearate and 2-tetradecylglycidate). 2-bromostearate is able to restore a normal insulin response to glucose in starved islets, and to inhibit the oxidation of exogenous palmitate, thus increasing its esterification into islet phospholipids [15]. Both 2-tetradecylglycidate (McN-3802) and its methyl ester (McN-3716) are potent and specific inhibitors of long-chain fatty-acid oxidation [34]. McN-3716 (0.1 mmol/l) is also known to inhibit islet oxidation of exogenous, as well as endogenous, palmitate [35]. It has been shown to reduce insulin response to glucose in the 5.6–11.1 mmol/l range but not at 16.7 mmol/l, and this inhibitory effect manifests itself after the drug has been present more than 60 min [35]. It is possible that even though glucose also reduces the oxidation of palmitate, the remaining fractional oxidation rate together with that of the sugar, contributes significantly to the generation of the energy required for a sustained secretory stimulation.

Altogether, the results show that the stimulation by glucose and palmitate of insulin secretion, "de novo" phospholipid synthesis, and ⁴⁵Ca²⁺-turnover is considerably impaired in starved islets and it may be completely prevented by inhibitors of fatty-acid oxidation. This positive correlation among the three phenomena argues in favour of the existence of a functional link among them. It might be suggested, as a working hypothesis, that palmitate amplifies the insulin secretory response of islets to glucose, through the stimulation of the "de novo" synthesis of acidic phospholipids, and the subsequent generation of inositol phosphates, which would contribute to accelerate calcium turnover.

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