Effect of 2-Bromostearate on Glucose-Phosphorylating Activities and the Dynamics of Insulin Secretion in Islets of Langerhans During Fasting

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SUMMARY

Glucose-phosphorylating activity and insulin secretion were measured in homogenates of isolated rat islets and of perfused rat pancreas, respectively. Fasting for 96 h produced a significant decrease of both low- and high-K_m glucose-phosphorylating activities and blocked the insulin secretory response to glucose. In the presence of glucose, 0.25 mM 2-bromostearate, a known inhibitor of fatty acid oxidation, partially restored the insulin response to glucose that was lost during fasting. This effect paralleled the restoration of glucose-phosphorylation activities (primarily the high-K_m component) seen when islets isolated from 96-h–fasted rats were preincubated with 0.25 mM 2-bromostearate.

It is concluded that fasting-induced adaptations of glucose-phosphorylating enzymes could account, at least in part, for the reduced insulin secretory response to glucose. 2-Bromostearate, an inhibitor of fatty acid oxidation, is able to restore both insulin secretory response and glucose-phosphorylating activities, suggesting possible interrelations among the correlated impairment in insulin secretion, islet glucose-phosphorylating activity, islet glucose metabolism, and the oxidation of fatty acids in the B-cell during fasting.

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The close parallels between extracellular glucose concentration, its metabolism in the B-cell, and its ability to stimulate insulin release are well known. Strong experimental evidence supports the hypothesis that glucose metabolism in the B-cell is a prerequisite for stimulation of insulin release.[1,2] It is well established that glucose transport is not a limiting factor for its metabolism in the B-cell.[3] It seems that the first rate-limiting step in glucose metabolism in this tissue is the phosphorylation of glucose to glucose-6-phosphate. There is experimental evidence to show that this step is controlled by three different enzymes: hexokinase with a low K_m for glucose, a "glucokinase"[4–6] with high K_m for glucose, and a glucose-6-phosphatase inhibited by glucose.[7]

Fasting is known to inhibit the insulin secretory response to glucose both in vivo and in vitro,[8–10] to decrease both glucose utilization and oxidation,[11,12] and to lower glucose-phosphorylating activity.[13,14] At the same time, there is an increase in lipolysis and enhanced fatty acid oxidation by the tissues.

The aim of this study was to relate glucose-phosphorylating activity in the islets of Langerhans to insulin secretory rate under the experimental conditions of fasting and feeding. To study possible interactions between glucose and fatty acid metabolism in the islets during fasting, we have used 2-bromostearate as an experimental tool to inhibit fatty acid oxidation in the B-cell.[15]

MATERIALS AND METHODS

Animals. Male, albino, Wistar rats (250–300 g) were used. Before the experiments, the animals had been fed a standard pellet diet ad libitum. During fasting, the animals had free access to water.

Perfusion of pancreas. The pancreas was isolated and perfused using the procedure of Sussman et al.[16] with some modifications.[17] In brief, the preparation consisted of the pancreas and of the attached duodenum. A nonrecirculating medium of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.5% bovine serum albumin (BSA) was continuously gassed with a mixture of 95% O_2/5% CO_2 and kept at 37°C. The buffer solution was pumped through the aorta and the total portal effluent was collected every 2 min. The perfusion pressure was 20 mm Hg and the perfusion flow was 2.5 ml/min.

A 10-min period of preperfusion was allowed before the start of the actual experiment, which was designated 0 min.

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Samples of 5 ml of perfusate were collected into chilled tubes that were stored at −20°C.

At the end of the perfusion period, a pulse of norepinephrine at a nonphysiologic dose (10 μg/ml) was infused to check the viability of the preparation by monitoring the elevation of the perfusion pressure.

Insulin secretion was measured by radioimmunoassay, using our own antibody and ^{125}I-insulin labeled by the chloramine-T method.[18]

Preparation of islets. Islets were isolated by the collagenase method.[19] The islets (250–300) were collected in cold Hanks' solution.[20]

Incubation of islets. Batches of 200–250 islets were incubated, with shaking, in 1 ml of bicarbonate-buffered salt solution,[21] pH 7.4, containing 1% BSA. The incubations were carried out at 37°C for 60 min and the islets were gassed with 95% O_2/5% CO_2 every 15 min.

2-Bromostearic acid was neutralized with NaOH and was solubilized by coupling it to BSA, reaching a final concentration of 7 mol fatty acid analogue/mol albumin, according to Garland et al.[22]

Islet homogenates. At the end of the incubation, the islets were centrifuged in a Beckman microfuge for 30 s. The islets were then rinsed several times with Krebs solution and suspended in 200–250 μl homogenization medium: 50 mM Hepes, 150 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, and 1 mM 2-mercaptoethanol. The islets were disrupted by sonication for intervals of 5 s, three times at position 10 (MSE, MK2 cell disrupter).

Assay of glucose-phosphorylating activities. In principle, the analysis involved assay at two glucose concentrations: the hexokinase at 0.5 mM glucose, at which concentration the glucokinase is essentially inactive; and at 25 mM glucose, at which concentration all phosphotransferase activity was measured. The glucokinase activity was, thus, obtained by difference.

A two-step isotopic method described for galactokinase assay[23] and modified for hexokinase assay[24] was used.

The assay mixture consisted of: 50 mM Hepes (pH 7.4), 150 mM KCl, 5 mM $MgCl_2$, 5 mM Mg-ATP, 10 mM NaF, and 0.5 mM or 25 mM D-(U-^{14}C)-glucose, with specific activities of 3.7 and 0.7 mCi/mmol, respectively.

The reaction was initiated by the addition of 20 μl of islet homogenate and was continued at 30°C for 20 min in a total volume of 100 μl. The reaction was terminated by the addition of 30 μl of 2 M glucose and 0.25 M EDTA, after which a 60-μl aliquot was spotted on a DEAE cellulose filter (DE-81, Whatman) that retains phosphoric esters. Subsequently, the filters were washed thoroughly with 300 ml deionized water, dried, and the radioactivity counted by liquid scintillation spectrometry. Blank incubations without homogenate and blank samples without ATP were included in each experiment.

All experiments were performed in duplicate. The rates of glucose phosphorylation were linear with time for at least 1 h and were proportional to the amount of islet homogenate used.

Protein content. The protein content of the islets was measured by the protein binding method,[25] using BSA as standard.

Chemicals. Crude collagenase was obtained from Worthington Biochemical Corp. (code CLS IV) (Freehold, New Jersey). Hepes (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) and ATP (adenosine-5′-triphosphate disodium salt) were obtained from Boehringer-Mannheim (FRG), and D-(U-^{14}C)-glucose (292 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks, United Kingdom). Bovine serum albumin (BSA, fraction V) was obtained from Sigma (St. Louis, Missouri). 2-Bromostearic acid was obtained from BDH Chemicals Ltd. (Poole, United Kingdom). Coomassie brilliant blue G-250 was obtained from Sigma. Other chemicals of analytic grade were obtained from E. Merck (Darmstadt, FRG).

RESULTS

Effect of fasting and glucose incubation on islet glucose-phosphorylating activities. Table 1 shows the glucose-phosphorylating activities in homogenates of islets of Langerhans isolated from fed and 48- or 96-h–fasted rats.

Under the assay conditions described above, the glucokinase activity was 61% of the total glucose-phosphorylating activity in islets from fed rats.

Fasting induced a decrease of both activities. The decrease was more evident in the total glucose-phosphorylating activity (51% after 48 h of fasting, 90% after 96 h of fasting) than in the hexokinase activity (58% after 48 h; 76%

TABLE 1
Effect of fasting on glucose-phosphorylating activities in islet extracts

	Total glucose-phosphorylating activity	Glucokinase activity	Hexokinase activity
	(pmol D-(U-^{14}C)-glucose/min/μg islet protein)		
Fed	4.77 ± 0.17 (11)	2.92 ± 0.06 (10)	1.90 ± 0.11 (10)
48-h–Fasted	2.34 ± 0.13 (10)*	1.52 ± 0.09 (11)*	0.81 ± 0.03 (10)*
96-h–Fasted	0.48 ± 0.03 (9)*	0.03 ± 0.005 (9)*	0.45 ± 0.05 (10)*

Batches of 250–300 islets were obtained from fed and 48-h– or 96-h–fasted rats and sonicated immediately. Portions of homogenate corresponding to 20 μg of islet protein were incubated at 30°C for 30 min with D-(U-^{14}C)-glucose (0.5 or 25 mM, 3.7 mCi/mmol and 0.7 mCi/mmol, respectively) and 5 mM Mg-ATP. The D-(U-^{14}C)-glucose phosphoric esters formed were assayed as described in the text. Glucokinase activity was obtained by the difference of individual values at 25 mM and 0.5 mM glucose. Results are given as mean ± SEM, with the number of observations in parentheses. Significance was determined by Student's *t* test.
*$P < 0.001$ versus fed control.

TABLE 2
Effect of previous incubation with glucose on islet glucose-phosphorylating activities

	Total glucose-phosphorylating activity	Glucokinase activity	Hexokinase activity
	(pmol D-(U-^{14}C)-glucose/min/μg islet protein)		
Fed controls	4.81 ± 0.20 (8)	2.95 ± 0.08 (8)	1.88 ± 0.09 (9)
Fed + incubation with glucose	4.99 ± 0.18 (10)†	2.97 ± 0.11 (10)†	2.03 ± 0.09 (10)†
96-h–Fasted	0.50 ± 0.03 (8)	0.04 ± 0.007 (8)	0.47 ± 0.03 (10)
96-h–Fasted + incubation with glucose	1.05 ± 0.12 (11)*	0.71 ± 0.07 (9)*	0.44 ± 0.06 (13)

Batches of 200–250 islets were incubated for 60 min at 37°C in 1 ml KRB, pH 7.4, in 1% BSA medium supplemented with 2.75 mM glucose (0 min to 30 min) and 16.7 mM (30 min to 60 min). After the incubation, the islets were disrupted and portions of homogenates corresponding to 20 μg of islet protein were incubated at 30°C for 20 min with D-(U-^{14}C)-glucose (0.5 or 25 mM, 3.7 mCi/mmol and 0.7 mCi/mmol, respectively) and Mg-ATP 5 mM. The D-(U-^{14}C)-glucose phosphoric esters were assayed as described in the text. Results are given as mean ± SEM with the number of observations in parentheses. Significance was determined by Student's *t* test.
*$P < 0.001$ versus 96-h–fasted rats.
†Not significant versus fed controls.

after 96 h). In contrast, the hexokinase activity disappeared completely after 96 h of fasting.

To test whether glucose exerted an induction on these activities, as has been shown for liver glucokinase,[26,27] we studied the glucose-phosphorylating activities in islets previously incubated for 60 min with 2.75 mM and 16.7 mM glucose (Table 2). The incubation with glucose did not modify the glucose-phosphorylating activities in islet extracts from fed rats.

In islets isolated from 96-h–fasted rats, the previous incubation with glucose exerted an important effect on the glucose-phosphorylating activities: total phosphorylating activity increased to 210%, glucokinase activity reached 24% of the value found in fed rats, while the hexokinase component was not altered under these experimental conditions.

Effect of incubation with 2-bromostearate on islet glucose-phosphorylating activities. Table 3 shows the effect of incubation with 0.25 mM 2-bromostearate and glucose on glucose-phosphorylating activities in islets from fed and 96-h–fasted rats. 2-Bromostearate (0.25 mM) produced a decrease in hexokinase (16%) in islet extracts from fed rats. Incubation with 0.25 mM 2-bromostearate and glucose restored hexokinase activity (108% of the activity in controls), decreased the glucokinase component by 12%, and did not alter the total glucose-phosphorylating activity. 2-Bromostearate (0.25 mM) alone partly restored the phosphorylating activities lowered during fasting, this effect being potentiated by the presence of glucose. 2-Bromostearate (0.25 mM) alone increased total phosphorylating activity up to 30% of the values in fed rats. This effect was due to an important

TABLE 3
Effect of previous incubation with 2-bromostearate and glucose on islet glucose-phosphorylating activities

	Total glucose-phosphorylating activity	Glucokinase activity	Hexokinase activity
	(pmol D-(U-^{14}C)-glucose/min/μg islet protein)		
Fed controls	4.81 ± 0.20 (8)	2.95 ± 0.08 (8)	1.88 ± 0.09 (9)
Fed + incubation with 0.25 mM 2-bromostearate	4.59 ± 0.17 (7)‡	2.97 ± 0.15 (7)†	1.58 ± 0.03 (8)*
Fed + incubation with 2-bromostearate + glucose	4.63 ± 0.13 (8)‡	2.59 ± 0.10 (8)*	2.03 ± 0.08 (8)‡
96-h–Fasted controls	0.50 ± 0.03 (8)	0.04 ± 0.007 (8)	0.47 ± 0.04 (10)
96-h–Fasted + incubation with 0.25 mM 2-bromostearate	1.43 ± 0.07 (7)†	1.02 ± 0.02 (6)†	0.47 ± 0.03 (6)§
96-h–Fasted + incubation with 0.25 mM 2-bromostearate + glucose	3.56 ± 0.17 (14)†	2.56 ± 0.12 (13)†	1.07 ± 0.05 (13)†

Batches of 200–250 islets were incubated for 60 min at 37°C in 1 ml KRB, pH 7.4, in 1% BSA medium supplemented with 0.25 mM 2-bromostearate or glucose 2.75 mM (0 min to 30 min) and 16.7 mM (30 min to 60 min) and 0.25 mM 2-bromostearate. After the incubation, the islets were disrupted and portions of homogenate corresponding to 20 μg of islet protein were assayed as described in the text. Results are given as mean ± SEM with the number of observations in parentheses. Significance was determined by Student's *t* test.
*$P < 0.05$ versus fed controls.
†$P < 0.0001$ versus 96-h–fasted rats.
‡Not significant versus fed controls.
§Not significant versus 96-h–fasted controls.

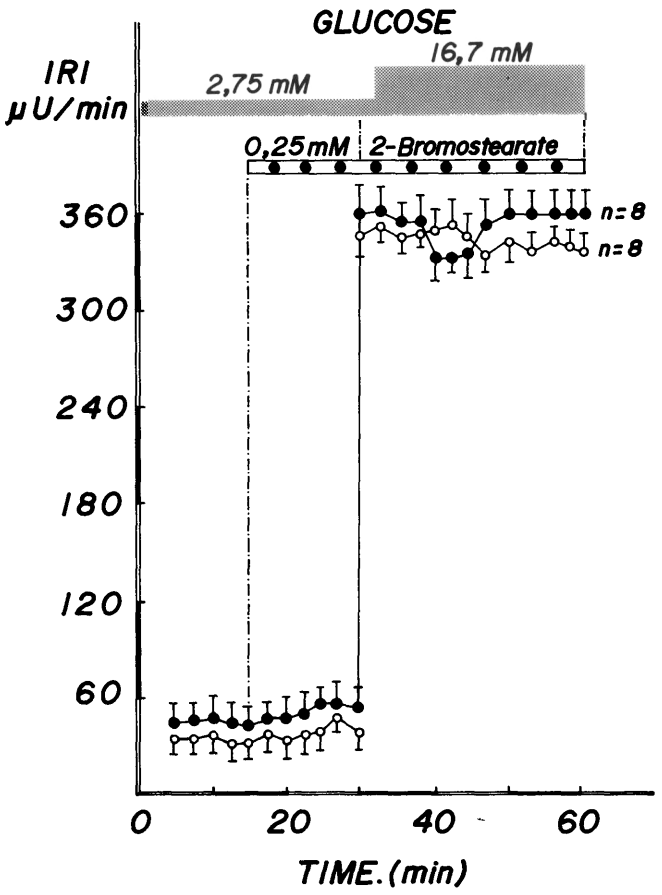

FIGURE 1. Effect of 0.25 mM 2-bromostearate and 2.75 mM or 16.7 mM glucose on insulin secretion in perfused pancreas from fed rats. (○——○) Without 0.25 mM 2-bromostearate, (●——●) with 0.25 mM 2-bromostearate. All values represent the mean ± SEM of the indicated number of experiments.

increment in glucokinase activity. Glucose potentiated the effect of 2-bromostearate: under these conditions, total phosphorylating activity reached 74% of the value in islets from fed rats. In this case, both glucokinase and hexokinase activities were significantly restored: 87% and 57% of the values in islets from fed rats, respectively.

Effect of 2-bromostearate on glucose-induced insulin release in perfused rat pancreas. Figure 1 shows the dynamics of insulin secretory response to 2.75 mM and 16.7 mM glucose in the absence and in the presence of 0.25 mM 2-bromostearate in the fed state.

2-Bromostearate (0.25 mM) did not modify the insulin secretory response to glucose in perfused pancreas from fed rats; this agrees with the lack of effect of 2-bromostearate on islet glucose-phosphorylating activities in this nutritional state. The insulin secretory response to glucose was partially blocked during fasting (compare Figure 2 with Figure 1). As shown in Figure 2, the presence of 0.25 mM 2-bromostearate in the perfusion medium partially restored the insulin secretory response to glucose, reaching about 50% of the secretory values seen in response to 16.7 mM glucose in perfused pancreas from fed rats.

Figure 3 shows that the relationship between the extracellular glucose concentration and the output of insulin was sigmoidal in the fed state. This response was not modified by the presence of 0.25 mM 2-bromostearate.

Fasting induced a loss of the sigmoidal insulin secretory pattern to glucose. The presence of 0.25 mM 2-bromostearate in the perfusion medium partially restored the sigmoidal response to glucose.

DISCUSSION

The results are discussed herein, assuming that the enzyme activities that we studied in islet homogenates are mainly present in the B-cells, which constitute the major component of the rat islet.[28] The possible contribution, however, of the other cell types must be kept in mind.

Fasting is a metabolic state characterized by diminished insulin response to glucose,[8-10] lowered glucose metabolism in the B-cell,[11] and a decrease of islet glucose-phosphorylating activities.[13]

2-Bromostearate (0.25 mM) is partially able to restore the insulin secretory response to glucose, and this effect is parallel to the restoring effect of incubation with 0.25 mM 2-bromostearate plus glucose on the high-K_m component of glucose-phosphorylating activities in islet homogenates from 96-h–fasted rats.

Malaisse et al.,[13] however, reported a decrease of 64%

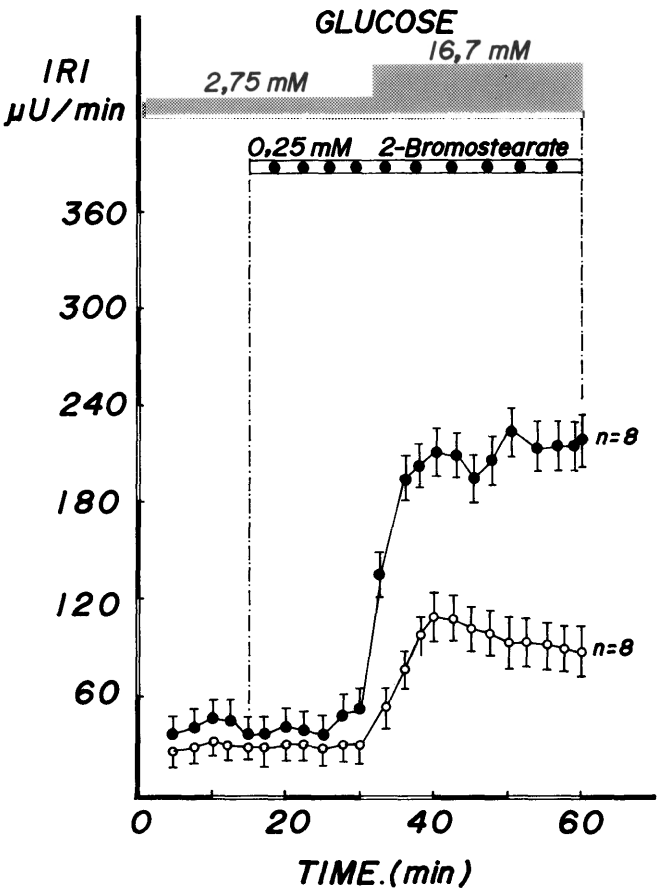

FIGURE 2. Effect of 0.25 mM 2-bromostearate and 2.75 or 16.7 mM glucose on insulin secretion in perfused pancreas from 96-h–fasted rats. (○——○) Without 0.25 mM 2-bromostearate, (●——●) with 0.25 mM 2-bromostearate. All values represent the mean ± SEM of the indicated number of experiments.

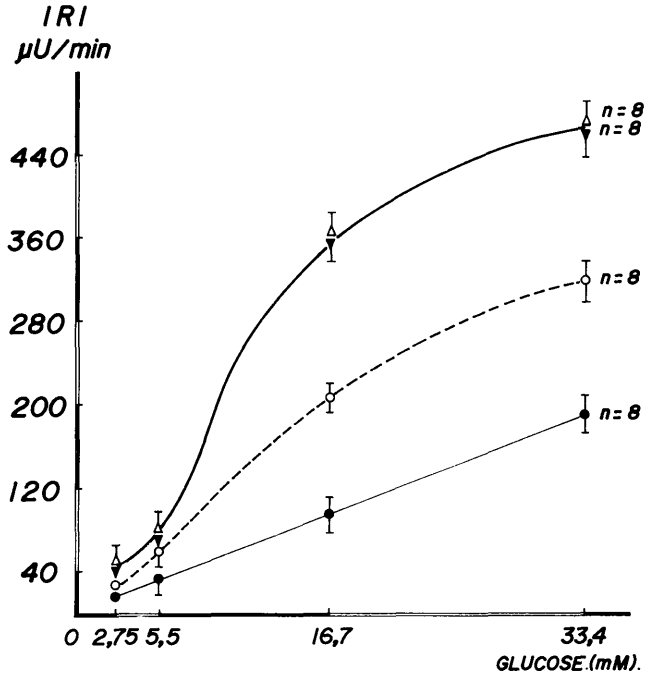

FIGURE 3. Kinetics of insulin secretion at different glucose concentrations in perfused pancreas from fed rats (△——△); from fed rats with 0.25 mM 2-bromostearate in the perfusion medium (▲——▲); from 96-h–fasted rats (●——●); and from 96-h–fasted rats with 0.25 mM 2-bromostearate in the perfusion medium (○——○). The pancreata were perfused as described in MATERIALS AND METHODS. All values represent the mean ± SEM of the indicated number of experiments. N refers to the number of pancreata perfused.

after 48 h fasting, and Burch et al.[14] reported a decrease of 45% after 72 h fasting. We found a decrease of 58% after 48 h fasting, while the activity disappeared after 96 h fasting.

The fact that we measured the total phosphotransferase activity at a nonsaturating concentration of glucose could explain these discrepancies.

These results are in agreement with the decrease in glucose utilization and oxidation seen during fasting[10–12] and lend support to the theory that glucose phosphorylation is a regulatory step in glucose metabolism in the B-cell.

The fact that the impairment of glucose-stimulated insulin secretion during fasting can be restored by refeeding[14] and, moreover, that actinomycin-D blocks the return to normal glucose-stimulated insulin release[30] leads us to think that this phosphorylating system can be restored by glucose itself. In this sense, the incubation of islets from 96-h–fasted rats for 60 min at two glucose concentrations exerts an important effect on both glucose-phosphorylating activities. This effect is more evident on the glucokinase component. This agrees with the result obtained in hepatocytes.[26] The activities found under this experimental condition, however, were lower than those obtained in islets from fed rats.

Free fatty acid concentration in blood reaches high levels during fasting;[31] free fatty acids and ketone bodies are the major metabolic fuel in this nutritional state.[32] Little is known, however, about the role of lipids as fuels for oxidative metabolism in the B-cell during fasting. Hammar and Berne[33] have demonstrated that the presence of B-hydroxyacyl-CoA dehydrogenase is necessary for the degradation of fatty acids in the B-cell. Moreover, Berne[15] demonstrated that pancreatic islets metabolize ketone bodies and fatty acids and that 2-bromostearate inhibits the oxidation of palmitate.

To study possible interactions between carbohydrate and fatty acid metabolism, we studied the effect of inhibition of fatty acid oxidation in islets by 2-bromostearate during fasting. Glucose-phosphorylating activities in fed rats were unaffected by incubation with 0.25 mM 2-bromostearate alone. This correlates well with the lack of effect of α-bromopalmitate on the uptake of glucose in hearts from fed rats.[34]

As shown in Figure 1, 0.25 mM 2-bromostearate did not exert an important effect on glucose-induced insulin release in perfused pancreas from fed rats, which is in agreement with the results obtained in perifused rat islets.[35]

The incubation of islets from 96-h–fasted rats with 0.25 mM 2-bromostearate partly restored glucose-phosphorylating activities. Incubation with 0.25 mM 2-bromostearate and 16.7 mM glucose, however, was able to restore the phosphorylating activities; this effect was more important regarding the high-K_m component.

The presence of glucose was necessary for this effect. A similar requirement was necessary for the restoring effect of 2-bromostearate on insulin secretion in perfused pancreas from 96-h–fasted rats.

Otte et al.[36] have reported that, during fasting, the inhibition of lipolysis with 3',5-dimethylisoxazole has an influence on glucose metabolism and on insulin secretion by isolated pancreatic islets; the oxidation of D-(U-^{14}C)-glucose by fasted islets was stimulated by 100% and insulin secretion was increased by about 40%, which was only 10% less than in fed rats.

These observations lend support to the hypothesis that the glucose effect is secondary to its metabolism and that the phosphorylation of glucose is a rate-limiting step in glucose metabolism in the B-cell.

Fasting induces a decrease in this phosphorylating activity, in agreement with the diminished sensitivity of the B-cell to glucose in this nutritional state. The fact that 2-bromostearate restored the phosphorylating activity and glucose-induced insulin release (Figure 3) allows us to substantiate a relationship between metabolism of glucose and fatty acids in the B-cell.

No effect of fatty acids on glucose metabolism in the B-cell has been reported, however. This is in contrast to the effect reported in other tissues, such as heart or skeletal muscle.[37] In addition, the fact that medium- and long-chain fatty acids stimulate the insulin secretory response to glucose in fed rats[38–40] and that increased free fatty acid levels in blood decrease glucagon and increase somatostatin levels (suggesting a possible intraislet action via a free fatty acid-mediated somatostatin release in fed rats[41]) indicates that the interaction between glucose and fatty acid metabolism in the B-cell remains an unsolved question.

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REFERENCES

[1] Hedeskov, C. J.: Mechanism of glucose-induced insulin secretion. Physiol. Rev. 1980; 60:442–509.

[2] Ashcroft, S. J. H.: Glucoreceptor mechanisms and the control of insulin secretion and biosynthesis. Diabetologia 1980; 18:5–15.

[3] Hellman, B., Sehlin, J., and Täljedal, I.-B.: Evidence for mediate transport of glucose in mammalian pancreatic B-cells. Biochem. Biophys. Acta 1971; 241:147–54.

[4] Matschinsky, F. M., and Ellerman, J. E.: Metabolism of glucose in the islets of Langerhans. J. Biol. Chem. 1968; 243:2730–36.

[5] Ashcroft, S. J. H., and Randle, P. J.: Enzymes of glucose metabolism in normal mouse islets. Biochem. J. 1970; 119:5–15.

[6] Sols, A.: Diabetes y regulación metabólica. Punto de vista de un bioquímico. Endocrinol. Clin. Metab. 1982; 1:43–48.

[7] Täljedal, I.-B.: Presence, induction and possible role of glucose-6-phosphatase in mammalian pancreatic islets. Biochem. J. 1969; 114:387–94.

[8] Malaisse, W. J., Malaisse-Lagae, F., and Wright, P. H.: Effect of fasting upon insulin secretion in the rat. Am. J. Physiol. 1967; 213:843–48.

[9] Fink, G., Gutman, R. A., Cresto, J. C., Selawry, H., Levine, R., and Recant, L.: Glucose-induced insulin release patterns: effect of starvation. Diabetologia 1974; 9:243–50.

[10] Wolters, G. H. J., Konijnendijk, W., and Bouman, P. R.: Effects of fasting on insulin secretion, islet glucose metabolism and the cyclic adenosine-3′,5′-monophosphate content of rat pancreatic islets in vitro. Diabetes 1977; 26:530–37.

[11] Hedeskov, C. J., and Capito, K.: The effect of starvation on insulin secretion and glucose metabolism in mouse pancreatic islets. Biochem. J. 1974; 140:423–33.

[12] Levy, J., Herchuelz, A., Sener, A., and Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. XX. Fasting: a model for altered glucose recognition by the B-cell. Metabolism 1976; 25:583–91.

[13] Malaisse, W. J., Sener, A., and Levy, J.: The stimulus-secretion coupling of glucose-induced insulin secretion: fasting-induced adaptation of key glycolytic enzymes in isolated islets. J. Biol. Chem. 1976; 251:1731–37.

[14] Burch, P. T., Trus, M. D., Berner, D. K., Leontire, A., Zawalich, K. G., and Matschinsky, F. M.: Adaptation of glycolytic enzymes: glucose use and insulin release in rat pancreatic islets during fasting and refeeding. Diabetes 1981; 30:923–28.

[15] Berne, C.: The metabolism of lipids in mouse pancreatic islets. Biochem. J. 1975; 152:661–66.

[16] Sussman, K. E., Waughan, G. D., and Timmer, R. F.: An in vitro method for studying insulin secretion in the perfused isolated rat pancreas. Metabolism 1966; 15:466–76.

[17] Goberna, R.: Ph.D. thesis. University of Valencia, 1968.

[18] Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 1962; 194:495–98.

[19] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from rat pancreas. Diabetes 1967; 16:35–39.

[20] Hanks, J. H., and Wallace, R. E.: Relation of oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. Exp. Biol. Med. 1949; 71:196–200.

[21] Krebs, H. A., and Henseleit, K.: Untersuchung uber die Harntoffbildung im Tierkorper. Hoppe Seylers Z. Physiol. Chem. 1932; 210:33–66.

[22] Garland, P. B., Newsholme, E. A., and Randle, P. J.: Regulation of glucose uptake by muscle. 9. Effects of fatty acids and ketone bodies and of alloxan-diabetes and starvation on pyruvate metabolism and on lactate/pyruvate and L-glycerol-3-phosphate/dihydroxyacetone-phosphate concentration ratios in rat heart and rat diaphragm muscle. Biochem. J. 1964; 93:665–78.

[23] Blume, K. G., and Beutler, E.: Galactokinase in human erythrocytes. *In* Methods in Enzymology, Vol. 42. Colowick, S. P., and Kaplan, N. O., Eds. New York, Academic Press, 1975:47–53.

[24] Sols, A., Aragón, J. L., Lazo, P. A., and González, J.: Enzymes in situ in animal cells and the regulation of glycolysis. Fed. Proc. 1978; 37:1428.

[25] Bradford, M. M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 1976; 72:248–56.

[26] Wakelam, M. J. O., and Walker, D. G.: The separate roles of glucose and insulin in the induction of glucokinase in hepatocytes isolated from neonate rats. Biochem. J. 1981; 196:383–90.

[27] Spence, J. T., Merrit, M. J., and Pitot, H. C.: Role of insulin, glucose and cyclic AMP in the regulation of glucokinase in cultured hepatocytes. J. Biol. Chem. 1981; 256:1598–1603.

[28] Orci, L.: The islet of Langerhans: a multihormonal microorgan. *In* Insulin and Metabolism. Bajaj, J. S., Ed. Amsterdam, Excerpta Medica, 1977:1–10.

[29] Matschinsky, F. M., Trus, M., Burch, M., Berner, D., Ghosh, A., Zawalich, W., and Weill, M. V.: Dual control of glucose phosphorylation in pancreatic islets. *In* Diabetes 1979. Waldausl, W. K., Ed. Amsterdam, Excerpta Medica ICS, 1979:154–59.

[30] Grey, N. J., Goldring, S., and Kipnis, D. M.: The effect of fasting, diet and actinomycin D on insulin secretion in the rat. J. Clin. Invest. 1970; 49:881–89.

[31] Smith, S. W., Odell, R. H., Marsh, D. J., and Yates, E. F.: Patterns of plasma free fatty acids concentration in fasting resting dogs. Am. J. Physiol. 1977; 232:E25–34.

[32] Cahill, G. F.: Starvation in man. N. Engl. J. Med. 1970; 282:668–75.

[33] Hammar, H., and Berne, C.: The activity of B-hydroxyacyl-CoA dehydrogenase in the pancreatic islets of hyperglycemic mice. Diabetologia 1970; 6:526–28.

[34] Burges, R. A., Butt, W. D., and Baggaley, A.: Some effects of α-bromopalmitate, an inhibitor of fatty acid oxidation on carbohydrate metabolism in the rat. Biochem. J. 1968; 109:38–39p.

[35] Tamarit-Rodríguez, J., Vara, E., and Tamarit, J.: Insulin, glucagon, and somatostatin response to palmitate by perifused rat islets and its modifications by 2-bromostearate. Diabetologia 1981; 21:334.

[36] Otte, H. M., Holze, S., and Hasselblatt, A.: Glucose metabolism and insulin secretion in rat pancreatic islets after treatment with an antilipolytic agent. Diabetologia 1981; 21:311.

[37] Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A.: The glucose fatty acid cycle. Lancet 1963; 1:785–90.

[38] Montague, W., and Taylor, K. N.: Regulation of insulin secretion by short chain fatty acids. Nature 1968; 217:853.

[39] Malaisse, W. J., and Malaisse-Lagae, E.: Stimulation of insulin secretion by non carbohydrate metabolites. J. Lab. Clin. Med. 1968; 72:438–48.

[40] Goberna, R., Tamarit, J., Jr., Osorio, J., Fussganger, R., Tamarit, J., and Pfeiffer, E. F.: Action of B-hydroxybutyrate, acetoacetate and palmitate on the insulin release in the perfused rat pancreas. Horm. Metab. Res. 1974; 6:256–60.

[41] Wasada, T., Howard, B., Dobbs, R. E., and Unger, R. A.: Evidence for a role of free fatty acids in the regulation of somatostatin secretion in normal and alloxan diabetic dogs. J. Clin. Invest. 1980; 66:511–16.