Pancreatic Islet Levels of Citrate Under Conditions of Stimulated and Inhibited Insulin Release

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SUMMARY

The possible significance of citrate for insulin release was elucidated by measuring the levels of this metabolite in isolated pancreatic islets from obese-hyperglycemic mice and their lean litter mates. The amount of islet citrate depended on the extracellular glucose concentration but was not affected by the insulin secretagogues, glibenclamide or dibutyryl cyclic-3,5-AMP. Increased β-cell levels of citrate do not necessarily result in stimulation of insulin release, as shown by the increased amounts of citrate in islets exposed to epinephrine. Octanoate (5 mM) had no effect on the level of citrate in islets exposed to 0.6 mg./ml. glucose. In the presence of 3 mg./ml. glucose, octanoate significantly depressed the citrate level. It seems unlikely that the citrate molecule has the important role in controlling insulin release that has previously been suggested. DIABETES *21*:999-1002, October, 1972.

Citrate is known to be an important regulator of intermediary metabolism in a variety of tissues. In pancreatic β-cells citrate has been thought to stimulate insulin secretion by diverting glucose-6-phosphate to the pentose phosphate pathway through inhibition of phosphofructokinase[1,2] or by affecting the zinc bond of the stored hormone.[3] If the metabolic events in muscle described by Randle[4] are applicable to the β-cells, the stimulatory action of free fatty acids on insulin release[1,2,5] could be explained by increased intracellular citrate levels consequent to the activation of citrate synthetase through a rise of the acetyl-CoA/CoA ratio.[1,2,4] The possible role of citrate in pancreatic β-cell function arises from the observation that the islet level of citrate is considerably higher in obese-hyperglycemic mice than in their lean litter mates.[6]

Knowledge of how the citrate content varies when pancreatic islets are exposed to different stimulators and inhibitors of insulin release might help to elucidate whether this metabolite plays the suggested regulatory role in the β-cells. The present communication describes such studies performed in vitro with islets microdissected from obese-hyperglycemic mice and their lean litter mates.

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MATERIALS AND METHODS

Animals. Forty-seven obese-hyperglycemic mice (gene symbol: *obob*) of both sexes and sixteen of their female lean litter mates were taken from a local colony[7] when five to seven months old. The animals were fasted overnight before being killed by decapitation under ether anesthesia.

Preparation of tissues. Pieces of pancreas were removed and eight to twelve islets were carefully dissected at +2° C. in a Krebs-Ringer bicarbonate buffer containing 0.6 mg./ml. glucose.[8] After the islets had been incubated under the desired conditions (see below) they were freeze-dried at —40° C. and 0.001 mm. Hg for twelve hours. The freeze-dried islets were weighed on a quartz fiber balance before being transferred to micro test tubes for further analyses.

Incubation procedure. The islets from the obese-hyperglycemic mice were individually incubated in 1 ml. Krebs-Ringer bicarbonate medium containing 0.5 per cent albumin. After preincubation for ten minutes at 37° C. at a glucose concentration of 0.6 mg./ml. the microdissected islet was exposed for thirty minutes to fresh bicarbonate medium containing either 0.6 or 3.0 mg./ml. glucose with or without one of the following additives: octanoate (5 mM), glibenclamide (50 μg./ml.), dibutyryl cyclic-3,5-AMP (250 μg./ml.) and epinephrine (2 μg./ml.). In one series of experiments $CaCl_2$ was omitted from the incubation medium and replaced by equimolar amounts of NaCl. In each experiment the same type of medium was used for three to four islets from one and the same animal. The incubation procedure differed for the islets from the lean mice

in not including the period of preincubation. In the latter case the islets were incubated for thirty minutes with 3 mg./ml. glucose in the presence or absence of 5 mM octanoate.

Chemical methods. A fluorometric microtechnic was used to measure the islet content of citrate. In the initial step three to four islets were pooled and heated for fifteen minutes at 60° C. in 0.05 N NaOH (1 μL. /μg. islet dry weight) to extract citrate and to destroy interfering tissue enzymes and pyridine nucleotides. Measurements of citrate in the extraction media were either made in triplicate by adding 5 μL. (obese-hyperglycemic mice) or in duplicate by adding 2 μL. (lean mice) to a reaction medium containing citrate lyase and malate dehydrogenase.[6] The serum albumin included in this medium had been thoroughly dialyzed to remove its citrate contamination. Exposure to the reaction media was followed by the destruction of remaining NADH by heating with HCl and subsequent measurements of the NAD^+ formed by means of enzymatic cycling.[9] The cycling medium contained 200 μg. glutamate dehydrogenase and 50 μg. of ox heart lactate dehydrogenase (purified with charcoal to remove pyridine nucleotide contamination) per milliliter medium. The cycling reaction was carried out for thirty minutes at 38° C., giving an approximate amplification of 1,500X. The final fluorometric readings were performed in an Aminco Bowman spectrophotofluorometer.

RESULTS

The amounts of citrate found after exposing the isolated islets to either a high or a low glucose concentration are shown in table 1. After thirty minutes of incubation with 0.6 mg./ml. glucose the islets from male and female obese-hyperglycemic mice contained an average of 6.5 and 6.7 mmoles citrate/kg. dry weight respectively. Significantly higher citrate contents were recorded when the same type of islets were exposed to 3.0 mg./ml. glucose. In the female mice the difference was equivalent to 1.34 mmoles ($t = 2.47$; $P < 0.02$) and in the male mice to 1.88 mmoles ($t = 3.24$; $P < 0.01$) citrate/kg. islet dry weight. Microdissected islets from female lean litter mates contained as much as 10 mmoles citrate/kg. dry weight when incubated with the high glucose concentration.

The effects of a number of modifications of the incubation medium known to influence β-cell function are shown in table 2. When tested in the presence of a high glucose concentration, 5 mM octanoate produced a significant reduction of the citrate content of islets

TABLE 1

Citrate content in islets from obese-hyperglycemic mice and their lean litter mates after thirty minutes of incubation with 0.6 or 3.0 mg./ml. glucose. The levels are calculated as mmoles citrate/kg. islet dry weight and represent mean values ± S.E.M. The numbers of animals studied are given within parentheses.

Mice		Glucose concentration	
Type	Sex	0.6 mg./ml.	3.0 mg./ml.
Lean	female	—	10.06 ± 0.31 (16)
Obese	female	6.71 ± 0.31 (13)	8.05 ± 0.36 (25)
Obese	male	6.45 ± 0.28 (12)	8.33 ± 0.28 (22)

microdissected from obese-hyperglycemic mice. The addition of two other insulin secretagogues, glibenclamide and dibutyryl cyclic-3,5-AMP, however, had no significant effects. Inhibition of the glucose-stimulated insulin release with epinephrine resulted in a substantial increase of the islet citrate content. Omission of Ca^{++} from the incubation medium, an alternative way of reducing insulin release, had no effect on islet citrate.

DISCUSSION

To obtain results representative of the β-cells, most studies were performed with islets microdissected from

TABLE 2

Changes of citrate content (mmoles/kg. islet dry weight) in islets from obese-hyperglycemic mice of both sexes and their female lean litter mates after modifying the incubation medium either by the addition of octanoate (5 mM), glibenclamide (50 μg./ml.), dibutyryl cyclic-3,5-AMP (DBcAMP, 250 μg./ml.) or epinephrine (2 μg./ml.) or by the omission of Ca^{++}. The statistical significance of effects were judged from the mean differences between paired test and control incubations using islets from a single animal in each pair. The numbers of animals studied are given within parentheses.

Type of mice	Modification of the incubation medium	Glucose concentration 0.6 mg./ml.	3.0 mg./ml.
Lean	Octanoate	—	−0.45 ± 0.33 (16)
Obese	Octanoate	+0.48 ± 0.29 (12)	−1.29 ± 0.23* (12)
Obese	Glibenclamide	+0.58 ± 0.45 (13)	−0.16 ± 0.33 (10)
Obese	DBcAMP	—	+0.39 ± 0.57 (10)
Obese	Epinephrine	—	+ 1.26 ± 0.21* (12)
Obese	Omission of Ca^{++}	—	+0.36 ± 0.36 (12)

* $P < 0.001$

obese-hyperglycemic mice. Whereas islets from lean control mice contain about 80 per cent β-cells, those from obese-hyperglycemic mice consist of more than 90 per cent of such cells.[7,10] The latter kind of islet material not only provides a fairly pure population of β-cells known to respond adequately to various insulin secretagogues but also has the advantage of being that most extensively defined from a metabolic point of view.[7,11-13]

The observation that citrate stimulates the respiration of microdissected islets from obese-hyperglycemic mice[14] makes it reasonable to assume that this molecule penetrates the β-cells. Citrate stimulation of the in vitro release of insulin has been recorded with pieces of duck[15] and rat[16] pancreas as well as with isolated rat islets.[1,2] A similar effect was seen in the cultured fetal rat pancreas when caffeine was included in the incubation medium.[17,18] It has furthermore been reported that citrate can overcome the blocking effect of 2-deoxyglucose on insulin release[19] and even elicits a more intense release in the presence than in the absence of 2-deoxyglucose.[16] Some authors have failed to observe any stimulatory effect of citrate on insulin release when using pieces of rabbit[20] or rat[21,22] pancreas. The absence of a clear-cut citrate effect under certain in vitro conditions might be attributed to interference with the Ca^{++} ions of the incubation medium.[22] It is generally believed that in one way or another this ion plays a major role in the sequence of events leading to the discharge of insulin from the β-cells.[23-25]

Previous in vivo analyses have shown that islets from obese-hyperglycemic mice contain 7 to 8 mmoles citrate/kg. dry weight, which is about twice as much as found in their lean litter mates.[6] The present in vitro approach failed to reveal such a difference when exposing islets from the two types of mice to 3.0 mg./ml. glucose. It was further observed that the levels of citrate increased when the glucose concentration of the incubation medium was increased from 0.6 to 3.0 mg./ml. These data suggest that the previously observed in vivo accumulation of citrate in the islets from obese-hyperglycemic mice[6] might be a consequence of their hyperglycemia.

No differences have been recorded in the level of serum free fatty acids between obese-hyperglycemic mice and their lean litter mates.[26] Nevertheless it was considered worthwhile to use our in vitro system to evaluate whether a "glucose-fatty acid cycle" similar to that described in muscle[4] could give rise to enhanced β-cell function. This was accomplished by measuring the citrate content in islets exposed to octanoate in a concentration known to stimulate insulin release in other in vitro systems.[1,2,5] The observation that the citrate content was not increased but was actually depressed by this treatment contradicts the previous assumption that citrate in the β-cells is regulated in the same way as has been postulated for muscle. This result makes it reasonable to question the previously suggested role of citrate in controlling insulin release. A similar view has been expressed by Matschinsky et al.[27] based on the in vivo observation that the islet levels of citrate remain remarkably constant under different nutritional conditions.

Glibenclamide and dibutyryl cyclic-3,5-AMP are effective stimulators of insulin release from the microdissected islets of obese-hyperglycemic mice[28] (Lernmark: personal communication). However, neither of these agents affected the islet citrate content. Adding epinephrine to, or omitting Ca^{++} from, the incubation medium represent alternative procedures for inhibiting insulin release in the present[29,30] and in other in vitro systems.[31] It should be noted that epinephrine caused a significant rise of the citrate content in our microdissected islets. This implies that increased levels of citrate in the β-cells do not necessarily result in a stimulation of insulin release but can actually be associated with an inhibition of this process.

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