Export of Metabolites From Pancreatic Islet Mitochondria as a Means to Study Anaplerosis in Insulin Secretion

Michael J. MacDonald

Recent evidence suggests that the mitochondrial synthesis (anaplerosis) of α-ketoglutarate or intermediates that can be converted to α-ketoglutarate, such as citrate or glutamate, are important for insulin secretion stimulated by metabolizable secretagogues. In order to focus on the productive role of mitochondria (anaplerosis) separate from the consumptive role of the cytosol (cataplerosis) in insulin secretion, pyruvate and other metabolites of insulin secretagogues were added to microgram amounts of mitochondria obtainable from rat or mouse pancreatic islets and the export of metabolites was surveyed. Cellular levels of metabolites in rat islets were also measured. The export of malate from mitochondria was the most responsive to various substrates. The export of citrate did not increase in the presence of pyruvate alone or pyruvate plus glutamate, but malate plus pyruvate caused citrate to be exported. Citrate levels in intact cells did not change with glucose. Glutamate levels did not increase in intact islets in the presence of glucose, thus not providing evidence for glutamate acting as a messenger in glucose-induced insulin secretion. The citrate level may not need to increase in order to provide increased malonyl-coenzyme A for signaling insulin secretion. Unlike many cells, insulin cells probably obtain cytosolic NADPH equivalents by exporting them from mitochondria to the cytosol via a pyruvate malate shuttle or an isocitrate shuttle. The current results suggest that the reason for anaplerosis in insulin secretion is quite complex and not fully explained by current knowledge.

GLUCOSE STIMULATES insulin release by aerobic glycolysis. The final metabolite of glycolysis is, of course, pyruvate and in pancreatic islets about half of glucose-derived pyruvate enters mitochondrial metabolism as oxaloacetate via the reaction catalyzed by pyruvate carboxylase and the other half enters the citric acid cycle as acetyl-coenzyme A after decarboxylation in the reaction catalyzed by the pyruvate dehydrogenase complex.[1-5] The high rate of pyruvate carboxylation indicates there is a net synthesis of citric acid cycle intermediates, which, therefore, also means that citric acid cycle intermediates are consumed in pathways besides the cycle. The requirement for the synthesis of citric acid cycle intermediates (anaplerosis) in insulin secretion is not well understood. However, excess citric acid cycle intermediates cannot remain inside the mitochondrion without altering mitochondrial function, and this indicates that the purpose of anaplerosis is the export of intermediates to the cytosol (cataplerosis) where they have supporting or signaling roles in insulin secretion. Recent work suggests that anaplerosis is important to provide α-ketoglutarate or 2 citric acid cycle intermediates, citrate and isocitrate, which are interconvertible with α-ketoglutarate.[6] Therefore, in the current work secretagogue-derived metabolites, pyruvate and other metabolites, were added to rat or mouse pancreatic islet mitochondria and the export of several citric acid cycle intermediates measured in an attempt to learn more about anaplerosis in the insulin cell. Measuring the export of metabolites from mitochondria could give information about anaplerosis that cannot be obtained from estimating total cellular levels of metabolites. For example, the cellular level of a metabolite might not change if the rate of its consumption equals the rate of its formation even though the rates of synthesis and export of the metabolite from mitochondria to the cytosol are quite high. Studies of metabolite levels in intact rat islets provided with glucose were also performed.

MATERIALS AND METHODS

Pancreatic Islet Isolation

Islets were isolated by collagenase digestion from pancreata of 250 to 400 g male and female Sprague Dawley rats or 2 different mouse strains allowed free access to food and water.[1-3] All experiments were performed with fresh un-preincubated islets.

Mitochondria

Mitochondria were isolated from about 2,000 rat pancreatic islets or 200 mouse islets as previously described.[3] Mitochondrial pellets were resuspended in 270 μL (rat) or 90 μL (mouse) of MSH (215 mmol/L mannitol, 65 mmol/L sucrose, and 5 mmol/L potassium Hepes buffer) containing 2 mmol/L K_2ADP, 3 mmol/L $MgCl_2$, 5 mmol/L potassium bicarbonate, and 5 mmol/L potassium phosphate, pH 7.3. The suspensions of mitochondria were then divided into 30- or 90-μL (rat) or 30-μL (mouse) portions to which substrates were added to bring the final concentration of each substrate to 5 mmol/L. Mitochondria were incubated at 37°C for zero minutes (reaction stopped 5 seconds before substrate added to companion test tubes) or 30 minutes, after which the mitochondrial suspensions were centrifuged at 14,000 × *g* for 2 minutes and the supernatant fractions removed and acidified with one-tenth volume of 6% perchloric acid. After centrifuging to remove protein, the resulting supernatant fraction was neutralized to about pH 7 with one-tenth volume of 0.92 mol/L KOH. The potassium perchlorate precipitate was removed by centrifugation and the neutralized extracts were used for metabolite assays. Each condition was repeated on many days.

Incubation of Islets

Islets were incubated in batches of 100 per test tube in 200 μL Krebs Ringer bicarbonate buffer, pH 7.3, containing an insulin secretagogue for zero minutes (reaction stopped 5 seconds before substrate was added to companion test tubes) or 15 or 30 minutes at 37°C. The Krebs Ringer solution was quickly removed and the islets washed once in the

From the University of Wisconsin Childrens Diabetes Center, Madison, WI.

Submitted July 1, 2002; accepted March 20, 2003.

Supported by NIH Grant No. DK28348, the Oscar C. Rennebohm Foundation, and the Robert Wood Johnson Family Trust.

Address reprint requests to Michael J. MacDonald, MD, Room 3459 Medical Science Center, 1300 University Ave, Madison, WI 53706.

0026-0495/03/5208-0006$30.00/0

doi:10.1016/S0026-0495(03)00149-5

Krebs Ringer solution. Fifty microliters of 6% perchloric acid was added to the islet pellet and it was vortexed vigorously and placed on ice. The perchloric acid extract was centrifuged to remove protein and neutralized to approximately pH 7 with about 7 μL 30% KOH. The perchloric acid pellet was saved for measurement of protein.

Assays of Metabolites

Metabolites exported from islet mitochondria and in intact islets were assayed enzymatically with alkali-enhanced fluorescence of NAD(P)(H) as previously described.[3,6,7] Total protein in islet and mitochondrial pellets was measured by the Lowry method.[8]

Statistics

Statistical significance was estimated by analysis of variance (ANOVA) and Student's *t* test.

RESULTS

Export of Metabolites From Islet Mitochondria

Mitochondria were incubated with pyruvate, the final premitochondrial metabolite of glucose, the most potent insulin secretagogue, as well as with glutamate, the metabolism of which is enhanced in leucine- or leucine-glutamine–induced insulin release. The export of metabolites from mitochondria was studied to discern if their export was increased to suggest that they might have signaling roles in insulin secretion. When rat islet mitochondria were incubated without an added substrate for up to 30 minutes, the export of malate was negligible. The unstimulated levels of aspartate and citrate in the medium were slightly higher than malate and increased very slightly by 30 minutes (Fig 1, lower panel). Adding glutamate and pyruvate separately or together did not cause the rate of citrate export from islet mitochondria to increase. Either pyruvate or glutamate alone caused malate export to increase and pyruvate plus glutamate caused malate export to increase even more (Table 1). Aspartate export was not influenced by pyruvate. Glutamate caused aspartate export to increase, but the presence of pyruvate in addition to glutamate did not augment aspartate export above that seen with glutamate alone (Table 1). Separate experiments that studied the time course of export of malate, citrate, and aspartate when pyruvate plus glutamate was added to islet mitochondria showed that citrate in the medium did not increase between 0 and 30 minutes compared to mitochondria incubated without an added substrate, whereas malate export increased 10-fold and aspartate export increased 3-fold (Fig 1, upper panel).

The pattern of export of metabolites from mouse islet mitochondria was similar to that from rat islet mitochondria. Pyruvate and α-ketoglutarate, which is produced from glutamate via transamination or oxidative deamination, each increased malate export. Succinate, which dramatically increased malate export from rat islet mitochondria, also caused a very large increase in malate efflux from mouse islet mitochondria. Glycerol-3-phosphate, which cannot be converted into a mitochondrial metabolite, but produces large amounts of adensoine triphosphate (ATP) especially in islet mitochondria, when added in the presence of pyruvate, augmented malate export above that with pyruvate alone. None of these substrates had any large effect on the export of citrate, aspartate, isocitrate, or glutamate. Succinate slightly increased aspartate and glutamate export (Table 2).

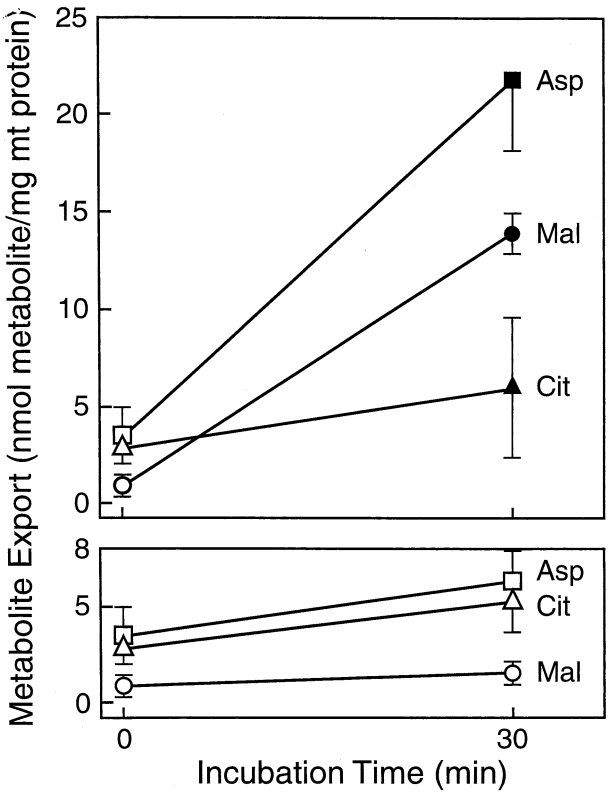

Fig 1. Synthesis and export of malate, aspartate and citrate from rat pancreatic islet mitochondria incubated with or without pyruvate + glutamate. After 5 minutes at 37°C, medium was rapidly separated from mitochondria and sampled for metabolites (0 time). Then pyruvate + glutamate (5 mmol/L of each, upper panel) was added (or not added, open symbols and lower panel) to the remaining medium containing mitochondria and the incubation continued for 30 minutes. Results are the mean ± SE from 8 replicate incubations.

The lack of export of citrate from mitochondria might be due to its inability to exit mitochondria because citrate transport requires that it exchange with an external anion in order for it to cross the inner mitochondrial membrane. Therefore, mitochondria were incubated with pyruvate and in addition malate, which can exchange with citrate on the tricarboxylate transporter. Malate alone caused citrate export to increase, but pyruvate plus malate increased citrate export even more (Table 3).

Effect of Glucose on Metabolite Levels in Intact Islets

The levels of metabolites in intact islets incubated in the presence of glucose were studied to discern if their levels were changed, suggesting the possibility of their having a signaling or supporting role in insulin secretion. Although glucose did not alter the level of any metabolite, as pointed out in the introductory section, if consumption of a metabolite equalled its production, its level would not be changed. This result supports the idea of studying isolated mitochondria to obtain

Table 1. Effect of Added Pyruvate and Glutamate on Export of Metabolites From Pancreatic Islet Mitochondria

	Exported Metabolite (nmol metabolite exported/mg mitochondrial protein/30 min)			
	Malate	Aspartate	Citrate	Glutamate
Substrate added				
None	0.2 ± 0.5 (10)	7.2 ± 1.5 (8)	7.2 ± 2.1 (5)	9.2 ± 0.8 (6)
Pyruvate	8.8 ± 1.5 (6)†	8.3 ± 2.7 (7)	8.7 ± 1.7 (5)	9.7 ± 1.4 (5)
Glutamate	6.8 ± 1.6 (5)*	23.5 ± 3.0 (10)†	8.1 ± 1.1 (5)	NM
Pyruvate + glutamate	12.8 ± 2.4 (8)†	23.2 ± 2.4 (19)†	10 ± 1.5 (10)	NM

NOTE. Mitochondria isolated from pancreatic islets of fed rats were incubated with pyruvate, glutamate, or pyruvate plus glutamate. Metabolites present in the medium were measured after 30 minutes. Results are the mean ± SE with the number of replicate incubations in parentheses.

*$P < .01$ and †$P < .001$ *v* no substrate added.

Abbreviation: NM, not measured.

clues about anaplerosis. In the absence of an insulin secretagogue, the levels of all metabolites, except aspartate, in intact rat islets remained essentially constant during the 30-minute incubation (Fig 2). The level of aspartate decreased from 35 ± 4 nmol aspartate/mg islet protein to 11 ± 1 nmol aspartate/mg islet protein over 30 minutes. Basal citrate and glutamate levels were about 50% and 3-fold higher, respectively, than basal malate levels. The initial basal aspartate level in intact islets at the beginning of the incubation period was much higher than the level of any other metabolite, but by 15 minutes aspartate had fallen to unremarkable levels. There was no apparent explanation for this initial high level of aspartate other than possibly cold ischemia that occurs during islet preparation. The addition of glucose (16.7 mmol/L) did not change the level of any metabolite measured over 30 minutes compared with the concentration observed with no secretagogue. Basal α-ketoglutarate and isocitrate levels were very low and were not increased by glucose. Basal malate levels were slightly higher than these 2 metabolites, but were not affected by glucose. Glutamate and citrate levels were higher than other metabolites and were also not increased by glucose (Fig 2).

DISCUSSION

During glucose-induced insulin release, pyruvate decarboxylation can provide all the substrate (acetyl-CoA) required for energy production by the citric acid cycle. However, in the insulin cell the amount of glucose-derived pyruvate that is carboxylated to 4-carbon cycle intermediates equals the amount of pyruvate decarboxylated.[1-6,9,10] This net synthesis of cycle intermediates suggests that certain intermediates may be exported from the mitochondria to the cytosol, where they could play major roles in signaling or supporting insulin secretion. It is likely that synthesized metabolites have extramitochondrial roles because excess levels of cycle intermediates inside mitochondria would disrupt the normal flux of the citric acid cycle. To focus on the mitochondrial production of metabolites involved in anaplerosis and cataplerosis metabolites of various insulin secretagogues, such as pyruvate (from glucose) and glutamate (analogous to leucine- or leucine-glutamine–stimulated insulin release) were added to mitochondria isolated from rat and mouse pancreatic islets and the export of metabolites was studied. The validity of this experimental approach to learn about anaplerosis in insulin secretion is supported by the fact that glucose, the most potent insulin secretagogue, did not alter the levels of any metabolite in intact islets (Fig 2). The current work surveyed the mitochondrial export of key intermediates, such as malate and citrate, recently implicated as having roles in insulin secretion.[3,9,10] In the presence of various added substrates, the export of malate from mitochondria of rat or mouse pancreatic islets increases more than that of other metabolites. Pyruvate alone or with glutamate did not affect citrate export from rat islet mitochondria. Since citrate exchanges with malate on the tricarboxylate transporter of the mitochondrial inner membrane, it is possible that pyruvate is unable to support citrate export when there is an insufficient level of an extramitochondrial exchangeable anion. This may be the case because adding malate plus pyruvate to the mitochondria permits citrate to be exported (Table 3). This indicates that in the beta cell during glucose-induced insulin secretion, anaplerosis from pyruvate via the pyruvate carboxylase plus pyruvate dehydrogenase reactions might produce significant citrate for export from mitochondria when malate is exported to the cytosol to

Table 2. Effect of Adding Various Substrates on Export of Metabolites From Mitochondria From Mouse Pancreatic Islets

	Exported Metabolite (pmol metabolite exported mitochondria from 25 islets/30 min)					
Substrate added	Malate	Citrate	Aspartate	Isocitrate	Glutamate	α-Ketoglutarate
None	52, 58	86, 131	84, 126	37, 93	46, 118	104, 118
Pyruvate	96, 120	71, 107	82, 111	84, 58	53, 96	230, 235
Succinate	890, 3700	65, 147	101, 173	64, 57	79, 132	11, 112
α-Ketoglutarate	100, 114	100, 65	58, 116	70, 64	11, 16	NM, NM
Pyruvate + glycerol-3-phosphate	184, 117	54, 109	54, 99	46, 53	71, 224	224, 257

NOTE. Mitochondria from pancreatic islets of BALB/cHeA mice (the first number of each pair) and from an F1 generation of a Mod-1/BALB/cHeA F1 cross (the second number of each pair) were incubated with each substrate for 30 minutes.

Abbreviation: NM, not measured.

Table 3. Effect of Various Substrates on Citrate Export From Rat Pancreatic Islet Mitochondria

Substrate Added	Citrate Export (nmol/mg mitochondrial protein)
None	1.3 ± 0.2 (8)
Pyruvate	1.3 ± 0.2 (4)
Malate	4.1 ± 0.6 (4)*
Pyruvate plus malate	5.3 ± 0.3 (8)†
Succinate	2.1 ± 0.3 (4)

NOTE. Mitochondria were incubated with 5-mmol/L concentrations of various substrates for 30 minutes at 37°C and citrate in medium was measured. Results are the mean ± SE with the number of observations in parentheses.

*$P < .01$ or †$P < 0.001$ *v* no addition or pyruvate alone

exchange back into the mitochondria. Although malate augmented citrate export in the presence of pyruvate in the experiments shown in Table 3, this is not always the case in our hands (unpublished data). In the experiments shown in Table 3, the control values for citrate export were lower than is often seen in many other experiments (Table 1, Fig 1, and unpublished data). (There is no adequate explanation for this difference except possibly that the islets used in the experiments shown in Table 3 were all from male rats that were about 50 to 100 g smaller than the female rats used to prepare islets for other experiments.) Since augmenting the mitochondrial α-ketoglutarate level with pyruvate plus glutamate did not augment citrate export (Fig 1), it is unlikely that a significant amount of citrate is synthesized from α-ketoglutarate via the reverse of the isocitrate dehydrogenase and aconitase reactions when α-ketoglutarate production is enhanced by the activation of glutamate dehydrogenase, such as during leucine-induced or leucine-glutamine–induced insulin release.[11,12]

In 1972, Hellman and Idahl[13] noted that glucose increased citrate levels less than 25% in pancreatic islets from the obese hyperglycemic mouse and concluded that citrate is unlikely to have an important role in controlling insulin release. However, another group has recently observed the citrate levels double in both purified rat islet beta cells[9] and INS-1 cells [10] incubated with a high concentration of glucose. It was hypothesized that an increased level of citrate-derived malonyl-CoA from the metabolism of glucose by inhibiting carnitine palmitoyl-CoA transferase should inhibit fatty acid transport into mitochondria.[9,10,14-16] This should cause the level of long-chain fatty acyl-CoAs to increase in the cytosol, which might influence the activity of various enzymes, such as glucokinase and the K_{ATP} channel.[16] Since the level of malonyl-CoA required for inhibition of carnitine palmitoyl-CoA transferase in islet mitochondria is lower than 1 μmol/L,[17] as in other mitochondria, and the basal level of citrate in the cell is 100- to 400-fold this level, it would seem unnecessary for the level of cytosolic citrate to increase in order to influence the formation of malonyl-CoA. In our hands cellular citrate levels and the levels of any citric acid cycle intermediate measured in numerous experiments with intact rat islets (Fig 2 and unpublished data) are not changed much by any insulin secretagogue. The basal levels of citrate (estimated to be 0.35 to 0.9 mmol/L based on protein content of islets[19]) in islets were higher than unstimulated malate (Table 1). In many tissues malate levels are about the same as citrate or slightly higher except in heart tissue where citrate levels are higher (0.4 to 1.3 mmol/L).[20] It is possible that citrate levels are high in normal islets. If citrate levels are increased by the islet isolation procedure, this is not associated with insulin release because unstimulated insulin release was low (<20 μU insulin/5 islets/h) and secretagogue-stimulated insulin release was high (>350 μU insulin/5 islets/h) in the islets used to study metabolites. In INS-1 cells we observed that citrate levels increase $\leq$100% in the presence of glucose and other secretagogues (unpublished data). Since there is ample evidence that malonyl-CoA levels increase in secretagogue-stimulated islets,[16] it is possible, if not likely, that this increase occurs by activation of acetyl-CoA carboxylase and without a significant increase in the cellular level of citrate. In this respect the insulin cell would resemble liver where activation of acetyl-CoA carboxylase by its dephosphorylation enhances cytosolic malonyl-CoA levels rather than skeletal muscle where increased cellular citrate enhances malonyl-CoA formation.[18] In fact, increased citrate levels in the cytosol during glucose-stimulated insulin secretion might be counterproductive because citrate inhibits phosphofructokinase and this would inhibit glycolysis and thus insulin secretion. It should also be mentioned that the absence of an increase in a cellular metabolite does not mean the synthesis of the metabolite is unimportant. An important metabolite may be rapidly synthesized, but just as rapidly consumed.

It was recently proposed that an increased level of glutamate formed from α-ketoglutarate via the reverse of the glutamate dehydrogenase reaction is a messenger in glucose-induced insulin secretion.[21] However, glucose (Fig 2) and other insulin secretagogues[11,12] do not increase glutamate levels in intact

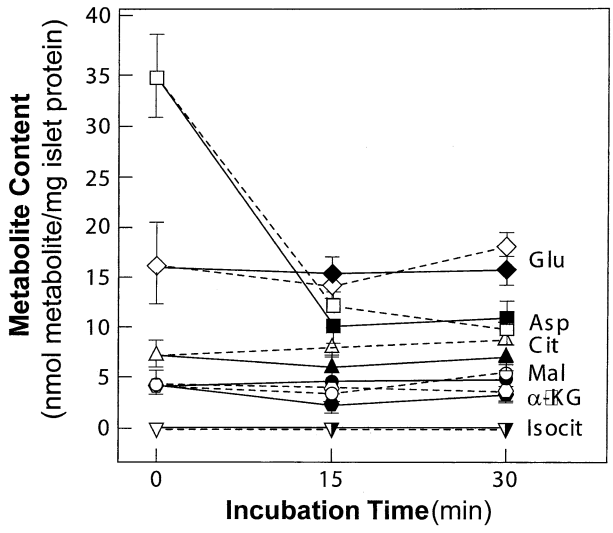

Fig 2. Time course of metabolite levels in rat pancreatic islets incubated with and without glucose. Islets (100/test tube) were incubated in Krebs Ringer bicarbonate buffer, pH 7.3 alone (open symbols and dotted lines) or with 16.7 mmol/L glucose (closed symbols and solid lines) for various times. Results are the mean ± SE of 4 replicate incubations for each condition except for aspartate and glutamate, which are from 8 replicate incubations.

islets. Other evidence against glutamate being a messenger is that glutamine by itself cannot stimulate insulin release from islets,[12,19] but it increases glutamate 11-fold in islets.[11,19] Since glutamate export does not increase in islet mitochondria supplied with pyruvate alone (Table 1), flux through the glutamate dehydrogenase reaction is most likely quiet during glucose-induced insulin secretion. The preponderance of evidence suggests that when there is flux through the glutamate dehydrogenase reaction, such as during leucine-induced insulin release, it is in the direction of α-ketoglutarate.[11,12,19,22,23] Flux through the glutamate dehydrogenase in the forward direction is facilitated because α-ketoglutarate dehydrogenase can form a complex with glutamate dehydrogenase enabling channeling of substrate from glutamate dehydrogenase to α-ketoglutarate dehydrogenase.[24] Thus α-ketoglutarate can be directly converted to succinyl-CoA, which is also an activator of glutamate dehydrogenase.

As might be expected, glutamate causes aspartate export from islet mitochondria to increase (Table 1) because glutamate can transaminate with oxaloacetate to form aspartate. However, malate is the metabolite most responsive to substrates added to islet mitochondria. The export of malate from islet mitochondria is negligible in the absence of a substrate and increases significantly in the presence of almost any added substrate. Increased malate export is most noticeable in the presence of added pyruvate or glutamate or pyruvate in combination with glutamate (Table 1). Succinate causes massive efflux of malate from mitochondria of the rat (unpublished data) and the mouse (Table 2). The responsiveness of malate export from beta-cell mitochondria to substrates, such as pyruvate (Table 1) and succinate,[6] which are metabolites of the insulin secretagogues glucose and methyl succinate, respectively, suggests that malate export from mitochondria is in some way important in insulin secretion. This might be because in the cytosol malate can undergo oxidation to pyruvate and CO_2 in the reaction catalyzed by malic enzyme. This would effectively transport NADPH equivalents and CO_2 out of the mitochondria.[3] The resulting pyruvate can re-enter mitochondrial pyruvate pools where it can be carboxylated in the pyruvate carboxylase reaction or decarboxylated to acetyl-CoA and CO_2 in the reaction catalyzed by pyruvate dehydrogenase. Acetyl-CoA can be metabolized to CO_2 in the citric acid cycle or condense with oxaloacetate and used to synthesize citrate, isocitrate or α-ketoglutarate, which cannot be formed from four carbon carboxylates alone.[6] The formation of cytosolic NADPH in the beta cell via a pyruvate malate shuttle might be important in the insulin cell where it is likely that very little NADPH is formed via the hexosemonophosphate pathway.[25-28] The need for such a shuttle is consistent with the fact that the cytosolic NADP/NADPH ratio of most types of cells is reduced while the same ratio in the mitochondria is relatively oxidized and that 6-aminonicotinamide, an agent that interferes with NADPH availability, causes hyperglycemia in rats[29] and inhibits glucose- and amino acid–induced insulin release in isolated pancreatic islets.[30,31]

Isocitrate levels in intact islets are essentially undetectable (Fig 2), which is not unexpected because isocitrate levels in many tissues are very low. However, it seems possible that significant NADPH equivalents are sometimes exported from islet mitochondria in the form of isocitrate, which can reduce NADP in the isocitrate dehydrogenase reaction in the cytosol. This would occur when pyruvate and malate (or oxaloacetate) are both increased in the mitochondrion. In the rat beta cell, all metabolizable insulin secretagogues are capable of forming pyruvate plus oxaloacetate or malate, due to the pyruvate malate shuttle, which can interconvert, pyruvate, oxaloacetate, and malate.[3] In the mouse beta cell, isocitrate export from mitochondria might be a necessary means of exporting NADPH equivalents to the cytosol because mouse pancreatic islets do not possess malic enzyme[6,32] and thus cannot possess the pyruvate malate shuttle.

ACKNOWLEDGMENT

The author thanks Heather Drought, Richard C. Raphael, and Julian Buss for technical assistance.

REFERENCES

1. MacDonald MJ: Metabolism of the insulin secretagogue methyl succinate by pancreatic islets. Arch Biochem Biophys 300:201-205, 1993

2. MacDonald MJ: Estimates of glycolysis, pyruvate (De)carboxylation, pentose phosphate pathway and methyl succinate metabolism in incapacitated pancreatic islets. Arch Biochem Biophys 305:205-214, 1993

3. MacDonald MJ: Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. J Biol Chem 270:20051-20058, 1995

4. Khan A, Ling ZC, Landau BR: Quantifying the carboxylation of pyruvate in pancreatic islets. J Biol Chem 271:2539-2542, 1996

5. Lu D, Mulder H, Zhao P, et al: ^{13}C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). Proc Natl Acad Sci USA 99:2708-2713, 2002

6. MacDonald MJ: Differences between mouse and rat pancreatic islets: Succinate responsiveness, malic enzyme and anaplerosis. Am J Physiol Endocrinol Metab 283:E302-E310, 2002

7. Passonneau JV, Lowry OH: Enzymatic Analysis, A Practical Guide. Totowa, NJ, Humana Press, 1993

8. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

9. Schuit F, De Vos A, Farfari S, et al: Metabolic fate of glucose in purified islet cells: Glucose-regulated anaplerosis in cells. J Biol Chem 272:18572-18579, 1997

10. Farfari S, Schulz V, Corkey B, et al: Glucose-regulated anaplerosis and cataplerosis in pancreatic beta-cells: Possible implication of a pyruvate/citrate shuttle in insulin secretion. Diabetes 49:718-726, 2000

11. Malaisse WJ, Sener A, Malaisse-Lagae F, et al: The stimulus-secretion coupling of amino acid-induced insulin release: Metabolic response of pancreatic islets of L-glutamine and L-leucine. J Biol Chem 257:8731-8737, 1982

12. Fahien LA, MacDonald MJ, Kmiotek EH, et al: Regulation of insulin release by factors that also modify glutamate dehydrogenase. J Biol Chem 263:13610-13614, 1988

13. Hellman B, Idahl L: Pancreatic islet levels of citrate under conditions of stimulated and inhibited insulin release. Diabetes 21:999-1002, 1972

14. Chen S, Oawa A, Ohneda M, et al: More direct evidence for a malonyl-CoA-carnitine palmitoyltransferase I interaction as a key event in pancreatic beta-cell signaling. Diabetes 43:878-883, 1994

15. Assimacopoulos-Jeannet F, Thumelin S, Roche E, et al: Fatty

acids rapidly induce the carnitine palmitoyltransferase i gene in the pancreatic β-cell line INS-1. J Biol Chem 272:1659-1664, 1997

16. Corkey BE, Deeney JT, Yaney GC, et al: The role of long-chain fatty acyl-CoA esters in β-cell signal transduction. J Nutr 130:299S-304S, 2000 (suppl 2)

17. Shrago E, MacDonald MJ, Woldegiorgis G, et al: The role of carnitine palmitoyltransferase and carnitine in the metabolism of pancreatic islets, in Borum PR (ed): Clinical Aspects of Human Carnitine Deficiency. New York, NY, Pergamon, 1986, pp 23-37

18. Saha AK, Laybutt DR, Dean D, et al: Cytosolic citrate and malonyl-CoA regulation in rat muscle in vivo. Am J Physiol 276: E1030-E1037, 1999

19. MacDonald MJ, Fahien LA: Glutamate is not a messenger in insulin secretion. J Biol Chem 275:34025-34027, 2000

20. Bergmeyer HU, Gawehn K (eds): Methods of Enzymatic Analysis, vol 4 (ed 2). New York, NY, Academic, 1974, pp 1625-2302

21. Maechler P, Wollheim CB: Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402:685-689, 1999

22. Bertrand G, Ishiyama N, Nenquin M, et al: The elevation of glutamate content and the amplification of insulin secretion in glucose-stimulated pancreatic islets are not causally related. J Biol Chem 277:32883-32891, 2002

23. Li C, Najafi H, Daikhin Y, et al: Regulation of leucine stimulated insulin secretion and glutamine metabolism in isolated rat islets. J Biol Chem 278:2853-2858, 2003

24. Fahien LA, MacDonald MJ, Teller JK, et al: Kinetic advantages of hetero-enzyme complexes with glutamate dehydrogenase and the alpha-ketoglutarate dehydrogenase complex. J Biol Chem 264:12303-12312, 1989

25. Matschinsky FM, Kauffman FC, Ellerman JE: Effect of hyperglycemia on the hexose monophosphate shunt in islets of Langerhans. Diabetes 17:475-480, 1968

26. Ashcroft SJH, Weerasinghe LCC, Bassett JM, et al: The pentose cycle and insulin release in mouse pancreatic islets. Biochem J 126: 525-532, 1972

27. Snyder PJ, Kashket S, O'Sullivan JB: Pentose cycle in isolated islets during glucose-stimulated insulin release. Am J Physiol 219:876-880, 1970

28. Giroix MH, Sener A, Malaisse WJ: Pentose cycle pathway in normal and tumoral islet cells. FEBS Lett 185:1-3, 1985

29. Ammon HPT, Steinke J: Effect of 6-aminonicotinamide on insulin release and C-14 glucose oxidation by isolated pancreatic rat islets: difference between glucose, tolbutamide and aminophylline. Endocrinology 91:33-38, 1972

30. Ammon HPT, Steinke J: 6-Amnionicotinamide (6-AN) as a diabetogenic agent: In vitro and in vivo studies in the rat. Diabetes 21:143-148, 1972

31. MacDonald MJ, Ammon HPT, Patel TN, et al: Failure of 6-aminonicotinamide to inhibit the potentiating effect of leucine and arginine on glucose-induced insulin release in vitro. Diabetologia 10:761-765, 1974

32. MacDonald MJ, Marshall LK: Survey of normal appearing mouse strain which lacks malic enzyme and NAD^+-linked glycerol phosphate dehydrogenase: normal pancreatic beta cell function, but abnormal metabolite pattern in skeletal muscle. Mol Cell Biochem 220:117-125, 2001