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High Level of ATP Citrate Lyase Expression in Human and Rat Pancreatic Islets

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Abstract

A comparison of an enzyme's level in pancreatic islets with its level in other body tissues can give clues about the importance of a metabolic pathway in the islets. ATP citrate lyase plays a key role in the pyruvate citrate shuttle, as well as for the synthesis of short chain acyl-CoAs and lipid, and its level in human and rat pancreatic islets relative to other tissues has not been previously reported. We compared the level of ATP citrate lyase mRNA and enzyme activity in pancreatic islets of humans and rats and the INS-1 832/13 cell line to levels in liver, a lipid synthesizing organ, and also kidney. The mRNA level was much higher in human islets and rat islets than in liver and kidney of the same genus and the enzyme activity was 8-fold and 12-fold higher in islets of humans and rats, respectively, compared to liver of the same genus. These data support other evidence that indicates ATP citrate lyase is important for the pyruvate citrate shuttle and lipid synthesis in insulin secretion.

Introduction

Over the years our laboratory has measured levels of enzymes in normal pancreatic islets [1-4] or islets from individuals with type 2 diabetes and found clues about which metabolic pathways might be important or less important for insulin secretion. In 1975 Berne [5] reported that the enzyme activity of ATP citrate lyase is considerably higher in pancreatic islets of the obese New Zealand (NZO) mouse than in the liver and kidneys of this mouse. This is the only study we know of where the level of ATP citrate lyase enzyme activity in islets was compared to its level in other tissues. The observation that the activity of ATP citrate lyase is very high in the islet is important because the enzyme is a component of the pyruvate citrate shuttle [6-9], which is important for synthesis of short chain acyl-CoAs that are the precursors for lipid synthesis in the cytosol. Confi rming a high level of this enzyme could support other evidence that the pancreatic islet is a lipid synthesizing tissue and that lipid remodeling is necessary for normal insulin secretion [8]. These ideas might be further relevant to the concept that an excess of lipids in the beta cells of the islet might contribute to beta cell toxicity in type 2 diabetes (the lipo-toxicity hypothesis). Because information on the relative level of ATP citrate lyase is available from only the mouse and the mouse that Berne studied was obese, which might have stimulated an insulin resistance-induced

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increase in beta cell enzymes necessary for insulin secretion, we investigated the generalizability of the ATP citrate lyase concept to other genera. We measured the mRNA that encodes the enzyme, as well as its enzyme activity, in islets of normal humans and rats, as well as its expression in the INS-1 832/13 insulin cell line, which is a cell line of glucose-responsive beta cells.

Materials and Methods

Quantitative PCR

Tissues were homogenized with a Qiashredder (Qiagen) (islets) or using a Potter–Elvehjem homogenizer (liver) and RNA was prepared using the RNeasy Mini kit (product number 74104, Qiagen). On-column DNase digestion was performed using the Qiagen RNase-Free DNase Set. cDNA was made with randomized primers with the Retroscript kit (AM1710) (Applied Biosystems). Quantitative PCR was performed on a BioRad MyIQ Real Time Detection System with SYBR Premix Ex Taq (RR041Q) (Takara). 18s RNA and mRNA that encodes glutamate dehydrogenase (Glud1) were measured as internal controls for Acly mRNA measurements. Nucleotide sequences of primers used are shown in Supplemental Table 1S. RNA was isolated from islets of human donors whose average BMI and age are shown in Table 1 and from islets and livers of rats whose ages and weights are also shown in Table 1. Islet mRNA levels were compared with the same genus liver mRNA levels. Human liver RNA was from a 51-year old male (Clontech, catalogue number 636531) and a liver surgical specimen from a person (of unknown age and gender due to privacy protection).

ATP citrate lyase activity

Subcellular fractionation of pancreatic islets and the other organs was as described previously [4]. Briefly, islets were homogenized in 220 mM mannitol, 70 mM sucrose, 5 mM potassium Hepes buffer, pH 7.5 (MSH) containing 1 mM dithiothreitol. The homogenate was centrifuged at 600*g* for 10 min to precipitate the nuclei and cell debris fraction, and the supernatant fraction was centrifuged at 5 500*g* for 10 min to precipitate the mitochondrial fraction. The resulting supernatant fraction was centrifuged at 20 000*g* for 20 min to generate a cytosolic fraction used to measure enzyme activity. Liver cytosol was prepared similarly from a homogenate (3 ml MSH-dithiothreitol buffer/1 g liver). Human liver samples used for measurements of enzyme activity were from surgical samples from 3 individuals of unknown age and gender. ATP citrate lyase activity was measured in a reaction mixture containing 5 mM citrate, 0.3 mM coenzyme A, 3 mM ATP, 0.15 mM NADH, 10 mM $MgCl_2$, 10 mM dithiothreitol, and 6 units/ml of malate dehydrogenase from pig heart mitochondria in 100 mM Tris chloride buffer, pH 8.5, at 37°C [9].

Results

Relative mRNA levels

Table 1 shows that the level of ATP citrate lyase (Acly) mRNA in human or rat pancreatic islets was much higher than in liver of the same genus. The level of Acly mRNA relative to Glud1 (glutamate dehydrogenase) mRNA and 18s RNA in the livers of older obese rats was higher than in the livers of younger and leaner rats. The average level of Acly mRNA relative to Glud1 mRNA (that is abundant in both islets and liver) or 18s RNA in young rats was assigned a value of 1. When the relative Acly mRNA levels in the livers of the young rats and old rats were averaged together and these averages were compared to the relative mRNA levels in the rat islets, the rat islet mRNA levels were 11- and 2.3-fold higher than the liver values normalized to Glud1 mRNA and 18s RNA, respectively. The Acly levels in human islets relative to human liver were even higher and were 49- and 24-fold higher in the islets compared to the livers normalized to Glud1 mRNA and 18s RNA, respectively.

Enzyme activity

Table 2 shows that the enzyme activity of ATP citrate lyase in rat pancreatic islets was 12-fold higher than rat liver and 17-fold higher than in rat kidney. Table 2 also shows the ATP citrate lyase activity in human liver compared to data from an extensive study in which the enzyme was measured in islets of nondiabetic humans and compared to its level in INS-1 832/13 cells and rat pancreatic islets [4]. In this previous study, the average ATP citrate lyase level in rat islets was 50 % higher than in the current study and it was observed that the level of ATP citrate lyase in human islets was one-fourth the level in rat islets and the INS-1 832/13 insulinoma cell line. In spite of this difference between human islets and rat islets or the insulin cell line, the level of ATP citrate lyase in human islets was 8-fold higher than in human liver and 4-fold and 6-fold higher than the level in rat liver and rat kidney, respectively.

Discussion and Conclusions

The high level of ATP citrate lyase mRNA and enzyme activity in pancreatic islets supports the idea that the enzyme is important for insulin secretion. In addition to the enzyme being involved in the pyruvate citrate shuttle, which uses oxaloacetate formed from mitochondrially-derived citrate in the ATP citrate lyase reaction for transporting redox equivalents of NAD(P)(H) between the mitochondrial matrix and the cytosol [3,6,7], the ATP citrate lyase reaction forms acetyl-CoA, which can be converted to malonyl-CoA and other short chain acyl-CoAs that can all be used for the synthesis of lipids in the cytosol [4,6-9]. Our recent work suggests that rapid lipid synthesis and remodeling in the beta cell are important for insulin secretion [8]. The formation of acyl-CoA precursors of lipid can occur via a pathway involving ATP citrate lyase as well as via another pathway that utilizes acetoacetyl-CoA synthetase to form short chain acyl-CoAs in the cytosol [4,9]. This redundant pathway appears to be especially important in the human pancreatic islets where pyruvate carboxylase and ATP citrate lyase are lower and the levels of enzymes of the alternate pathway are higher than in rat islets [4]. Interestingly, even though the level of ATP citrate lyase is high in rat islets and in beta cell lines, our data [9] and those of Joseph et al. [10] show that severely knocking down the level of ATP citrate lyase in the rat insulinoma INS-1 832/13 cell line and/or islets with siRNA technology does not inhibit glucose-stimulated insulin secretion. The knockdown of ATP citrate lyase without inhibiting insulin release also supports the idea that a redundant pathway that forms short chain acyl-CoAs is present in the cytosol of the beta cell [4,9].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Relative ATP citrate lyase mRNA levels in human and rat islets are much higher than in liver of the same genus.

Genus (n)	Acly/Glud1	Acly/18s
Young rat livers (2)	1 (1.01, 0.99)	1 (1.07, 0.93)
Old rat livers (2)	4.4 (4.5, 4.3)	5.4 (5.0, 5.7)
Average all rat livers (4)	2.7 ± 0.9	3.2 ± 1.1
Rat islets (4)	30 ± 4.4[a]	7.3 ± 1.8
Rat islet/Rat liver (4)	11	2.3
Human livers (2)	1 (083, 1.17)	1 (0.53, 1.47)
Human islets (9)	49 ± 7.8[a]	30 ± 2.7[a]

Islet AcLy mRNA was estimated by qRT-PCR and is expressed relative to values from liver samples from 4 rats (two 7-week old male rats (weighing 200 g and 207 g) and two 11-month-old female retired breeder rats (weighing 327 g and 339 g)) or the average of 2 human liver samples. RNA was isolated from 4 batches of islets with each batch from eight 200–250 g rats. The mean ± SE BMI and age of the 9 human donors whose islets were used for the mRNA measurements were 30 ± 1.8 kg/m ^{2} and 46.3 ± 5.4 years. Values were normalized to glutamate dehydrogenase (Glud1) mRNA and 18 s RNA as internal controls. The average relative Acly mRNA levels of livers from the younger rats and human livers were assigned a value of 1 and the levels of other livers and islets are expressed relative to these averages. Results are the mean ± SE with the number of samples in parentheses for n > 2. Individual values are shown in parentheses when there were 2 samples.

[a] $p < 0.001$ vs. same genus average liver value.

Table 2

High level of ATP citrate lyase enzyme activity in human and rat pancreatic islets compared to liver and kidney.

Tissue	ATP Citrate lyase activity (nmol oxaloacetate formed/min/mg cytosol protein)
Rat islets	64 ± 2.9 (4)[a]
Rat liver	5.4 ± 0.7 (4)
Rat kidney	3.8 ± 0.7 (4)
Human islets	24 ± 1.9 (21)[a,b,c]
Human liver	3.2 ± 0.4 (3)
INS-1 832/13 Cell	104 ± 3.6 (31))[a,b]
Rat islets	96 ± 9.0 (13))[a,b]

Results are the mean ± SE (n) of measurements from 4 batches of islets from 2 rats each and livers and kidneys from 4 of the rats used for islet isolation. The table also shows previously published data on ATP citrate lyase activity measured in an extensive study of human islets from 21 donors compared to the INS-1 832/13 cell line and rat islets measured as controls within the same assays (see Table 3 of reference [4]) for comparison with the enzyme activity measured in human livers in the current study

[a] $p < 0.001$ vs. rat liver or rat kidney

[b] Values are from Table 3 of reference [4]

[c] $p < 0.001$ vs. human liver