Do Pancreatic Islets Contain Significant Amounts of Phosphoenolpyruvate Carboxykinase or Ferroactivator Activity?

MICHAEL J. MacDONALD AND CHENG-MIN CHANG

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/3/246/353713/34-3-246.pdf by guest on 23 July 2024

SUMMARY

Phosphoenolpyruvate carboxykinase activity was measured in rat pancreatic islet cytosol and mitochondria. No carboxykinase activity was detected under a variety of conditions, including those that increase phosphoenolpyruvate carboxykinase activity in nonislet tissues, such as starving animals or incubating the islet extracts with Fe^{2+} or Mn^{2+} before assaying for enzyme activity. The amounts of islet cytosol protein used exceeded those of liver in companion assays used as controls. It was calculated that if islet phosphoenolpyruvate carboxykinase activity was 0.005 that of liver, or 1×10^{-5} as high as pyruvate kinase activity in islets, it should have been detected in the assays used. Ferroactivator is a protein that permits Fe^{2+} to activate phosphoenolpyruvate carboxykinase and it is ubiquitous to many tissues that do and even do not contain the carboxykinase. Ferroactivator activity was not detectable in pancreatic islets. Pyruvate kinase, an enzyme that catalyzes a reaction that is essentially the opposite of that catalyzed by phosphoenolpyruvate carboxykinase (i.e., phosphoenolpyruvate formation), is plentiful in islet cytosol. Therefore, even if phosphoenolpyruvate carboxykinase activity is present in pancreatic islets, it is so low that it is unlikely that phosphoenolpyruvate formation would be favored and the contribution of the carboxykinase to intracellular carbohydrate metabolism must be quantitatively unimportant. DIABETES 1985; 34:246–50.

It is probable that glucose, the most potent physiologic insulin secretagogue, needs to be metabolized (as opposed to its mere binding to a receptor) to stimulate insulin release from the pancreatic beta cell.[1] Furthermore, there is evidence that part of the glycolytic pathway, between the triose phosphates and pyruvate, or a pathway branching from this part of the glycolysis pathway, plays an important role in insulin secretion. The oxidation of glucose via the tricarboxylic acid cycle by itself appears insufficient for stimulating insulin secretion. If metabolism of substrate via the tricarboxylic acid cycle was sufficient for signaling and supporting insulin secretion, pyruvate (which can be metabolized in the tricarboxylic acid cycle but not to triose phosphates, unless the beta cell contains pyruvate carboxylase and phosphoenolpyruvate carboxykinase) should be as potent an insulin secretagogue as glyceraldehyde (which can be phosphorylated to glyceraldehyde phosphate, which can be metabolized in a number of pathways as described below). However, even though both pyruvate and glyceraldehyde are oxidized to carbon dioxide by pancreatic islets, only glyceraldehyde is a potent stimulator of insulin release.[1–4]

If it was known that pancreatic islets contained phosphoenolpyruvate carboxykinase as well as pyruvate carboxykinase, it might alter current thinking regarding pathways of carbohydrate metabolism in islets. If islets contained these two "glyconeogenic" enzymes, pyruvate could be metabolized to triose phosphates that could participate in the glycerol phosphate shuttle[5,6] and undergo conversion to phospholipids,[7] both of which could be important regulators of insulin secretion. The ability of mixtures of amino acids to stimulate insulin release[8–10] would be partially explained. Any amino acid that could be metabolized to phosphoenolpyruvate could be further metabolized similarly to any phosphorylated, three-carbon glycolytic intermediate and should stimulate insulin secretion similarly to glucose or glyceraldehyde. It is also known that phosphoenolpyruvate inhibits Ca^{2+} accumulation in islet mitochondria.[11] Phosphoenolpyruvate carboxykinase would influence the islet content of phosphoenolpyruvate.

Phosphoenolpyruvate carboxykinase activity has been reported to be "rather high" in mouse pancreatic islets.[12] This report describes our attempts to measure phosphoenolpyruvate carboxykinase and phosphoenolpyruvate carboxy-

From the Department of Pediatrics, University of Wisconsin Medical School, Madison, Wisconsin.
Address reprint requests to Michael J. MacDonald, Department of Pediatrics, University of Wisconsin Medical School, Room 3459, 1300 University Avenue, Madison, Wisconsin 53706.
Received for publication 3 February 1984 and in revised form 4 June 1984.

kinase ferroactivator activity in rat pancreatic islets. Ferroactivator is a recently discovered cytosol protein that permits Fe^{2+} to activate phosphoenolpyruvate carboxykinase.[13,14]

MATERIALS AND METHODS

Tissue preparation and subcellular fractions. Pancreatic islets were isolated from pancreata of 200–250-g Sprague-Dawley rats (King Animal Laboratory, Madison, Wisconsin) by collagenase digestion of the pancreas[15] and by centrifuging the digested pancreata in a discontinuous gradient of Ficoll.[16] Islets were then picked manually with glass micropipettes from the upper layers of the Ficoll gradient. Only islets that were undamaged and completely free of acinar and connective tissue were selected. Islets were washed twice in 0.23 M mannitol, 0.07 M sucrose, 5 mM potassium-Hepes buffer, pH 7.5, and 1 mM dithiothreitol and then homogenized in the same solution. Islet cytosol was the supernatant fraction after centrifuging a homogenate of 1500 islets in 200 μl of the mannitol-sucrose solution at 105,000 × *g* for 1 h.[17]

Liver cytosol was prepared as previously described[14] by centrifuging a 1:3 (wt/vol) homogenate of liver in the mannitol-sucrose solution at 105,000 × *g* for 1 h.

Mitochondria were isolated from the islets as previously described[17] by centrifuging the original homogenate at 600 × *g* for 10 min and then centrifuging the resulting supernatant fraction at 5500 × *g* for 10 min. The pellet from the higher speed centrifugation contained the mitochondria and was washed twice with the mannitol-sucrose solution by centrifuging at 5500 × *g* for 10 min.

Phosphoenolpyruvate carboxykinase activity. Phosphoenolpyruvate carboxykinase was assayed in the direction of phosphoenolpyruvate formation essentially as previously described[13,14,18–24] except that assay conditions were modified to permit detection of low amounts of enzyme activity. Before assaying for enzyme activity, a tissue extract was incubated for 10 min at ice temperature without or with 30 μM $FeCl_2$ or 100 μM $MnCl_2$ in 100 μl of 1 mM dithiothreitol and 5 mM potassium-Hepes buffer, pH 7.5. Ninety-five microliters of this mixture was added to the enzyme reaction mixture to give final concentrations of 1.5 mM oxalacetate, 2 mM ITP, 3 mM $MgCl_2$, and 50 mM potassium-Hepes buffer, pH 7.5, in 0.8 ml. After incubating 10 min at 30°C, the reaction was stopped by the addition of 10 mg of KBH_4. After 2 min, the mixture was acidified with 0.4 ml of 6% $HClO_4$ and then neutralized with about 35 μl 30% KOH. The phosphoenolpyruvate formed by the reaction was measured by a standard enzymic assay.[25]

Phosphoenolpyruvate carboxykinase activity was also measured in the direction of oxalacetate formation by a continuous spectrophotometric assay at 340 nm and at 30°C in 0.6 ml of reaction mixture containing 50 mM potassium-Hepes, pH 7.5, 1 mM $MnCl_2$, 1 mM IDP, 2 mM phosphoenolpyruvate, 50 mM $NaHCO_3$, 0.15 mM NADH, and 7 U of malic dehydrogenase.[19,24]

Ferroactivator assay. The ability of islet and liver cytosol to permit Fe^{2+} to activate purified phosphoenolpyruvate carboxykinase was measured as previously described.[13,14,19,27] Briefly, before assaying for enzyme activity in the direction of phosphoenolpyruvate formation, aliquots of serially diluted tissue extracts were incubated with 2 μg of purified phosphoenolpyruvate carboxykinase (sp act 14 U/mg of protein when assayed at 25°C) for 10 min at ice temperature in a final volume of 100 μl of 1 mM dithiothreitol, 5 mM potassium-Hepes, pH 7.5, and 30 μM $FeCl_2$. These preliminary incubations were started by adding $FeCl_2$ and terminated by adding the incubation mixture to the enzyme reaction mixture. The assayable activity in blanks that contained cytosol and Fe^{2+} but not purified phosphoenolpyruvate carboxykinase was subtracted from total enzyme activities to give the rates attributable to the purified enzyme. A plot of the activities of purified phosphoenolpyruvate carboxykinase versus the log of the cytosol protein concentrations is linear.[13,14,19,27] The activity of purified phosphoenolpyruvate carboxykinase when incubated without $FeCl_2$ is defined as 100% enzyme activity, as in Figure 1.

Pyruvate kinase activity. Pyruvate kinase activity was measured at 37°C in a final volume of 1 ml reaction mixture containing 80 mM triethanolamine chloride buffer, pH 7.5, 7 mM phosphoenolpyruvate, 5 mM $MgSO_4$, 100 mM KCl, 2.5 mM ADP, 0.14 mM NADH, 1 mM dithiothreitol, and 2.5 U of lactate dehydrogenase. NADH oxidation was monitored spectrophotometrically at 340 mm.

Enzyme rates. Enzyme rates are expressed on the basis of cytosol protein rather than tissue weight because pancreatic islets cannot be conveniently and accurately weighed.

Protein. Protein was measured by the method of Lowry et al.[28] after precipitating protein with 10% trichloroacetic acid and washing each sample once with the acid.

Materials. Auxiliary enzymes were from Boehringer Mannheim, Indianapolis, Indiana. Na_3ITP, Na_2IDP, Na_2ADP, NADH, and dithiothreitol were from P-L Biochemicals, Inc., Milwaukee, Wisconsin, and potassium phosphoenolpyruvate and Hepes were from the Sigma Chemical Co., St. Louis, Missouri. Phosphoenolpyruvate carboxykinase was purified from rat liver cytosol according to the procedure of Philippidis et al.[29] with further purification by agarose-GTP affinity chromatography.[30]

RESULTS

Table 1 lists the conditions used in attempts to increase phosphoenolpyruvate carboxykinase activity in pancreatic islets. Assay conditions, such as incubation time, temperature, and volumes, were modified slightly to permit the detection of small amounts of enzyme activity. These assay procedures had previously been used with success in measuring low amounts of carboxykinase activity, such as in adipose tissue.[24] For the assays listed in Table 1, more islet cytosol protein than liver cytosol protein was used. The assays were designed so that islet carboxykinase activity as low as 0.005 that of the liver enzyme should have been discernible from the activity in control cuvettes to which no enzyme was added (i.e., zero or background activity). Incubating cytosol from liver, kidney, or adipose tissues with 30 μM Fe^{2+} or 100 μM Mn^{2+} before assaying for activity increases the maximal activity of the carboxykinase 3–4-fold when assayed in the direction of phosphoenolpyruvate formation.[13,14,19,24,27] Starving rats for 24–48 h increases the enzyme activity in liver, kidney, and adipose tissue 2–5-fold.[14,31–33] None of these manipulations of conditions separately or in combination increased islet phosphoenolpyruvate

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/3/246/353713/34-3-246.pdf by guest on 23 July 2024

TABLE 1
Assay conditions in which no pancreatic islet phosphoenolpyruvate carboxykinase activity was detected

Assay conditions
Assayed in the direction of phosphoenolpyruvate formation
Cytosol from islets of fed rats
No additions (24)
+ Fe^{2+} (20)
+ Mn^{2+} (20)
+ 3-mercaptopicolinic acid (5)*
+ Fe^{2+} + 3-mercaptopicolinic acid (15)*
Cytosol from islets of rats starved 48 h
No additions (8)
+ Fe^{2+} (8)
Mitochondria from islets of fed rats
No additions (6)
+ Fe^{2+} (6)†
+ Mn^{2+} (6)†
Assayed in the direction of oxalacetate formation
Cytosol from islets of fed rats (4)

Up to 112 μg of islet cytosol protein and 55 μg of islet mitochondria protein per assay were used. Five to eighty micrograms of liver cytosol protein from fed rats gave enzyme rates between 15 and 27 nmol product formed/min/mg of protein when assayed in the absence of effectors. These rates for liver are in the range previously reported.[14] Enzyme rates as low as 0.075 nmol product formed/min/mg islet or liver cytosol protein should have been detectable. Starving an animal for 48 h increases the enzyme activity 3–5-fold in nonislet tissues[14,31–33] and incubating the cytosol with Fe^{2+} or Mn^{2+} before assaying for enzyme activity increases the activity another 3–4-fold.[13,14,27] The number of observations on islet tissues is shown in parentheses.

*3-Mercaptopicolinic acid was added to the enzyme reaction mixture.

†Fe^{2+} and Mn^{2+} were incubated with the subcellular fraction before assaying for enzyme activity.

carboxykinase activity to detectable levels. Homogenates of islet mitochondria did not contain detectable phosphoenolpyruvate carboxykinase activity. When the enzyme activity was measured in the direction of oxalacetate formation no activity was detected.

Since the enzyme in homogenates of adipose tissue is inactivated within 24 h in the absence of a thiol-reducing agent[24] and the enzyme from liver when purified is susceptible to inactivation by oxidation of its thiol groups,[27,30] all homogenates of islets were made in dithiothreitol. This, however, did not permit the detection of phosphoenolpyruvate carboxykinase activity. When islet cytosol was added to purified carboxykinase and the enzyme activity assayed after 1 h, the enzyme was fully active, demonstrating that islet cytosol did not inactivate the enzyme.

Adding 3-mercaptopicolinic acid, a potent inhibitor of phosphoenolpyruvate carboxykinase, to the enzyme reaction mixture did not decrease the activity below that of the control without added islet cytosol; nor did adding 3-mercaptopicolinic acid to islet cytosol that was previously incubated with Fe^{2+} decrease the enzyme activity. In liver from a variety of species[19–23] and in adipose tissue from the rat,[24] phosphoenolpyruvate carboxykinase exposed to Fe^{2+} is made more susceptible to inhibition by 3-mercaptopicolinate.

Ferroactivator, as originally described, is a liver cytosol protein that permits Fe^{2+} to activate purified liver phosphoenolpyruvate carboxykinase.[13,27] Since ferroactivator protein was originally described, a structurally different protein from the erythrocyte[34] and another protein from liver[35] as well as small molecules not yet completely characterized have been found to have ferroactivator activity. Figure 1 shows that there was no detectable ferroactivator activity of any kind in islet cytosol, even though more islet protein than liver cytosol protein was used in the assay for ferroactivator activity. The activation of purified phosphoenolpyruvate carboxykinase in the presence of islet cytosol was no greater than that due to the Fe^{2+} alone (average of 51%). The assay for ferroactivator activity is very sensitive and can detect activity in extremely small amounts of sample from various organs.[24]

Pancreatic islet cytosol contains ample pyruvate kinase activity (0.99 ± 0.11 μmol pyruvate formed/min/mg cytosol protein [N = 4] versus 0.73 ± 0.09 [N = 6] in liver [means ± SD]).

DISCUSSION

The results of the current study show that, if pancreatic islets have phosphoenolpyruvate carboxykinase, its enzyme activity is too low to have any great quantitative influence on carbohydrate metabolism in islets. In numerous experiments, enough islet tissue was used to detect phosphoenolpyruvate carboxykinase activity that was 0.005 as high as that in liver or 1×10^{-5} as high as pyruvate kinase activity in islets. By using the assay methods described above, we have had no difficulty detecting phosphoenolpyruvate carboxykinase activity and demonstrating its activation by metal ions in tissues in which phosphoenolpyruvate carboxykinase activity is low, such as in adipose tissue.[24] Furthermore, no islet phosphoenolpyruvate carboxykinase activity could be detected when the islet cytosol or mitochondria were incubated in the presence of Mn^{2+} or Fe^{2+}, which are potent activators of cytosolic phosphoenolpyruvate carboxykinase in other tissues. Rat tissues are not known to contain mitochondrial phosphoenolpyruvate carboxykinase, but to make sure that islets do not differ in this respect from other tissues, the

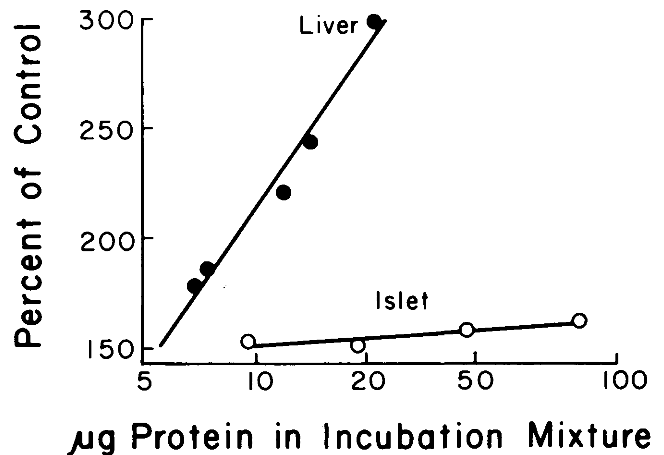

FIGURE 1. Ferroactivator assay: effect of pancreatic islet cytosol and liver cytosol on the activity of purified phosphoenolpyruvate carboxykinase. Various amounts of cytosol were incubated with 30 μM $FeCl_2$ and purified enzyme before assaying for enzyme activity as described for the ferroactivator assay procedure in the text. Results are expressed as a percent of the activity of purified carboxykinase incubated in the routine incubation mixture without $FeCl_2$ and cytosol (100%). The activity of the purified enzyme in the presence of $FeCl_2$ averaged 151% of the control.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/3/246/353713/34-3-246.pdf by guest on 23 July 2024

enzyme activity was measured in islet mitochondrial fractions.

If we had succeeded in detecting a low amount of phosphoenolpyruvate carboxykinase activity in islets, it would still be necessary to explain its function in islets, since pyruvate kinase activity is very plentiful in islets. Any phosphoenolpyruvate synthesized would, more than likely, be rapidly converted to pyruvate by pyruvate kinase. Pyruvate kinase from liver is widely known to undergo cAMP-enhanced phosphorylation with a resulting inhibition of its activity. Although we have tried extensively to prove inhibition of islet pyruvate kinase by either Ca^{2+} or cAMP-dependent phosphorylation, we have been unsuccessful.[1] We[1] and others[36] have shown with both kinetic and immunologic studies that islet pyruvate kinase is the M_2 isoenzyme. The M_2 isoenzyme is not known to undergo inhibition by phosphorylation in mammalian tissues.

Recently, Lardy and co-workers described several proteins that they named ferroactivators because of their ability to permit Fe^{2+} to activate purified phosphoenolpyruvate carboxykinase. Ferroactivator activity is plentiful in tissues containing high amounts of phosphoenolpyruvate carboxykinase, such as liver and kidney, and it is present in several other tissues as well.[13,14] Although ferroactivator is plentiful in the erythrocyte, which contains no phosphoenolpyruvate carboxykinase,[13,14,34] the function of ferroactivator in the erythrocyte is unknown at this time. Very little or no ferroactivator activity in pancreatic islets was detected when up to 15-fold more islet cytosol protein than liver cytosol protein was used in the assay for ferroactivator activity (Figure 1).

An absence of phosphoenolpyruvate carboxykinase activity or an extremely low activity of the enzyme in the beta cell may explain why glyceraldehyde and glucose are potent insulin secretagogues and pyruvate is not. Pyruvate is metabolized to carbon dioxide by islets slightly less efficiently than is glyceraldehyde (metabolism of 30 mM pyruvate by islets yields CO_2 comparable to metabolism of 10 mM glyceraldehyde[1-4]), but not less efficiently enough to explain the complete inability of pyruvate to stimulate insulin release. (Thirty millimolar pyruvate does not stimulate insulin release at all, but 10 mM glyceraldehyde stimulates insulin release almost as well as glucose.) If pyruvate could be converted to phosphoenolpyruvate, several reactions that are available to glyceraldehyde or glucose should also be available to pyruvate. For example, phosphoenolpyruvate decreases the net uptake of calcium by islet mitochondria,[11] which could increase the cytosolic calcium concentration to activate secretory processes. Also, phosphoenolpyruvate, by its conversion to triose phosphate via the reversible reactions of the glycolysis pathway, could participate in the glycerol phosphate shuttle or undergo conversion to phospholipid. The glycerol phosphate shuttle by virtue of the very high activity of the mitochondrial glycerol phosphate dehydrogenase in insulin-secreting tissue[5,6] appears to be important for insulin secretion. There is a great deal of evidence suggesting that phospholipid metabolism plays a major role in regulating insulin secretion.[7]

Hedeskov and Capito[12] have reported "the presence in mouse islets of rather high activities" of phosphoenolpyruvate carboxykinase. However, their data show that the carboxykinase activity was 0.0006 that of pyruvate kinase. In our hands, the assayable activity of phosphoenolpyruvate carboxykinase activity of rat islets was no greater than that of blanks without added cytosol in enzyme assays performed on numerous islet preparations. Species differences may explain the difference between their results and ours. It is possible that they used an enzyme assay for phosphoenolpyruvate carboxykinase that was either more sensitive than the ones we used or one that detected activity that was not due to phosphoenolpyruvate carboxykinase. However, they did not describe the assay procedure they used. Duff and Snell[37] have thoroughly described how some of the assay procedures for phosphoenolpyruvate carboxykinase can provide false-positive evidence for the enzyme in tissues in which its activity is low or nonexistent. We conclude that even if mouse or rat islets contain some phosphoenolpyruvate carboxykinase activity, its activity seems too low to be quantitatively important in phosphoenolpyruvate formation. The high activity of pyruvate kinase should strongly favor phosphoenolpyruvate's conversion to pyruvate.

ACKNOWLEDGMENTS

The authors thank Robert Mertz for technical assistance.

This work was supported by NIH grant AM-28348 and by a grant from the Juvenile Diabetes Foundation International.

REFERENCES

[1] Zawalich, W. S.: Intermediary metabolism and insulin secretion from isolated rat islets of Langerhans. Diabetes 1979; 28:252–60.

[2] Ashcroft, S. J. H., Weerasinghe, L. C. C., and Randle, P. J.: Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. Biochem. J. 1973; 132:223–31.

[3] Hellman, B., Idahl, L.-A., Lernmark, A., Sehlin, H., and Taljedal, I.-B.: The pancreatic β-cell recognition of insulin secretagogues: comparisons of glucose with glyceraldehyde isomers and dihydroxyacetone. Arch. Biochem. Biophys. 1974; 162:448–57.

[4] Sener, A., Kawazu, S., Hutton, J. C., Boschero, A. C., Devis, G., Somers, G., Herchuelz, A., and Malaisse, W. J.: The stimulus-secretion coupling of glucose induced insulin release: effect of exogenous pyruvate on islet function. Biochem. J. 1978; 176:217–32.

[5] MacDonald, M. J.: High content of mitochondrial glycerol-3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. J. Biol. Chem. 1981; 256:8287–90.

[6] MacDonald, M. J., Warner, T. F., and Pellett, J. R.: Increased mitochondrial glycerol phosphate dehydrogenase activity in insulinomas of two hypoglycemic infants. J. Clin. Endocrinol. Metab. 1983; 57:662–64.

[7] Best, L., and Malaisse, W. J.: Phospholipid and islet function. Diabetologia 1983; 25:299–305.

[8] Floyd, C. J., Fajans, S. S., Conn, J. W., Knopf, R. F., and Rull, J.: Stimulation of insulin secretion by amino acids. J. Clin. Invest. 1966; 45:1487–1502.

[9] Lambert, A. E., Kanazawa, Y., Orci, L., and Christensen, H. N.: Stimulation of insulin release by natural amino acids and their non-metabolized analogues. Diabetologia 1970; 6:635–36.

[10] Pagliara, A. S., Stillings, S. N., Hover, B., Martin, D. B., and Matschinsky, F. M.: Glucose modulation of amino acid-induced glucagon and insulin release in the isolated perfused rat pancreas. J. Clin. Invest. 1974; 54:819–32.

[11] Sugden, M. C., and Ashcroft, S. J. H.: Effects of phosphoenolpyruvate, other glycolytic intermediates and methylxanthines on calcium uptake by a mitochondrial fraction from rat pancreatic islets. Diabetologia 1978; 15:173–80.

[12] Hedeskov, C. J., and Capito, K.: Pancreatic islet metabolism of pyruvate and other potentiators of insulin release: effects of starvation. Horm. Metab. Res. 1980; 10 (Suppl.):8–13.

[13] Bentle, L. A., and Lardy, H. A.: P-enolpyruvate carboxykinase ferroactivator: purification and some properties. J. Biol. Chem. 1977; 252:1431–40.

[14] MacDonald, M. J., Bentle, L. A., and Lardy, H. A.: P-enolpyruvate carboxykinase ferroactivator: distribution and the influence of diabetes and starvation. J. Biol. Chem. 1978; 253;116–24.

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

[16] Scharp, D. W., Kemp, C. B., Knight, M. J., Ballinger, W. F., and Lacy, P. E.: The use of Ficoll in the preparation of viable islets of Langerhans from the rat pancreas. Transplantation 1973; 16:686–89.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/3/246/353713/34-3-246.pdf by guest on 23 July 2024

[17] MacDonald, M. J.: Evidence for the malate aspartate shuttle in pancreatic islets. Arch. Biochem. Biophys. 1982; 213:643–49.

[18] Seubert, W., and Huth, W.: On the mechanism of gluconeogenesis and its regulation. II. The mechanism of gluconeogenesis from pyruvate and fumarate. Biochem. Z. 1965; 343:176–91.

[19] MacDonald, M. J., and Lardy, H. A.: 3-Aminopicolinate: a synthetic metal-complexing activator of phosphoenolpyruvate carboxykinase. J. Biol. Chem. 1978; 253:2300–2307.

[20] MacDonald, M. J., and Lardy, H. A.: Structural requirements for pyridine carboxylate effects on phosphoenolpyruvate carboxykinase. Bioorganic Chem. 1978; 7:251–62.

[21] MacDonald, M. J.: Effect of Fe^{2+} and Mn^{2+} on 3-mercaptopicolinate inhibition of cytosolic and mitochondrial phosphoenolpyruvate carboxykinase of five species. Biochim. Biophys. Acta 1978; 526:293–98.

[22] MacDonald, M. J.: Evidence that Fe^{2+} complexes of 3-aminopicolinate and 3-mercaptopicolinate activate and inhibit phosphoenolpyruvate carboxykinase. Biochem. Biophys. Res. Commun. 1979; 90:741–49.

[23] MacDonald, M. J.: Rapid inactivation of rat liver phosphoenolpyruvate carboxykinase by microsomes and reversal by reductants. Biochim. Biophys. Acta 1980; 615:223–36.

[24] MacDonald, M. J., and Grewe, B. K.: Inhibition of phosphoenolpyruvate carboxykinase, glyceroneogenesis and fatty acid synthesis in rat adipose tissue by quinolinate and 3-mercaptopicolinate. Biochim. Biophys. Acta 1981; 663:302–13.

[25] Bergmeyer, H. U., and Gawehn, K.: Methods of Enzymatic Analysis. New York, Academic Press, 1973:1446–51.

[26] Holton, D. D., and Nordlie, R. C.: Comparative studies of catalytic properties of guinea pig liver intra- and extramitochondrial phosphoenolpyruvate carboxykinase. Biochemistry 1965; 4:723–31.

[27] Bentle, L. A., Snoke, R. E., and Lardy, H. A.: A protein factor required for activation of phosphoenolpyruvate carboxykinase by ferrous ions. J. Biol. Chem. 1976; 251:2922–28.

[28] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phoenol reagent. J. Biol. Chem. 1951; 193:265–75.

[29] Philippidis, H., Hanson, R. W., Reshef, L., Hopgood, M. G., and Ballard, F. J.: The initial synthesis of proteins during development: phosphoenolpyruvate carboxykinase in rat liver at birth. Biochem. J. 1972; 126:1127–34.

[30] Colombo, G., Carlson, G. M., and Lardy, H. A.: Phosphoenolpyruvate carboxykinase (guanosine triphosphate) from rat liver cytosol: separation of homogeneous forms of the enzyme with high and low activity by chromatography on agarose-hexane-guanosine triphosphate. Biochemistry 1978; 17:5321–29.

[31] Reshef, L., Hanson, R. W., and Ballard, F. J.: Glyceride-glycerol synthesis from pyruvate: adaptive changes in phosphoenolpyruvate carboxykinase and pyruvate carboxylase in adipose tissue and liver. J. Biol. Chem. 1969; 244:1994–2001.

[32] Shrago, E., Lardy, H. A., Nordlie, R. C., and Foster, D. O.: Metabolic and hormonal control of phosphoenolpyruvate carboxykinase and malic enzyme in rat liver. J. Biol. Chem. 1963; 238:3188–92.

[33] Hopgood, M. F., Ballard, F. J., Reshef, L., and Hanson, R. W.: Synthesis and degradation of phosphoenolpyruvate carboxykinase in rát liver and adipose tissue: changes during a starvation–re-feeding cycle. Biochem. J. 1973; 134:445–53.

[34] Chee, P. P., and Lardy, H. A.: Isolation from erythrocytes of a green hemeprotein with ferroactivator activity. J. Biol. Chem. 1981; 256:3865–70.

[35] Merryfield, M. L., Kramp, D. C., and Lardy, H. A.: Purification and characterization of a rat liver ferroactivator with catalase activity. J. Biol. Chem. 1982; 257:4646–54.

[36] Chatterton, T. A., Reynolds, C. H., Lazarus, N. R., and Pogson, C. I.: Immunological and kinetic properties of pyruvate kinase in rat pancreatic islets. Biochem. J. 1982; 204:605–608.

[37] Duff, D. A., and Snell, K.: Limitations of commonly used spectrophotometric assay methods for phosphoenolpyruvate carboxykinase activity in crude extract of muscle. Biochem. J. 1982; 206:147–52.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/3/246/353713/34-3-246.pdf by guest on 23 July 2024