Starvation and Diabetes Reduce the Amount of Pyruvate Dehydrogenase Phosphatase in Rat Heart and Kidney

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The pyruvate dehydrogenase complex (PDC) is inactivated in many tissues during starvation and diabetes to conserve three-carbon compounds for gluconeogenesis. This is achieved by an increase in the extent of PDC phosphorylation caused in part by increased pyruvate dehydrogenase kinase (PDK) activity due to increased PDK expression. This study examined whether altered pyruvate dehydrogenase phosphatase (PDP) expression also contributes to changes in the phosphorylation state of PDC during starvation and diabetes. Of the two PDP isoforms expressed in mammalian tissues, the Ca2+-sensitive isoform (PDP1) is highly expressed in rat heart, brain, and testis and is detectable but less abundant in rat muscle, lung, kidney, liver, and spleen. The Ca2+-insensitive isoform (PDP2) is abundant in rat kidney, liver, heart, and brain and is detectable in spleen and lung. Starvation and streptozotocin-induced diabetes cause decreases in PDP2 mRNA abundance, PDP2 protein amount, and PDP activity in rat heart and kidney. Refeeding and insulin treatment effectively reversed these effects of starvation and diabetes, respectively. These findings indicate that opposite changes in expression of specific PDK and PDP isoenzymes contribute to hyperphosphorylation and therefore inactivation of the PDC in heart and kidney during starvation and diabetes. Diabetes 52:1371–1376, 2003

RESEARCH DESIGN AND METHODS

Materials. Male Wistar rats were from Harlan Industries (Indianapolis, IN). Protamine-zinc insulin for the treatment of diabetic rats was from Anthony Products (Arcadia, CA). Ultraspec RNA isolation system was from Biotex Laboratories (Houston, TX). [γ-32P]ATP, [α-32P]dCTP, and 125I-Protein A were obtained from NEN Life Science Products (Boston, MA). High prime DNA labeling kit was from Roche Applied Science (Indianapolis, IN). QuickHyb hybridization solution and sonicated salmon sperm DNA were from Stratagene (La Jolla, CA). Rat multiple tissue Northern blot and Chroma spin hybridization solution and sonicated salmon sperm DNA were from Stratagene (La Jolla, CA). Rat multiple tissue Northern blot and Chroma spin labeling kit was from Roche Applied Science (Indianapolis, IN). Other chemicals were from Sigma (St. Louis, MO). The Biochemistry Biotechnology Facility (Indianapolis, IN) synthesized and purified the phosphorylated peptide used in the phosphatase assay.

Animals. Male Wistar rats weighing ~200 g were maintained in a temperature- and light-controlled animal room and fed with rodent laboratory chow ad libitum. Animals were from Harlan Industries (Indianapolis, IN) and received food and water ad libitum. Animals were housed under temperature- and light-controlled conditions and fed with rodent laboratory chow ad libitum.

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E1α, a subunit of pyruvate dehydrogenase component of pyruvate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphatase.
Ser(P)-Met-Ser-Asp-Pro-Gly-Val-Ser(P)-Tyr-Arg-NH2] was synthesized according to the PDP assay. Version 3.02, from SPSS (Chicago, IL) was used for data analysis. SCAN-IT Software, Version 4.1, from Silk Scientific obtained in Western and Northern blot analysis were quantified with UNSCAN-IT Software, Version 4.1, from SILK Scientific (Orem, UT). SigmaPlot, Version 3.02, from SPSS (Chicago, IL) was used for data analysis.

RESULTS

Tissue distribution of PDP1 and PDP2 messages in the rat. By Northern blot analysis, PDP1 mRNA was present in all tissues examined but was expressed most abundantly in heart, brain, and testis (Fig. 1). PDP1 was also the predominant form in skeletal muscle, even though the basal level of PDP1 expression was low in this tissue relative to the levels present in heart, brain, and testis. In contrast, mRNA for PDP2 was very abundant in heart, brain, liver, and kidney (Fig. 1). PDP2 message was also present in rat spleen and lung but was below the level of detection in rat skeletal muscle and testis. These findings provide evidence for different patterns of PDP1 and PDP2 expression in tissues, which may allow for tissue-specific regulation of PDC activity in different physiological states. Interestingly, two bands corresponding to PDP2 mRNA were detected in all tissues examined. Two bands for PDP1 were likewise detected in brain and to a much lesser extent in heart. Alternative splicing may be responsible for this heterogeneity but has not been investigated.

Effect of starvation on relative abundance of PDP1 and PDP2 protein and mRNA in rat heart and kidney. Starvation for 48 h induced significant decreases in the amounts of PDP2 protein expressed in the heart (1.00 ± 0.09 in well-fed rats vs. 0.25 ± 0.03 in starved rats, n = 4; P < 0.01) and the kidney (1.00 ± 0.04 in well-fed rats vs. 0.45 ± 0.09 in starved rats, n = 4; P < 0.01) (Figs. 2A and 3A). Corresponding decreases in PDP2 message abundance occurred in the heart (1.00 ± 0.07 in well-fed rats vs. 0.41 ± 0.01 in starved rats, n = 4; P < 0.01) and the kidney (1.00 ± 0.09 in well-fed rats vs. 0.57 ± 0.07 in starved rats, n = 4; P < 0.01) (Fig. 2B and 3B). Refeeding of starved rats for 48 h resulted in nearly complete recoveries of PDP2 protein and message in both tissues (Figs. 2 and 3).

Although PDP1 is expressed in relatively large amounts in the heart, starvation for 48 h had no effect on the protein and message levels of this phosphatase in rat heart (Fig. 2). In the kidney, where expression of PDP1 is very low relative to PDP2, starvation induced a further decrease in PDP1 protein (1.00 ± 0.05 in well-fed rats vs. 0.58 ± 0.13 in starved rats, n = 4; P < 0.01; Fig. 3A) but had little if any effect on PDP1 message (Fig. 3B). Refeeding partially reversed the effect of starvation on the level of PDP1 protein (Figs. 3A).

Starvation of rats for 48 h was required to observe the effects shown in Figs. 2 and 3 on PDP2 message and protein in kidney and heart. Starvation for 48 h did not induce significant changes in the PDP2 expression in these tissues.

Effect of streptozotocin-induced diabetes on PDP2 protein amount and mRNA abundance in rat heart and kidney. Induction of diabetes by streptozotocin was
confirmed by the measurement of blood glucose levels (390 ± 25 mg/dl at 48 h after administration of the drug vs. 95 ± 8 mg/dl in controls). Treatment of the diabetic rats with long-lasting insulin at 12-h intervals for 48 h was effective in lowering blood glucose levels to the normal control range (<100 mg/dl). Diabetes induced by streptozotocin caused substantial decreases in the levels of PDP2 protein in heart (1.00 ± 0.03 in control rats vs. 0.33 ± 0.04 in diabetic rats, n = 6; P < 0.01) and kidney (1.00 ± 0.05 in control rats vs. 0.63 ± 0.07 in diabetic rats, n = 6; P < 0.01) (Fig. 4A).

Corresponding decreases in PDP2 mRNA levels occurred in the heart (1.00 ± 0.07 in control rats vs. 0.37 ± 0.04 in diabetic rats, n = 6; P < 0.01) and the kidney (1.00 ± 0.07 in the control rats vs. 0.66 ± 0.04 in the diabetic rats, n = 6; P < 0.01) (Fig. 4B). These effects were completely reversed by insulin treatment (Fig. 4A and B, respectively). Levels of PDP1 protein in these tissues remained relatively constant under these experimental conditions (data not shown).

**Effect of starvation and diabetes on PDP activity in mitochondria isolated from rat heart and kidney.** Starvation induced modest but significant decreases in PDP activity in both heart (18%) and kidney (32%) (Table 1).

Refeeding the animals induced complete restoration of PDP activity in both tissues (Table 1). Diabetes caused 26 and 28% reductions in PDP activity in heart and kidney,
reserves body protein (16,17). Inhibition of pyruvate oxidation is achieved by a combination of regulatory mechanisms that virtually eliminate nonneuronal cell pyruvate dehydrogenase activity during starvation. Most important among these is covalent modification of the complex by phosphorylation. The balance between the relative activities of the PDKs and pPDPs determines the extent to which the complex is inhibited by phosphorylation. It is well established that increased PDK expression in starvation shifts the balance toward greater phosphorylation of the complex. Whether stable changes in PDP activity also occur is much less certain. Some of the increase in PDK activity induced by starvation is due to elevated concentrations of free fatty acids and ketone bodies. Catabolism of these compounds increases the levels of NADH and acetyl-CoA (18,19), which in turn increase PDK activity by reduction and acetylation of the lipoyl residues of the E2 component of the complex (20,21). Complementing this mechanism is a marked increase in PDK4 expression in many tissues (1–5), along with some increase in PDK2 expression in a few tissues (3,5). Changes in blood levels of glucocorticoids, insulin, and free fatty acids signal increased PDK4 expression in starvation (22).

Although a role for greater PDK activity in promoting PDC phosphorylation during starvation is well established and appropriate in this physiological state (1–5), it is obvious that a decrease in PDP activity could induce the same effect. Because Ca$^{2+}$ stimulates PDP1 activity (9), factors that lower mitochondrial calcium levels decrease phosphatase activity. Although higher Ca$^{2+}$ levels are likely involved in activation of the PDC in heart by epinephrine and increased work load (rev. in 10), evidence is lacking that would implicate changes in Ca$^{2+}$ concentration in regulation of PDP activity during starvation. On the other hand, rapid activation of the PDC by insulin, purportedly by activation of PDP, has been reported in many studies (rev. in 23). Because insulin levels are decreased in starvation, it follows that PDP activity could be decreased for want of insulin. Indeed, recent evidence suggests a role for stimulation of protein kinase C6 in insulin-mediated PDP activation (14,24). However, the activating effect of insulin on the PDC lasts only a few minutes (14,24), making it uncertain whether the decrease in insulin during starvation would have a lasting effect upon the activity state of the complex by this mechanism.

**DISCUSSION**

Survival during starvation is dependent upon mechanisms that limit oxidative loss of pyruvate in nonneuronal tissues of the body. Pyruvate and other three-carbon compounds that can be converted to glucose must be conserved during starvation. Efficient recycling of these compounds is particularly important in limiting utilization of glucogenic amino acids that otherwise would have to be used to maintain glucose levels during starvation. Limiting amino acid turnover helps keep proteolysis in check and con-
The possibility that stable changes in PDP activity could be induced has been examined in the past with mixed results. The recent finding that two isoforms of this enzyme are expressed in a tissue-specific manner, one sensitive and one insensitive to Ca\(^{2+}\) stimulation (8), revealed an unexpected level of complexity that might help to explain why changes in PDP activity have been seen in response to starvation in some studies but not in others. The present study provides additional information about tissue specificity of PDP isoform expression. The liver and the kidney express PDP2 and almost no PDP1, the testes and skeletal muscle express PDP1 but perhaps no PDP2, and the heart and the brain express both PDP1 and PDP2. Assuming that protein levels of the PDPs correspond to message levels, Ca\(^{2+}\) activation of PDP1 activity is likely important in the heart, skeletal muscle, brain, and testes, whereas Ca\(^{2+}\)-independent mechanisms for regulation of PDP2 activity are likely present in the liver and kidney and perhaps the heart and brain. Failures to detect effects of different metabolic states on phosphatase activity in heart and adipose tissue have been reported (19,25,26). In contrast, clear evidence for decreased PDP activity in heart and lactating mammary gland in response to starvation has been reported (6,7,27). The present study confirms that a decrease in kidney PDP activity is induced by starvation and also provides evidence that this can be due, at least in part, to decreased expression of the PDP2 protein. A modest decrease in PDP activity in response to starvation was also found with rat heart, consistent with our finding of a significant decrease in the level of PDP2 expression in this tissue.

Diabetes caused by chemical destruction of pancreatic \(\beta\)-cells is also known to cause hyperphosphorylation and therefore inactivation of the pyruvate dehydrogenase complex in many tissues of the rat (rev. in 28). As in starvation, increased PDK activity (29,30) occurs that can be explained at least in part by increased PDK4 expression (1) as a consequence of increased levels of free fatty acids and glucocorticoids and decreased levels of insulin (22). As would be predicted from the findings with starvation, diabetes also resulted in decreased PDP activity and decreased PDP2 expression in kidney and heart. From these findings it appears likely that concurrent upregulation of PDK expression and downregulation of PDP expression contribute to hyperphosphorylation of PDC in diabetes. This, of course, conserves three-carbon compounds for hepatic gluconeogenesis and contributes to the hyperglycemia characteristic of diabetes.

Streptozotocin is a cytotoxic molecule that induces diabetes by destroying pancreatic \(\beta\)-cells. Although the action of streptozotocin is quite selective for \(\beta\)-cells, cytotoxic effects do occur in other tissues (31), raising the possibility that streptozotocin-induced decrease in PDP2 expression may be due to a direct toxic effect rather than the diabetic state. However, starvation, which like diabetes lowers insulin levels, caused the same effects on PDP2 expression as streptozotocin, and the effects of starvation were reversed completely by refeeding, which increases insulin levels. Moreover, treatment of rats with streptozotocin with the same conditions used in this study causes the opposite change in PDK4 expression, i.e., causes a marked increased in its level (1,2). And most important, treating streptozotocin-diabetic rats with insulin completely restored normal levels of PDP2 (this study) and PDK4 (1,2). These findings strongly suggest that the changes in the expression of the PDC regulatory enzymes are a result of the diabetic state rather than a general toxic effect of streptozotocin.

In general, changes in PDP activity followed changes in PDP2 mRNA and protein. However, the overall decrease of total PDP activity in rat heart in response to starvation and diabetes was smaller than the decrease of PDP2 protein and message. This may reflect background phosphatase activity caused by the presence of other phosphoprotein phosphatases in the mitochondrial extracts. Although a cocktail of phosphatase inhibitors was included in the phosphatase assay to minimize the contribution of alkaline phosphatase and other serine/threonine phosphatase, it may not be possible to suppress the activities of the contaminating phosphatase without inhibiting PDP2 activity. Other members of the protein phosphatase 2C family may also provide background activities to PDP, particularly since they are capable of dephosphorylating PDC (rev. in 32). PDP1 is very abundant in rat heart, but without change under starvation and diabetes it may also contribute to the background activity. The observed decrease in PDP activity in rat kidney was closer to the observed decrease in PDP2 protein, consistent with the observation that PDP2 is the dominant PDP isoenzyme expressed in this tissue.

The finding that insulin treatment efficiently reverses the effect of diabetes on PDP2 protein and message suggests that insulin may be involved in long-term regulation of its expression. Regulation at the level of either transcription or message stability probably accounts for the reasonably good agreement between changes in message and protein levels.

No attempt was made to examine PDP levels and activity in the different cell types and mitochondrial subpopulations that exist in heart and kidney. This is of interest and will be pursued in future studies. Significant differences are known to exist in regulation of PDK expression in different muscle fiber types (5,33).

In conclusion, this study demonstrates that starvation and diabetes decrease PDP2 expression in rat kidney, a tissue in which PDP2 is the dominantly expressed phosphatase, and rat heart, a tissue that also expresses large amounts of PDP1. These findings suggest that opposite changes in PDP2 and PDK4 expression contribute to the hyperphosphorylation and inhibition of PDC activity in these tissues by starvation and diabetes. Whether regulation of PDP expression is an important mechanism in other tissues remains to be established.

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