

Regulation of Pyruvate Dehydrogenase Kinase Expression by Peroxisome Proliferator-Activated Receptor- α Ligands, Glucocorticoids, and Insulin

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Pyruvate dehydrogenase kinase (PDK) catalyzes phosphorylation and inactivation of the pyruvate dehydrogenase complex (PDC). Two isoforms of this mitochondrial kinase (PDK2 and PDK4) are induced in a tissue-specific manner in response to starvation and diabetes. Inactivation of PDC by increased PDK activity promotes gluconeogenesis by conserving three-carbon substrates. This helps maintain glucose levels during starvation, but is detrimental in diabetes. Factors that regulate PDK2 and PDK4 expression were examined in Morris hepatoma 7800 C1 cells. The peroxisome proliferator-activated receptor- α (PPAR- α) agonist WY-14,643 and the glucocorticoid dexamethasone increased PDK4 mRNA levels. Neither compound affected the half-life of the PDK4 message, suggesting that both increase gene transcription. Fatty acids caused an increase in the PDK4 message comparable to that induced by WY-14,643. Insulin prevented and reversed the stimulatory effects of dexamethasone on PDK4 gene expression, but was less effective against the stimulatory effects of WY-14,643 and fatty acids. Insulin also decreased the abundance of the PDK2 message. The findings suggest that decreased levels of insulin and increased levels of fatty acids and glucocorticoids promote PDK4 gene expression in starvation and diabetes. The decreased level of insulin is likely responsible for the increase in PDK2 mRNA level in starvation and diabetes. *Diabetes* 51:276–283, 2002

The mitochondrial pyruvate dehydrogenase complex (PDC) catalyzes irreversible decarboxylation of pyruvate to acetyl-CoA. In the well-fed state, PDC is relatively active in generating acetyl-CoA for fatty acid synthesis in liver and adipose tissue and ATP production in most other tissues. In the starved state, PDC has to be inactivated to conserve three-carbon compounds for gluconeogenesis. The rela-

tive activity of PDC therefore serves as an important metabolic switch for fuel selection by many tissues of the body (1). The reversible phosphorylation of PDC by specific kinases and phosphatases is primarily responsible for regulation of PDC activity. Phosphorylation of the pyruvate dehydrogenase component of PDC (E1 α) by pyruvate dehydrogenase kinase (PDK) inhibits the activity of the complex. Inactivation is catalyzed by four PDK isozymes that are expressed in varying amounts in different tissues (2). Reactivation is achieved by two phosphatase isozymes that are also expressed in a tissue-dependent manner (3).

Starvation and diabetes enhance phosphorylation of PDC and therefore lower its activity in most tissues (4–8). This decrease in activity is attributable at least in part to increased PDK activity (4,5,7,8), which in turn is attributable to increased PDK4 expression in heart (9), skeletal muscle (10,11), liver, kidney, and lactating mammary gland (12). Starvation also increases the amount of PDK2 in liver and kidney (8,12,13). Insulin treatment and refeeding reverse the effect of diabetes and starvation, respectively, on PDK4 expression in skeletal muscle (10), heart (9), and liver (P.W., R.A.H., unpublished observations). Treatment of rats with WY-14,643 increases PDK4 expression in skeletal muscle (10). Given that WY-14,643 is a peroxisome proliferator-activated receptor- α (PPAR- α) activator (14,15), we have proposed that activation of this nuclear hormone receptor promotes PDK4 expression (10). Because fatty acids function as endogenous PPAR- α agonists (16,17), increased levels of free fatty acids (FFAs) may promote PDK4 expression during starvation and diabetes.

The present study was initiated to learn more about the physiologically important factors that regulate the expression of PDK2 and PDK4. The slow-growing, well-differentiated rat Morris hepatoma 7800 C1 cell line was chosen as the model system. These cells have been used previously to study regulation of peroxisomal enzyme expression by dexamethasone, insulin, and fatty acids (18,19). In rats bearing the solid tumor from which these cells were originally derived (Morris hepatoma 7800), starvation inactivates PDC in the tumor to the same degree as in the host liver (20), suggesting that the regulatory mechanisms for the activity state of PDC are retained in these cells. Using this model system, in this study we present evidence that 1) WY-14,643 and dexamethasone increase PDK4 mRNA and protein; 2) FFAs also increase PDK4 expression; 3) insulin effectively blocks and reverses the effects of dexamethasone, but is less effective in opposing WY-

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E1 α , a subunit of pyruvate dehydrogenase component of PDC; EC₅₀, half-maximum effect; FFA, free fatty acid; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PPAR- α , peroxisome proliferator-activated receptor- α .

14,643 and FFAs; 4) the half-life of the PDK4 message is relatively short (~ 1.5 h); and 5) insulin also downregulates the PDK2 message.

RESEARCH DESIGN AND METHODS

Materials. Dr. Jon Bremer (University of Oslo, Norway) kindly provided the Morris hepatoma 7800 C1 cells. Cell culture reagents were obtained from Life Technologies (Grand Island, NY); WY-14,643, from Biomol Research Laboratories (Plymouth Meeting, PA); human recombinant insulin, from Calbiochem-Novabiochem (La Jolla, CA); radioactive nucleotides, from NEN Life Science Products (Boston, MA); and the randomly primed DNA labeling kit, from New England BioLabs (Beverly, MA). The albumin-bound fatty acid solutions were prepared according to a previously published procedure (21) and standardized with an assay kit from Roche Molecular Biochemicals (Indianapolis, IN).

Rat studies. Male Wistar rats (~ 200 g) were obtained from Harlan Industries (Indianapolis, IN). One group was fed Purina chow fortified with 0.1% WY-14,643 for 3 days. Another group was injected intraperitoneally with dexamethasone (5 mg/kg body wt) 6 h before being killed. Procedures for tissue processing have been previously described (12).

Cell culture. Cells were grown in Ham's F-10 nutrient mixture supplemented with 10% (vol/vol) horse serum, 3% (vol/vol) heat-inactivated calf serum, penicillin (50 units/ml), and streptomycin (50 μ g/ml) on 60-mm dishes at 37°C in 5% CO₂ and 95% air. The medium was changed every other day until cells grew to the end of log phase. Cells were rinsed with Dulbecco's PBS and maintained for 12 h in medium with 10% (vol/vol) charcoal-stripped bovine calf serum. Hormones in aqueous solutions, chemical compounds in DMSO, or fatty acids in defatted serum albumin were added at the concentrations indicated.

Northern blot analysis. Total RNA was extracted with an Ultraspec RNA isolation system (Biotecx Laboratories, Houston, TX). Northern blotting was performed as previously described (12). PDK2 and PDK4 mRNA were detected with corresponding ³²P-labeled cDNA probes for PDK2 and PDK4 (12). A ³²P-labeled 28S rRNA antisense oligonucleotide probe (22) was used as a control for loading and transfer.

Western blot analysis. Cells were rinsed twice with ice-cold PBS and lysed with extraction buffer containing 50 mmol/l HEPES (pH 7.5), 100 mmol/l NaF, 3 mmol/l EDTA, 2% BSA, and 1% Triton X-100. Extracts were centrifuged at 12,000 rpm for 10 min, and 4 μ g of total soluble protein were used for Western blot analysis with antisera against recombinant rat PDK4 (12).

Image quantification and data analysis. Relative band densities were determined with UN-SCAN-IT Software, Version 4.1, from Silk Scientific (Orem, UT). SigmaPlot, Version 3.02, from SPSS (Chicago, IL) was used for data analysis.

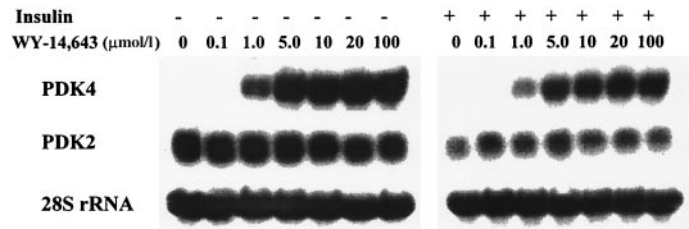
RESULTS

WY-14,643 increased PDK4 mRNA and protein in 7800 C1 cells and the intact liver. Incubation of 7800 C1 cells with WY-14,643 caused a dosage-dependent increase in the abundance of the PDK4 message (Fig. 1A and B). The concentration required for half-maximum effect (EC₅₀) was ~ 1 μ mol/l, which is comparable to that producing half-maximal binding of WY-14,643 (0.6 μ mol/l) to mouse PPAR- α in CV-1 cells (16). WY-14,643 concentrations of 1 and 100 μ mol/l caused greater than 7- and 15-fold increases in the PDK4 message, respectively. A sevenfold increase in PDK4 mRNA accumulation occurred in response to WY-14,643 (10 μ mol/l) after only 1 h (14 ± 3 [treated] vs. 2.0 ± 0.8 [not treated]; $P < 0.05$; three independent observations) (Fig. 1C). A plateau level of 10-fold induction occurred after only 4 h. At 200 μ mol/l, clofibrate, another well-established PPAR- α ligand, had an effect on PDK4 expression that was comparable in magnitude to that produced by 10 μ mol/l WY-14,643 (data not shown).

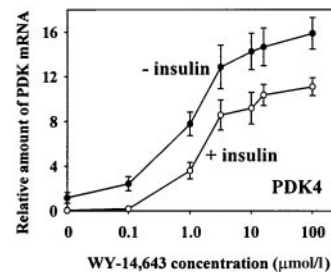
Incubation of 7800 C1 cells with WY-14,643 also produced a substantial increase in PDK4 protein (Fig. 2). A fivefold increase in PDK4 protein occurred after a 16-h incubation with this PPAR- α agonist.

Rats were also fed for 3 days on a diet supplemented

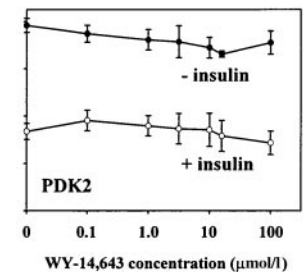
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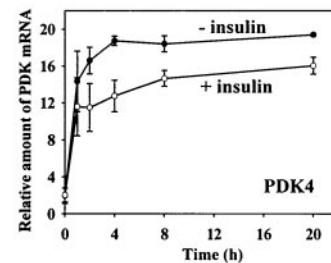
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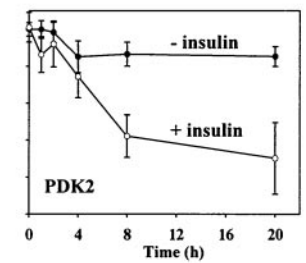


FIG. 1. Effect of WY-14,643 on the abundance of PDK4 and PDK2 mRNAs in 7800 C1 cells. **A:** Representative autoradiograph of Northern blot of PDK4 and PDK2 messages in total RNA (10 μ g) prepared from cells cultured for 20 h with indicated concentrations of WY-14,643 with and without 100 nmol/l insulin. **B:** Quantitative changes in abundance of PDK4 mRNA in cells cultured as described in **A**. **C:** Time course of the effect of 10 μ mol/l WY-14,643 with and without 100 nmol/l insulin on the abundance of PDK4 mRNA. **D:** Quantitative changes in abundance of PDK2 mRNA in cells cultured as described in **A**. **E:** Time course of the effect of 10 μ mol/l WY-14,643 with and without 100 nmol/l insulin on the abundance of PDK2 mRNA. Data points are means \pm SE of three independent experiments.

with WY-14,643 to determine whether this compound has the same effect on PDK4 expression in the intact liver. As was found with 7800 C1 cells, WY-14,643 induced large increases relative to controls in the abundance of PDK4 message (7.4 ± 0.6 vs. 1.0 ± 0.1 , respectively; $P < 0.01$) (Fig. 3A) and the amount of PDK4 protein (4.2 ± 0.9 vs. 1.0 ± 0.2 , respectively; $P < 0.05$) (Fig. 3B) in the intact liver.

WY-14,643 does not affect expression of PDK1, 2, or 3 in 7800 C1 cells. mRNAs for PDK1 and PDK3 are almost undetectable in 7800 C1 cells, and their abundance was not affected by incubation with WY-14,643 (data not shown). Expression of the PDK2 message was high in 7800 C1 cells relative to the other PDKs (Fig. 1A), making it the dominant PDK expressed in the basal condition. In contrast to its effects on PDK4, WY-14,643 had no effect on PDK2 gene expression in 7800 C1 cells, regardless of the dosage (Fig. 1A and D) or time (Fig. 1E).

Dexamethasone increased PDK4 mRNA and protein in 7800 C1 cells. Incubation of 7800 C1 cells with

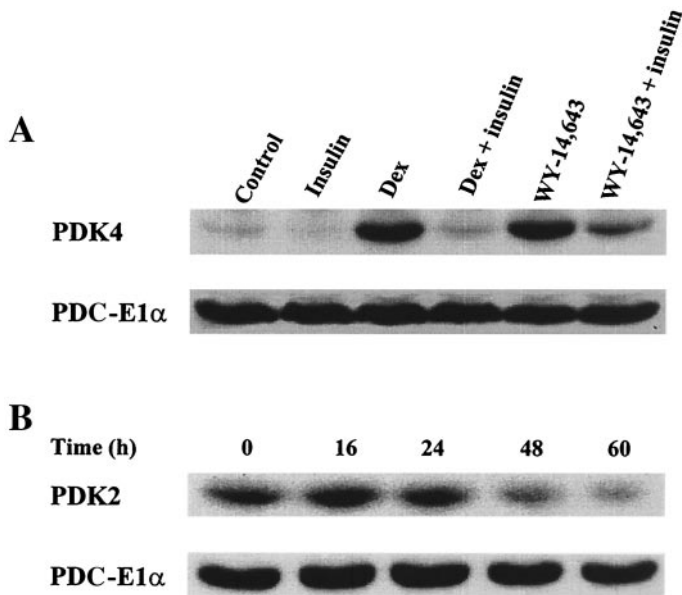


FIG. 2. Effect of WY-14,643, dexamethasone (Dex), and insulin on PDK4 and PDK2 protein in 7800 C1 cells. Cell lysates prepared from cells treated with WY-14,643 (100 $\mu\text{mol/l}$), dexamethasone (1 $\mu\text{mol/l}$), or insulin (100 nmol/l) were subjected to Western blot analysis. **A:** Autoradiograph of PDK4 protein after 16 h of treatment. **B:** autoradiograph of PDK2 protein after indicated hours of exposure of cells to insulin. Medium was changed every 24 h. Lanes correspond to 40 μg of total extract protein. Antisera against PDK4, PDK2, and the PDC-E1 α subunit, respectively. None of the treatments produced a significant change in the amount of PDC-E1 α . Similar results were obtained in three independent experiments.

dexamethasone also caused a large, dosage-dependent increase in PDK4 mRNA abundance (Fig. 4A and B), with 7- and 11-fold increases in the PDK4 message occurring at concentrations of 10^{-8} and 10^{-6} mol/l, respectively. The EC_{50} value was $\sim 10^{-8}$ mol/l, which is comparable to the k_d of 7×10^{-9} mol/l for the glucocorticoid receptor (23). The antagonist mifepristone (10^{-6} mol/l) (24) completely blocked the stimulatory effect of dexamethasone (10^{-6} mol/l) (data not shown). Corticosterone, the dominant rat glucocorticoid, produced effects at 10^{-6} mol/l that were comparable to those of dexamethasone at the same concentration (data not shown). Significant accumulation of PDK4 mRNA required only 1 h of incubation with 10^{-6} mol/l dexamethasone (9.3 ± 1.8 [treated] vs. 1.0 ± 0.2 [not treated]; $P < 0.05$; three independent observations) (Fig. 4C).

Incubation of 7800 C1 cells with dexamethasone also produced a substantial increase in PDK4 protein (Fig. 2). A sixfold increase in PDK4 protein occurred after 16 h with this steroid. We also injected rats (200 g males) with dexamethasone (5 mg/kg; 6 h before they were killed) to determine whether it exerts the same effect on PDK4 expression in the intact liver. As expected from the findings with 7800 C1 cells, the steroid produced a dramatic increase in PDK4 expression in the intact liver (data not shown). In other experiments, we also found upregulation of PDK4 expression by dexamethasone in cultured primary hepatocytes (P.W., R.A.H., unpublished observations). Thus the effects observed with 7800 C1 are very likely relevant to how expression of PDK4 is normally regulated in vivo.

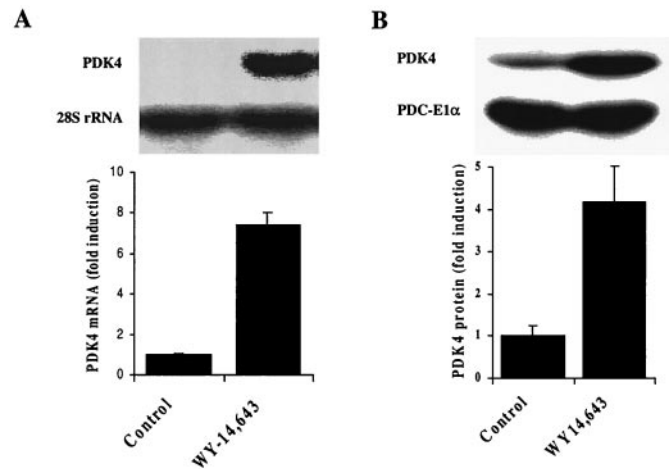


FIG. 3. Effect of WY-14,643 on PDK4 mRNA and protein amount in the liver of intact rats. Male Wistar rats were fed a diet with and without 0.1% WY-14,643 for 3 days. **A:** Autoradiograph of representative Northern blot performed with liver total RNA. Lanes correspond to 20 μg of total RNA. Blots were sequentially hybridized with ^{32}P -labeled rat PDK4 cDNA and a 28S rRNA oligonucleotide probe (loading control). Bar graph gives quantification of the relative abundance of PDK4 mRNA for three animals (means \pm SE). **B:** Autoradiograph of representative Western blot performed with hepatic mitochondrial protein isolated from rats in the indicated groups. Lanes correspond to 15 μg of protein. Antisera against PDK4 and PDC were used to determine amounts of PDK4 and the PDC-E1 α subunit (loading control), respectively. Bar graph gives quantification of the amount of PDK4 protein for four animals (means \pm SE).

Dexamethasone did not affect expression of PDK1, 2, or 3 in 7800 C1 cells. Dexamethasone did not affect the low basal level of PDK1 and PDK3 expression in 7800 C1 cells (data not shown). The steroid likewise produced no effect on the high basal level of PDK2 expression, regardless of dosage (Fig. 4D) or time (Fig. 4E).

WY-14,643 and dexamethasone did not affect PDK4 mRNA stability. It was hypothesized that increased PDK4 message levels in response to these compounds could result from an increase in PDK4 mRNA stability. To examine this possibility, the decrease in PDK4 mRNA abundance caused by inhibition of transcription by actinomycin D was measured with and without WY-14,643 and dexamethasone (Fig. 5). Because basal PDK4 expression is low, the cells were preincubated with WY-14,643 (Fig. 5A and B) or dexamethasone (Fig. 5C) to upregulate the PDK4 message. The cells were then washed and the media changed so that part were incubated with actinomycin D (10 $\mu\text{g/ml}$ of culture medium) without WY-14,643 or dexamethasone and part were incubated with a combination of actinomycin D and WY-14,643 (10 $\mu\text{mol/l}$) or actinomycin D and dexamethasone (1 $\mu\text{mol/l}$). WY-14,643 caused no change in the half-life of PDK4 mRNA under these conditions (1.3 ± 0.4 [without] vs. 1.2 ± 0.4 h [with] WY-14,643; $P = 0.76$; four independent observations). A trend was found for a slightly longer half-life with 10^{-6} mol/l dexamethasone (1.4 ± 0.3 [without] vs. 1.8 ± 0.4 h [with]; four independent determinations), but the difference was not statistically significant ($P = 0.45$). Thus a change in PDK4 mRNA stability did not appear to be responsible for the increase in the PDK4 message induced by WY-14,643 or dexamethasone.

The half-life of the PDK2 mRNA was found to be considerably longer than that of PDK4 (>6 vs. 1.5 h,

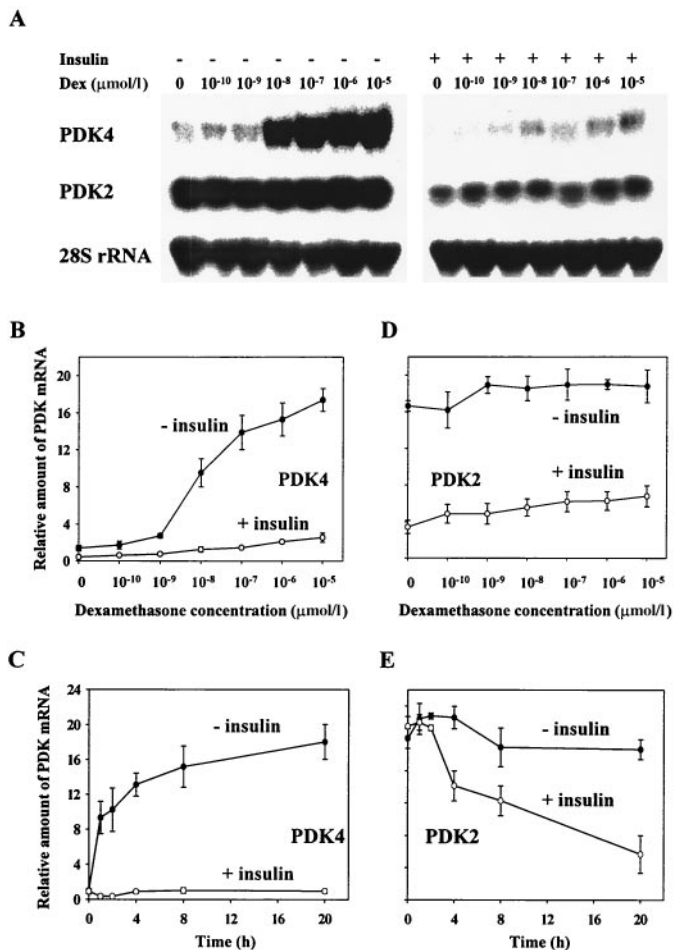


FIG. 4. Effect of dexamethasone (Dex) on the abundance of PDK4 and PDK2 mRNAs in 7800 C1 cells. **A:** Autoradiograph of representative Northern blot of PDK4 and PDK2 messages in total RNA (10 μg) prepared from cells cultured for 20 h with dexamethasone at the indicated concentrations with and without 100 nmol/l insulin. **B:** Quantitative changes in abundance of PDK4 mRNA in cells cultured as described in **A**. **C:** Time course of the effect of 1 $\mu\text{mol/l}$ dexamethasone with and without 100 nmol/l insulin on the abundance of PDK4 mRNA. **D:** Quantitative changes in the abundance of PDK2 mRNA in cells cultured as described in **A**. **E:** Time course of the effects of 1 $\mu\text{mol/l}$ dexamethasone with and without 100 nmol/l insulin on the abundance of PDK2 mRNA. Data points are means \pm SE of three independent experiments.

respectively, in three independent experiments). Neither dexamethasone nor WY-14,643 altered the stability of the PDK2 mRNA (data not shown).

Insulin decreased PDK4 and PDK2 mRNAs and proteins in 7800 C1 cells. Insulin at an initial concentration of 100 nmol/l partially prevented induction of PDK4 expression at all WY-14,643 concentrations tested (14 ± 2 [10 $\mu\text{mol/l}$ WY-14,643] vs. 9.0 ± 1 [10 $\mu\text{mol/l}$ WY-14,643 plus 100 nmol/l insulin]; $P < 0.05$; three independent observations) (Fig. 1A and B) and also over the entire culture time from 1 to 20 h (19 ± 1 [after 4 h with WY-14,643] vs. 13 ± 2 [after 4 h with WY-14,643 plus insulin]; $P < 0.05$; three independent observations) (Fig. 1C). Insulin was also partially effective in reversing increased PDK4 gene expression caused by WY-14,643 (Fig. 6A and B). For an unknown reason, insulin was more effective in the first few hours after its addition. After 4 h of exposure to insulin, the level of the PDK4 message increased significantly and then stabilized at about 25% below the initial level (Fig. 6A

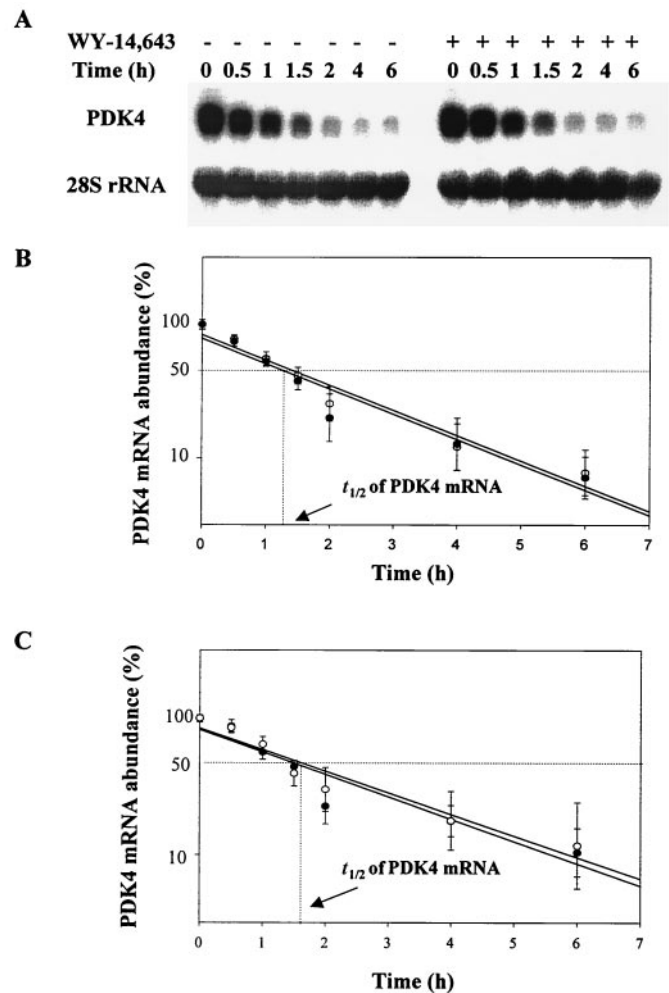


FIG. 5. Effect of WY-14,643 and dexamethasone on the stability of PDK4 mRNA in 7800 C1 cells. **A:** Autoradiograph of representative Northern blot of PDK4 message in total RNA (10 μg) prepared from cells cultured initially for 16 h in the presence of 10 $\mu\text{mol/l}$ WY-14,643 and then for the indicated lengths of time in the medium containing actinomycin D (10 $\mu\text{g/ml}$) with and without 10 $\mu\text{mol/l}$ WY-14,643. **B:** Quantitative changes of relative abundance of PDK4 mRNA in cells cultured as described in the Northern blot given in **A** without WY-14,643 (\circ) and with 10 $\mu\text{mol/l}$ WY-14,643 (\bullet). **C:** Quantitative changes of relative abundance of PDK4 mRNA in the cells cultured initially for 16 h in the presence of dexamethasone (1.0 $\mu\text{mol/l}$) and then for the indicated lengths of time in the medium containing actinomycin D (10 $\mu\text{g/ml}$) with (\circ) and without (\bullet) dexamethasone (1 $\mu\text{mol/l}$). Lines were drawn by linear-regression analysis. Data points correspond to means \pm SE of four independent experiments.

and B). This time course was highly reproducible and not likely explainable by the degradation of insulin, because, as is discussed below, insulin remained effective over a 20-h incubation in antagonizing the effects of dexamethasone on PDK4 expression. The effect of insulin during the first 4 h of incubation was dosage dependent, with an EC_{50} value of about 1 nmol/l (Fig. 6C).

As expected from its effect on message levels, insulin also partially prevented the increase in PDK4 protein induced by WY-14,643 (52% less protein in the presence of insulin; average of three independent experiments) (Fig. 2).

In contrast to the marginal and complex pattern of insulin's effect on PDK4 expression in the presence of WY-14,643, insulin effectively prevented PDK4 expression over a wide span of dexamethasone concentrations ($15 \pm$

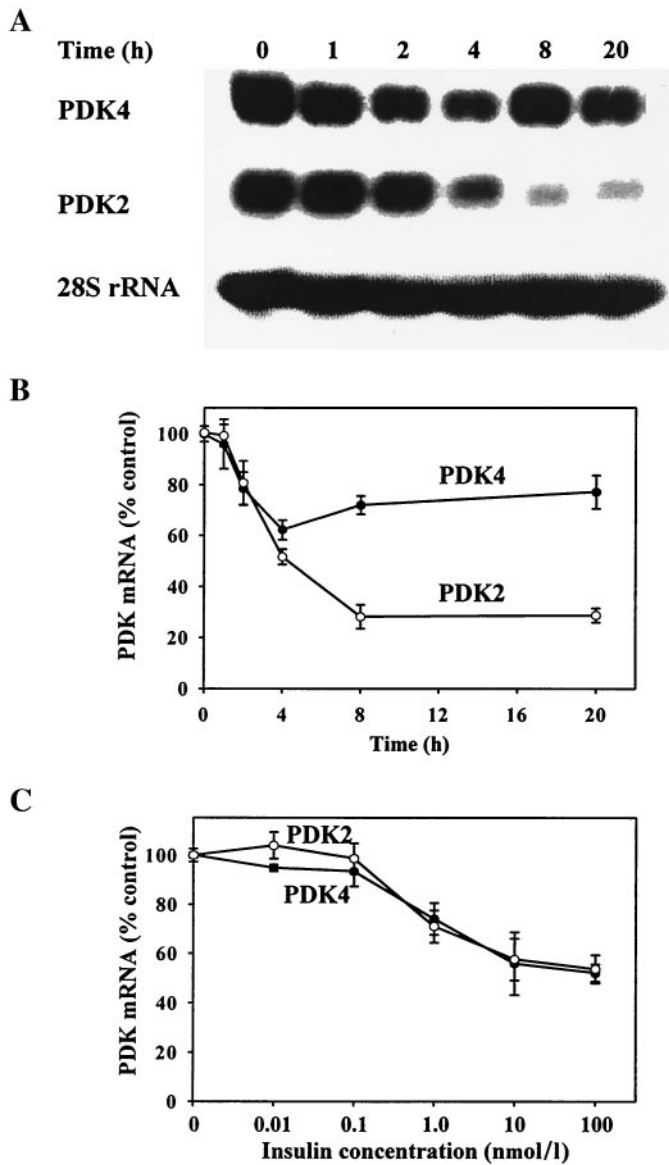


FIG. 6. Time course and concentration study of the effect of insulin on the abundance of PDK4 and PDK2 mRNAs in 7800 C1 cells pretreated with WY-14,643. **A**: Autoradiograph of representative Northern blot of PDK4 and PDK2 messages in total RNA (10 μ g) prepared from cells cultured initially for 16 h with 10 μ mol/l WY-14,643 and then for the indicated lengths of time with 100 nmol/l insulin. **B**: Quantitative changes in abundance of PDK2 and PDK4 mRNAs induced by insulin under the conditions described in **A**. **C**: Quantitative changes in abundance of PDK2 and PDK4 mRNAs in cells cultured initially for 16 h with 10 μ mol/l WY-14,643 and then for an additional 4 h with varying concentrations of insulin. Data points are means \pm SE of three independent experiments.

2 [10^{-6} mol/l dexamethasone] vs. 2.1 ± 0.2 [10^{-6} mol/l dexamethasone and 100 nmol/l insulin]; $P < 0.05$; three independent observations) (Fig. 4A and B) and over the entire time period of 1–20 h (18 ± 2 [after 20 h with dexamethasone] vs. 1.0 ± 0.2 [after 20 h with dexamethasone plus insulin]; $P < 0.05$; three independent observations) (Fig. 4C). Insulin also effectively suppressed PDK4 mRNA levels induced by dexamethasone in a concentration- (Fig. 7A and B) and time-dependent manner (Fig. 7C). Insulin antagonized the effect of dexamethasone, with an EC_{50} of $\sim 0.5 \times 10^{-9}$ mol/l (Fig. 7A and B), which is comparable to the maximum insulin concentration found

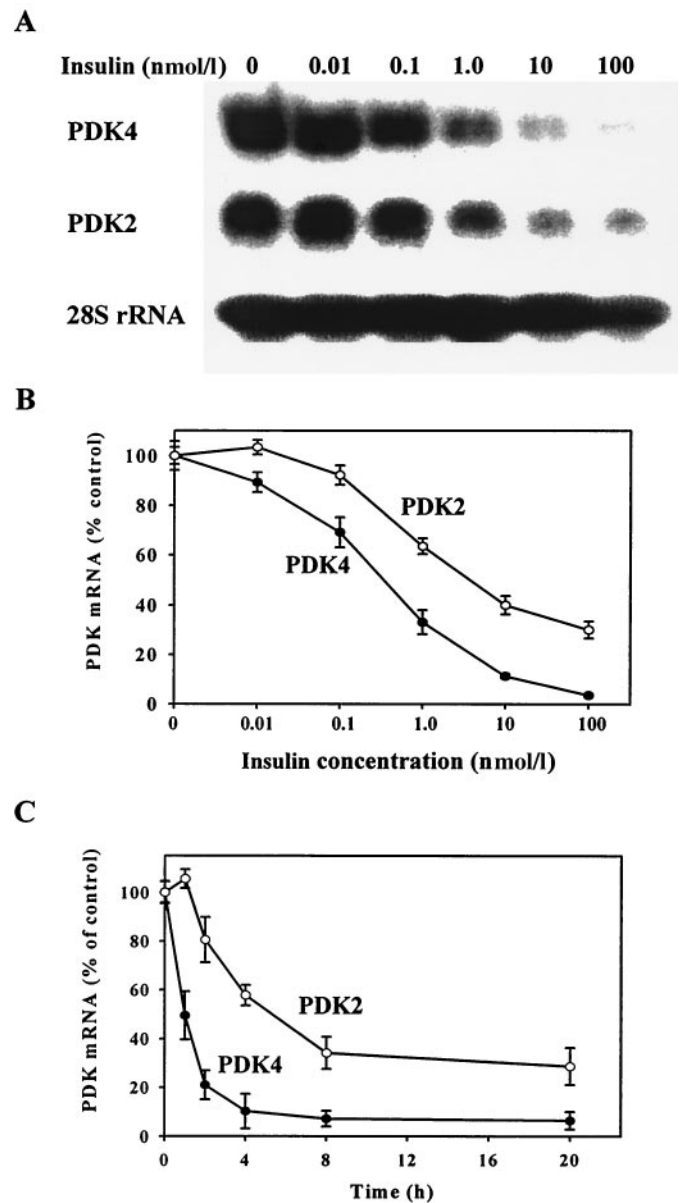


FIG. 7. Time course and concentration study of the effect of insulin on the abundance of PDK2 and PDK4 mRNAs in 7800 C1 cells pretreated with dexamethasone. **A**: Autoradiograph of representative Northern blot of PDK4 and PDK2 messages in total RNA (10 μ g) prepared from cells cultured initially for 16 h with 1.0 μ mol/l dexamethasone and then for an additional 6 h after supplementation of the medium with the indicated concentrations of insulin. **B**: Quantitative changes in abundance of PDK2 and PDK4 mRNAs induced by varying concentrations of insulin under the conditions described in **A**. **C**: Time study of changes in abundance of PDK2 and PDK4 mRNAs in cells cultured for 16 h in the presence of 1.0 μ mol/l dexamethasone followed by supplementation of the medium with 100 nmol/l insulin at t_0 . Data points given are means \pm SE of three independent experiments.

in the portal vein of the rat (25). The half-life of the PDK4 message after the addition of insulin was ~ 1.1 h (Fig. 7C), which is similar to the half-life observed after inhibition of transcription with actinomycin D.

As expected from its effects on message levels, insulin also effectively prevented the increase in PDK4 protein induced by dexamethasone in 7800 C1 cells (83% less protein in the presence of insulin; average of three independent experiments) (Fig. 2).

Insulin was also effective in downregulating the basal

level of the PDK2 message (Figs. 1D, 1E, 4D, 4E, 6, and 7). A 50% reduction in the PDK2 message occurred after 4–5 h of incubation with insulin (Figs. 1E, 4E, 6B, 7C). When directly compared, insulin was more effective in reducing the PDK4 message than the PDK2 message (Fig. 7B), and the extent to which the PDK4 message was lowered by insulin was greater than that of PDK2 (95 vs. 70%, respectively) (Fig. 7C). Nevertheless, insulin significantly decreased the level of PDK2 message at physiologically relevant concentrations. Downregulation of PDK2 gene expression by insulin was not affected by WY-14,643 (Fig. 1D) or dexamethasone (Fig. 4D). On the other hand, the decreased effectiveness of insulin on PDK4 expression caused by WY-14,643 resulted in insulin being almost equally effective in downregulating PDK2 and PDK4 messages in the presence of this PPAR- α agonist after 4 h of incubation (Fig. 6C). When the incubation period was extended to 20 h, insulin was more effective in lowering the PDK2 than the PDK4 message (data not shown).

As expected from insulin's effect on PDK2 message levels, insulin also induced downregulation of the amount of PDK2 protein in 7800 C1 cells (55% less protein after 60 h; average of three independent experiments) (Fig. 2B). This effect required a long incubation time, presumably because of the relatively long half-lives of both message and protein.

Fatty acids increased the abundance of PDK4 mRNA in 7800 C1 cells. It was hypothesized that if WY-14,643 produces its effects via PPAR- α , the capacity of FFAs to activate this receptor/transcription factor should have the same effect on expression of PDK4. Indeed, incubation of 7800 C1 cells with palmitate or oleate at initial concentrations of 0.35 mmol/l caused 7.5- and 9-fold increases, respectively, in the abundance of PDK4 mRNA (Fig. 8). As found with WY-14,643, insulin was only partially effective in preventing the increase in PDK4 message induced by fatty acids.

DISCUSSION

The goal of this study was to identify factors that regulate expression of the genes encoding PDK2 and PDK4. This is important because tissue-dependent increases in PDK2 and PDK4 expression during starvation (9–12) contribute to greater phosphorylation and, therefore, a lower activity state of PDC. Nearly complete inactivation of the complex in major tissues is critical in the body's strategy to conserve glucose and gluconeogenic substrates. In part because of PDC inactivation, the liver changes from an organ that uses glucose and three-carbon compounds for fatty acid synthesis into an organ dedicated to the conversion of three-carbon compounds into glucose. PDC inactivation in other tissues also conserves three-carbon compounds and maximizes their availability for gluconeogenesis.

Increased expression of PDK4 mRNA and protein occurs in many tissues in response to starvation and chemical-induced diabetes (9–12). Although less dramatic, starvation also increases PDK2 gene expression in the liver and kidney (8,11,12). Conditions that affect expression of the other two known PDK isoforms, PDK1 and PDK3, have not been found.

Morris hepatoma 7800 C1 cells were chosen because of

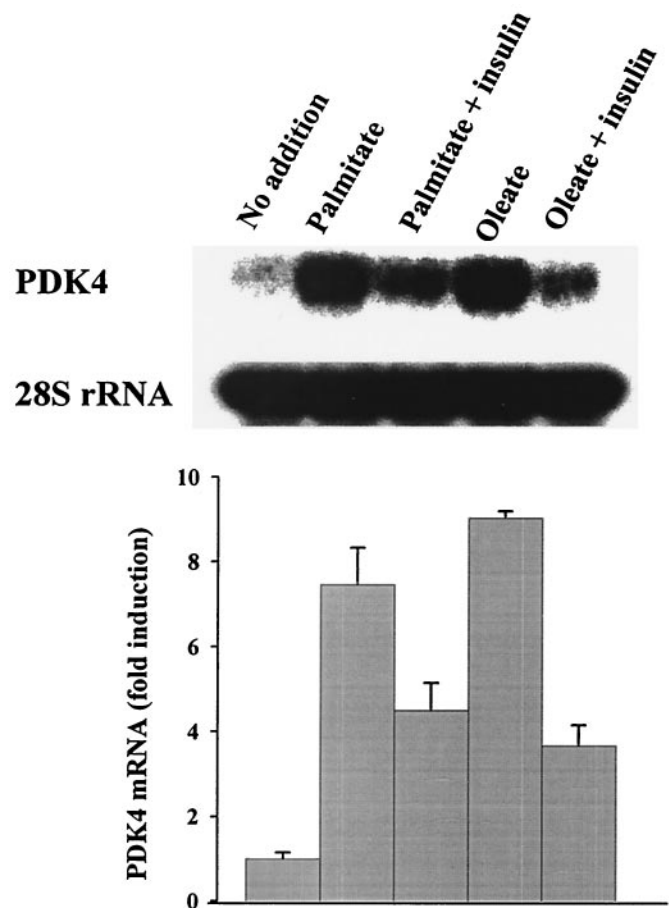


FIG. 8. Effect of palmitate and oleate on the abundance of PDK4 mRNA in 7800 C1 cells. *Upper panel:* Autoradiograph of representative Northern blot of PDK4 message in total RNA (10 μ g) prepared from cells cultured for 4 h with 350 μ mol/l palmitate or oleate with and without 100 nmol/l insulin. Culture medium of all cells was supplemented with 0.5% defatted BSA. *Lower panel:* Quantitative changes in abundance of PDK4 mRNA under the conditions given in *upper panel*. Bars correspond to means \pm SE of three independent experiments.

their responsiveness to glucocorticoids, PPAR- α ligands, and insulin, as previously demonstrated (18,19,26). These are factors that change substantially during the starve/feed cycle and therefore might be expected to affect expression of the PDK2 and PDK4 genes. 7800 C1 cells are well differentiated and contain mitochondria in amounts comparable to that found in normal rat liver (27). These cells are therefore more like liver in their dependence on mitochondrial processes than more rapidly growing and less-well-differentiated hepatoma cells. Most importantly, the solid tumor from which these cells were derived responds to starvation of its host much like normal liver does (20). This includes inactivation of its PDC by phosphorylation (20), a likely consequence of upregulation of PDK4 activity.

WY-14,643 and dexamethasone stimulated PDK4 gene transcription in 7800 C1 cells. The respective seven- and ninefold increases over basal levels of PDK4 message with these compounds occurred within just 1 h of incubation. Their effects on PDK4 expression were additive (data not shown), suggesting independent signaling mechanisms. The half-life of the PDK4 message is quite short (<1.5 hours) relative to most other eukaryotic mRNAs, but in the same range as that of other important regulatory enzymes,

such as PEPCK (28). In the current study, insulin was as effective as actinomycin D in preventing and reversing the increase in PDK4 mRNA caused by dexamethasone. On the other hand, insulin was less effective in preventing and reversing PDK4 induction by WY-14,643.

The basal level of PDK2 mRNA is much greater and its half-life is considerably longer than that of PDK4 mRNA. Dexamethasone and WY-14,643 could not be shown to have effects on the PDK2 message level. Insulin, however, was quite effective in diminishing the level of the PDK2 message.

The increased expression of PDK4 in response to dexamethasone likely reflected a physiologically important effect of glucocorticoids. It occurred in the intact liver in response to administration of dexamethasone, and probably in other tissues as well, given that glucocorticoid receptors are present in many cells of the body. The induction of PDK4 expression by the increase in blood levels of glucocorticoids during starvation promotes gluconeogenesis by blocking irreversible loss of pyruvate carbon to the tricarboxylic acid cycle. For the same reason, overexpression of PDK4 in response to excess glucocorticoids very likely contributes to diabetes induced by excessive therapeutic use of steroids and comorbid with Cushing's syndrome (29). In contrast, glucocorticoid deficiency most likely leads to less than normal expression of PDK4 and therefore a higher PDC activity state in major tissues. This could account for the loss of control of flux through PDC in starved, adrenalectomized rats (30) and likely contributes to the fasting hypoglycemia of patients with Addison's disease (31).

The response observed in this study to the PPAR- α ligand WY-14,643 suggests that fatty acids may also be important factors in the regulation of PDK4 expression in starvation and diabetes. Fatty acids function as endogenous PPAR- α ligands (17), and plasma FFAs are elevated in conditions thus far identified to increase PDK4 expression. These include starvation (9–12), diabetes (9,10), high-fat-diet feeding (32), hibernation (33), carnitine deficiency (34), and exercise (35). WY-14,643 induces PDK4 expression in skeletal muscle (10), kidney (36), heart (36), and liver (this study) of normal mice, but not in tissues of PPAR- α -null mice (36). Expression of PDK4 in response to starvation is also blunted in PPAR- α -null mice. These findings clearly indicate that WY-14,643 acts via PPAR- α in causing its effect on PDK4 expression, and further suggest a role for PPAR- α activation by fatty acids in the induction of PDK4 expression during starvation. Our finding that fatty acids have the same effect as WY-14,643 on PDK4 mRNA expression in 7800 C1 cells provides additional support for this mechanism.

Insulin was relatively ineffective in preventing or reversing induction of PDK4 by WY-14,643 or FFAs. This inability to completely oppose the effect of PPAR- α activation may be a consequence of insulin resistance that occurs when FFAs are elevated (37).

Regulation of PDK2 and PDK4 expression by insulin also appears physiologically important. PDK2 and PDK4 are both increased in conditions in which insulin levels are low (e.g., starvation and diabetes). It is also likely that insulin resistance is a contributing factor to the increase in PDK4 expression in rats fed high-fat diets (32).

In conclusion, our findings suggest that glucocorticoids, FFAs, and insulin play important roles in setting the level of PDK expression. This level in turn determines the phosphorylation state and therefore the activity state of PDC. Thus the inactivation of PDC that occurs in most of the major tissues of the body during starvation and diabetes is likely explained by the effects that a decrease in insulin and the increase in glucocorticoids and FFAs have on PDK4 expression in these conditions.

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