Vanadate Treatment Restores the Expression of Genes for Key Enzymes in the Glucose and Ketone Bodies Metabolism in the Liver of Diabetic Rats

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Abstract

Oral administration of vanadate to diabetic streptozotocin-treated rats decreased the high blood glucose and D-3-hydroxybutyrate levels related to diabetes. The increase in the expression of the *P*-enolpyruvate carboxykinase (PEPCK) gene, the main regulatory enzyme of gluconeogenesis, was counteracted in the liver and the kidney after vanadate administration to diabetic rats. Vanadate also counteracted the induction in tyrosine aminotransferase gene expression due to diabetes and was able to increase the expression of the glucokinase gene to levels even higher than those found in healthy animals. Similarly, an induction in pyruvate kinase mRNA transcripts was observed in diabetic vanadate-treated rats. These effects were correlated with changes on glucokinase and pyruvate kinase activities. Vanadate treatment caused a decrease in the expression of the liver-specific glucose transporter, GLUT-2. Thus, vanadate was able to restore liver glucose utilization and block glucose production in diabetic rats. The increase in the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCoAS) gene, the key regulatory enzyme in the ketone bodies production pathway, observed in diabetic rats was also blocked by vanadate. Furthermore, a similar pattern in the expression of PEPCK, GLUT-2, HMGCoAS, and the transcription factor CCAAT/enhancer-binding protein α genes has been observed. All of these results suggest that the regulation of the expression of genes involved in the glucose and ketone bodies metabolism could be a key step in the normalization process induced by vanadate administration to diabetic rats. (*J. Clin. Invest.* 1993. 92:4–11.) Key words: *P*-enolpyruvate carboxykinase • glucokinase • pyruvate kinase • GLUT-2 • CCAAT/enhancer-binding protein α

Introduction

The main alterations related to the diabetic process are hyperglycemia and ketoacidosis. The increase in blood glucose is mainly the result of the impairment of either liver or peripheral tissues to metabolize glucose and the activation of gluconeogenesis in the liver and the kidney (1). Hepatocytes are able to respond to fluctuations in blood glucose levels by parallel changes in the rate of glucose phosphorylation. The key enzyme in the regulation of this process is glucokinase. The regulation of glucokinase activity has been strongly correlated with corresponding changes of mRNA and with rates of transcription of the glucokinase gene (2–6). In diabetic animals, where glycolysis is inoperative and gluconeogenesis is increased, glucokinase mRNA is very low (3, 5–10). Treatment of diabetic rats with insulin induces glucokinase gene transcription (8). Another key step in liver glucose metabolism is pyruvate kinase, which plays a central role in the regulation of the glycolytic pathway (11). These controls include short-term regulation, such as inactivation of the L-type enzyme by phosphorylation (12–14), and long-term regulation by changes in the amount of the enzyme. The alterations in the amount of this enzyme are primarily due to changes in the level of transcription of its gene (15, 16). Thus, mRNA concentrations, which are decreased in starvation and diabetes, are restored to normal by refeeding the animals a high carbohydrate diet and insulin administration (17, 18).

The cytosolic form of *P*-enolpyruvate carboxykinase (PEPCK)[1] is the main regulatory enzyme of gluconeogenesis (19). PEPCK has no known allosteric modifiers and responds to changes in metabolic flux over the gluconeogenic pathway by a rapid increase in enzyme protein (19). PEPCK activity is regulated by different agents through the modification of the synthesis rate of the enzyme (19). During the diabetic process, the expression of the liver PEPCK gene is markedly increased by the lack of insulin and the rise of glucagon levels (20–22). Insulin administration to streptozotocin-induced diabetic rats results in a decrease of liver PEPCK mRNA levels to those levels found in normal animals. The insulin effect (23–25) is mainly due to an inhibition of PEPCK gene transcription. On the other hand, insulin has no effect on kidney PEPCK mRNA levels, which are mainly controlled by the body acid–base status. The production of β-hydroxybutyrate and acetoacetate in diabetic acidosis results in an induction of kidney PEPCK mRNA concentration (26, 27). Liver ketogenesis supplies metabolic fuels for energy to peripheral tissues. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCoAS) is the key enzyme in the regulation of ketone bodies synthesis (28–30). The gene for HMGCoAS has been cloned (31); its expression is rapidly changed in response to AMP, insulin, dexamethasone, and refeeding and is greatly increased by starvation and diabetes (32).

Several reports have shown that vanadate acts as an insulin-like effector in vivo by normalizing blood glucose levels in diabetic animals (33–36) and by preventing the decline in cardiac performance due to this pathological process (33). Vanadate is

also able to modify carbohydrate metabolism in both the intact animal (34, 37) and in isolated cells (38–43) and tissues (44). Furthermore, vanadate has been found to be a powerful inhibitor of PEPCK and tyrosine aminotransferase (TAT) gene expression in hepatoma cells (45). However, vanadate induces the expression of L-pyruvate kinase and glucokinase genes in hepatocytes in primary culture (46). The 5' flanking region of the PEPCK gene contains regulatory elements that are responsive to cAMP (47–52), glucocorticoids (48–50), insulin (24, 53), and vanadate (45). No insulin or vanadate regulatory elements have been described in glucokinase and pyruvate kinase promoters. It has been suggested that CCAAT/enhancer-binding protein α (C/EBPα), a transcription factor that is highly expressed in liver and adipose tissue, could have a physiological function regulating the transcription of genes coding for enzymes of energy metabolism (54). The promoters of genes involved in this metabolism have been found to be *trans*-activated by C/EBPα in vitro (52, 54, 55).

The aim of this work is to analyze the effects of oral treatment of diabetic rats with vanadate in the expression of the genes for regulatory enzymes involved in liver glucose and ketone bodies metabolism. We have found that vanadate, like insulin, restores liver performance. Furthermore, since the mechanism(s) by which vanadate provokes these effects are unknown, we have investigated the effects of diabetes and vanadate treatment on the expression of C/EBPα.

Methods

Materials. The random primed DNA-labeling kit was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). [^{32}P]dCTP (3,000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Gene Screen Plus was purchased from DuPont-New England Nuclear (Boston, MA). Sodium orthovanadate was obtained from Sigma Chemical Co. (St. Louis, MO). The other reagents used in this study were of the highest purity available. *P*-enolpyruvate carboxykinase cDNA was provided by Dr. R. W. Hanson (Case Western Reserve University, Cleveland, OH), and HMGCoAS cDNA by Dr. F. G. Hegardt (University of Barcelona, Barcelona, Spain). Glucokinase cDNA was provided by Dr. P. B. Iynedjian (University of Geneva, Geneva, Switzerland). Tyrosine aminotransferase cDNA was a gift from Dr. Günter Schutz (Institut für Zell und Tumorbiologie, Heidelberg, Germany). Pyruvate kinase cDNA was a gift from Dr. A. Kahn (Institut Cochin de Genetique Moleculaire, Paris, France). Liver-specific glucose transporter (GLUT-2) cDNA was provided by Dr. G. Bell (University of Chicago, Chicago, IL). C/EBPα cDNA was provided by S. L. McKnight (Carnegie Institution of Washington, Baltimore, MD).

Treatment of animals. Male Sprague-Dawley rats (180–230 g) were used. Diabetic animals were obtained by applying a single intravenous injection of streptozotocin (60 mg/kg body wt), dissolved in a 10 mM sodium citrate solution with 0.9% NaCl, pH 4.5, immediately before administration. Diabetic rats were used 7 d after streptozocin treatment. Each diabetic state was assessed by measuring glucosuric and ketonuric levels (Gluketur Test; Ames). The animals were divided into three groups: control, diabetic, and vanadate-treated diabetic. Each group consisted of five rats. The controls and diabetic rats drank a 0.5-g/100 ml NaCl solution. The vanadate-treated animals drank a 0.5-g/100 ml NaCl solution containing freshly prepared sodium orthovanadate (0.7 mg/ml). 15 d after treatment the animals were killed, blood was collected, and livers and kidneys were removed and quickly frozen in liquid N_2. This protocol was repeated three times. Finally, a second series of experiments was performed in which the animals were divided into four groups, each one consisting of five animals: control, vanadate-treated control, diabetic, and vanadate-treated diabetic. This

procedure was repeated twice and the protocol followed was as described above.

Enzyme and metabolite assays. Glucose was measured by the hexokinase method (GlucoQuant system; Boehringer Mannheim Biochemicals). D-3-hydroxybutyrate was determined by the D-3-hydroxybutyrate dehydrogenase technique (Boehringer Mannheim Biochemicals). Serum alanine aminotransferase (SALT) activity was assayed enzymatically (Boehringer Mannheim Biochemicals). Serum insulin concentration was quantified by radioimmunoassay (system INSIK-5; Soren Biomedica, Saluggia (Vercelli), Italy). To determine pyruvate kinase activities, the liver was homogenized in an ice-cold buffer containing 100 mM potassium fluoride, 15 mM EGTA, and 50 mM glycilglycine, pH 7.4. To measure glucokinase activity, liver samples were homogenized in an ice-cold buffer, pH 7.4, containing 50 mM Tris-HCl, 300 mM sucrose, 100 mM KCl, 1 mM EDTA, and 0.7 μl/ml β-mercaptoethanol. These activities were analyzed in 12,000 g as described in references 56 and 57, respectively. Pyruvate kinase activity was determined at 0.15 mM *P*-enolpyruvate (active form) and at 5 mM *P*-enolpyruvate (total activity) (56). Glucokinase activity was calculated as the difference between the glucose phosphorylation capacity at 100 and 0.5 mM glucose (57).

Northern analysis. Total RNA was extracted from tissues by the guanidine isothiocyanate method (58), and the RNA samples (20 μg) were subjected to electrophoresis on 1% agarose gel containing 2.2 M formaldehyde. Northern blots were hybridized to ^{32}P-labeled cDNAs. The PEPCK cDNA probe corresponded to a 1.1-kb PstI-PstI fragment from the 3' end of the PEPCK cDNA; TAT cDNA corresponded to a 600-b PstI-PstI fragment that included the 3' end of the TAT cDNA; HMGCoAS, a 1,500-bp EcoRI-EcoRI fragment from the cDNA; glukokinase cDNA, a 2.3-kb EcoRI-EcoRI fragment containing the full-length cDNA; pyruvate kinase cDNA, a 1.6-kb PstI-PstI fragment from the cDNA; GLUT-2 cDNA, a 1.5-kb EcoRI-EcoRI fragment from the cDNA; C/EBPα cDNA, a 1.9-kb BamHI-HindIII fragment from the cDNA. The β-actin probe corresponded to a 1.3-kb EcoRI-EcoRI fragment of β-actin cDNA. These probes were labeled using [α-^{32}P]dCTP, by following the method of random oligopriming as described by the manufacturer. The specific activity of the DNA probe labeled in this manner was $\sim$ 10^9 cpm/μg DNA. Hybridization and washing conditions have been described previously (45). The membranes were exposed to Kodak XAR-5 film, and densitometric analysis of autoradiograms was performed at nonsaturating exposures with a scanning densitometer. The actin signal was used to correct for loading inequalities as an internal standard.

Presentation of the data and statistical analysis. Any variability attributable to the experimental procedures was ruled out because in studies of individual RNA samples the correlation coefficient between increasing amounts of input RNA and signal intensity was 0.99 for all transcripts. To analyze the effects of perturbations independently of variability in basal gene expression, the densitometric readings of experimental samples were expressed as a percentage of readings in paired samples of nontreated control rats (always tested in the same Northern blot) after correction for loading differences with the actin signal. The data were summarized with the mean±SEM. Statistical analysis was carried out by analysis of variance followed by the Newman–Keuls test for multiple comparisons (59). Differences were considered statistically significant at $P < 0.05$.

Results

Effects of vanadate on serum parameters. In agreement with previous reports (33–36), a 15-d treatment of streptozotocin diabetic rats with vanadate decreased the blood glucose levels, which had been augmented in the diabetic state (Table I). This glucose normalization was a result of vanadate action since no increase in blood insulin was detected in diabetic vanadate-treated animals (Table I). In addition, vanadate restored body weight and fluid intake of diabetic animals (Table I). Further-

Table I. Effects of Vanadate Treatment on Body Weight, Fluid Intake, and Serum Parameters

| Group	Weight gain	Fluid intake	Blood glucose	SALT	β-Hydroxy-butyrate	Insulin
	g	*ml/d*	*mg/dl*	*U/liter*	*mM*	*ng/ml*
Control						
Nontreated (*n* = 25)	53±9	46±6	136±10	49±1	0.10±0.03	20.5±1.8
Vanadate treated (*n* = 10)	38±9*	30±9*	130±11	48±2	0.13±0.05	18.3±2.0
STZ-diabetic						
Nontreated (*n* = 25)	2±3§	290±18§	501±25§	105±10§	1.48±0.35§	6.9±1.6§
Vanadate treated (*n* = 25)	13±5‡	35±10	195±17*	52±5	0.36±0.16*	6.1±1.5§

Glucose, SALT, D-3-hydroxybutyrate, and insulin were measured as described in Methods. Results are expressed as means±SEM for the number of animals in parentheses and the significance of differences was tested by Student's *t* test: * *P* < 0.05, ‡ *P* < 0.01, § *P* < 0.001 vs. nontreated controls.

more, vanadate induced a strong reduction (~ 75%) in the increased levels of D-3-hydroxybutyrate caused by diabetes (Table I). These results agreed with a normalization in the metabolic state in the diabetic animals after treatment.

We also measured SALT activity, since it is one of the most commonly used parameters to detect hepatic damage. As shown in Table I, streptozotocin treatment induced a significant increase in this enzymatic activity in serum. Vanadate administration to these animals resulted in a decrease of SALT activity to the levels of healthy animals, suggesting that this compound was able to restore the liver tissue to normal status in diabetic rats.

Effects of vanadate on glucokinase and pyruvate kinase gene expression and enzyme activity in normal and diabetic rats. Since vanadate was able to normalize blood glucose levels when administered to diabetic rats, a possible mechanism to explain this action might involve an induction in glucokinase gene expression. Total RNA was isolated from the liver of normal and diabetic animals and analyzed by Northern blotting. Although no significant differences were detected in vanadate-treated control animals (96±15% of nontreated controls), oral treatment of diabetic rats with vanadate caused an induction of liver glucokinase mRNA concentrations, which increased from 10±5% in nontreated diabetic rats to 255±72% in vanadate-treated diabetic rats (*P* < 0.01), related to control nontreated animals (Fig. 1 *A*). A similar pattern was observed when glucokinase activity was determined in the liver of the animals. As shown in Table II, oral treatment with vanadate of control rats did not cause any significant modification of glucokinase activity compared with control nontreated animals. The glucokinase activity of diabetic rats was very low, but a great increase in the enzyme activity was noted in diabetic rats treated with van-
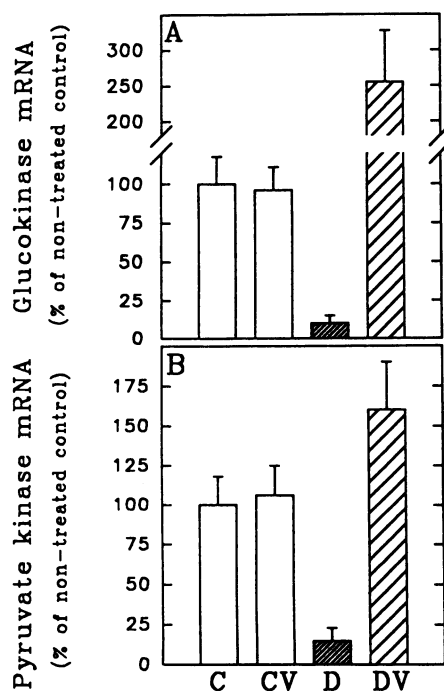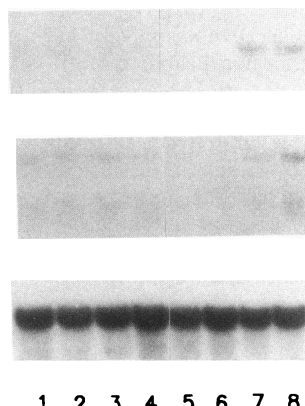
Figure 1. Effects of vanadate treatment on glucokinase and pyruvate kinase mRNA concentrations. (*Left*): The concentrations of mRNA for glucokinase (*A*) and pyruvate kinase (3.2-kb transcript) (*B*) were determined by Northern analysis of total RNA (20 μg per sample) isolated from the liver of nontreated control rats (*C*) (*n* = 25), control rats treated with vanadate (*CV*) (*n* = 10), nontreated diabetic rats (*D*) (*n* = 25), and diabetic rats treated with vanadate (*DV*) (*n* = 25) as described in Methods. Signal intensity was quantified by densitometry and the readings obtained were expressed as a percentage of readings in paired nontreated control animals after correction for loading inequalities with the β-actin signal. Each bar represents the mean±SEM. (*Right*): Northern blots from two representative animals of each group (C, lanes *1* and *2*; CV, lanes *3* and *4*; D, lanes *5* and *6*; DV, lanes *7* and *8*) are shown.

adate (Table II). This increase was even higher than that observed in control animals ($\sim 60\%$).

An increase of glucose utilization through hepatic glycolysis could also contribute in decreasing hyperglycemia. When L-type pyruvate kinase gene expression was determined, very low concentrations of pyruvate kinase mRNA ($15\pm8\%$ of the nontreated control) were detected in diabetic livers. However, a $160\pm30\%$ induction in the three pyruvate kinase mRNA transcripts of 3.2-, 2.2-, and 2.0-kb was observed after vanadate treatment of diabetic rats (Fig. 1 B). No significant changes were observed in the concentration of pyruvate kinase mRNA in control animals treated with vanadate compared with control nontreated rats ($106\pm19\%$). The changes in pyruvate kinase activity showed a pattern similar to that detected in mRNA concentrations. Both pyruvate kinase activities at low (active form) and high (total activity) concentrations of the substrate were not modified by vanadate treatment of control animals, whereas the effector was able to counteract the inhibition of the enzyme caused by diabetes (Table II).

Effects of vanadate on PEPCK and TAT gene expression in normal and diabetic rats. To decrease blood glucose levels, in addition to stimulating glucose use, vanadate may decrease glucose production through gluconeogenesis. In this regard, we studied the effects of vanadate treatment on the expression of the PEPCK gene in the liver and kidney of healthy and diabetic animals, since the activity of PEPCK is regulated at the level of the transcription of its gene. Fig. 2 shows that diabetes caused an increase of $660\pm118\%$, related to control rats, in the expression of the liver PEPCK gene. The administration of vanadate produced a significant reduction of these mRNA concentrations up to $240\pm80\%$ of the control animals. However, no significant modification was observed on PEPCK mRNA concentrations in the liver of control rats treated with vanadate (data not shown). In addition, we also detected a $550\pm100\%$ increase in the kidney PEPCK mRNA concentrations in diabetic rats compared with control animals and a reduction to $220\pm35\%$ after treatment with vanadate (Fig. 3). These results show that the administration of vanadate to diabetic animals was able to affect the expression of the PEPCK gene in different tissues.
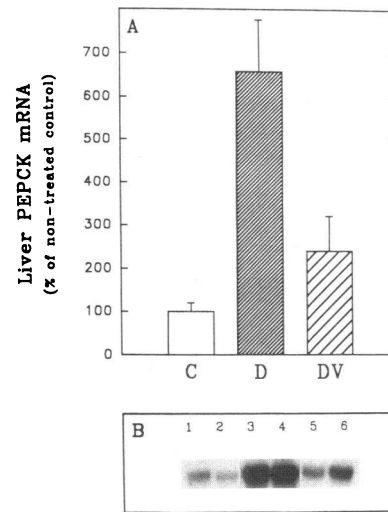

Figure 2. Effects of vanadate treatment on liver PEPCK mRNA concentrations. (*A*) The concentration of mRNA for PEPCK was determined by Northern analysis of total RNA isolated from the liver of control rats (*C*), diabetic rats (*D*), and diabetic rats treated with vanadate (*DV*). Each experimental group consisted of 15 animals. The data are expressed as a percentage of the nontreated control animals. Results represent the mean $\pm$SEM. (*B*) Northern blots from two representative animals of each group (C, lanes *1* and *2*; D, lanes *3* and *4*; DV, lanes *5* and *6*) are shown.

We also investigated the effects of vanadate on TAT, another enzyme related to gluconeogenesis and regulated at the level of gene expression in a similar manner as PEPCK (60–62). cAMP and glucocorticoids induce the expression of the gene whereas insulin inhibits TAT gene expression (63, 64). In this study a similar pattern of modification ($480\pm70\%$ induction caused by diabetes and reduction to $180\pm38\%$ after vanadate treatment) of the expression of the gene for TAT was detected (Fig. 4).

Effects of vanadate on liver glucose-transporter GLUT-2 gene expression. GLUT-2, the liver- and pancreas-specific glucose transporter, is involved in glucose uptake and release processes (65). Insulin treatment of diabetic animals decreases the amount of protein and mRNA of GLUT-2 (66). However, little is known about the regulation of the expression of this glucose transporter in the liver. We analyzed the effects of diabetes and vanadate administration on the expression of this glucose transporter. Fig. 5 shows that although diabetes caused
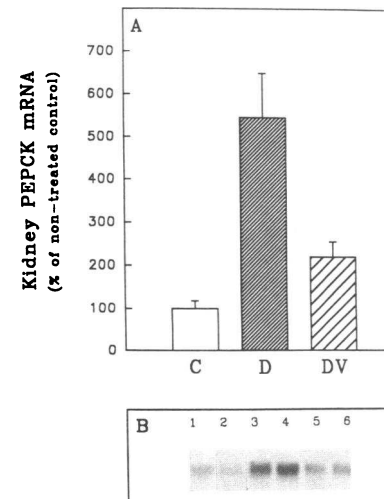
Table II. Effects of Vanadate Treatment on Liver Glucokinase and Pyruvate Kinase Activities

Group	Glucokinase	Pyruvate kinase	
		Active	Total
		U/g liver	
Control			
Nontreated ($n = 12$)	3.1 ± 0.7	23.5 ± 1.9	46.8 ± 4.1
Vanadate treated ($n = 10$)	2.9 ± 0.8	25.1 ± 2.0	48.3 ± 4.8
STZ-diabetic			
Nontreated ($n = 25$)	$0.1\pm0.1^\S$	$1.9\pm0.2^\S$	$12.1\pm1.7^\S$
Vanadate treated ($n = 10$)	$5.2\pm0.9^*$	$34.7\pm2.3^\ddagger$	$65.5\pm4.5^\ddagger$

Glucokinase and piruvate kinase activities were determined as indicated in Methods. Results are expressed as means$\pm$SEM for the number of animals in parentheses and the significance of differences was tested by Student's *t* test: *$P < 0.05$, $\ddagger P < 0.01$, $\S P < 0.001$ vs. nontreated controls.

Figure 3. Effects of vanadate treatment on kidney PEPCK mRNA concentrations. (*A*) The concentration of mRNA for PEPCK was determined by Northern analysis of total RNA isolated from the kidney of control rats (*C*), diabetic rats (*D*), and diabetic rats treated with vanadate (*DV*). Each experimental group consisted of 15 animals. The data are expressed as a percentage of the nontreated control animals and results are mean$\pm$SEM. (*B*) Northern blots from two representative animals of each group are shown as described in Fig. 2.
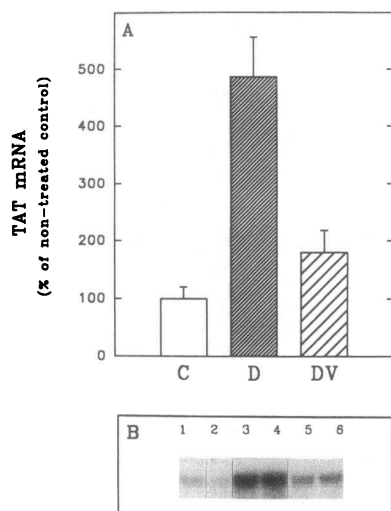
Figure 4. Effects of vanadate treatment on liver TAT mRNA levels. (*A*) The concentration of mRNA for TAT was determined by Northern analysis of total RNA isolated from the liver of control rats (*C*), diabetic rats (*D*), and diabetic rats treated with vanadate (*DV*). Each experimental group consisted of 15 animals. The data are expressed as a percentage of the nontreated control animals and results are mean±SEM. (*B*) Northern blots from two representative animals of each group (C, lanes *1* and *2*; D, lanes *3* and *4*; DV, lanes *5* and *6*) are shown.

Figure 6. Effects of vanadate treatment on liver mitochondrial HMGCoAS mRNA concentrations. (*A*) The concentration of mRNA for HMGCoAS was determined by Northern analysis of total RNA isolated from the liver of control rats (*C*), diabetic rats (*D*), and diabetic rats treated with vanadate (*DV*). Each experimental group consisted of 15 animals. The data are expressed as a percentage of the nontreated control animals and results are mean±SEM. (*B*) Northern blots from two representative animals of each group are presented as described in the Fig. 2 legend.

a 220±45% induction in GLUT-2 gene expression, vanadate treatment of diabetic rats reduced the mRNA concentrations to those detected in the liver of healthy animals (85±18%). No changes of the GLUT-2 mRNA concentrations were noted in control animals treated with vanadate compared with control nontreated animals (data not shown).

Effects of vanadate on mitochondrial HMGCoAS gene expression. As already described in Table I, vanadate treatment provoked a 75% reduction in the increased levels of D-3-hydroxybutyrate caused by diabetes. Thus, we next investigated the effect of vanadate treatment on the expression of the mitochondrial HMGCoAS gene. Fig. 6 shows that although diabetes produced an increase (270±52% over the control nontreated rats) in specific HMGCoAS mRNA concentrations, vanadate administration to diabetic rats caused a decrease of these concentrations (to 120±28% of nontreated control animals), which resulted in a normalization of the expression of the gene in these animals.

Effects of vanadate administration to the C/EBPα gene expression. To investigate a role of C/EBPα in the regulation of the expression of the genes involved in key steps in the regulation of carbohydrate and ketone bodies metabolism, we further analyzed the expression of this gene in the liver of diabetic and diabetic vanadate-treated rats. Fig. 7 shows that although vanadate treatment did not produce any significant effect on C/EBPα mRNA concentrations in control rats, diabetes caused an induction in the expression of liver C/EBPα (142±28% of nontreated control animals). However, when the animals were treated with vanadate, a decrease of C/EBPα mRNA concentrations was detected compared with diabetic rats. The concentration of C/EBPα mRNA reached in diabetic treated animals was lower than in control nontreated animals (70±14%).

Discussion

In this study, we have investigated the role of vanadate in restoring the normal metabolic state in diabetic animals. In the last few years, many reports have been available regarding the insulin-like actions of vanadate in regulating glucose metabolism either in vivo (33–37) or in vitro (38–44). Our results indicate
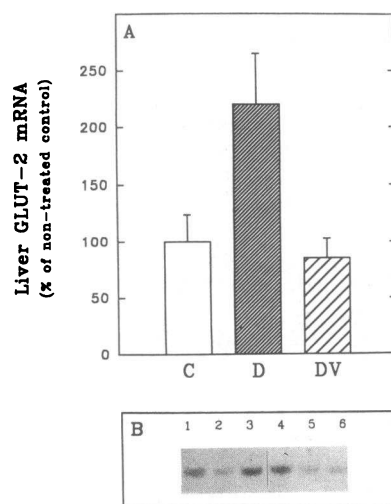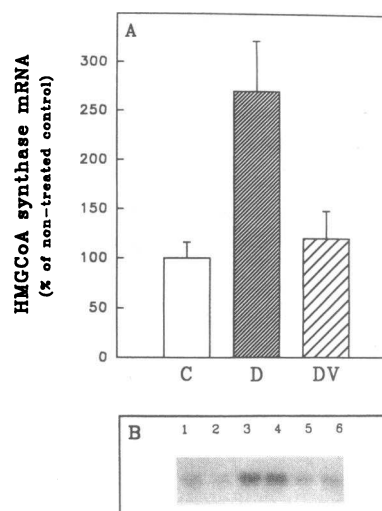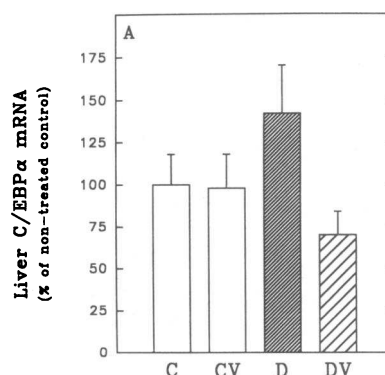

Figure 5. Effects of vanadate treatment on liver GLUT-2 mRNA concentrations. (*A*) The concentration of mRNA for GLUT-2 was determined by Northern analysis of total RNA isolated from the liver of control rats (*C*), diabetic rats (*D*), and diabetic rats treated with vanadate (*DV*). Each experimental group consisted of 15 animals. The data are expressed as a percentage of the nontreated control animals and results are mean±SEM. (*B*) Northern blots from two representative animals of each group (C, lanes *1* and *2*; D, lanes *3* and *4*; DV, lanes *5* and *6*) are shown.

Figure 7. Effects of vanadate treatment on liver C/EBPα transcription factor mRNA levels. The level of mRNA for C/EBPα was determined by Northern analysis of total RNA isolated from the liver of control rats (*C*) (*n* = 25), control rats treated with vanadate (*CV*) (*n* = 10), diabetic rats (*D*) (*n* = 25), and diabetic rats treated with vanadate (*DV*) (*n* = 25). The data are expressed as a percentage of nontreated control animals and results are mean±SEM.
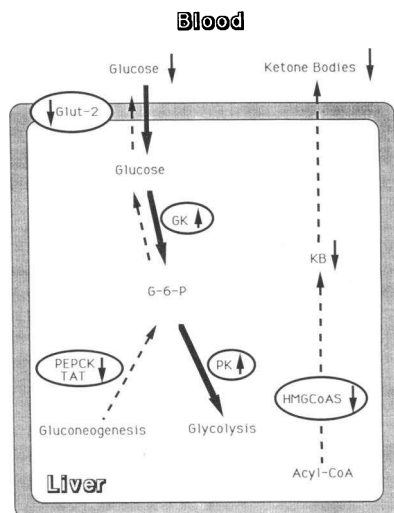
Blood

Figure 8. Schematic representation of the pathways of hepatic glucose and ketone bodies metabolism. The dark arrows indicate the pathways activated by vanadate. The broken-line arrows depict the pathways inhibited by vanadate. *KB,* ketone bodies; *GK,* glucokinase; *PK,* pyruvate kinase; *HMGCoAS,* HMGCoA synthase.

that vanadate is also able to normalize blood ketone bodies levels, which results in a decrease of the acidosis state in the diabetic animal. In addition, SALT activity was normalized after vanadate treatment. Presumably, an initial increase in SALT activity could be an indication of liver damage arising from the toxic effects of the streptozotocin injection. However, 3 wk after the injection of the drug, the metabolic acidosis might provoke hepatic damage, which would induce the high levels of SALT activity that we have detected in diabetic rats.

These metabolic effects of vanadate could probably be the result of changes in the expression of genes coding for regulatory enzymes, as is shown in the present study. A decrease of glucose production could be due to the inhibition of PEPCK gene expression. Vanadate decreased PEPCK mRNA levels in livers of diabetic rats, whereas it did not provoke any specific effect in normal animals (data not shown). Vanadate counteracted the effects mediated by cAMP in the liver, probably acting through regulatory elements on the PEPCK promoter that respond to cAMP. We have previously shown sequences responsive to vanadate between −109 and −68 on the 5′ flanking region of the PEPCK gene (45). The most likely target for vanadate in that region is cAMP regulatory element-1, which maps from −91 to −84 (51). This indicates a major difference in the site of action of vanadate and insulin, since an insulin-responsive element has been located between −416 and −407 on the PEPCK promoter (67). However, the synthesis of this enzyme in the rat kidney is not responsive to cAMP (26, 27) and its mRNA abundance is not altered by cAMP or starvation (68). The injection of triamcinolone, as well as the induction of metabolic acidosis by NH_4Cl, caused a marked enhancement of renal PEPCK mRNA (27, 68). In diabetic animals we observed a fivefold induction in the expression of this gene in the kidney, probably because of the increase of blood 3-hydroxybutyrate, resulting from the activation of the HMGCoAS in the liver of diabetic rats (69, 70). In addition, the expression of HMGCoAS is induced during diabetes (32). Vanadate treatment decreased HMGCoAS mRNA concentrations caused by diabetes to levels observed in healthy animals. Vanadate treatment was shown to be able to counteract the increase in kidney PEPCK mRNA levels, probably as a result of either the reduction of ketone bodies levels and the normalization of

the body acid–base balance and/or a direct effect on the PEPCK promoter. However, no data are available regarding the action of vanadate on the PEPCK promoter in the kidney.

In our study, the expression of the gene for TAT, an enzyme also related with gluconeogenesis, was regulated like PEPCK. TAT gene expression is restricted to parenchymal cells of the liver (71) and it is activated by glucocorticoids and glucagon (60–62). TAT expression is undetectable during gestation but increases rapidly within the first hours after birth as a result of the increase in the glucagon/insulin ratio in newborn animals (72). The increase in this ratio could be the cause of the high expression of the TAT genes in diabetes. Vanadate treatment was able to block the induction of TAT mRNA levels, suggesting that vanadate may interfere with the cAMP-mediated activation of the TAT promoter. However, no data are available regarding the effects of vanadate on specific transcription factors involved in the control of this promoter.

Like insulin, vanadate not only decreases gene expression, it is also able to induce the expression of the genes for glucokinase and pyruvate kinase in primary cultured hepatocytes (46). The results presented in this paper indicate that vanadate treatment also induces the expression of these genes in the intact animal, which is impaired by the diabetic process. However, no changes in the expression of both genes are detected in healthy rats treated with vanadate. Furthermore, vanadate treatment of diabetic rats also causes an induction in glucokinase and pyruvate kinase mRNA concentrations. This effect is also found in cultured hepatocytes, where the induction of these genes is higher in the presence of glucose (46). Blood glucose levels detected in vanadate-treated diabetic animals are 1.5-fold higher than in controls, which might be responsible for the increase in the expression of both genes. Despite several reports describing glucokinase and pyruvate kinase gene promotor regulatory regions, the mechanism by which vanadate regulates the expression of both genes is still not known and responsive elements to this effector have not yet been described. The effects of vanadate on glucokinase and pyruvate kinase mRNA concentrations are directly related with glucokinase and pyruvate kinase activities. These results are in agreement with previous reports that indicate that vanadate administration to diabetic rats restores 6-phosphofructo-2-kinase and glucokinase activities (34).

Regarding glucose transport, the liver plays a dual role in that glucose uptake occurs from circulation when gluconeogenesis and glycogenolysis are low, but it releases glucose when gluconeogenesis and glycogenolysis are activated, such as in the insulin-deficient diabetic state. Diabetic rats presented an increase in the amount of protein and mRNA for GLUT-2 (66). Insulin treatment of these animals decreased the amount of protein and mRNA toward levels observed in nondiabetic animals (66). A great efflux of glucose occurs from the liver in the absence of insulin and the glucose transporter plays an important role in this condition. However, it has recently been suggested that the increase in GLUT-2 mRNA levels observed in a diabetic liver could be attributed to high glucose concentrations rather than to insulin deficiency (73).

It has been suggested that regulation of energy balance could be channeled through the liver-enriched transcription factor C/EBPα. The concentration of C/EBPα increased dramatically in the liver of the fetal rat 3 d before birth (74). The expression of PEPCK (75) and TAT (73) genes starts a few

hours after birth. Moreover, C^{14CoS} albino-mutant mice, which lack the "hepatocyte-specific developmental regulation" (*hsdr-1*) locus on chromosome 7, showed a strong reduction of transcription rates of the C/EBPα gene shortly before birth and failed to activate a group of hepatic enzymes perinatally (54, 76). One subset of such affected genes includes PEPCK, serin dehydratase, glucose 6-phosphatase, TAT, and aldolase B, which are involved in gluconeogenesis. These mice died a few hours after birth, presumably as a consequence of hypoglycemia. C/EBPα has been shown to transactivate PEPCK in vitro (54, 76) and our results indicated that this transcription factor was induced by diabetes, suggesting that C/EBPα could affect PEPCK gene expression in vivo. Similarities were found between C/EBPα and PEPCK, HMGCoAS, and GLUT-2 genes regarding their pattern of expression. Vanadate may act on these subsets of genes involved in energy metabolism at least in part through the regulation of C/EBPα. The observations that C/EBPα avidly binds to cAMP response element-1 (CRE-1) on the PEPCK promoter, which overlaps the sequence described to be responsive to vanadate (42), and that there is an inhibition of both C/EBPα and PEPCK gene expression in diabetic rats after vanadate administration, suggested that vanadate may act on the expression of the PEPCK gene through modifications of the C/EBPα transcription factor in vivo.

In conclusion, the increase of the glucokinase and L-pyruvate kinase mRNA concentrations and the inhibition of PEPCK gene expression may contribute to the decrease of blood glucose levels observed after oral treatment with vanadate. As summarized in Fig. 8, the induction of glucokinase will augment glucose entry into metabolic pathways. The rise of L-pyruvate kinase will allow the use of the newly synthetized glucose 6-phosphate through glycolysis. The inhibition of PEPCK will block liver glucose production, dramatically increased during diabetes, which correlates with the decrease in GLUT-2 expression. On the other hand, the inhibition of HMGCoAS gene expression will result in a decrease on ketone bodies production, which is reflected in the reduction of blood D-3-hydroxybutyrate levels. Finally, vanadate might restore the carbohydrate and ketone bodies metabolism at least in part through the regulation of specific transcription factors, including members of the C/EBP family involved in the control of energy metabolism.

Acknowledgments

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