Intrinsic Gluconeogenesis Is Enhanced in Renal Proximal Tubules of Zucker Diabetic Fatty Rats

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Recent studies indicate that renal gluconeogenesis is substantially stimulated in patients with type 2 diabetes, but the mechanism that is responsible for such stimulation remains unknown. Therefore, this study tested the hypothesis that renal gluconeogenesis is intrinsically elevated in the Zucker diabetic fatty rat, which is considered to be an excellent model of type 2 diabetes. For this, isolated renal proximal tubules from diabetic rats and from their lean nondiabetic littermates were incubated in the presence of physiologic gluconeogenic precursors. Although there was no increase in substrate removal and despite a reduced cellular ATP level, a marked stimulation of gluconeogenesis was observed in diabetic relative to nondiabetic rats, with near-physiologic concentrations of lactate (38%), glutamine (51%) and glycerol (66%). This stimulation was caused by a change in the fate of the substrate carbon skeletons resulting from an increase in the activities and mRNA levels of the key gluconeogenic enzymes that are common to lactate, glutamine, and glycerol metabolism, *i.e.*, mainly of phosphoenolpyruvate carboxykinase and, to a lesser extent, of glucose-6-phosphatase and fructose-1,6-bisphosphatase. Experimental evidence suggests that glucocorticoids and cAMP were two factors that were responsible for the long-term stimulation of renal gluconeogenesis observed in the diabetic rats. These data provide the first demonstration in an animal model that renal gluconeogenesis is upregulated by a long-term mechanism during type 2 diabetes. Together with the increased renal mass (38%) observed, they lend support to the view so far based only on *in vivo* studies performed in humans that renal gluconeogenesis may be stimulated by and crucially contribute to the hyperglycemia of type 2 diabetes.

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Type 2 diabetes is a heterogeneous metabolic disorder that is becoming a global epidemic with major health consequences. It is characterized by defects in both insulin secretion and tissue sensitivity to insulin (1,2), leading to hyperglycemia. It is well accepted that part of this hyperglycemia results from the abnormal persistence of hepatic glucose production during the absorptive state and by an increase to excessive levels of hepatic glucose production in the postabsorptive state (3). Thus, an elevated glucose production in type 2 diabetes has until recently be generally attributed exclusively to the liver. This is not necessarily justified because it is well established that, on a gram-for-gram basis, the kidney can synthesize glucose at rates several times higher than those observed in the liver (4). In line with this, studies that were performed *in vivo* during the past decade suggested that the synthesis of glucose by the kidneys of postabsorptive humans may explain 5 to 25% of the total glucose formed by gluconeogenesis in the body (5–8). Human renal gluconeogenesis has also been demonstrated to be almost equal to hepatic gluconeogenesis in obese patients who undergo a prolonged fasting (9); moreover, this metabolic process has been shown to be stimulated in patients with type 2 diabetes (10,11) and inhibited by insulin (8,13,14). Despite the latter observations and the potential pathophysiologic importance of renal gluconeogenesis in type 2 diabetes, an intrinsic stimulation of renal gluconeogenesis to our knowledge has not been reported in any animal model of type 2 diabetes. This lack of an appropriate model that spontaneously displays increased renal gluconeogenesis so far has represented a significant limitation in the understanding of the mechanisms by which the kidney might be involved in the hyperglycemia observed in type 2 diabetes.

Therefore, to test the hypothesis that intrinsic renal gluconeogenesis is stimulated in type 2 diabetes, we conducted a study in which renal gluconeogenesis in Zucker diabetic fatty (ZDF) rats, an excellent model for the study of type 2 diabetes (15,16), was compared with that in lean nondiabetic Zucker rats. For this, isolated renal proximal tubules, the exclusive nephron segments that contain the key gluconeogenic enzymes (17,18), were isolated and incubated with lactate or glutamine or glycerol, three physiologic substrates that are taken up by the human and rat kidney *in vivo* (19–21). We also measured the activities of the key gluconeogenic enzymes that are common to lactate, glutamine, and glycerol gluconeogenesis and the expression of the genes that code for these enzymes. In addition, we attempted to identify factors that are responsible for the changes observed.

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Materials and Methods

Reagents

L-Glutamine, glycerol, and glutaminase (grade V) were from Sigma (Saint Quentin-Fallavier, France). L-Lactate, other enzymes, coenzymes, and oligo dT were purchased from Roche (Meylan, France). Superscript II reverse transcriptase (RT), platinum Taqpolymerase, and dNTP were obtained from Invitrogen (Pontoise, France). The mRNA extraction kit was purchased from Dynal (Oslo, Norway), and primers were obtained from Genset SA (Paris, France). L-[U-^{14}C]lactate (5.62 GBq/mmol) was from Amersham (Little Chalfont, UK).

Rats

All experiments were approved by the Institutional Animal Care and Use Committee of the Lyon 1 University. Male ZDF rats (ZDF/Gmi: fa/fa) and lean control rats (ZDF/Gmi-lean:fa/+ or +/+) were obtained from Charles River (Saint Germain sur l'Arbresle, France) at 13 wk of age. They were given a Purina 5008 Chow (IPS Product Supplies Inc., London, England) *ad libitum* and had free access to water. For testing their possible role in regulating renal gluconeogenic enzyme activities in diabetic rats, either dexamethasone or cAMP (25 or 50 mg/kg body wt, respectively) was injected intraperitoneally in nondiabetic rats either 6 or 18 h before the preparation of isolated kidney tubules. The control, nondiabetic rats were treated intraperitoneally with the vehicle (tricaprylin in the case of dexamethasone and saline in the case of cAMP).

Preparation of Renal Proximal Tubules and Incubations

At 14 to 17 wk of age, the fed rats were anesthetized with sodium pentobarbital (35 mg/kg intraperitoneally). After catheterization of a carotid artery, blood was withdrawn for measurement of blood acid-base parameters with a blood microsystem acid base analyzer (model BMS 3; Radiometer, Copenhagen, Denmark) and blood ketone-body levels as described previously (21); plasma was also separated by centrifugation at 4°C for measurement of cAMP and corticosterone levels. Then, the kidneys were removed and weighed and placed in ice-cold Krebs-Henseleit medium. Renal proximal tubules were prepared by collagenase treatment of renal cortex slices as described by Baverel *et al.* (22). Incubations were performed for 60 min in 25-ml stoppered Erlenmeyer flasks in an atmosphere of O_2/CO_2 (19:1). Tubules were incubated in 4 ml of Krebs-Henseleit medium (24) with or without 1 and 2 mM lactate or glutamine and 0.5 and 2 mM glycerol. The flasks were prepared in duplicate for all experimental conditions. Incubation was stopped by adding perchloric acid (3% [vol/vol] final concentration) to each flask. In all experiments, zero-time flasks with and without substrates were prepared by adding perchloric acid before the tubules. After removal of the denatured protein by centrifugation, the supernatant was neutralized with 20% (wt/vol) KOH for metabolite determination. For testing their influence, insulin and glucose were added to the medium of tubules from diabetic and nondiabetic rats incubated for 60 min with 1 mM [U-^{14}C]lactate (approximately 2000 Bq/flask); in these experiments, incubation and collection of the $^{14}CO_2$ that was formed were carried out as described previously (24).

Metabolite Assays

All of the metabolites studied were determined by the methods described by Passonneau and Lowry (25). The ^{14}C-glucose that was formed from [U-^{14}C]lactate was isolated as described by Katz *et al.* (26) and counted by liquid scintillation. The dry weight of the tubules that were added to each flask and blood metabolites and acid-base parameters were determined as described previously (21,22). Plasma cAMP and corticosterone levels were measured by competitive immunoassay kits provided by R&D Systems Europe (Lille, France).

Measurement of Enzyme Activities

Preparation of renal proximal tubule homogenates and measurement of phosphoenolpyruvate carboxykinase (EC 4.1.1.32), fructose-1,6-bisphosphatase (EC 3.1.3.11), and glucose-6-phosphatase (EC 3.1.3.9) were conducted by methods previously described in detail (27).

Semiquantitative Analysis of mRNA Expression

To determine the glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, and fructose-1,6-bisphosphatase mRNA levels in the kidney cortex of diabetic rats and their control littermates, we performed semiquantitative RT-PCR as described previously (27). Levels of each mRNA of interest were related to those of the housekeeping β-actin gene transcripts. Gene-specific oligonucleotide primers (20 nucleotides) were selected from the published cDNA sequences of mouse and rat glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, and fructose-1,6-bisphosphatase. As an internal control, a primer pair was selected from the cDNA sequence of rat β-actin. Forward and reverse primers that were chosen for glucose-6-phosphatase and phosphoenolpyruvate carboxykinase were those that were used for the mouse kidney in a previous study (27). The fructose-1,6-bisphosphatase (accession no. M86240) primers were as sense 5′-TGTTTTGATCCCCTC-GATGG-3′ and antisense 5′-TCCAGCATGAAGCAGTTGAC-3′. The β-actin (accession no. NM_031144) primers were sense 5′-GAAGTGT-GACGTTGACATCC-3′ and antisense 5′-AATCTCCTTCTGCATC-CTGT-3′, giving a PCR product of 103 bp. The β-actin was amplified for 24 cycles with glucose-6-phosphatase, 23 cycles with phosphoenolpyruvate carboxykinase, and 22 cycles with fructose-1,6-bisphosphatase, each cycle using the following parameters: 94°C for 30 s, 60°C for 45 s, and 72°C for 60 s. The linearity of amplification was verified in each experiment. PCR products were separated electrophoretically on a 2% (wt/vol) agarose gel (Invitrogen, Paisley, Scotland) for glucose-6-phosphatase and fructose-1,6-bisphosphatase or on a 2% (wt/vol) agarose −1000 gel for phosphoenolpyruvate carboxykinase (Invitrogen) and stained with SYBR Green I (Molecular Probes Europe, Leiden, The Netherlands). The gel then was scanned using a fluorescence laser scanner (Molecular Dynamics, Sunnydale, CA). Band intensities were quantified using the Image Quant software. mRNA levels are reported relative to β-actin.

Statistical Analyses

Net substrate utilization and product formation by kidney tubules were calculated as the difference between the total contents of the flask (tissue + medium) at the start (zero-time incubations) and after 1 h of incubation. The metabolic rates are expressed in nanomoles of substance removed or produced per hour per milligram of tubule dry weight, and the enzyme activities are reported as micromoles of substrate used or product formed per milligram of tubule protein per hour; they are given as means ± SEM. The conversion of [U-^{14}C]lactate into ^{14}C-glucose was calculated by dividing the radioactivity in glucose by the specific radioactivity of ^{14}C-lactate. The results were analyzed by the *t* test for unpaired data, comparing values obtained in diabetic with those in nondiabetic rats.

Results

As shown in Table 1, the body weight of the nondiabetic rats was slightly but significantly lower than that of the diabetic animals. Striking, the weight of the two kidneys of the diabetic rats exceeded by 38% that of the nondiabetic rats. Table 1 also

Table 1. Baseline characteristics of nondiabetic lean Zucker rats and ZDF rats

	Nondiabetic	Diabetic
Body weight (g)	369 ± 7 (n = 11)	398 ± 13[b] (n = 10)
Weight of the two kidneys	2.6 ± 0.1 (n = 11)	3.6 ± 0.1[b] (n = 10)
Blood glucose concentration (mM)	8.4 ± 0.3 (n = 11)	28.3 ± 1.0[b] (n = 10)
Blood pH	7.38 ± 0.02 (n = 4)	7.37 ± 0.02 (n = 4)
Plasma bicarbonate (mM)	26.3 ± 1.2 (n = 4)	25.4 ± 1.9 (n = 4)
Blood β-hydroxybutyrate concentration (mM)	0.17 ± 0.01 (n = 4)	0.37 ± 0.20[b] (n = 4)
Blood acetoacetate concentration (mM)	0.11 ± 0.01 (n = 4)	0.32 ± 0.11[b] (n = 4)
Plasma cAMP concentration (nM)	10 ± 2 (n = 6)	18 ± 2[b] (n = 6)
Plasma corticosterone concentration (ng/ml)	368 ± 44 (n = 5)	674 ± 69[b] (n = 5)

[a] Values are means ± SEM. for the number (n) of rats shown in parentheses. ZDF, Zucker Diabetic Fatty.

[b] $P < 0.05$ nondiabetic *versus* diabetic, unpaired t test.

shows that there was no metabolic acidosis in the diabetic rats despite their significantly higher blood concentrations of both β-hydroxybutyrate and acetoacetate. It is interesting that the plasma cAMP and corticosterone levels were markedly elevated (80 and 84%, respectively) in diabetic rats when compared with nondiabetic rats.

Metabolism of Lactate, Glutamine, and Glycerol in Renal Proximal Tubules from Lean Zucker Rats and ZDF Rats

Table 2 shows that the removal of lactate but not glutamine and glycerol was inhibited in tubules from diabetic rats when compared with that in tubules from control rats. With lactate, glutamine, and glycerol as substrate, there was no change in the accumulation of pyruvate, glutamate, and lactate, respectively. Striking, mean glucose synthesis was stimulated by 38% from lactate, 51% from glutamine, and 66% from glycerol, despite a fall of cellular ATP levels. Accumulation of intermediates of neither the tricarboxylic acid cycle nor aspartate, alanine, 3-glycerophosphate, or glycogen was observed in any of the experimental conditions used. Assuming that all of the products that were found to accumulate arose from added substrates, the complete oxidation of lactate, glutamine, and glycerol, which can be estimated by carbon balance, was greatly reduced—even suppressed in the case of glycerol—in tubules from diabetic rats.

At zero-time, the tubules from five control rats and those from four diabetic rats contained 1.5 ± 1.1 and 3.8 ± 0.7 nmol/mg dry wt glycogen (in glycosyl equivalents), respectively (NS). After 60 min of incubation in the presence of 1 mM lactate, the glycogen content was 2.2 ± 1.1 and 3.5 ± 0.7 nmol/mg dry wt, respectively (NS). Glucose synthesis in the absence of exogenous substrate was 6 ± 1 and 15 ± 1 nmol/mg dry wt per h (114%; $P < 0.01$) in tubules from control (n = 16)

Table 2. Metabolism of 1 mM lactate and glutamine and of 0.5 mM glycerol in isolated renal proximal tubules from nondiabetic lean Zucker rats and ZDF rats[a]

Substrate	Rats	Amount of Tubules per Flask (mg dry wt)	ATP Concentration (nmol/mg dry wt)	Metabolite Removal (−) or Production			Carbon Balance
				Lactate	Pyruvate	Glucose	
Lactate	Nondiabetic (n = 11)	5.3 ± 0.4	12.6 ± 2 (n = 4)	−406 ± 26	34 ± 6	45 ± 4	282 ± 22
	Diabetic (n = 7)	6.8 ± 0.9	7.1 ± 0.9[b] (n = 4)	−311 ± 16[b]	14 ± 11	62 ± 5[b]	173 ± 6[b]
				Glutamine	Glutamate	Glucose	
Glutamine	Nondiabetic (n = 11)	5.3 ± 0.4	12.1 ± 2.4 (n = 4)	−298 ± 18	110 ± 7	41 ± 4	106 ± 10
	Diabetic (n = 10)	6.8 ± 0.8	7.1 ± 0.9[b] (n = 4)	−243 ± 17	85 ± 11	62 ± 6[b]	34 ± 14[b]
				Glycerol	Lactate	Glucose	
Glycerol	Nondiabetic (n = 5)	5.3 ± 0.7	11.8 ± 2.1 (n = 4)	−130 ± 13	18 ± 1	44 ± 6	24 ± 14
	Diabetic (n = 5)	6.2 ± 0.3	5.8 ± 1.2[b] (n = 4)	−159 ± 16	26 ± 3	73 ± 7[b]	−13 ± 7[b]

[a] Results (nmol/mg dry wt per h) are reported as means ± SEM for the number (n) of experiments given in parentheses. With lactate, glutamine, and glycerol as substrate, carbon balance was calculated as the difference between the substrate removed on the one hand and the sum of twice the glucose produced and the pyruvate or glutamate or lactate accumulated on the other hand, respectively.

[b] $P < 0.05$ nondiabetic *versus* diabetic rats, unpaired t test.

Table 3. Effect of insulin and glucose on the metabolism of 1 mM [U-^{14}C]lactate in isolated renal proximal tubules from nondiabetic lean Zucker rats and ZDF rats[a]

Experimental Condition	Rats	Glucose Produced	[U-^{14}C]Lactate Converted into ^{14}C-Glucose	Percentage of Radioactive Glucose Synthesized
1 mM [U-^{14}C]lactate	Nondiabetic	39 ± 1	54 ± 3	69 ± 6
	Diabetic	59 ± 3[b]	73 ± 7[b]	62 ± 3
1 mM [U-^{14}C]lactate + 100 nM insulin	Nondiabetic	38 ± 2	54 ± 2	71 ± 5
	Diabetic	60 ± 4[b]	74 ± 6[b]	62 ± 3
1 mM [U-^{14}C]lactate + 5 mM glucose	Nondiabetic	—	55 ± 2	—
	Diabetic	—	75 ± 7[b]	—
1 mM [U-^{14}C]lactate + 25 mM glucose	Nondiabetic	—	52 ± 3	—
	Diabetic	—	75 ± 7[b]	—

[a] Kidney tubules (5.8 ± 0.8 and 8.6 ± 0.4 mg dry wt per flask for nondiabetic and diabetic rats, respectively) were incubated as described in the Materials and Methods section. Results (nmol/mg dry wt per h) are reported as means ± SEM for four experiments with nondiabetic rats and four experiments with diabetic rats. The percentage (means ± SEM) of radioactive glucose synthesized was calculated as half the [U-^{14}C]lactate converted into ^{14}C-glucose divided by the glucose produced.

[b] $P < 0.05$ nondiabetic *versus* diabetic rats, unpaired *t* test.

and diabetic ($n = 15$) rats, respectively. After 60 min of incubation without any exogenous substrate, the glycogen content of these tubules was 1.1 ± 0.6 and 2.4 ± 0.5 nmol/mg dry wt, respectively (NS).

In tubules from diabetic rats when compared with those from nondiabetic rats, glucose synthesis from 2 mM lactate, glutamine, and glycerol was stimulated by 69, 56, and 50%, respectively. The corresponding values were 91 ± 10 ($n = 10$) *versus* 54 ± 4 ($n = 11$) nmol/mg dry wt per h ($P < 0.05$), 81 ± 8 ($n = 10$) *versus* 52 ± 5 ($n = 11$) nmol/mg dry wt per h ($P < 0.05$), and 78 ± 1 ($n = 5$) *versus* 52 ± 3 ($n = 5$) nmol/mg dry wt per h ($P < 0.05$).

Effect of Insulin and Glucose on Conversion of ^{14}C-Lactate into ^{14}C-Glucose

Table 3 shows that addition of 100 nM insulin or 5 or 25 mM glucose did not alter the conversion of [U-^{14}C]lactate into ^{14}C-glucose in tubules either from nondiabetic or from diabetic rats. It also shows that, irrespective of the experimental condition, such conversion was higher in tubules from diabetic than in tubules from nondiabetic rats. As seen in Table 2, glucose production was also higher in tubules from diabetic than in those from nondiabetic rats (Table 3). In the presence of 1 mM [U-^{14}C]lactate plus 5 or 25 mM glucose, it was not possible to measure enzymatically the glucose synthesized from lactate in a reliable manner because of the high glucose concentration. For the same reason, no substantial glucose removal could be measured enzymatically with 5 and 25 mM glucose as sole substrate, and lactate accumulation was negligible (results not shown).

Activities of Phosphoenolpyruvate Carboxykinase, Fructose-1,6-Bisphosphatase, and Glucose-6-Phosphatase in Renal Proximal Tubules from Lean Zucker Rats and ZDF Rats

Table 4 shows that diabetes stimulated in a statistically significant manner the activity of the three key gluconeogenic enzymes that are common to lactate and glutamine gluconeogenesis; it greatly stimulated the activity of phosphoenolpyruvate carboxykinase (2.2-fold) and, to a much lesser extent, those of glucose-6-phosphatase (1.4-fold) and of fructose-1,6-bisphosphatase (1.2-fold).

Phosphoenolpyruvate Carboxykinase, Fructose-1,6-Bisphosphatase, and Glucose-6-Phosphatase mRNA Levels in the Renal Cortex from Lean Zucker Rats and ZDF Rats

Figure 1 clearly shows that a statistically significant elevation of the renal cortical cellular levels of phosphoenolpyruvate carboxykinase (3.0-fold increase), glucose-6-phosphatase (2.3-fold increase), and fructose-1,6-bisphosphatase (1.5-fold in-

Table 4. Activity of key gluconeogenic enzymes that are common to lactate and glutamine gluconeogenesis in renal proximal tubules from nondiabetic lean Zucker and ZDF rats[a]

Rats	Phosphoenolpyruvate Carboxykinase	Fructose-1,6-Bisphosphatase	Glucose-6-Phosphatase
Nondiabetic ($n = 5$)	1.1 ± 0.1	2.0 ± 0.1	5.1 ± 0.1
Diabetic ($n = 6$)	2.4 ± 0.1[b]	2.4 ± 0.1[b]	7.1 ± 0.4[b]

[a] Values, expressed in μmol/mg protein per h, are presented as means ± SEM for the number (n) of experiments given in parentheses.

[b] $P < 0.05$ nondiabetic *versus* diabetic rats, unpaired *t* test.

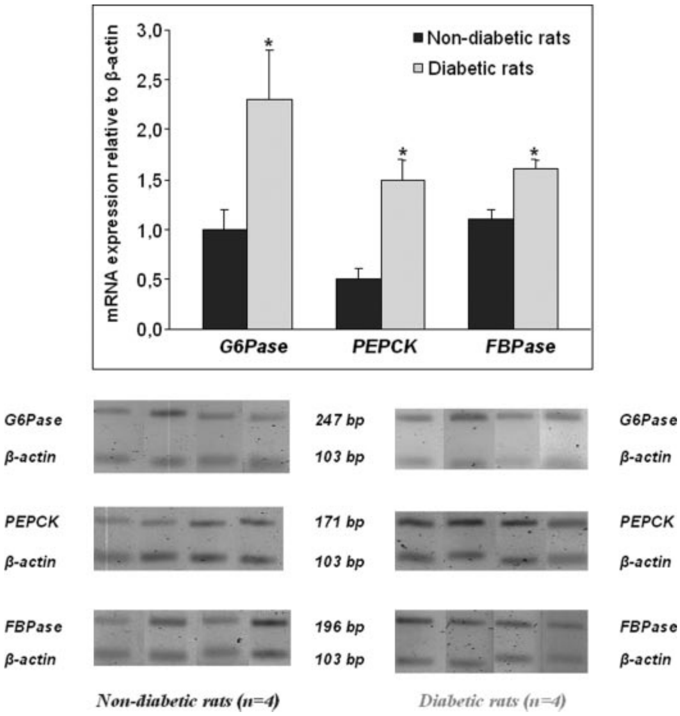

Figure 1. mRNA levels of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (FBPase) in diabetic rats and their nondiabetic littermates. The mRNA levels were analyzed by semi-quantitative reverse transcription–PCR analysis, as described in Materials and Methods. The amplified cDNA were separated by agarose-gel electrophoresis, as shown in the lower part of the figure. Band intensities were quantified and are reported relative to the β-actin band. The values are means ± SEM for four diabetic rats and four nondiabetic rats. The statistical difference between diabetic and lean rats was measured by the unpaired *t* test: $^*P < 0.05$.

crease) mRNA occurred in diabetic rats when compared with those in control animals.

Effect of Dexamethasone and Dibutyryl-cAMP Administration on Activity of Key Gluconeogenic Enzymes in Renal Proximal Tubules from Nondiabetic Lean Zucker Rats

Table 5 shows that the intraperitoneal administration of 25 mg/kg body wt dexamethasone 18 h earlier stimulated the activity of phosphoenolpyruvate carboxykinase (63%) and, to a much lesser extent, of glucose-6-phosphatase (12%) but not that of fructose-1,6-bisphosphatase. Therefore, at least one other factor was responsible for the stimulation of the activity of the last enzyme. It is interesting that the activities of fructose-1,6-bisphosphatase and glucose-6-phosphatase were increased by 21 and 19%, respectively, in renal proximal tubules of lean rats at the same time point (18 h) after an intraperitoneal injection with 50 mg/kg body wt dibutyryl cAMP (Table 5); under the same conditions, the activity of phosphoenolpyruvate carboxykinase was only slightly (9%) but in a statistically significant manner stimulated in these tubules (Table 5). Given its weak effect on phosphoenolpyruvate carboxykinase activity, the effect of cAMP was studied 6 h after its injection; this resulted in a much more pronounced stimulation of the three enzyme activities (Table 5).

Discussion

Unlike type-2 diabetic patients *in vivo* (10,11), no animal model of type 2 diabetes has been reported until now to display an increased renal gluconeogenesis. To our knowledge, this study is the first to demonstrate such an elevation *in vitro* from three physiologic substrates that are taken up by the human and rat kidney *in vivo* (19–21).

Biochemical and Molecular Mechanisms for Long-Term Stimulation of Renal Gluconeogenesis in Diabetic Zucker Rats

Increased renal gluconeogenesis in diabetic rats was due to a change in the fate of lactate, glutamine, and glycerol carbon skeleton; indeed, gluconeogenesis was favored at the expense of complete oxidation (Table 2). It should be emphasized that increased glucose synthesis in tubules from diabetic rats was confirmed by using radioactive lactate as substrate even in the presence of a high glucose concentration (Table 3). Note that the distribution of lactate between glucose synthesis and oxidation that was observed in this work was similar to that found by Krebs *et al.* (28) in one study but different from what they reported in another study (29). It is also important to underline that lactate, glutamine, and glycerol gluconeogenesis was greater in tubules from diabetic rats despite a lower concentration of ATP in these tubules when compared with those of control rats. Because ATP is a key compound in gluconeogenesis, its lower availability was compensated by other mechanisms that led to a stimulation of glucose synthesis. Note that the fall in ATP concentration, which can be only a consequence but not the cause of the increased gluconeogenesis observed in these tubules, is in agreement with the diminution of substrate oxidation.

In agreement with the elevation of renal gluconeogenesis in our diabetic rats, we found a stimulation of the three key gluconeogenic enzymes that are common to gluconeogenesis from both lactate and glutamine. In keeping with the stimulation of the activities of fructose-1,6-bisphosphatase and glucose-6-phosphatase, the synthesis of glucose from glycerol was also found to be greatly stimulated. It is interesting that the elevations of the corresponding mRNA levels in the renal cortex of diabetic rats establish that the stimulation of the activities of the three enzymes mentioned above occurred by a long-term regulation involving the upregulation of the genes that code for these enzymes. Note here that the expression of the β-actin gene, which we used as a reference for the expression of the genes for key gluconeogenic enzymes, has been shown to remain unaltered in the kidney of diabetic rats (30).

Factors Responsible for Long-Term Stimulation of Renal Gluconeogenesis in Diabetic Zucker Rats

Metabolic Acidosis, Insulin, and Leptin. Because our diabetic rats were not acidotic, the role of metabolic acidosis,

Table 5. Effect of dexamethasone and dibutyryl-cAMP on the activity of key gluconeogenic enzymes in renal proximal tubules from nondiabetic lean Zucker rats

Experimental Condition	Phosphoenolpyruvate Carboxykinase	Fructose-1,6-Bisphosphatase	Glucose-6-Phosphatase
Control	1.6 ± 0.1	1.9 ± 0.2	4.2 ± 0.5
Dexamethasone (25 mg/kg body wt, 18 h earlier)	2.6 ± 0.1[b]	1.9 ± 0.2	4.7 ± 0.5[b]
Control	1.1 ± 0.07	1.4 ± 0.2	3.1 ± 0.6
cAMP (50 mg/kg body wt, 18 h earlier)	1.2 ± 0.08[b]	1.7 ± 0.2[b]	3.7 ± 0.8[b]
Control	0.9 ± 0.1	1.7 ± 0.1	3.3 ± 0.3
cAMP (50 mg/kg body wt, 6 h earlier)	1.9 ± 0.2[b]	2.5 ± 0.2[b]	4.9 ± 0.3[b]

[a] Values, expressed in μmol/mg protein per h, are presented as means ± SEM for four experiments in each experimental series.

[b] $P < 0.05$. Because each experiment involved one control and one treated rat, statistical difference between the dexamethasone- or the dibutyryl-cAMP–treated rats and the control rats was measured by the paired *t* test.

which is responsible for the stimulation of gluconeogenesis in renal cortical slices prepared from rats that have type 1 diabetes (31–33), can be ruled out. Although insulin has been shown repeatedly to inhibit gluconeogenesis in the human kidney *in vivo* (7,9,13,14) and in one study in the rat kidney *in vivo* (34), it is unlikely that it did so by a direct mechanism that altered gene expression because experiments that have been performed in the rat kidney indicate that insulin has no direct effect on the activity or synthesis of phosphoenolpyruvate carboxykinase in the kidney of diabetic rats (32,35). Moreover, our study shows that insulin does not inhibit glucose synthesis from lactate in isolated kidney tubules (Table 3). Therefore, disappearance *in vivo* of a possible direct inhibitory effect of insulin on renal gluconeogenesis in our diabetic rats, which are known to be insulinopenic at 14 to 17 wk of age, was probably not involved in the intrinsic stimulation of *in vitro* renal gluconeogenesis that we observed in these rats. It also should be pointed out that leptin resistance, which is characteristic of our diabetic rats, could not be involved in the effects observed because leptin receptors in the rat kidneys are localized exclusively in the renal inner medulla (36). Similarly, glucagon, whose plasma levels have been shown to be elevated in patients with type 2 diabetes (37), was probably not involved directly in the stimulation of renal gluconeogenesis, because this hormone does not stimulate the production of cAMP in the renal proximal tubule (38).

Glucocorticoids and cAMP. By contrast, it is conceivable that corticosterone, whose circulating concentration was elevated in our diabetic Zucker rats like in a previous study by other authors (39), was responsible, at least in part, for the stimulation of renal glucose synthesis in our diabetic rats. Indeed, glucocorticoids have been shown to upregulate (*1*) the expression of the phosphoenolpyruvate carboxykinase gene (33,40,41), (*2*) the enzymatic activities of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (35,42–44), and (*3*) the capacity of rat renal cortical slices to synthesize glucose from pyruvate and succinate (45) but not the expression of the glutaminase gene (41) or the activity of fructose-1,6-bisphosphatase (43) in the rat kidney. In agreement with the last observations, the activities of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase but not that of fructose-1,6-bisphosphatase were stimulated in renal proximal tubules of lean Zucker rats that were treated with dexamethasone (Table 5). The absence of effect of dexamethasone on the activity of fructose-1,6-bisphosphatase, in agreement with the absence of a glucocorticoid response element in the corresponding gene (46), suggests, therefore, that at least another factor was responsible for the upregulation of the expression of the fructose-1,6-biphosphate gene. In this respect, our data presented in Table 5 strongly suggest that cAMP, which has been shown to stimulate the transcription of the fructose-1,6-bisphosphatase gene in liver (46), had the same effect in the kidney of our diabetic rats, which contains the same fructose-1,6-bisphosphatase subunit as the rat liver (47). In agreement with our findings, cAMP stimulates the transcription of the phosphoenolpyruvate carboxykinase gene (41,43) but not that of glutaminase in the rat kidney (41). Note that it is conceivable that the diabetic hyperglucagonemia, resulting in part from the disappearance of the insulin-induced inhibition of glucagon secretion, *via* the elevation of circulating cAMP levels, was indirectly responsible *in vivo* for the stimulation of intrinsic renal gluconeogenesis that we observed.

Thus, our results strongly suggest that, in our diabetic rats, at least circulating glucocorticoids and cAMP acted in conjunction to stimulate renal gluconeogenesis. In agreement with this view and our finding that plasma cAMP levels were increased by 80% in our diabetic rats is the demonstration by Nakae *et al.* (48) that dexamethasone plus cAMP stimulated the expression of the phosphoenolpyruvate carboxykinase and glucose-6-phosphatase genes in LLCPK1-FBPase+ cells.

Conclusion

Renal proximal tubules from ZDF rats synthesized much more glucose from lactate, glutamine, and glycerol than tubules from their lean nondiabetic littermates. This stimulation correlates with the increased activities and mRNA level of key gluconeogenic enzymes. These data, together with the observation that the weight of the kidneys of our diabetic rats was augmented by 38% when compared with that of the nondiabetic animals, strongly suggest that renal gluconeogenesis may contribute crucially to the hyperglycemia and the elevated systemic glucose production observed in these animals (49). Among the factors that potentially are involved in the probably multifactorial long-term stimulation of renal gluconeogenesis that we observed, our results suggest that both glucocorticoids and cAMP might play a substantial role by augmenting the expression of key gluconeogenic genes

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