Glycemia-lowering effect of cobalt chloride in the diabetic rat: role of decreased gluconeogenesis

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Glycemia-lowering effect of cobalt chloride in the diabetic rat: role of decreased gluconeogenesis. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E984–E991, 1998.—Results of previous studies indicated that treatment of diabetic rats (induced by streptozotocin) with cobalt chloride (CoCl2) resulted in a significant decrement in serum glucose concentration. The present study was designed to determine the potential role of enhanced glucose uptake vs. decreased glucose production in the above response. The rate of systemic appearance of glucose, measured under fasting conditions using [3-3H]glucose tracer, was reduced from 35.5 ± 2.5 to 17.5 ± 1.8 µmol·kg−1·min−1 in diabetic rats treated with 2 mM CoCl2 added to the drinking water for 10–14 days (P < 0.01). Tissue accumulation of intravenously administered 2-deoxy-[14C]glucose was significantly reduced in kidney and eye of diabetic rats treated with CoCl2, whereas the uptake remained unchanged in several other tissues including cerebrum, red and white skeletal muscle, heart, and liver. The relative content of phosphoenolpyruvate carboxykinase (PEPCK) mRNA increased 3.1-fold in livers of diabetic compared with normal rats (P < 0.001), and treatment of diabetic rats with CoCl2 decreased hepatic PEPCK mRNA levels to normal. The content of PEPCK mRNA in the liver was decreased by 33% in CoCl2-treated normal rats (P < 0.05). Treatment with CoCl2 resulted in no change in cAMP levels in the livers of either diabetic or normal rats. These results suggest that the glycemia-lowering effect of CoCl2 is mediated by reductions in the rate of systemic appearance of glucose and hepatic gluconeogenesis.

Materials.

CoCl2 and other standard chemicals were obtained from Sigma Chemical (St. Louis, MO). Reagent-grade ether was obtained from Fisher (Pittsburgh, PA). The cAMP kit was obtained from Amersham (Arlington Heights, IL). [3-3H]glucose (10.4 Ci/mmol), 2-deoxy-[U-14C]glucose (294 Ci/mmol), and deoxy-[α-32P]cytidine (3,000 Ci/mmol) were obtained from DuPont-NEN Research Products (Boston, MA). PE-50 polyethylene nontoxic tubing (ID, 0.58 mm; OD, 0.965 mm) was obtained from Becton-Dickinson (Sparks, MD). The rodent sling jacket was obtained from Harvard Bioscience (South Natick, MA). QuickPrep total RNA extraction kit and Quikhyb were purchased from Pharmacia Biotech (Piscat-
Diabetic group were then placed on 2 mM CoCl₂ in the mM). One-half of the group of normal rats and one-half of the vein to ensure the presence of diabetes (serum glucose (31). After 1 wk, a sample of blood was obtained from the tail cycle. The protocol was approved by the Institutional Animal Laboratory Animals. They had free access to rat chow (Purina), and water was administered on a 12:12-h light-dark

Diabetes was induced by injection of a freshly prepared solution of STZ in saline at 60 mg/kg body wt in the tail vein (31). After 1 wk, a sample of blood was obtained from the tail vein to ensure the presence of diabetes (serum glucose >25 mM). One-half of the group of normal rats and one-half of the diabetic group were then placed on 2 mM CoCl₂ in the drinking water for 12–16 days. During the ~2-wk period, normal and diabetic rats gained ~30 and ~15 g of weight, respectively, and treatment with CoCl₂ resulted in no change in weight gained by diabetic rats. In a previous study employing various concentrations of CoCl₂, we found that a similar group of diabetic rats treated with up to 4 mM CoCl₂ for 7 wk gained ~100 g in body weight, a rate that was identical to that in diabetic rats not treated with CoCl₂ (31). On the basis of the daily water intake, we estimate that diabetic and normal rats received a total dose of ~2 and ~1 mmol of CoCl₂, respectively. Values for serum glucose, electrolytes, and other constituents summarized in Table 1 were measured in the hospital laboratory.

Measurement of Rₐ of glucose and of glucose uptake by tissues. These experiments were performed in different sets of animals from the ones described above; diabetes and treatment with CoCl₂ were as indicated above. Indwelling arterial and venous catheters were placed 3–4 days before the tracer study. The animals were anesthetized with an intramuscular injection (0.1 ml/100 g body wt) of ketamine-acepromazine mixture (90 mg ketamine and 1 mg acepromazine per ml). PE-50 catheters were inserted in the right external jugular and left carotid artery under sterile conditions. The catheters were filled with an anticoagulant mixture of polyvinylpyrrolidone and heparin in isotonic saline (polyvinylpyrrolidone, 0.75 g; heparin, 25 units in 1 ml of isotonic saline). This polymer allows catheter patency for a week or more. The catheters were tunneled subcutaneously, and the distal end of the catheter was sutured to the dorsum of the rat’s neck. The free end of the catheter was sealed, and the rat was placed in a Harvard rodent jacket sling, thus allowing the animal to move freely during the postoperative period. After surgery, rats were housed in individual cages and had free access to a standard diet (Purina rat chow) and water or CoCl₂ solution as indicated.

Table 1. Effect of treatment with CoCl₂ on serum concentration of certain constituents in diabetic and normal rats

<table>
<thead>
<tr>
<th></th>
<th>Diabetic (n = 6)</th>
<th>Diabetic+ CoCl₂ (n = 6)</th>
<th>Control (n = 4)</th>
<th>Control + CoCl₂ (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>35.4 ± 2.8</td>
<td>21.2 ± 2.2*</td>
<td>8.6 ± 0.3</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>Na⁺, meq/l</td>
<td>123 ± 3</td>
<td>127 ± 4</td>
<td>128 ± 1</td>
<td>131 ± 1</td>
</tr>
<tr>
<td>HCO₃⁻, meq/l</td>
<td>27 ± 1</td>
<td>23 ± 1*</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Ca²⁺, mM</td>
<td>2.98 ± 0.03</td>
<td>2.38 ± 0.05</td>
<td>2.50 ± 0.05</td>
<td>2.50 ± 0.05</td>
</tr>
<tr>
<td>Mg²⁺, mM</td>
<td>1.63 ± 0.04</td>
<td>1.56 ± 0.04</td>
<td>1.90 ± 0.06</td>
<td>1.84 ± 0.04</td>
</tr>
<tr>
<td>Phosphate, mg/dl</td>
<td>8.7 ± 0.2</td>
<td>7.9 ± 0.3*</td>
<td>9.9 ± 0.4</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.30 ± 0.04</td>
<td>0.37 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>3.4 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>108 ± 30</td>
<td>61 ± 6</td>
<td>57 ± 4</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>760 ± 406</td>
<td>117 ± 31</td>
<td>88 ± 17</td>
<td>59 ± 12</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>236 ± 20</td>
<td>300 ± 30</td>
<td>248 ± 25</td>
<td>25 ± 12</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE; n = no. of rats. Nofasting diabetic rats were killed at 9:00 AM, and blood was obtained for analysis. Rats in both control (non diabetic) groups were fasted for 24 h before death. Measurement of serum glucose was performed, however, on 200 µl of blood obtained from tail vein 2 days before death. Nofasting insulin and glucagon levels are from a previous study using different but similar groups of rats (31). ND, not done. *P < 0.05 compared with respective CoCl₂-untreated group.
The integral of the specific activity of 2-deoxy-[14C]glucose in the plasma was calculated by establishing the best-fit exponential curve for the plasma specific activity measurements. No correction was made for lump constant, the correction factor for the discrimination against 2-deoxy-[14C]glucose in glucose transport and phosphorylation pathways (6). It was assumed that the lump constant will be the same in control and experimental animals.

Measurement of CAMP levels in the liver. Groups of CoCl2-treated and untreated normal and diabetic rats separate from those used above were employed. Rats were decapitated after CO2-inhalation anesthesia. Livers were removed promptly and frozen immediately using liquid nitrogen or dry ice.

Data on all four experimental groups, i.e., diabetic rats, diabetic rats treated with CoCl2, normal rats, and normal rats treated with CoCl2, are presented throughout. However, analysis of the potential effects of CoCl2 on any given parameter was performed in diabetic rats and normal rats treated (vs. not treated) with the agent. This was done because diabetes itself is associated with changes in some of the parameters being examined.

All experimental results are expressed as means ± SE. Unpaired Student’s t-test was employed, and P < 0.05 was considered significant (27).

RESULTS

Effect of CoCl2 on the concentration of glucose and selected other constituents in the blood of diabetic and normal rats. Normal and diabetic rats gained ≈30 and ≈15 g, respectively, during the ~2 wk of study, and treatment with CoCl2 resulted in no change in the rate of weight gain by diabetic rats. Table 1 summarizes the effect of 10 days of treatment with CoCl2 on the concentration of several serum constituents in the four groups of rats. Rats in both diabetic groups were not fasted before obtaining blood samples. In both groups of normal rats, with the exception of serum glucose and insulin, values are from rats after a 24-h period of food deprivation. Blood glucose was determined under nonfasting conditions, because it was found in preliminary studies that blood glucose concentration in diabetic rats decreased to normal levels after a 24- to 30-h period of food deprivation. In accordance with our previous observations (31), treatment with CoCl2 resulted in a dramatic reduction of nonfasting glycemia of diabetic rats from 35.4 ± 2.8 to 21.2 ± 2.2 mM glucose (P < 0.01), whereas the agent resulted in no significant change in the serum glucose concentration of normal rats. In diabetic rats treated with CoCl2, there was a significant decrease in the concentration of bicarbonate and phosphate compared with diabetic rats not treated with the agent; the decrements in serum cholesterol and triglyceride in CoCl2-treated diabetic rats were not significant, although two diabetic rats that had not been treated with CoCl2 had lipemic serum. Treatment of normal rats with CoCl2 resulted in no measurable change in the concentration of any of the serum constituents. The concentrations of insulin and glucagon in the serum of diabetic rats treated or not treated with CoCl2 (both reported previously (31)) have also been included in Table 1. After 10 days of treatment with CoCl2, hematocrit values in both normal and diabetic rats rose equally from 37 ± 1 to 40 ± 1%.

Effect of CoCl2 on the Ra of glucose in diabetic and normal rats. The Ra was determined in all four experimental groups after 24–30 h of food deprivation; the fasting period resulted in a normalization of serum glucose concentration in both groups of diabetic rats. Serum glucose concentrations during measurement of Ra were 6.37 ± 0.22 and 6.04 ± 0.42 mM in diabetic and CoCl2-treated diabetic rats, respectively. Ra, expressed either as micromoles per minute (data not shown) or as micromoles per minute per kilogram body weight, was ~50% lower in diabetic animals treated with CoCl2 (35.5 ± 2.5 vs. 17.5 ± 1.8 μmol·kg⁻¹·min⁻¹; P < 0.01; Fig. 1). Ra also was lower by ~35% in normal rats treated with CoCl2 (from 47.5 ± 8.5 to 33.0 ± 3.0 μmol·kg⁻¹·min⁻¹), but the change was not significant (Fig. 1). Comparison of glucose Ra values in diabetic vs. normal rats [both groups not treated with CoCl2] reveals no significant difference between the two groups. Because the concentration of glucose in the blood was constant during the 3-h period of measurement of Ra, the experimental results can be used to calculate the clearance of glucose from the circulation. Glucose clearance was 0.89 ± 0.22 and 0.59 ± 0.07 ml·100 g body wt⁻¹·min⁻¹ in normal and CoCl2-treated normal rats, respectively (P > 0.1). It was 0.53 ± 0.04 and 0.26 ± 0.04 ml·100 g body wt⁻¹·min⁻¹ in diabetic and CoCl2-treated diabetic rats, respectively (P < 0.01).

Effect of CoCl2 on the rate of glucose uptake by selected tissues in diabetic and normal rats. After determination of Ra, glucose uptake by several tissues of the same
rats was measured by previously described methods (6). Figure 2 summarizes the amount of 2-deoxy-[14C]glucose 6-phosphate accumulated in tissues of diabetic rats not treated or treated with CoCl2. It should be noted that accumulation of glucose in tissues is a composite function of transport and phosphorylation steps acting sequentially. Among the tissues examined in the diabetic rat, heart and cerebrum exhibited high rates of uptake (expressed as nmol·g tissue wt⁻¹·min⁻¹). Because only a small fraction of the eye represents the metabolically highly active cells of the retina, this tissue probably manifests the highest rate of glucose uptake and metabolism. The kidney had an intermediate rate of uptake, whereas the red and white gastrocnemius muscle exhibited lower rates. Treatment of diabetic rats with CoCl2 tended to increase the rate of uptake of 2-deoxy-[14C]glucose by heart and liver, whereas the uptake by cerebrum, kidney, muscle, and eye was decreased; of the aforementioned changes, only the decrements in uptake by the kidney and eye were significant.

Results of measurement of 2-deoxy-[14C]glucose uptake by the indicated tissues in normal rats not treated or treated with CoCl2 are summarized in Fig. 3. Compared with diabetic rats, rates of glucose uptake in normal rats are somewhat higher in cerebrum, liver, and muscle and lower in heart, kidney, and eye; none of the changes are significant. Treatment of normal rats with CoCl2 resulted in a slight to moderate decrease in the rate of glucose uptake by all the tissues examined, although none of the changes reached significance. It is probable, however, that the sum of uptake values by all the tissues combined is reduced as a result of treatment with CoCl2.

Effect of CoCl2 on the content of PEPCK mRNA in the livers of diabetic and normal rats. We next examined the possibility that the activity of PEPCK, the key enzyme in the control of gluconeogenesis, might be suppressed as a result of exposure to CoCl2. Because PEPCK activity closely parallels PEPCK mRNA con-
tent (13), we measured the effect of CoCl₂ treatment on the concentration of PEPCK mRNA in the liver. Two experimental protocols were employed. In the first, the relative abundance of PEPCK mRNA was measured in livers of nonfasted normal rats, diabetic rats, and diabetic rats treated with CoCl₂ (Fig. 4A). Rats were not fasted because food deprivation increases PEPCK expression in normal rats. The content of PEPCK mRNA in the liver of diabetic rats was increased to 3.1-fold over that found in the liver of nontreated rats (P < 0.001). Treatment of diabetic rats with CoCl₂ resulted in a significant reduction in the content of PEPCK mRNA in the liver to levels similar to those found in livers of normal rats not treated with CoCl₂ (P > 0.4). In a separate set of experiments, we found that treatment of diabetic rats with CoCl₂ under nonfasting conditions also decreased liver PEPCK mRNA content by approximately threefold (P < 0.05; data not shown). In the second protocol, the effect of CoCl₂ on the level of PEPCK mRNA in the liver of nondiabetic animals was determined. In this experiment, normal and CoCl₂-treated normal rats were deprived of food for 24 h to elicit an upregulation of PEPCK gene expression before study (Fig. 4B). The relative abundance of hepatic PEPCK mRNA was decreased by 33% in normal rats treated with CoCl₂ compared with the control group (P < 0.05). The effect of CoCl₂ was also determined in nonfasted normal and CoCl₂-treated normal rats. PEPCK mRNA content was present at low levels in liver of fasted normal rats and decreased to below detectable levels in CoCl₂-treated rats (data not shown).

Effect of CoCl₂ on the levels of cAMP in the livers of diabetic and normal rats. It is well established that the expression of the gene for the cytosolic form of PEPCK in liver is highly regulated by several hormones, including glucagon, insulin, growth hormone, and cortisol, and by the nutritional status of the animal (10). Hence the reduction in the levels of PEPCK mRNA in livers of CoCl₂-treated diabetic and normal animals summarized above may well be mediated by alterations in one or a combination of the above regulators. Because of the dominant and opposing roles of glucagon (acting through cAMP) and insulin on the regulation of PEPCK expression (9, 17) and because of our finding that the concentration of serum insulin is not changed as a result of treatment with CoCl₂ in either diabetic or normal rats (Table 1), we examined the possibility that the concentration of hepatic cAMP is reduced in CoCl₂-treated rats (Fig. 5). Under nonfasting conditions, the concentration of cAMP is slightly (but not significantly) lower in the livers of diabetic compared with normal rats, and decreases slightly as a result of CoCl₂ treatment. Similarly, there was no significant change in cAMP levels in 24-h food-deprived normal rats treated with CoCl₂ (Fig. 5B). Food deprivation in normal rats, however, was associated with a significant increase in hepatic cAMP levels from 625 ± 77 to 823 ± 40 pmol/g wet wt (P < 0.05).

DISCUSSION

The present study was prompted from the earlier observation that treatment of STZ-induced diabetic rats with CoCl₂ results in a significant reduction in the serum glucose concentration (31). In principle, the
glycemia-lowering effect of CoCl₂ could be secondary to decreased systemic glucose production, increased tissue glucose uptake, or a combination of the two mechanisms. The observed induction of GLUT-1 mRNA in several tissues of CoCl₂-treated rats suggested that enhanced glucose uptake may well play a dominant role in the above effect, although GLUT-1 expression and glucose transport were not measured in that study (31). The results of studies reported herein, however, indicate that the glycemia-lowering effect of CoCl₂ is mediated by a reduction in the $R_a$ of glucose and possibly gluconeogenesis. It is worth emphasizing that the results of the present study are internally consistent, i.e., the decrease in glycemia of CoCl₂-treated diabetic rats is associated with a significant reduction in $R_a$ and a decrease in liver PEPCK mRNA content. A reduction in calorie intake as the explanation for the reduction in glucose production is excluded by the observation that, in accordance with previous results (31), diabetic rats treated or not treated with CoCl₂ gained weight at an equal rate during the 2-wk period of treatment.

Previous reports indicate that glucose $R_a$ values in non-insulin-dependent diabetic subjects are higher than in normal controls (22), although the higher $R_a$ values in diabetic subjects are documented under conditions in which the blood glucose concentrations are significantly higher than for nondiabetic controls (28). $R_a$ values measured in the present study, whether expressed per animal or per unit body weight, were somewhat but not significantly lower in the diabetic animals. The reasons for this apparent discrepancy are unknown but may reflect the fact that the model employed in the present study is one of insulin-dependent diabetes. Moreover, unlike previous protocols, diabetic and normal rats in this study were deprived of food for 24–30 h before measurement of glucose $R_a$, a condition which served to markedly reduce the serum glucose concentration of the diabetic group to levels approximating those in food-deprived normal rats. A profound depressing effect of a prolonged period of food deprivation (72 h) on hepatic glucose production in the rat has been reported previously (1). Treatment of diabetic rats with CoCl₂ resulted in ~50% reduction in glucose $R_a$. If glucose $R_a$ is decreased by a similar extent under nonfasting conditions, then this change alone would be expected to result in a significant decrease in the glycemia of diabetic rats.

There was a tendency toward lower 2-deoxy-[$^{14}$C]glucose uptake in tissues of CoCl₂-treated rats, although only in the case of kidney and eye in diabetic rats was the decrease significant. It is possible that the marked reduction in glycemia of diabetic rats during the period of food deprivation results in a lower rate of tissue glucose uptake and thereby leads to a masking of any further decrement in glucose uptake by treatment with CoCl₂. In keeping with this premise, it has been reported that food deprivation for 72 h in normal rats results in a decrease in sensitivity of peripheral tissues to the actions of insulin (1). Nevertheless, the summation of tissue uptake values, reflecting the uptake by the organism as a whole, is apt to be reduced in CoCl₂-treated animals. It should also be noted that the decreased clearance of glucose from the circulation of diabetic rats treated with CoCl₂ might help explain the finding that the glycemia of these rats is not lower than that of diabetic rats not treated with CoCl₂.

Because the measurements of $R_a$ and 2-deoxy-[$^{14}$C]glucose uptake in various tissues were performed after a fasting period of 24–30 h, it is highly likely that $R_a$ of glucose closely reflects gluconeogenesis rather than glycogenolysis. We therefore explored the possibility that treatment with CoCl₂ reduces gluconeogenesis by the liver, a process that is controlled in part by the level of PEPCK activity (29). Because PEPCK activity closely parallels the level of its mRNA (13), we measured the relative abundance of PEPCK mRNA in livers of diabetic and normal rats treated with CoCl₂. In accordance with previous findings, the abundance of PEPCK mRNA was increased in livers of diabetic rats compared with normal controls under nonfasting conditions (7). In addition, treatment with CoCl₂ significantly reduced hepatic PEPCK mRNA levels in diabetic rats under fasting or nonfasting conditions. CoCl₂ treatment also significantly reduced PEPCK mRNA levels in the livers of normal rats. However, a direct correspondence between PEPCK mRNA and hepatic glucose production may not always exist, especially under differing experimental and nutritional conditions. It is worth emphasizing that the decrease in PEPCK mRNA content does not represent a nonspecific or toxic effect of the agent because diabetic rats treated with up to 4 mM CoCl₂ for 7 wk demonstrated no reduction in weight gain compared with diabetic controls not treated with CoCl₂ (31), and the abundances of other mRNAs such as those encoding erythropoietin (8, 30) and GLUT-1 and GLUT-2 (31) are increased in CoCl₂-treated rats.

The mechanism by which CoCl₂ modifies the level of PEPCK mRNA in the livers of diabetic rats is of interest. When CoCl₂ is added to cells in the presence of oxygen, many of its effects on gene expression mimic the effects noted in response to lowered oxygen concentration (8). For example, transcription of the gene coding for GLUT-1 is stimulated in response to hypoxia by oxygen-sensing molecules that can also be activated by cobalt (2). Cobalt is thought to alter gene transcription by increasing the level of hypoxia-induced factor (HIF)-1, a transcription factor that binds to a regulatory element (CGT GCTG) in the promoter of a number of genes, most notably erythropoietin and vascular endothelial growth factor genes (30). The steady-state concentration of HIF-1 is induced by hypoxia or CoCl₂ by a mechanism that involves the stabilization of the protein against degradation, resulting in an accumulation of the transcription factor (24). The gene for PEPCK has also been shown to respond to changes in the redox state of liver cells in culture (11). This gene is expressed in the liver in a decreasing gradient from the perportal to the pericentral region (14), presumably because of higher levels of oxygen and nutrients supplied to hepatocytes in the periportal region. Previous
studies by Hellkamp et al. (11) have also demonstrated that glucagon-induced transcription from the PEPCK promoter is inhibited by reducing the concentration of oxygen from 16 to 8%. The hypoxia-inducible DNA element noted in the 3′-flanking region of the erythropoietin gene is also present in the PEPCK promoter at −129 to −121 kb (from the transcription start site), a position that is immediately 5′ to a control region that includes the cAMP and the nuclear factor-1 regulatory elements (−120 to −80 kb), which are critical for both basal and cAMP-induced transcription of the PEPCK gene (18, 21). However, T. Kietzmann (personal communication) has implicated a site in the PEPCK gene between −277 and −174 kb as being required for the negative effect of redox state on transcription from the PEPCK promoter in primary hepatocytes in culture (15). Although it is possible that the effect of CoCl2 noted in the present study is due to an inhibition of transcription of the PEPCK gene in the liver via a mechanism involving HIF-1, it is likely that there are multiple elements in the PEPCK promoter involved in the effect of cobalt on PEPCK gene expression.

Cytosolic PEPCK gene expression in the liver is highly controlled by a number of hormones and physiological conditions and most importantly by glucagon and insulin mediation of positive and negative regulation, respectively. Results of previous studies indicate that treatment with CoCl2 does not alter the concentration of insulin in the blood of normal rats (3, 31) and does not increase the extremely low levels of insulin in the blood of STZ-induced diabetic rats (31). It has also been reported that livers of CoCl2-treated nondiabetic rats are relatively “insensitive” to glucagon action, since the release of glucose by the liver in response to glucagon is decreased both in vivo and in vitro (4); the lower release of glucose is present, although the livers of CoCl2-treated rats contain higher levels of glycogen (12). Moreover, although the blood of STZ-induced diabetic rats (31). It has also been reported that livers of CoCl2-treated nondiabetic rats are relatively “insensitive” to glucagon action, since the release of glucose by the liver in response to glucagon is decreased both in vivo and in vitro (4); the lower release of glucose is present, although the livers of CoCl2-treated rats contain higher levels of glycogen (3). Because of the critical regulation of PEPCK gene transcription by glucagon and cAMP, we elected to measure the levels of this nucleotide in liver of diabetic and normal rats treated or not treated with CoCl2. The results showed no systematic change among any of the groups examined except for the finding that food deprivation of normal rats resulted in a significant increase in the level of CAMP in the liver. It has been reported that CoCl2-treated rats appear to be more “sensitive” to the action of insulin in enhancing glucose disposal from the blood after a glucose load (3). If the liver of CoCl2-treated rats is also more sensitive to actions of insulin, then such a finding might help explain the lower glucose R3 in such animals. This explanation, however, cannot be extended to diabetic rats, given the extremely low levels of insulin in these animals. Further studies are required to gain a better understanding of the mechanisms mediating the apparent reduction of gluconeogenesis in CoCl2-treated diabetic and normal rats.

This study was supported, in part, by grants from the Diabetes Association of Greater Cleveland and the National Institutes of Health (DK-45945 to F. Ismail-Beigi, DK-25541 to R. W. Hanson, and HD-11089 to S. C. Kalhan). P. Leahy and F. Saker were trainees on the National Institute of Diabetes and Digestive and Kidney Diseases Metabolism Training Grant DK-07319. F. Saker and J. Ybarra contributed equally to this study.

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Received 22 October 1997; accepted in final form 20 February 1998.

REFERENCES


