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Dichloroacetate-Induced Changes in Liver of Normal and Diabetic Rats (38905)

JAMES W. ANDERSON, DENNIS KAROUNOS, AND TATSUO YONEYAMA
(Introduced by J. W. Hollingsworth)

Endocrine-Metabolic Section and Laboratory Service, Veterans Administration Hospital and Departments of Medicine and Pathology, University of Kentucky Medical School, Lexington, Kentucky 40507

In 1962 Lorini and Ciman (1) reported that diisopropylammonium dichloroacetate (DIPA), a vasodilator, reduced hyperglycemia in diabetic rats but did not alter the blood glucose concentration of fed, nondiabetic animals. Subsequently it was demonstrated that dichloroacetate (DCA) appears to be the active component of DIPA which produces these effects (2). The influence of DIPA and DCA on glucose metabolism of heart, skeletal muscle, liver, and adipose tissue of normal, 24-hr starved and diabetic rats has been extensively studied (2–5), but the mechanism of action has not been clarified. Since the liver has an important role in glucose metabolism in both diabetic and normal animals (6) and since the influence of DCA on hepatic metabolism of normal or diabetic rats has not been characterized, the current studies were done to evaluate its effect on hepatic morphology, glycogen content, and the activities of certain enzymes of glucose and pyruvate metabolism.

Methods. Animals. Male Sprague-Dawley rats weighing 150–300 g were fed Purina Chow ad lib. before initiating the experiment. During all experiments, animals were pair-fed by placing 25 g of Purina Chow in their feeder at 4 PM. Diabetes was produced by the intravenous injection of 50 mg of streptozotocin per kg rat wt after a 12-hr fast. Animals were sacrificed at 21–35 days after induction of diabetes. Normal rats that did not receive streptozotocin are designated "nondiabetic."

DCA administration. All DCA-treated animals received 1000 mg DCA per kg body wt orally for 7 days. This dose of DCA is similar to that administered by previous workers (2, 3, 5). The DCA was neutralized to pH 7.0 with sodium hydroxide and added to the chow pellets and allowed to dry before feeding it to treated animals. Additionally, one series of rats (Group B) received intraperitoneal injections of 100 mg DCA per kg rat weight at 8:30 AM and 4:30 PM on the day prior to sacrifice and at 7:30 AM on the day of sacrifice, and were killed 2 hr later. Animals that received DCA are designated treated nondiabetic or treated diabetic rats and those that did not receive DCA are referred to as untreated nondiabetic or untreated diabetic rats.

Grouping of animals. Group A includes nondiabetic and diabetic animals that received oral DCA and untreated nondiabetic and diabetic rats. Mild diabetic rats had blood glucose values below 300 mg/dl while moderate diabetic rats had values above 300 mg/dl prior to treatment. Group B includes treated diabetic rats that received oral and intraperitoneal DCA as well as untreated nondiabetic and untreated diabetic rats. All diabetic rats in Group B had blood glucose values above 300 mg/dl prior to treatment.

Sacrifice procedure. Animals were anesthetized with ether and exsanguinated by cardiac puncture. The right lobe of the liver was removed for enzyme assays, glycogen estimations, light and electron microscopy. Jejunal mucosa (7) was removed for enzyme assays in certain rats.

Blood glucose measurements. Two or three blood glucose values were obtained in diabetic rats at 10–15 days after streptozotocin injections; the mean of these values were taken as the pretreatment (Pre-Rx) value. Blood was withdrawn from the tail vein of animals under light ether anesthesia and glucose measurements, in duplicate, were performed by the glucose oxidase method as previously described (8). Diabetic animals with similar mean blood glucose values were matched with one animal from each pair receiving DCA and the other animal receiving no treatment. Blood was obtained for glucose estimations on the day prior to

TABLE I. DCA-TREATED NONDIABETIC AND DIABETIC RATS.[a]

	DCA	No.	Rat weights (g) Initial	Rat weights (g) Terminal	Liver Wet weight (g/100 g)	Liver Glycogen (mg/g)	Serum triglyceride (mg/dl)[b]	Blood glucose (mg/dl) Pre-Rx	Blood glucose (mg/dl) Terminal
Group A									
Nondiabetic	0	10	242	282	4.16	40.7	40.3	—	129
			3	7	0.08	2.7	4.0		4
Nondiabetic	+	10	241	280	4.92[b]	45.4	41.0	—	124
			2	6	0.31	4.9	9.0		6
Mild diabetic	0	6	251	310	—	53.7	50.3	154	150
			7	9		4.0	7.7	10	16
Mild diabetic	+	5	253	300	—	54.7	46.8	156	130
			4	7		8.0	11.6	8	12
Moderate diabetic	0	6	253	200	—	21.0	55.3	456	420
			7	11		3.3	22.6	46	39
Moderate diabetic	+	6	247	211	—	17.8	71.8	443	353
			9	4		3.1	11.5	39	15
Group B									
Nondiabetic	0	12	160	284	3.57	36.5	53.8	—	134
			3	7	0.12	2.7	8.5		6
Diabetic	0	12	157	161	4.71	11.3	319	334	396
			2	5	0·14	1.7	82	9	9
Diabetic	+	12	157	170	6.16[b]	2.6[b]	175	330	316[c]
			4	7	0.16	1.0	29	11	14

[a] All DCA-treated rats, indicated by +, were given 1000 mg DCA/kg day; additionally, Group B animals received intraperitoneal injections of 100 mg DCA/kg at 25, 17, and 2 hr before sacrifice. Mean values are given with SEM indicated on next line below.

[b] differs from untreated rats, $P < 0.001$; [c] differs from untreated rats, $P < 0.05$.

sacrifice and on the day of sacrifice and the means of these values are designated the terminal blood glucose values. On the day prior to sacrifice most diabetic rats in Group B had blood glucose measurements before the intraperitoneal injection of DCA and 2 hr later; blood was obtained from treated and untreated diabetic animals in this fashion. Plasma triglycerides were extracted using the Dole extraction procedure (9), and glycerol was liberated and estimated colorimetrically by the Dade method (10).

Tissue measurements. Measurements of glucokinase, hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, and malic enzyme activities of the 105,000*g* supernatant obtained from homogenates of liver or jejunal mucosa were performed as previously described (11). The glycogen content of liver was measured as previously described (8) and protein concentrations were measured by the method of Lowry *et al.* (12). All chemicals unless otherwise noted were obtained from Sigma Chemical Corporation, St. Louis, MO.

Morphologic studies. Samples of liver from representative animals from each group were fixed in 10% formalin, embedded, sectioned, and stained by hematoxylin and eosin for light-microscopy studies. Small samples of liver were fixed in glutaraldehyde and post-fixed in cacodylate-buffered osmium tetraoxide solution. The specimens were dehydrated in a graduated acetone series and embedded in Spurr epoxy resin (13). Thin sections were stained with uranyl acetate and lead citrate and examined with a Phillips EM-300 electron microscope.

Results. Food intake and weight gain. All groups of animals ate virtually all of the food presented to them. Occasionally untreated nondiabetic animals left small amounts of food and the average intake for these animals in Group A was 24.3 ± 0.2 g/day (mean ± SEM). All treated animals ate the pellets containing DCA and their food consumption

was identical to that of their untreated counterparts. The weight gains of treated nondiabetic and mildly diabetic animals were virtually identical to those of the untreated animals (Table I). When moderately diabetic animals were treated with DCA those in group A lost slightly less weight than untreated animals and those in group B gained slightly more weight than the untreated diabetic animals.

Liver weight and glycogen content. DCA therapy was associated with a significant increase in the wet weight of liver in nondiabetic animals and in the diabetic animals in group B (Table I). The dry weight of liver and the total protein content of liver were virtually identical in DCA-treated and untreated nondiabetic animals and also when values from DCA-treated diabetic rats were compared with those of untreated diabetic animals. DCA treatment was not associated with a significant change in liver glycogen content of nondiabetic animals or of mildly or moderately diabetic animals in group A. A significant reduction in glycogen content, however, was observed in DCA-treated diabetic animals in group B.

Blood glucose and plasma triglyceride values. As others have noted (1, 4, 14) DCA treatment was not accompanied by significant alterations in the blood glucose concentrations of fed nondiabetic animals. However, in diabetic rats DCA therapy was accompanied by lower blood glucose values. A greater reduction in blood glucose values in both mild and moderate diabetics in group A was seen when values were compared with those of the untreated diabetic animals (Table I). A greater rise in blood glucose values was observed in the untreated diabetic rats in group B but differences were more apparent when blood glucose values were measured at 2 hr after the intraperitoneal injection of DCA. Eight untreated diabetic animals had a mean increase of 14 $\pm$ 18 mg/dl (mean $\pm$ SEM) when blood was obtained 2 hr after the initial morning determination. Ten DCA-treated diabetics had a mean reduction of plasma glucose of 48 $\pm$ 8 mg/dl at 2 hr after DCA injection (P vs untreated rats <0.001).

DCA therapy was not associated with a significant alteration in plasma triglyceride values in the nondiabetic rats or the diabetic animals in group A. The diabetics in group B had hypertriglyceridemia and DCA therapy was accompanied by lower plasma triglyceride values.

Liver enzyme activities. DCA therapy was accompanied by significant reductions in glucokinase and pyruvate kinase activities and by remarkable increases in glucose-6-phosphate dehydrogenase and malic enzyme activities in both nondiabetic and diabetic animals (Table II). Although nondiabetic animals showed no alteration in blood glucose values with DCA therapy, their hepatic enzyme showed greater changes than observed for the diabetic animals. The most remarkable changes were in the two enzymes of NADPH production where glucose-6-phosphate dehydrogenase was 3-fold higher and malic enzyme was 10-fold higher in DCA-treated nondiabetic animals as compared to untreated animals. Mildly diabetic animals that received DCA showed changes which were similar in direction but smaller in magnitude. The most prominent change observed in moderately diabetic animals in group A and in the diabetic animals in group B was a 5-fold increase in malc enzyme activity after DCA treatment. Whereas significant alterations in glucokinase (type IV hexokinase) were observed, no alterations in other isozymes of hexokinase were observed. These enzyme alterations could not be related to changes in protein concentrations since the protein concentration of the 105,000g supernatant fractions were virtually identical in DCA-treated and untreated animals.

The diabetics in group B differed, somewhat, from the moderate diabetics in group A in that those in group B had lower initial weights and lower initial blood glucose values. However, at death the untreated diabetics in group B had lower liver glycogen values, significantly higher serum triglyceride values, and lower hepatic glucokinase, pyruvate kinase, and malic enzyme activities than those of group B. Nevertheless, the enzyme changes (Table II) in those animals (group B) that received oral plus intraperitoneal DCA were very similar to those observed in the moderately diabetic animals (group A) that received only oral DCA.

Jejunal mucosal enzyme activities. Glucose-

TABLE II. LIVER ENZYME ACTIVITIES IN DCA-TREATED RATS.[a]

	DCA	No.	Glucokinase	Hexokinase	Pyruvate kinase	G6PDH	Malic enzyme
Group A							
Nondiabetic	0	10	21.0	—	366	26.6	10.7
			1.8		45	2.1	0.8
Nondiabetic	+	10	14.4[c]	—	221[b]	72.4[b]	110[b]
			0.9		32	10.8	11
Mild diabetic	0	6	23.9	—	505	23.4	8.36
			1.6		19	5.2	0.86
Mild diabetic	+	5	19.2[e]	—	348[b]	37.3[e]	72.3[b]
			0.7		20	2.5	11.8
Moderate diabetic	0	6	2.77	—	252	14.1	5.32
			0.86		33	1.6	1.35
Moderate diabetic	+	6	2.33	—	207	18.6	27.4[b]
			0.66		12	2.9	5.0
Group B							
Nondiabetic	0	12	12.7	3.33	321	20.6	9.15
			1.4	0·39	20.2	1.8	0.98
Diabetic	0	12	0.83	2.28	81.2	13.3	2.84
			0.12	0.14	9·0	0.5	0.29
Diabetic	+	12	0.35[c]	2.25	68.4	16.0[d]	14.0[b]
			0.01	0.18	13.5	1.0	1·7

[a] Animals were treated as indicated in Table 1. Specific enzyme activity is expressed as nmoles/min/mg protein with SEM indicated on next line below.

[b] a differs from untreated rats, $P < 0.001$; [c] differs from untreated rats, $P < 0.005$; [d] differs from untreated rats, $P < 0.01$; [e] differs from untreated rats, $P < 0.05$.

6-phosphate dehydrogenase and malic enzyme activities were measured in the 105,000*g* supernatant of jejunal mucosa from the nondiabetic rats and from the mildly diabetic rats in group A. No significant alterations were observed in DCA-treated rats. Glucose-6-phosphate dehydrogenase activity was 74.0 ± 12 (mean ± SEM) in untreated and 68.6 ± 13.3 nmoles/min/mg protein in DCA-treated nondiabetic animals. Malic enzyme activity was 23.8 ± 3.9 in untreated and 24.7 ± 4.0 nmoles/min/mg protein in DCA-treated nondiabetic animals. In the mildly diabetic animals glucose-6-phosphate dehydrogenase activity was 46.4 ± 16.2 in the untreated group and 61.4 ± 25.4 in the treated group while malic enzyme was 35.2 ± 3.4 in the untreated and 27.6 ± 3.5 nmoles/min/mg protein in the DCA-treated animals. Thus, while dramatic increases in these enzyme activities were observed in the livers of DCA-treated animals, no alterations were observed in jejunal mucosa.

In vitro effects of DCA on enzyme activities. To determine if incubation of purified glucose-6-phosphate dehydrogenase or tissue extracts containing malic enzyme was influenced by *in vitro* addition of DCA the following studies were performed. A highly purified preparation of glucose-6-phosphate dehydrogenase obtained from yeast (Sigma type XV) was assayed in the presence of DCA. No significant alterations in enzyme activity were observed when assays were carried out in the presence of DCA concentrations ranging from 1 m*M* to 14 m*M*. Likewise, when the 105,000*g* supernatant fraction of liver was assayed for malic enzyme activity in the presence of DCA concentrations ranging from 1 m*M* to 20 m*M*, no alterations in enzyme activity were observed.

Morphologic studies. With light microscopic observations there were no differences between DCA-treated diabetic animals and untreated diabetic animals from group B. Since diabetes, per se, leads to distinct alterations in hepatic morphology (14) electron microscopic studies were not performed in diabetic animals. Accordingly, extensive

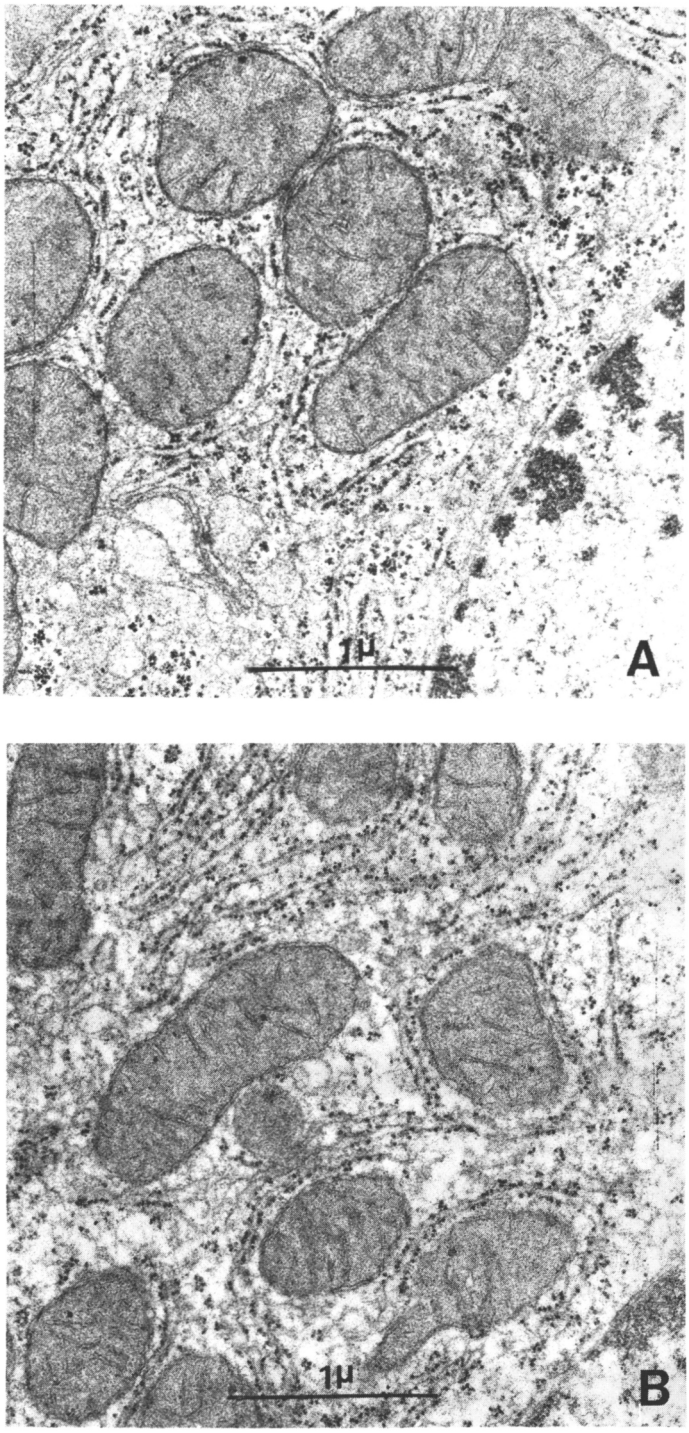

FIG. 1. Electron microscopic photograph of liver cell from untreated normal (nondiabetic) animal (A) and from DCA-treated nondiabetic animal (B). Double stained with lead citrate and uranyl acetate. × 40,000.

comparisons of the livers of DCA-treated and untreated nondiabetic animals (Group A) were performed. No differences between these two groups were observed by light microscopy. Furthermore, we were unable to detect any ultrastructural differences between DCA-treated and untreated nondiabetic rats; representative electron micrographs are presented in Fig. 1.

Discussion. Our current studies indicate that DCA therapy lowers the blood glucose concentration of diabetic animals but does not consistently alter the plasma triglyceride values or liver glycogen content. Although DCA therapy does not alter the blood glucose of fed nondiabetic rats it is accompanied by remarkable changes in hepatic enzyme activities. The activities of two glycolytic enzymes, glucokinase and pyruvate kinase, were significantly lower whereas the activity of two enzymes of NADPH production, glucose-6-phosphate dehydrogenase and malic enzyme, were grealy increased in these normal rats; similar changes, although smaller in magnitude, were observed in diabetic animals treated with DCA. These changes occur in the absence of alterations in the morphology of the liver by either light or electron microscopy.

Many investigators (2–5) have examined the effects of DCA or DIPA therapy on intact animals and on the metabolism of several tissues. Despite these extensive studies, no specific mechanism of action of DCA has been identified. Administration of DCA or DIPA lowers the blood glucose concentration of diabetic (1, 4, 14) and 24-hr starved (6) rats, but not of fed rats (1, 4). These changes in diabetic rats are accompanied by a reduction in glycosuria (1), increased survival (15), and an increase in the respiratory quotient (1) suggesting that glucose metabolism is increased but fat metabolism is decreased in the intact diabetic animal. These compounds do not alter insulin secretion *in vitro* (15) and lower the plasma insulin concentration of 24-hr starved rats (5).

Biochemical measurements of perfused tissues, tissues incubated with DCA or DIPA, or in tissues after treatment of the intact animals have yielded interesting, but difficult to interpret, data. Treatment with DCA or DIPA leads to increased glucose oxidation in diaphragm (2) and adipose tissue (2) while fatty acid oxidation is reduced in diaphragm (2) and apparently in liver (5). One major effect of these compounds appears to be an increase in pyruvate dehydrogenase activity with a virtual complete conversion of the inactive form to the active form in the heart of normal rats (4). DIPA also appears to decrease gluconeogenesis in liver of normal rats (5, 16). Glucose formation from lactate, pyruvate, and amino acids was reduced by incubating normal liver cells with DIPA, but glucose production from fructose, glycerol, xylitol, and sorbitol was not altered (16).

Certain effects of DCA may be highly specific for certain tissues since the dramatic changes in the activities of glucose-6-phosphate dehydrogenase and malic enzyme that we observed in the liver were not noted in jejunal mucosa. Thus, our own observations that glucokinase and pyruvate kinase activities were significantly decreased in DCA-treated rats suggest that anaerobic glycolysis may be reduced in the liver whereas studies of the diaphragm (2) and adipose tissue (2) suggest that glucose oxidation is increased in these tissues. However, our observations in the liver of DCA-treated animals are consistent with those of previous workers (5). The previously observed (5) reduction in the concentration of glucose-6-phosphate in liver is consistent with our observations that glucokinase activity is reduced and glucose-6-phosphate dehydrogenase is increased in the liver of DCA-treated animals. Additionally, since both glucose-6-phosphate dehydrogenase and malic enzyme lead to NADPH production, the increased activity of these enzymes may alter the redox state of the cell, a finding which is consistent with that reported previously (5).

Malic enzyme is a bifunctional enzyme (17) which catalyzes the conversion of malate to pyruvate (the reaction we measured) and also converts oxaloacetate to pyruvate. In the currently accepted concept

of gluconeogenesis (6), pyruvate is converted to oxaloacetate in the mitochondria by pyruvate carboxylase and emerges from the mitochondria as either malate or aspartate to be reconstituted as oxaloacetate in the cytoplasm for further conversion to phosphoenolpyruvate. Increased malic enzyme activity would thus reverse the gluconeogenic sequence and tend to inhibit gluconeogenesis. An increase in pyruvate dehydrogenase activity (4) which converts pyruvate to acetyl CoA would also inhibit gluconeogenesis by decreasing the availability of pyruvate to be converted to oxaloacetate. Thus, DCA may act at a critical step in pyruvate metabolism and lead to a reduction in gluconeogenesis; consequently hepatic glucose production would be reduced and the blood glucose value would be lowered. In fed animals gluconeogenic rates are low and the blood glucose concentration is maintained by ingested glucose and by glycogenolysis. However, in starved or diabetic rats, glycogen levels are low and rates of gluconeogenesis are high (6); under these circumstances a decrease in gluconeogenesis would rapidly lead to a reduction in the blood glucose concentration. It is noteworthy that ethanol, which inhibits gluconeogenesis, does not lower the blood glucose concentration in fed animals but produces significant hypoglycemia in starved animals (18).

Thus, our own studies and those of others (4, 5, 16) suggest that DCA may directly alter pyruvate metabolism in such a way to inhibit gluconeogenesis. DCA appears to produce this effect in normal, starved, and diabetic rats, but hypoglycemia results only when glucose is unavailable from the diet or from glycogen stores.

Summary. Dichloroacetate (DCA) was administered orally to normal (nondiabetic) and streptozotocin-diabetic rats in a dose of 1000 mg/day/kg rat wt. One group of diabetic animals received DCA both orally and intraperitoneally. DCA therapy lowered the blood glucose values of diabetic animals but did not alter values in nondiabetic rats. The hepatic activity of glucokinase and pyruvate kinase were significantly lower in both DCA-treated nondiabetic and DCA-treated diabetic animals than values observed for untreated animals. However, DCA therapy was accompanied by remarkable increases in the activities of glucose-6-phosphate dehydrogenase and malic enzyme in both nondiabetic and diabetic animals. Glucose-6-phosphate dehydrogenase was 3-fold higher in DCA-treated nondiabetic animals whereas malic enzyme activity was 10-fold higher in the treated animals than observed in the untreated animals. Similar changes, although smaller in magnitude, were observed for these enzymes in the DCA-treated diabetic animals. Although DCA therapy was accompanied by a significant increase in the wet weights of the liver for both nondiabetic and diabetic animals, no morphological changes were seen by light or electron microscopy. Our observations coupled with those of previous investigators suggest that DCA therapy may have an important role in pyruvate metabolism and may lower the blood glucose concentration by inhibiting hepatic gluconeogenesis.

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