Contribution of the Liver and Extrasplanchnic Tissues to the Hypoglycemic Action of Dichloroacetate in the Conscious Dog

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SUMMARY

In a previous study, administration of sodium dichloroacetate (DCA) to 48-h fasted dogs had no effect on glucose production (Ra), utilization (Rd), or clearance, possibly because the direct effects of DCA were offset by the consequences of the fall in plasma insulin that it caused. To examine this possibility, dichloroacetate was infused (0.4 mg/kg·min) for 5 h into 48-h fasted conscious dogs whose insulin and glucagon levels were "clamped" at basal values using a technique that involved the peripheral infusion of somatostatin to inhibit the endocrine pancreas and intraportal replacement of insulin (273 μU/kg·min) and glucagon (1 ng/kg·min). Metabolite balances across the liver were measured using an A-V difference technique, glucose turnover was determined using a tracer technique, and gluconeogenesis was assessed by measuring the conversion of alanine and lactate into glucose.

In the presence of fixed concentrations of insulin (14 μU/ml) and glucagon (162 pg/ml), DCA caused the conversion of alanine and lactate to glucose to fall by 73 ± 10% ($P < 0.01$), glucose production to fall by 19 ± 9% (NS), glucose utilization to fall by 17 ± 8% (NS), glucose clearance to increase by 55 ± 16% ($P < 0.05$), and the plasma glucose level to fall from 107 ± 10 to 56 ± 6 mg/dl ($P < 0.001$) after 300 minutes. Thus, with the plasma insulin level fixed, DCA induced hypoglycemia through an effect on both glucose production and clearance. Since the effects of DCA in the above experiment were undoubtedly attenuated by the hypoglycemia that occurred, the experiment was repeated with the exception that glucose was infused to maintain euglycemia (113 ± 11 mg/dl). In this case, the conversion of alanine and lactate to glucose fell by 76 ± 1% ($P < 0.01$), endogenous glucose production fell 46 ± 11% ($P < 0.01$), glucose utilization rose 39 ± 14% ($P < 0.05$) and glucose clearance increased 40 ± 18% ($P < 0.05$). Normalization of the alanine and lactate levels during the last 90 min of the latter experiment failed to significantly change glucose production and enhanced gluconeogenic conversion only slightly. Thus, in the conscious 48-h-fasted dog, we conclude that: (1) DCA acts to reduce the insulin/glucagon molar ratio such that the production of hypoglycemia is prevented, (2) when the effect of the compound on the pancreas is prevented, DCA causes hypoglycemia by a reduction in hepatic glucose production and a stimulation of peripheral glucose uptake, and (3) the effect of DCA on glucose production is in part due to reduced gluconeogenic substrate supply, but is principally related to the action of DCA on intrahepatic enzymatic processes. **DIABETES 31:326–332, April 1982.**

In a previous study conducted using 48-h-fasted conscious dogs, dichloroacetate (DCA) caused a decline in the arterial alanine and lactate levels, a decrease in the hepatic uptake of these gluconeogenic precursors, and a reduction in their intrahepatic conversion to glucose.[1] Despite such changes, however, overall glucose production and the plasma glucose level remained unchanged. In addition, although DCA has been reported to stimulate glucose uptake,[2] the compound failed to alter the rate of glucose utilization.[1] It is conceivable that the explanation for this paradox may lie in the fact that the plasma insulin level also fell in response to DCA infusion. Such a fall would have had a stimulatory effect on glucose production and may thereby have masked the effect of DCA on the liver. Similarly, it would have inhibited glucose clearance and may thereby have masked the effect of the compound on glucose utilization by extrahepatic tissues. The aim of the present study was to determine whether this hypothesis was correct and to ascertain the importance of the liver (glucose production) versus extrahepatic (glucose uptake) tissues in the action of the compound. To accomplish this, DCA was in-

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fused into conscious 48-h-fasted dogs whose insulin and glucagon levels had been "clamped" at basal concentrations by administering somatostatin to inhibit the endocrine pancreas and concurrently replacing insulin and glucagon intraportally in basal amounts.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were carried out on eight mongrel dogs (20–25 kg) that had been fed a high protein diet (30% crude protein, 44% carbohydrate, 12% moisture, 9% fat, and 5% crude fiber; Wayne Dog Chow, Wayne Meat Ration, Allied Mills, Inc., Chicago, Illinois) for 2–3 wk prior to use and then fasted for 48 h. Catheters were inserted in the hepatic vein, portal vein, splenic vein, and femoral artery under general anesthesia as previously described[3] 17 days prior to the experiment.

Two weeks after surgery, blood was withdrawn to determine the leukocyte count and hematocrit of the animal. Only animals having: (1) a leukocyte count below 16,000 mm^3, (2) a hematocrit above 0.38, (3) a good appetite (consuming ⅔ of their daily ration), and (4) normal stools were used.

On the day of an experiment, the free ends of the catheters were removed from the subcutaneous pockets through small skin incisions made under local anesthesia (2% Lidocaine, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts). Pancreatic hormones were subsequently infused through the splenic catheter while blood samples were intermittently withdrawn from the other three catheters. Angiocaths (18 gauge, Abbott Labs., North Chicago, Illinois) were inserted percutaneously into a saphenous vein for infusion of somatostatin and a cephalic vein for infusion of [^{14}C]-alanine, [^{3}H]-glucose, and indocyanine green. In each of four dogs, an additional angiocath was inserted into the other saphenous vein for the infusion of unlabeled alanine and lactate and into the other cephalic vein for infusion of unlabeled glucose. After completion of these procedures, the dog was allowed to stand calmly in a Pavlov harness for 20–30 min prior to the start of the experiment. Following all experiments, an autopsy was performed and the position of the catheter tips were confirmed.

Experimental design. Each experiment consisted of a 90 min (t = −120 to t = −30) tracer equilibration and hormone adjustment period, a 30-min (t = −30 to t = 0) control period, and a 300-min study period. The primed constant infusion of [3-^{3}H]-glucose (0.60 μCi/min), the constant infusion of [U-^{14}C]-alanine (0.07 μCi/kg·min), and the constant infusion of indocyanine green (0.075 mg/m^2·min) were begun at t = −120 min and continued throughout the study. The priming dose of [3-^{3}H]-glucose equaled the amount of tracer infused in 140 min. In both groups, an infusion of somatostatin (0.8 μg/kg·min) was started at −90 min to inhibit endogenous insulin and glucagon secretion[4] and, simultaneously, intraportal replacement infusions of insulin (300 μU/kg·min) and glucagon (1.0 ng/kg·min) were started. The plasma glucose level was monitored every 5 min and the infusion rate of insulin was adjusted to maintain euglycemia. The last alteration in the insulin infusion rate was made at least 15 min prior to the start of the control period and the final infusion rate averaged 273 ± 68 μU/kg·min (range 140–450 μU/kg·min). The infusion rates of both insulin and glucagon then remained fixed so that their plasma levels did not change in response to DCA administration. Two types of experiments were carried out. In group one, an infusion of DCA was started at t = 0 and hypoglycemia was allowed to develop. In group two, DCA was infused from t = 0 and euglycemia was maintained by means of a variable glucose infusion. In addition, in the latter group, alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) were infused beginning at 210 min to restore the concentrations of these metabolites to control levels for the last 90 min of the study.

Arterial, portal vein, and hepatic vein blood samples were taken every 10 min throughout the control period and every 30 min during the study period. In group two, blood samples were taken at 15-min intervals during the last 3 h of the study.

Processing of blood samples. Blood samples were drawn for determination of the cold concentration and radioactivity of both plasma glucose and alanine as well as whole blood lactate according to previously described techniques.[1,5–7] Immunoreactive glucagon (IRG) was assayed using 30K antiserum as previously described.[8] Immunoreactive insulin (IRI) was measured by the Sephadex bound-antibody procedure.[9] Indocyanine green (Hynson, Westcott and Dunning, Inc., Baltimore, Maryland) was measured spectrophotometrically at 810 nm to estimate hepatic blood flow.[10] Catecholamines were measured using the method of Passor and Peuler.[11]

Materials. [3-^{3}H]-Glucose (New England Nuclear, Boston, Massachusetts) was used as the glucose tracer (500 μCi/0.005 mg) and [U-^{14}C]-alanine (New England Nuclear) was used as the labeled gluconeogenic precursor (1 mCi/0.53 mg). Insulin and glucagon were purchased from Eli Lilly and Company (Indianapolis, Indiana), Phadebas Insulin Radioimmunoassay kits were purchased from Pharmacia Fine Chemicals, Inc., (Piscataway, New Jersey), and Trasylol was obtained from FBA Pharmaceutical, Inc. (New York). Glucagon 30K antiserum was obtained from the University of Texas, Southwestern Medical School, and the standard glucagon and [^{125}I]-glucagon were obtained from Novo Research Institute (Copenhagen, Denmark). L-Alanine and L-lactic acid were obtained from Sigma Co. (St. Louis, Missouri). Sodium dichloroacetate was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All hormone solutions were prepared with normal saline and contained 0.3% bovine serum albumin.

Tracer methods and calculations. The conversion of alanine and lactate into glucose was determined by dividing the production rate of [^{14}C]-glucose (dpm/min) (calculated using the hepatic arteriovenous difference method) by the combined specific activity of the alanine and lactate delivered to the liver as previously described.[1] Gluconeogenic efficiency was calculated as the ratio of net hepatic ^{14}C-glucose production to the net hepatic uptake of ^{14}C-alanine and ^{14}C-lactate as described elsewhere.[12]

The rates of glucose production, clearance, and utilization were determined using established tracer methodology as described previously.[4] In the second protocol, the rate of endogenous glucose production was calculated as the tracer-determined glucose production rate minus the unlabeled glucose infusion rate. The rate of appearance of alanine in plasma, as well as the net balance of substrates across the liver and gut, were estimated as previously outlined.[4]

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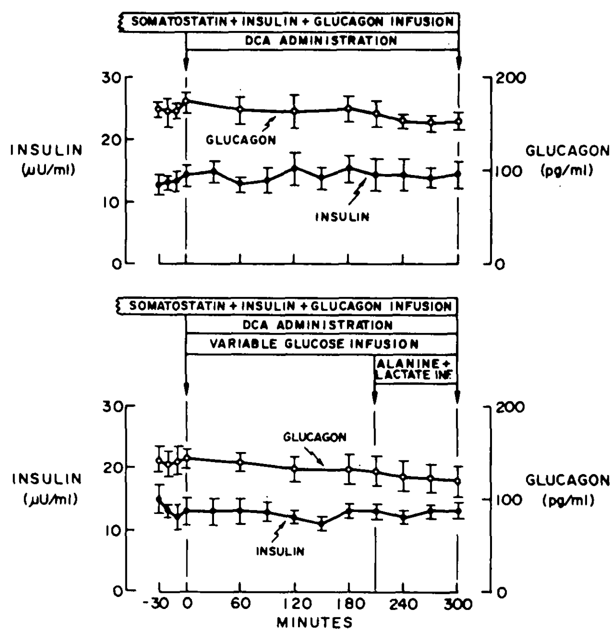

FIGURE 1. Plasma insulin and glucagon levels in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min) and glucagon (1 ng/kg·min) both without (group 1) and with (group 2) variable glucose infusion (to maintain euglycemia) as well as alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) replacement.

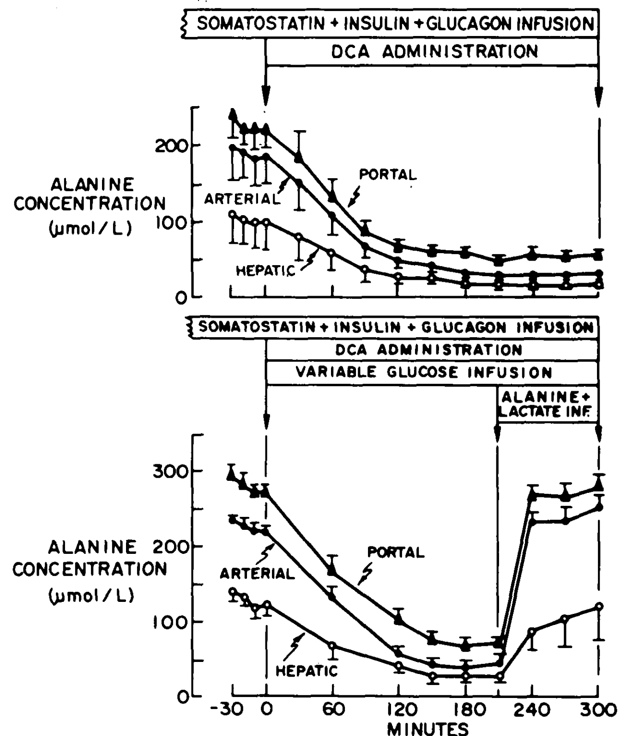

FIGURE 2. Arterial, portal vein, and hepatic vein plasma alanine levels in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min), both without (group 1) and with (group 2) variable glucose infusion (to maintain euglycemia) as well as alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) replacement.

Statistical significance was determined using the Student's *t* test or paired *t* test.[13]

RESULTS

Pancreatic hormone levels. Figure 1 shows that the plasma insulin level remained unchanged in both protocols (14 ± 2 and 13 ± 1 μU/ml, respectively). Similarly the plasma glucagon levels were stable throughout each type of experiment (162 ± 9 and 133 ± 13 pg/ml, respectively).

Alanine metabolism. In both protocols, DCA markedly lowered the plasma alanine level (Figure 2), the load of alanine presented to the liver, and the net hepatic uptake of the amino acid (Table 1). In the second protocol, infusion of alanine during the last 90 min of the study restored both the plasma level and hepatic uptake of the amino acid to control values (Figure 2, Table 1). The hepatic fractional extraction of alanine was similar during the control period of each protocol (0.56 ± 0.10 vs. 0.53 ± 0.03, respectively) and did not change significantly during hypoglycemia (0.63 ± 0.09) or when euglycemia was preserved, regardless of whether (0.58 ± 0.05) or not (0.62 ± 0.10) alanine and lactate were replaced.

Lactate metabolism. DCA infusion markedly lowered the whole blood lactate level (Figure 3), the load of lactate delivered to the liver, and the hepatic uptake of lactate in both groups (Table 1). Infusion of lactate during the latter part of the second protocol restored the concentration and hepatic uptake of this metabolite to control values (Figure 3, Table 1). The fractional extraction of lactate by the liver was similar during the control period of each protocol (0.49 ± 0.03 and 0.50 ± 0.02, respectively) and declined slightly when hypoglycemia occurred (0.38 ± 0.03) but was unchanged when euglycemia was preserved regardless of whether (0.53 ± 0.04) or not (0.46 ± 0.05) alanine and lactate were replaced.

Gluconeogenic conversion. The rate of conversion of alanine and lactate into glucose declined markedly in response to DCA such that it fell by 73 ± 10% and 76 ± 1% in the first and second protocols, respectively. Following restoration of the control alanine and lactate levels in the second protocol, gluconeogenic conversion was partially restored but remained suppressed by 34 ± 13% (Figure 4). The decreased conversion of alanine and lactate to glucose during DCA administration was also reflected by a fall in the gluconeogenic efficiency. The ratio of ^{14}C-glucose leaving the liver to ^{14}C-alanine and ^{14}C-lactate entering the organ declined both when hypoglycemia occurred (47 ± 17%) and when euglycemia was maintained, regardless of whether (54 ± 9%) or not (57 ± 13%) alanine and lactate were replaced (Table 2).

Effect of DCA on glucose metabolism. In the first protocol, DCA caused the arterial glucose concentration to fall from 107 ± 10 to 56 ± 6 mg/dl by the end of the study ($P < 0.01$, Figure 5). This fall was associated with a slight (19 ± 9%) decrease in glucose production. The rate of glucose utilization declined gradually and was decreased by 17 ± 8% at the end of the experiment. Glucose clearance, on the other hand, increased throughout the experiment and was markedly elevated (55 ± 16%; $P < 0.05$) during the last 2 h of the study.

In the second protocol, when euglycemia was maintained (113 ± 11 mg/dl) by glucose infusion, DCA caused a 46 ± 11% fall in glucose production (2.3 ± 0.3 to 1.3 ± 0.3

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TABLE 1
Effect of DCA infusion (0.4 mg/kg·min) on the splanchnic and hepatic uptakes of alanine and lactate in 48-h-fasted dogs (N = 4) maintained on infusions of somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min). In group I, hypoglycemia was allowed to develop while in group 2, euglycemia was maintained. In addition, for the final 90 min of the study, alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) were replaced

		Splanchnic uptake (μmol/kg·min)							
		Control	60 min	120 min	180 min	210 min	240 min	270 min	300 min
Group 1	Alanine	1.84 ± 0.06	1.37 ± 0.26	0.55 ± 0.08	0.43 ± 0.04	0.27 ± 0.04	0.28 ± 0.09	0.18 ± 0.07	0.32 ± 0.13
	Lactate	3.85 ± 0.45	2.07 ± 0.39	1.34 ± 0.40	1.49 ± 0.97	1.15 ± 0.30	1.07 ± 0.35	1.23 ± 0.31	0.78 ± 0.72
Group 2	Alanine	1.76 ± 0.09	1.14 ± 0.16	0.30 ± 0.05	0.22 ± 0.03	0.33 ± 0.07	2.77 ± 0.40	2.51 ± 0.36	2.57 ± 0.27
	Lactate	4.79 ± 0.64	2.02 ± 2.01	0.54 ± 0.19	0.53 ± 0.24	0.19 ± 0.19	6.55 ± 0.54	6.17 ± 0.51	7.05 ± 0.73
		Hepatic uptake (μmol/kg·min)							
		Control	60 min	120 min	180 min	210 min	240 min	270 min	300 min
Group 1	Alanine	2.40 ± 0.08	1.82 ± 0.18	0.94 ± 0.15	0.95 ± 0.16	0.72 ± 0.10	0.89 ± 0.13	0.79 ± 0.06	0.85 ± 0.16
	Lactate	5.43 ± 0.40	3.46 ± 0.28	2.04 ± 0.54	2.79 ± 0.32	2.46 ± 0.36	2.46 ± 0.18	2.56 ± 0.55	2.16 ± 0.37
Group 2	Alanine	2.47 ± 0.16	1.61 ± 0.22	0.90 ± 0.16	0.74 ± 0.08	0.67 ± 0.08	3.25 ± 0.40	2.92 ± 0.31	2.86 ± 0.24
	Lactate	6.88 ± 0.55	3.66 ± 2.08	2.85 ± 0.21	2.63 ± 0.10	1.96 ± 0.14	7.19 ± 0.84	7.12 ± 0.70	8.18 ± 0.72

mg/kg·min, $P < 0.05$, Figure 6). Restoration of the alanine and lactate levels at the end of the experiment did not enhance glucose output. Similar findings were apparent when glucose production was calculated using the arteriovenous difference technique rather than the tracer technique. Net hepatic glucose output fell from 2.3 ± 0.3 to 1.3 ± 0.3 mg/kg·min and again did not rise when alanine and lactate levels were restored. During the last 90 min of the second protocol, glucose utilization was elevated by 61 ± 19% ($P < 0.01$). The rate of glucose clearance increased throughout the experiment, finally being elevated by 66 ± 26% ($P < 0.01$).

Catecholamines. The mean basal plasma epinephrine and norepinephrine levels in the first protocol were 94 ± 16 and 170 ± 27 pg/ml, respectively (Table 3). During the last hour

FIGURE 3. Arterial, portal vein, and hepatic vein whole blood lactate levels in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min), both without (group 1) and with (group 2) variable glucose infusion (to maintain euglycemia) as well as alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) replacement.

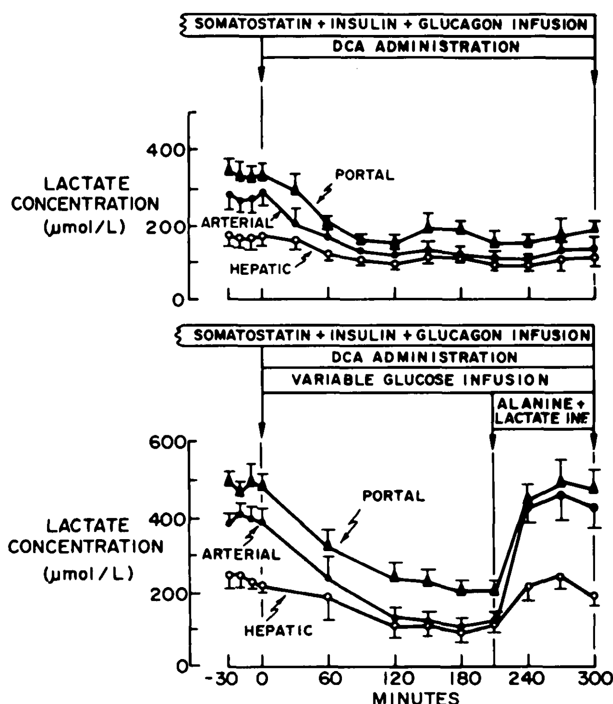

FIGURE 4. The percent change in the basal rate of conversion of circulating alanine and lactate to glucose in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min) and glucagon (1 ng/kg·min), both without (group 1) and with (group 2) variable glucose infusion (to maintain euglycemia) as well as alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) replacement.

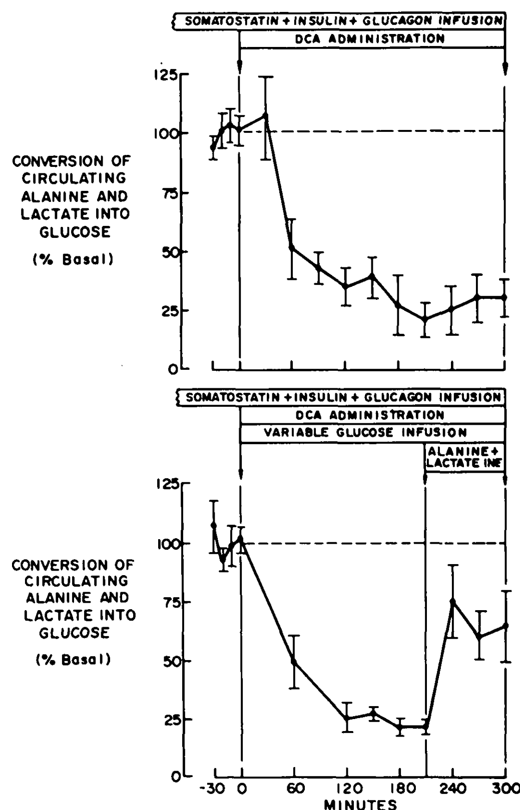

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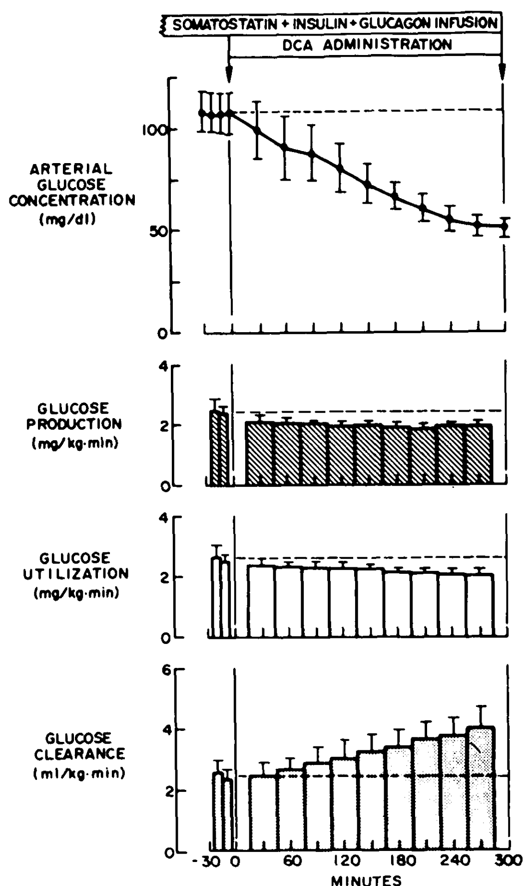
TABLE 2
Ratio of radioactivity released from the liver in the form of ^{14}C-glucose to the radioactivity taken up by the liver in the form of ^{14}C-Alanine and ^{14}C-lactate during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min) both without (group 1) or with (group 2) variable glucose infusion (to maintain euglycemia) as well as alanine (6 μmol/kg·min) and lactate (23.75 μmol/kg·min) replacement

	Group 1	Group 2
−30	0.47 ± 0.18	0.57 ± 0.14
−20	0.52 ± 0.16	0.45 ± 0.12
−10	0.49 ± 0.10	0.58 ± 0.15
0	0.57 ± 0.15	0.59 ± 0.14
30	0.62 ± 0.14	—
60	0.23 ± 0.05	0.25 ± 0.04
90	0.36 ± 0.11	—
120	0.25 ± 0.04	0.26 ± 0.07
150	0.26 ± 0.05	0.23 ± 0.01
180	0.15 ± 0.05	0.21 ± 0.03
210	0.17 ± 0.06	0.23 ± 0.04
240	0.22 ± 0.10	0.23 ± 0.06
270	0.24 ± 0.05	0.17 ± 0.03
300	0.27 ± 0.04	0.24 ± 0.10

of the study, the epinephrine level increased to 273 ± 82 pg/ml ($P < 0.05$) while the norepinephrine concentration remained unchanged (185 ± 24 pg/ml).

FIGURE 5. Arterial plasma glucose concentration, glucose production rate, glucose utilization rate, and glucose clearance rate in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min) and glucagon (1 ng/kg·min).



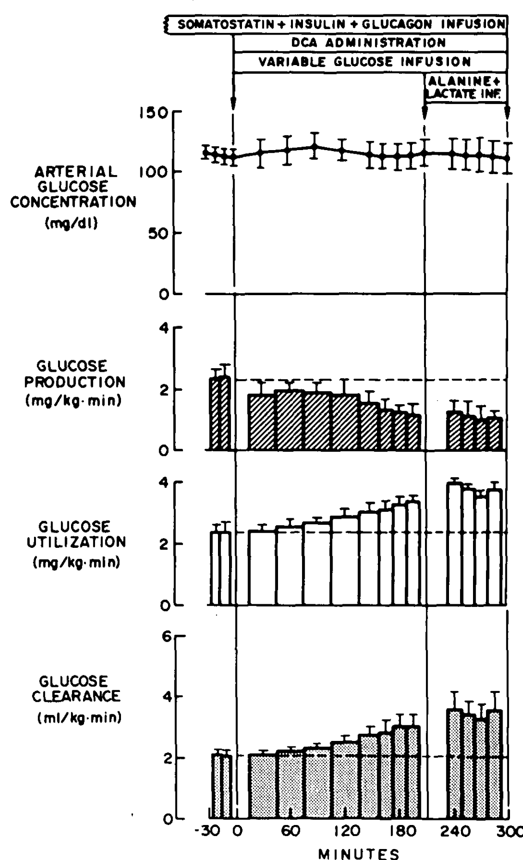


FIGURE 6. Arterial plasma glucose concentration, glucose production rate, glucose utilization rate, and glucose clearance rate in conscious 48-h-fasted dogs (N = 4) during infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min) in the presence of euglycemia achieved by variable glucose infusion both before and after restoration of the basal circulatory levels of alanine and lactate (by infusion of these metabolites at rates of 6 and 23.75 μmol/kg·min, respectively).

DISCUSSION

In the present study, administration of dichloroacetate to 48-h-fasted dogs in which insulin and glucagon had been "clamped" at basal levels was associated with a marked fall in the blood glucose level. In contrast, in a previous study, administration of DCA to dogs in which the level of the pancreatic hormones were not controlled was not associated with hypoglycemia.[1,12] It thus appears that the failure of DCA to cause hypoglycemia in normal 48-h-fasted dogs can be attributed to the effects of the compound on the endocrine

TABLE 3
Effects of infusion of DCA (0.4 mg/kg·min), somatostatin (0.8 μg/kg·min), insulin (average 273 ± 68 μU/kg·min), and glucagon (1 ng/kg·min) on epinephrine and norepinephrine levels in 48-h-fasted dogs (N = 4) in which hypoglycemia occurred

	Control*	DCA Administration†
Epinephrine (pg/ml)	94 ± 16	273 ± 82
Norepinephrine (pg/ml)	170 ± 27	199 ± 42

* Mean ± SEM of −30- and 0-min samples.
† Mean ± SEM of 270- and 300-min samples.

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pancreas. It is thus apparent that the modest fall in the insulin/glucagon molar ratio normally caused by DCA is adequate to produce metabolic effects which offset the direct effects of the compound itself.

It is of interest to examine the mechanism by which hypoglycemia occurs when pancreatic hormones are controlled. There are three possibilities: a reduction in glucose production, a stimulation of glucose utilization, or a combination of the two. With regard to the first possibility, it is apparent that glucose production fell 19 ± 9% in response to DCA. This undoubtedly represents an underestimate of DCA's effect on hepatic glucose output, however, since hypoglycemia occurred and either directly or indirectly (via epinephrine release) stimulated hepatic glucose production.[14,15] With regard to glucose uptake, the fall in the plasma glucose level cannot be explained by a rise in glucose utilization since the latter fell by almost 20%. The observed reduction in glucose utilization does not provide a good reflection of DCA's effect on glucose metabolism by extrahepatic tissues, since there was a concurrent elevation of plasma epinephrine and marked hypoglycemia. Glucose clearance, however, did indeed rise in response to DCA, but the accuracy of this parameter has been questioned in hypoglycemic states.[16,17] Nevertheless, the effect of DCA on glucose clearance is important in determining the magnitude of the hypoglycemia, as explained below. Were the fall in glucose production to be the only cause for hypoglycemia, the blood sugar level would have declined only 20%, and glucose utilization would then have dropped proportionally in response to the reduced glucose level. Since the glucose level fell nearly 50% to 56 ± 6 mg/dl, it is apparent that enhanced glucose clearance must have played a major role in DCA induced hypoglycemia. That is to say, glucose utilization was inappropriate for the ambient plasma glucose concentration. Thus, the plasma glucose level had to decline further to bring glucose utilization into balance with glucose production.

In view of the ability of hypoglycemia and catecholamines to modify the compound's effects on glucose production and utilization, it was necessary to "clamp" not only the insulin and glucagon concentrations in order to accurately evaluate DCA's effect on these processes, but also to prevent the plasma glucose level from changing. When the pancreatic hormones were "clamped" and euglycemia was maintained, a direct effect of DCA on glucose production was evident from both the tracer and arteriovenous difference data. Using each method, glucose production fell from 2.3 ± 0.3 to 1.3 ± 0.3 mg/kg·min. This inhibition could be due to either an inhibition of glycogenolysis and/or gluconeogenesis. It would seem most likely to be the result of the later for several reasons. First these dogs were fasted 48 h so that their hepatic glycogen levels were low (approximately 15 ± 5 mg/g liver[1]), and secondly, DCA has previously been shown to have little effect on glycogen breakdown.[1,18] A decrease in gluconeogenesis, on the other hand, is supported by both a decline in the gluconeogenic ratio (which reflects the efficiency of the intrahepatic gluconeogenic processes) and a decrease in the rate of conversion of alanine and lactate into glucose (which reflects hepatic substrate uptake as well as the efficiency of intrahepatic processes). These findings are consistent with earlier reports that DCA inhibits gluconeogenesis in vivo[12,19] and in vitro.[20-22] This decline in gluconeogenesis could have been due to a decrease in substrate supply to the liver and/or an inhibition of intrahepatic gluconeogenesis. The former possibility is supported by the fact that DCA depletes circulating levels of alanine, lactate, and pyruvate.[1,23-26] There was however a substantial fall in the rate of glucose production prior to the fall in hepatic substrate uptake, suggesting that DCA may also inhibit gluconeogenesis directly. This possibility is further supported by the finding that alanine and lactate replacement during the period of DCA administration only partially restored the conversion of alanine and lactate to glucose, did not alter the gluconeogenic efficiency of the liver and did not have a significant effect on glucose production. The latter finding is not surprising when one considers that an 8-μmol/kg·min increase in gluconeogenic precursor uptake would only increase glucose output by 0.2 mg/kg·min if conversion were 25% efficient. This direct effect of DCA is most likely due to the ability of the compound to inhibit pyruvate carboxylase[22,27] and/or to activate pyruvate dehydrogenase.[28,29] Regardless of the mechanism by which it works, DCA's inhibition of gluconeogenesis in vivo appears to be primarily due to its inhibitory effect on intrahepatic processes rather than to a decrease in substrate supply to the liver.

In addition to its effects on glucose production, DCA also increases extrahepatic glucose utilization. Earlier studies have shown that DCA can increase glucose oxidation in muscle,[2,30] and have suggested that the compound produces hypoglycemia by increasing peripheral glucose uptake.[2] In the present study, such an effect was clearly evident in the second protocol in which the drug caused a marked increase in glucose utilization (61 ± 19%) in the presence of both fixed basal insulin levels and euglycemia. These data support the idea that changes in glucose uptake in vivo can be regulated by intracellular glucose metabolism, as well as by glucose transport,[31] since DCA is not known to alter the latter process. Furthermore, since DCA has a marked action on muscle, and since glucose uptake by muscle in 48-h-fasted dogs represents only a small percent of total glucose utilization, an increase in overall glucose uptake of the magnitude observed probably represents a marked (severalfold) increase in muscle glucose uptake. The fate of this carbon within muscle remains unclear, but it undoubtedly increases tricarboxylic acid cycle flux secondary to the compounds activation of pyruvate dehydrogenase.[28,29]

In summary, the in vivo effects of DCA on carbohydrate metabolism in the 48-h-fasted dog can be explained by combining the effects of this compound at three sites, the pancreas, liver, and peripheral tissues. At the pancreas, this compound is able to decrease insulin secretion and perhaps enhance glucagon release, at the liver it inhibits gluconeogenesis and reduces glucose production, and in peripheral tissues it both decreases the release of alanine and lactate and stimulates the uptake of glucose. Thus the hypoglycemic effect of DCA appears to result primarily from the stimulation of peripheral glucose uptake, as well as by the inhibition of hepatic glucose production. However, with regard to the latter, the rate-limiting step appears to be a direct inhibitory effect on net gluconeogenesis within the liver rather than a decrease in gluconeogenic substrate availability, even though the latter does occur.

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