Mechanisms by Which Dichloroacetate Lowers Lactic Acid Levels: The Kinetic Interrelationships between Lactate, Pyruvate, Alanine, and Glucose (43675)

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Abstract. **Dichloroacetate (DCA) is gaining use as an alternative to bicarbonate therapy in the treatment of lactic acidosis. To determine the mechanism(s) by which DCA lowers blood lactate levels, we studied its effect on the kinetic interrelationships between pyruvate, lactate, alanine, and glucose in the hindlimb of dogs during hormonal stimulation of pyruvate production (Ra) and its conversion to lactate. Three groups of dogs (n = 6) were infused with 1-^{13}C-pyruvate to measure whole body pyruvate Ra, and pyruvate Ra and utilization (Rd) across the hindlimb during either a 4-hr infusion of saline (controls), or somatostatin, glucagon, and epinephrine (SGE), or SGE plus dichloroacetate (SGE + DCA). Pyruvate Ra was used as an index of rate of glycolysis and Rd as an index of pyruvate oxidation. In the controls, all kinetic parameters were constant during the saline infusion. Hindlimb pyruvate Ra and Rd were almost equal, and lactate release negligible. Compared to controls, SGE administration significantly increased ($P < 0.05$) wholebody pyruvate Ra (48.5 ± 6.2 vs 33.6 ± 2.4 μmol/kg/min) and blood lactate levels ($P < 0.05$). Hindlimb pyruvate Ra increased by ~150%, but Rd remained unchanged resulting in marked increases in lactate and alanine effluxes. Adding DCA to the SGE infusion significantly reduced wholebody pyruvate Ra ($P < 0.05$) and blood lactate levels ($P < 0.01$). In the hindlimb, however, there was no decrease in lactate output, despite a 91% increase in pyruvate utilization because pyruvate Ra also increased. These results suggest that during stimulation of rate of glycolysis, DCA lowers lactate levels by reducing the overall availability of pyruvate for lactate synthesis. This is accomplished by suppressing the rate of glycolysis in tissues other than skeletal muscle and stimulating pyruvate oxidation.** [P.S.E.B.M. 1994, Vol 205]

It is believed that most lactic acid is produced by the peripheral tissues and removed by the liver (1). Lactic acid levels increase when there is an imbalance between its rate of production and utilization. Several pathological conditions such as severe trauma, sepsis, fulminant liver failure, and untreated diabetes mellitus are characterized by increased blood lactic acid levels in the absence of circulatory failure or hypoxia (2, 3, 4, 5). Accumulation of lactate eventually causes metabolic acidosis which can lead to significant myocardial dysfunction and hypotension (6).

Recent studies have shown that in conditions of marked metabolic acidosis, such as sepsis and liver transplantation, dichloroacetate (DCA), because of its lactate-lowering effect, is more effective than standard bicarbonate therapy (3, 7). At present there is uncertainty about the exact mechanism(s) by which DCA lowers lactate levels *in vivo*. Early *in vitro* studies demonstrated that DCA stimulates pyruvate dehydrogenase (PDH) activity, thereby increasing the propor-

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tion of pyruvate disposed via acetyl CoA and the TCA cycle, relative to the conversion of pyruvate to lactate (8). Other studies showed that DCA also reduces lactate formation by inhibiting rate of glycolysis in muscle tissues, thereby reducing the availability of pyruvate (9–11). However, more recent *in vivo* studies in normal human volunteers (4) and in the perfused rat hindquarter (12) have failed to demonstrate an inhibition of rate of glycolysis by DCA, and in the perfused rat heart, rate of glycolysis was even stimulated by DCA (13). Furthermore, DCA markedly reduced plasma lactate levels without reducing efflux of lactate from the leg of normal human volunteers (14).

In this study we aimed to determine the mechanisms by which DCA reduces lactate levels by examining its effect on the kinetics of pyruvate, lactate, alanine, and glucose in skeletal muscle tissues. The effect of DCA on wholebody pyruvate kinetics was also measured. A dog model was used, in which the rate of glycolysis and the non-oxidative disposal of pyruvate as lactate and alanine were stimulated by decreasing the insulin to glucagon ratio (I/G ratio), and increasing epinephrine levels with the combined infusion of somatostatin, glucagon, and epinephrine (SGE).

Materials and Methods

Animals. Adult male dogs of mixed breed weighing 29.2 ± 1.4 kg were obtained from a USDA licensed dealer (Holco, Westfork, AR). The animals were housed in pens and fed Purina Lab Canine Diet 5006 (Purina Mills, Richmond, IN) and water *ad libitum*. They were conditioned for three weeks when they were treated for endo- and ecto-parasites, and immunized against distemper, hepatitis, leptospirosis, rabies, and parainfluenza.

Materials. The isotope 1-^{13}C-pyruvate (99% enriched, Merck, Dorvael, Canada) was used to measure pyruvate kinetics. Sodium dichloroacetate was obtained from Continental Trading Company Organics (Atlanta, GA). Sterile solutions of the isotope and DCA were prepared in 0.45% saline and passed through a 0.22 μm Millipore filter into sterile evacuated containers. Solutions of somatostatin (Bachem, Torrance, CA), and glucagon (Eli Lily, Indianapolis, IN) plus epinephrine (Park Davis, Morris Plains, NJ) were prepared in 0.45% saline immediately before the start of an infusion. For each isotope infusion, an aliquot of the infusate was analyzed spectrophotometrically for the exact 1-^{13}C-pyruvate concentration in order to calculate the actual infusion rate for each experiment.

Experimental Procedure. There were three experimental protocols and six animals were studied in each protocol. The animals were studied under anaesthesia after a 14-hr overnight fast. They were anaesthetized with 120.8 μmol/kg pentobarbital and a polyethylene catheter was placed in the jugular vein for infusion of the isotope and saline (protocol 1), or isotope and somatostatin (S), glucagon (G) and epinephrine (E) (protocol 2), or isotope plus SGE and DCA (protocol 3). Another catheter was placed in the carotid artery for sampling arterial blood. A specially designed T-shaped catheter was placed in the common femoral vein, just above the caudal femoral tributary, and used to sample blood leaving the limb and to measure blood flow rate through the limb. To ensure that this catheter did not impede normal blood flow through the limb, its internal diameter closely matched that of the femoral vein of the animal. Rate of blood flow through the limb was obtained by mechanically collecting the volume of blood flowing through the T-shaped catheter into a graduated tube in a timed interval. This procedure required that the catheter be clamped off at a point cranial to the outlet tubing simultaneously as the outlet tubing was opened to the graduated tube and the timer started. After ~2 sec, the clamp was removed from the catheter to allow femoral venous flow to continue, the collected blood was reinfused (to minimize changes in hematocrit), and the outlet tubing was flushed and re-clamped. Sampling catheters were kept patent by periodic flushing with a heparin-saline solution. Additional doses of pentobarbital (4 μmol/kg/h) were given throughout the experiment to maintain a constant level of anaesthesia. At the end of each experiment the animals were sacrificed by injection of euthanizing agent T-61 (Hoechst-Roussel Agri-Vet Co., Summerville, NJ). This study was approved by the Animal Welfare Committee of the University of Texas Medical Branch.

Protocol 1. The aim of protocol 1 was to establish control values. All the relevant parameters such as whole-body pyruvate Ra, hindlimb pyruvate Ra and Rd, and net balance of lactate, alanine, and glucose across the hindlimb, were measured during 4 hr of saline infusion.

Solutions of 1-^{13}C-pyruvate and saline were infused at 15 ml/hr for 4 hr via the jugular vein catheter. After injecting a priming dose of 22.5 μmol/kg of 1-^{13}C-pyruvate the isotope was infused continuously for 4 hr at 1.5 μmol/kg/min. Before the infusions started and at 1-hr intervals during the infusions, blood samples were drawn simultaneously from the carotid artery and the femoral vein catheters. Blood flow through the femoral vein was measured at the same time. During the last hour, four 2 ml blood samples were also drawn at 15-min intervals from the arterial catheter (in order to calculate whole-body pyruvate Ra).

Protocol 2. The aim of protocol 2 was to stimulate pyruvate production and its conversion to lactic acid, by infusing somatostatin, glucagon, and epinephrine. The same kinetic parameters were measured by

infusing 1-^{13}C pyruvate as described in protocol 1. In addition, a solution of somatostatin (0.1 μg/kg/min), glucagon (5 ng/kg/min), and epinephrine (10 ng/kg/min) was infused at 15 ml/hr into the jugular vein. Blood was sampled as per protocol 1.

Protocol 3. The aim of protocol 3 was to assess the effect of DCA on the same kinetic parameters during stimulation of pyruvate production and lactic acid synthesis with SGE. The isotope and hormones were infused as per protocol 2. In addition at the start of the infusions and 1.5 hr later, DCA was infused via the jugular vein at a rate sufficient to deliver a dose of 35 mg/kg over 30 min. Blood was sampled as per protocols 1 and 2.

Analysis of Blood Samples. Six milliliter ml blood samples were placed in ice-cold heparinized tubes. Glucose and lactate concentrations were determined immediately on a 0.5 ml aliquot. The rest of the sample was aliquoted as follows: 1 ml was pipetted into a tube containing 4 ml ethyl acetate plus 50 μl 12 *M* HCl for analysis of lactate isotopic enrichment, 0.1 ml into a tube containing 0.4 ml of lithium citrate–3.75% sulfosalicylic acid buffer, for determination of amino acid concentrations, 2 ml in a tube containing EDTA/trasylol for determination of insulin and glucagon concentrations, 2 ml in a tube containing 4 ml of 8% $HClO_4$ for pyruvate concentrations. The solutions were centrifuged at the earliest opportunity and the supernatant stored at −70°C for later analysis.

Amino acid concentrations were determined by column chromatography on a Beckman 6300 Autoanalyser (Beckman Instruments, Palo Alto, CA). Glucose concentrations were measured by the glucose oxidase reaction (Beckman Glucose Analyzer 2, Fullerton, CA) and lactate concentrations by the lactate oxidase reaction on a YSI model 23L Lactate Analyser (Yellow Springs Instrument, Yellow Springs, OH). Pyruvic acid concentrations were determined spectrophotometrically utilizing the oxidation of NADH in the lactate dehydrogenase reaction. Plasma insulin was measured by radioimmunoassay using a kit (Incstar Inc., Stillwater, MN) and glucagon by radioimmunoassay at the Mayo Medical Laboratories (Rochester, MN).

The isotopic enrichment of lactate was measured on a Hewlett-Packard 5985B GC-MS system (Hewlett-Packard, Palo Alto, CA). Its (bis) trimethylsilyl derivative was analyzed using chemical ionization and monitoring ions at m/e 219.1 and 220.1.

Calculations and Statistics. All kinetic parameters are expressed per kilogram of body weight. The standard steady state equation was used to calculate whole-body pyruvate production:

$$\text{Whole-body Pyruvate Ra} = \frac{E_{Inf} \times D}{E_{Art}}$$

where E_{Inf} and E_{Art} are the tracer/tracee ratios (enrichments) of pyruvate in the infusate, and lactate in arterial blood respectively, D is the rate of infusion of the isotope in μmol/kg body wt/min, and the units of Ra are μmol/kg body wt/min.

Net balance of substrates across the hindlimb was calculated according to:

$$\begin{aligned}\text{Net Balance} &= \text{Influx(FA)} - \text{Efflux(FV)} \\ &= \text{F(A} - \text{V)}\end{aligned}$$

where F is hindlimb blood flow rate (ml/kg body wt/min), and A and V are arterial and venous whole blood concentrations (μmol and nmol/ml) of the substrates.

Pyruvate Production (Ra) in the hindlimb was calculated from the equation:

$$\text{Hindlimb Pyruvate Ra} = \text{F(A)(Ea/Ev} - 1)$$

where Ea and Ev are the arterial whole blood and venous whole blood tracer/tracee ratios of lactate, A is the arterial concentration of lactate plus pyruvate in blood, and the units of Ra are μmol/kg body wt/min.

Although labeled pyruvate was infused, the enrichment of lactate was used to calculate pyruvate kinetics. This action is based on the previous finding that lactate and pyruvate enrichments were almost identical because of rapid isotopic equilibration (15). That is, lactate enrichment reflected that of the pyruvate pool (from which all lactate is derived). Although the amount of pyruvate in the blood is negligible compared to the amount of lactate, nevertheless, the sum of lactate and pyruvate concentrations is used to calculate hindlimb pyruvate Ra because label enters and leaves the pyruvate pool in the limb in both compounds.

Because Net Balance is the difference between Production (Ra) and Utilization (Rd), hindlimb Pyruvate Rd was obtained from:

$$\text{Hindlimb Pyruvate Rd} = \text{F(A} - \text{V)} + \text{Ra}$$

where A and V are the arterial and venous blood concentrations of lactate plus pyruvate, and the units of Rd are μmol/kg body wt/min.

Pyruvate Ra was used as an index of glycolysis as alanine contributes less than 10% of all pyruvate produced (15), Rd as an index of pyruvate oxidation, because pyruvate is not used for gluconeogenesis in skeletal muscle and only about 7% of all pyruvate produced is used for alanine synthesis (4). Lactate plus alanine effluxes from the hindlimb were considered an index of non-oxidative disposal of pyruvate in the limb.

Results are presented as mean ± SEM. Statistical evaluation of the data was performed using two-way ANOVA to test for overall differences among the three protocols. If the F-test revealed a significant difference, this was followed by Fisher's PLSD test to determine significance between means. The paired

T-test was used to compare measurements within a protocol (e.g., baseline vs 4 hr). Significance of difference was assumed at $P < 0.05$.

Results

Hormone Concentrations. In the controls baseline plasma insulin (11 ± 2 μU/ml) and glucagon (92 ± 4 pg/ml) concentrations did not change (14 ± 2 μU/ml and 102 ± 13 pg/ml respectively) after 4 hr of saline infusion. In protocols 2 and 3 the infusion of SGE and SGE + DCA respectively, caused baseline plasma insulin to decrease by ~50% (from 9 ± 1 and 12 ± 2 to 4 ± 0.6 and 6 ± 1 μU/ml respectively) and glucagon concentrations to increase ~3-fold from 118 ± 13 and 121 ± 20 to 480 ± 25 and 490 ± 30 pg/ml, respectively.

Glucose Concentration and Balance. In the controls arterial and femoral venous blood glucose concentrations were unchanged during 4 hr of saline infusion (Fig. 1). SGE elicited significant increases ($P < 0.05$) over the baseline and the time-matched control values. The addition of DCA did not significantly alter this increase in glucose concentration for 3 hr. After 4 hr, however, both arterial and venous concentrations declined to values that were not different from the time-matched control or the baseline values (Fig. 1). In all three protocols baseline glucose uptake across the hindlimb was ~1.8 μmol/kg body wt/min (Fig. 1). This did not change significantly during saline and SGE administration. When DCA was administered with SGE, although there was a transient increase in glucose uptake, this was not significant (Fig. 1).

Pyruvate Kinetics. Whole-body pyruvate Ra was 33.6 ± 2.4 μmol/kg body wt/min in the controls, and increased significantly ($P < 0.05$) to 48.5 ± 6.2 μmol/kg body wt/min in response to the administration of SGE (Fig. 2), indicating an overall stimulation of rate of glycolysis. Adding DCA to the SGE infusion caused a 50% decrease ($P < 0.05$) in pyruvate Ra to 24.5 ± 3 μmol/kg body wt/min. This value was significantly lower ($P < 0.05$) than the control value by 27%.

In the hindlimb baseline pyruvate Ra in the controls was 3.09 ± 0.3 μmol/kg body wt/min and remained unchanged during the saline infusion (Fig. 3). Compared to the time-matched control value, pyruvate Ra increased significantly ($P < 0.05$) by ~150% when SGE was administered, indicating a marked stimulation of rate of glycolysis in the muscle tissues of the hindlimb. The addition of DCA caused a further increase in pyruvate Ra which was significantly greater ($P < 0.05$) than the corresponding saline, and SGE values after 4 hr.

In the controls, baseline pyruvate Rd in the hindlimb (3.27 ± 0.4 μmol/kg body wt/min) was almost identical to Ra and remained unchanged (Fig. 3), indicating that production and utilization of pyruvate in the limb were almost equal. SGE administration elicited only a modest increase in pyruvate Rd, but the addition of DCA caused a significant increase ($P < 0.01$) in pyruvate Rd (Fig. 3), indicating a marked stimulation of pyruvate oxidation.

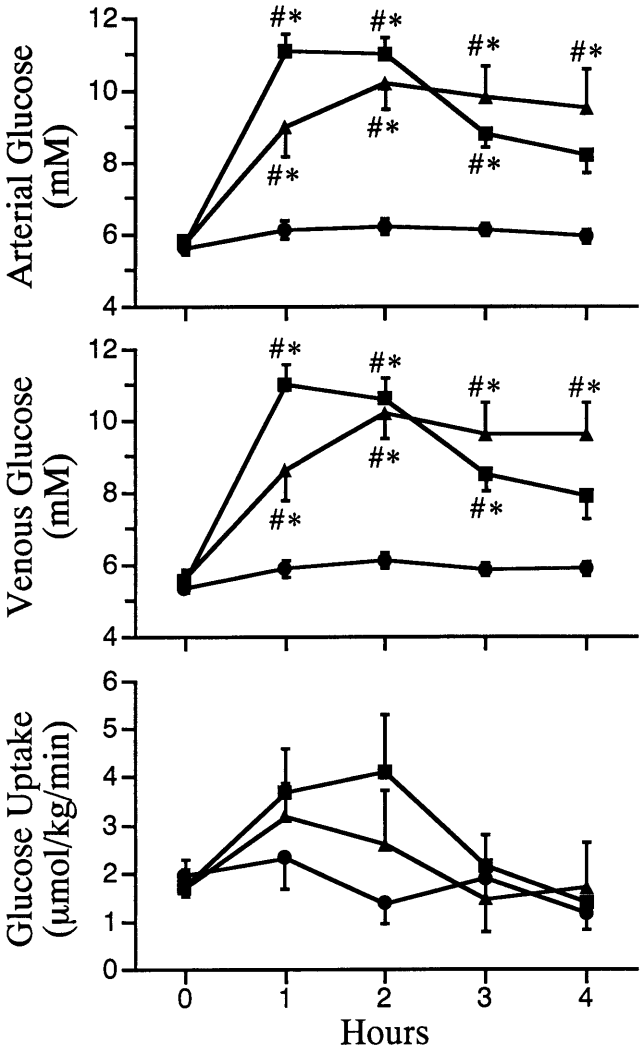

Figure 1. Arterial and femoral venous glucose concentrations and hindlimb glucose uptake during infusions of saline (-●-); somatostatin, glucagon, and epinephrine (-▲-); and somatostatin, glucagon, and epinephrine, plus dichloroacetate (-■-) in three groups ($n = 6$) of postabsorptive dogs. #*Indicate that the value is significantly different ($P < 0.05$) from the baseline and the time-matched control values, respectively.

Lactic Acid Concentration and Balance. Baseline arterial and venous blood lactate concentrations were similar in all three groups (Fig. 4). Both arterial and venous blood lactate concentrations increased modestly and to the same extent during the saline infusion. Hence, lactate balance across the hindlimb was always near zero, changing from a negligible uptake at 0 hr to a negligible release after 4 hr. When SGE was administered venous lactate concentration increased significantly ($P < 0.05$) over baseline by ~100%, as there was a marked efflux of lactate from the hindlimb (Fig. 4). The amount of lactate released after 4 hr was 3.98 ± 0.45 μmol/kg body wt/min, and accounted for

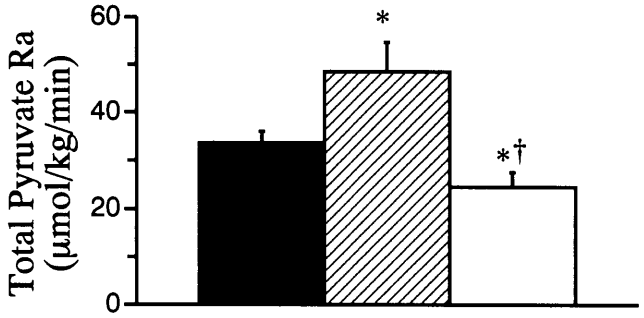

Figure 2. Wholebody pyruvate production rate (Ra) during infusions of saline (■), somatostatin, glucagon, and epinephrine (▨); and somatostatin, glucagon, and epinephrine, plus dichloroacetate (□) in three groups (n = 6) of postabsorptive dogs. Measurements were made during the last hour of a 4-hr infusion. *·†Indicate significantly different ($P < 0.05$) from the time-matched control and SGE values, respectively.

most of the pyruvate produced that was not utilized in the limb (Table I). After 2 hr, the DCA administration caused arterial blood lactate concentrations to decrease significantly ($P < 0.05$) when compared to baseline values, and both arterial and venous lactate concentrations decreased significantly ($P < 0.05$) when compared to the time-matched SGE values (Fig. 4). However, arterial concentration decreased to a greater

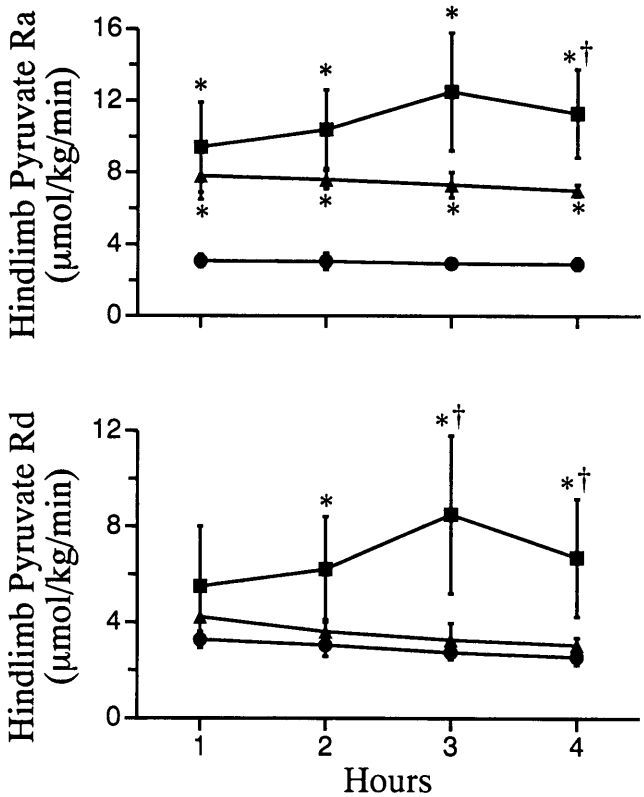

Figure 3. Hindlimb pyruvate production (Ra) and disposal (Rd) during infusions of saline (-●-); somatostatin, glucagon, and epinephrine (-▲-); and somatostatin, glucagon, and epinephrine, plus dichloroacetate (-■-) in three groups (n = 6) of postabsorptive dogs. *·†indicate that the value is significantly different ($P < 0.05$) from the time-matched control and SGE values, respectively.

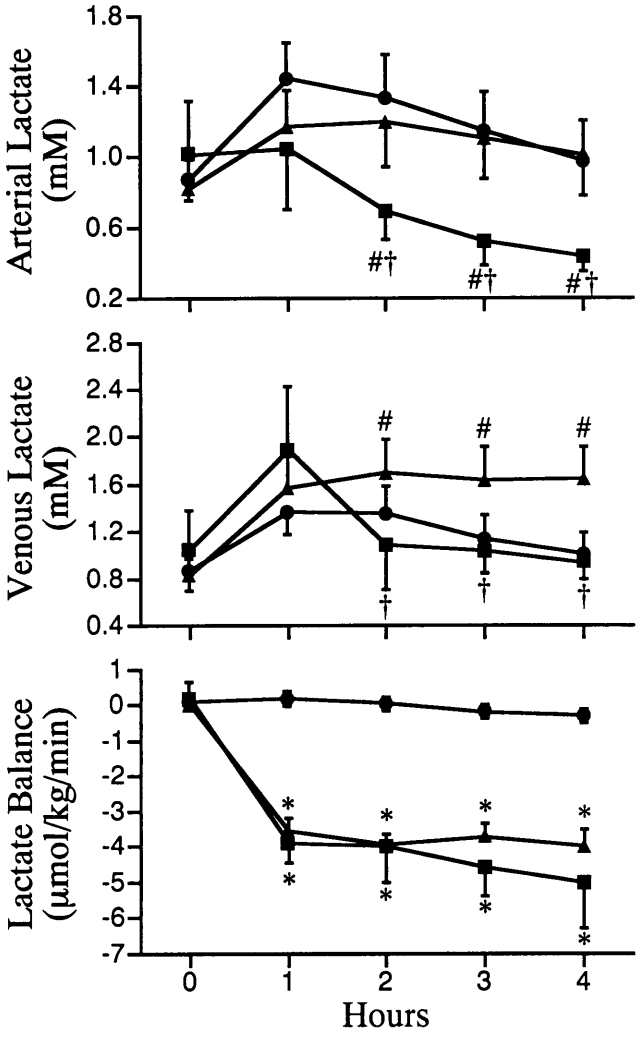

Figure 4. Arterial and femoral venous lactate concentrations, and lactate balance across the hindlimb during infusions of saline (-●-); somatostatin, glucagon, and epinephrine (-▲-); and somatostatin, glucagon, and epinephrine, plus dichloroacetate (-■-) in three groups (n = 6) of postabsorptive dogs. *·†Are significantly different ($P < 0.05$) from the time-matched control and SGE values, respectively; # is significantly different ($P < 0.05$) from the baseline value.

extent than venous concentration, resulting in no change in the rate of lactate release from the hindlimb (Fig. 4).

Alanine Concentration and Balance. Baseline arterial and femoral venous blood alanine concentrations were similar in all three groups (Fig. 5). During the saline infusion, there was a tendency for alanine concentration to decrease with time, but alanine rate of release from the hindlimb was constant. SGE administration prevented such a decrease in blood alanine concentration, and elicited a significant increase ($P < 0.05$) in the rate of alanine release from the hindlimb when compared to baseline or to time-matched control values (Fig. 5). The addition of DCA significantly reduced ($P < 0.05$) alanine concentrations, and its rate of release from the hindlimb ($P < 0.05$).

Table I. The Fate of Pyruvate Produced in the Hindlimb[a]

Experimental group	Pyruvate Ra (μmol/kg/min)	Pyruvate Rd (μmol/kg/min)	Lactate efflux (μmol/kg/min)
Saline	3.0 ± 0.3	2.90 ± 0.3 (97%)	0.05 ± 0.1 (1.7%)
SGE	7.42 ± 0.2*	3.53 ± 0.2 (48%)	3.80 ± 0.1* (51%)
SGE + DCA	10.9 ± 0.6*†	6.73 ± 0.6* (62%)	4.0 ± 0.28* (37%)

[a] Values shown are means ± SEM. The units are μmol/kg/min. The values within the parentheses are pyruvate Rd and lactate efflux expressed as percentages of Pyruvate Ra. Ra and Rd, rates of production and utilization; SGE, somatostatin, glucagon and epinephrine; SGE + DCA, somatostatin, glucagon, epinephrine and dichloroacetate.
*,† Indicates significantly different ($P < 0.05$) from the saline and SGE values, respectively.

Fate of Hindlimb Pyruvate. In the hindlimb of the controls, 97% of the pyruvate produced was utilized (Table I). When SGE was administered, because Ra increased by ~150%, while pyruvate Rd remained unchanged, only 45% of pyruvate produced was utilized in the limb. The increased lactate release accounted for 51%, and the increased alanine release accounted for 4%. When DCA was given, despite a further 110% increase in the amount of pyruvate produced by the hindlimb, lactate efflux did not change because a greater proportion (62%) of pyruvate produced was utilized within the limb. The proportion released as lactate was reduced to 38%. It can be assumed that all of this increase in utilization represents pyruvate oxidation in the limb, as alanine release decreased, suggesting no increased synthesis from pyruvate.

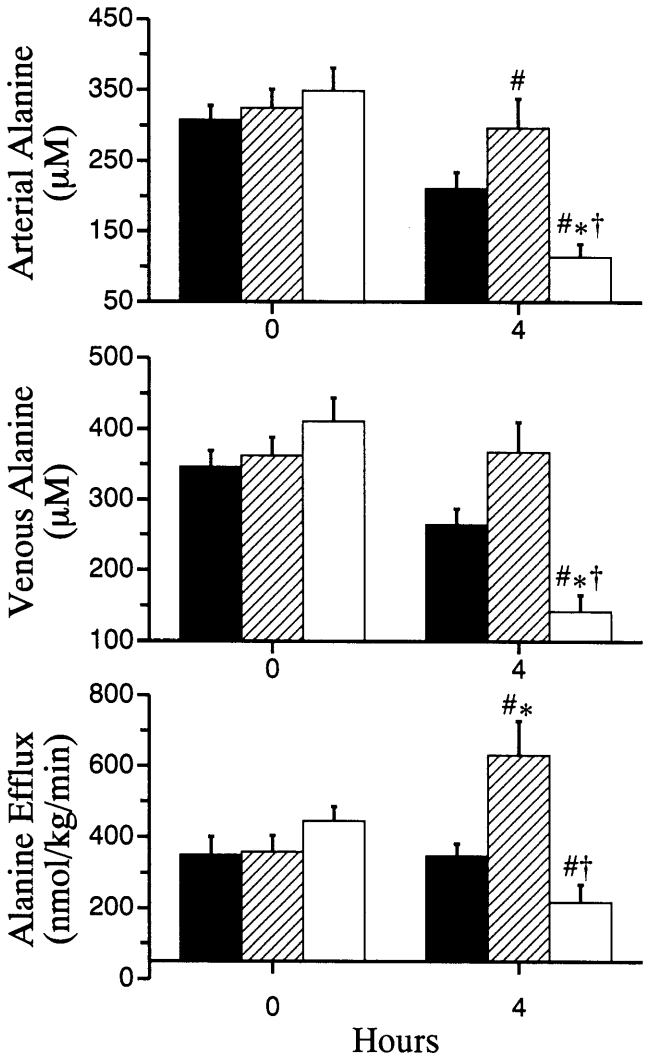

Figure 5. Arterial and femoral venous alanine concentrations, and efflux of alanine during infusions of saline (■); somatostatin, glucagon, and epinephrine (▨); and somatostatin, glucagon, and epinephrine, plus dichloroacetate (□). *,†Are significantly different ($P < 0.05$) from the time-matched control and SGE values, respectively; # is significantly different ($P < 0.05$) from the baseline value.

Discussion

The results of this study demonstrate that the rate of whole-body pyruvate production and its conversion to lactate is increased by simultaneously decreasing the plasma I/G ratio and increasing epinephrine levels. At the whole body level DCA prevented this hormonally-stimulated increase in pyruvate production, decreasing it below control values, and there was a marked reduction in lactic acid levels. In the hindlimb, DCA did not decrease lactate output, despite increasing the percent of pyruvate oxidized, because pyruvate production also increased. These findings suggest that DCA reduces lactic acid levels by inhibiting the rate of glycolysis in some tissue(s) other than skeletal muscle, and stimulating the rate of oxidation of pyruvate, thereby decreasing the availability of pyruvate for lactate synthesis.

The lactate-lowering effect of DCA can be due to either an overall decrease in lactate production, an increased rate of removal, or a combination of the two. The rate of production of lactate is determined by the availability of pyruvate, and by the cytoplasmic $NADH/NAD^+$ ratio which influences the equilibrium relation between lactate and pyruvate (2, 16). Most pyruvate is metabolized by the PDH reaction to acetyl-CoA, which is subsequently oxidized to carbon dioxide in the tricarboxylic (TCA) cycle (17). The PDH reaction requires NAD^+ and ATP (16, 17). Therefore, conditions that impair PDH activity, such as diabetes mellitus (8) or mitochondrial reoxidation of NADH to NAD^+, such as hypoxia (16) will increase the availability of pyruvate for conversion to lactate.

Pyruvate availability is also determined by the rate of glycolysis. A stimulated rate of glycolysis will generate pyruvate faster than it can be utilized by PDH, which will favor its conversion to lactate (2, 15, 16). This is best illustrated by the effects of hypoxia. Because hypoxia causes an increase in the NADH/NAD^+ ratio and a decrease in the ATP/ADP ratio, it simultaneously increases pyruvate production, via stimulation of phosphofructokinase by ADP (18), inhibits PDH activity (17), and stimulates lactic acid dehydrogenase activity, thereby increasing both pyruvate availability and its conversion to lactate (2, 16). Hence, inhibiting rate of glycolysis and/or stimulating pyruvate oxidation will reduce lactate production. The present findings suggest that DCA uses both mechanisms to lower lactic acid levels. There is an increase in the proportion of pyruvate oxidized, which decreases the proportion disposed via the non-oxidative pathways to lactate and alanine. Simultaneously, the overall rate of glycolysis is inhibited.

In agreement with the findings of other studies (4, 8, 10, 13), DCA stimulated pyruvate oxidation in the hindlimb. This is expected because DCA is known to stimulate PDH activity (8). At the whole body level of normal human subjects, the DCA-induced stimulation of pyruvate oxidation is associated with a 60% reduction in lactate production (4). Therefore in the present study, the failure of lactate release from the hindlimb to decrease, despite the increased oxidation of pyruvate, was unexpected. Because of the simultaneous increase in hindlimb pyruvate production, although the percent of pyruvate oxidized was increased by 91%, and the percent converted to lactate decreased (Table I), there was no change in the rate of lactate release from the limb. This finding is in agreement with those of Brown *et al.* (14) who showed that DCA elicited an increase in glucose oxidation rate and markedly reduced plasma lactate levels in normal human subjects. However, the reduction in plasma lactate occurred in the absence of any changes in lactate output from the leg. Our present findings and those of Brown *et al.* (14) suggest that the reduction of lactate production by DCA occurs exclusively in tissue(s) other than skeletal muscle. This is surprising because it is generally accepted that the skeletal muscle bed is the primary site of lactate production. Such a proposal, however, was based on the consistent finding that plasma lactic acid levels always increased during exercise (19). More recent evidence suggests that such a proposal is misleading. For example, it has now been shown that 66% of all the lactic acid produced in normal human subjects is from non-muscle tissues (20).

The present findings across the hindlimb is in agreement with those of McAllister *et al.* (13) who showed that in rat hearts perfused with glucose, DCA stimulated the rate of glycolysis and increased lactate output, despite an increased rate of glucose oxidation. On the contrary, Clarke *et al.* (9, 10) made the opposite observation that DCA inhibited glycolysis in normal and burned rat muscles. These different observations could be due to the dissimilar conditions under which the experiments were performed. In Clarke's *in vitro* studies (9, 10), skeletal muscles of burned and normal rats were incubated in media containing supraphysiological levels of glucose and insulin; conditions that will stimulate glucose uptake and its utilization for both glycolysis and glycogenesis. This was confirmed by their data which showed that DCA lowered pyruvate production because glucose uptake was preferably utilized by glycogen synthesis rather than for glycolysis (10). On the other hand, in the present *in vivo* study, although glucose concentration was increased to approximately the same level as in Clarke's study (10), plasma insulin levels were lowered and epinephrine levels increased, conditions that will promote the preferential utilization of glucose for glycolysis (18, 21, 22). It should be noted that in the present study DCA elicited a transient increase in glucose uptake, which, though not significantly different from the corresponding SGE value, may be responsible for the simultaneous increase in hindlimb pyruvate production.

The inhibition of wholebody rate of glycolysis by DCA is in agreement with its effect in burned patients, burned rats, and lactic acidotic guinea pigs (4, 10, 11). For example, in burned patients, the reduced conversion of pyruvate to lactate by DCA, was associated with an increased rate of pyruvate oxidation and an 18% reduction in rate of glycolysis. Similarly, Clarke *et al.* (10) concluded that the DCA-induced reduction in lactate output from the skeletal muscles of burned rats was mostly accounted for by the simultaneous reduction in pyruvate production.

There is some evidence to suggest that DCA reduces lactate levels by also stimulating rate of lactate removal from the circulation (1, 13). For example Graf *et al.* (1) showed that DCA lowered lactate levels by simultaneously inhibiting its production and stimulating its hepatic uptake in hypoxic lactic acidotic dogs, and McAllister *et al.* (13) reported an increased extraction of lactate by the heart after DCA administration to dogs.

Our present findings also suggest that during hormonal stimulation of glycolysis, the increased rate of lactate release from the hindlimb was not due to an impairment of PDH activity because pyruvate oxidation rate was not inhibited. Rather, the increased lactate (and alanine) output was a direct consequence of the increased availability of pyruvate. It can be calculated from the data in Table I that most (86%) of the extra pyruvate produced by the SGE administration can be accounted for by the lactate released. Similar findings have been reported in severely burned pa-

tients (4). For example, there was a 3-fold stimulation of rate of glycolysis in postabsorptive burned patients, and although a greater proportion of the pyruvate produced was oxidized in the patients (68% versus 57% in normals), there was still a 2-fold increase in the rate of lactate production (4). The similarities between the findings in burn injury (4, 9, 10) and in the present study, suggest that the alterations in pyruvate and lactate metabolism elicited by severe injury are mediated by the accompanying alterations in the hormonal milieu.

It is interesting to note that despite the 3-fold increase in pyruvate oxidation when DCA was given, lactate rate of release from the experimental limb did not decrease, but rate of alanine release decreased significantly. Because most alanine (~80%) is produced from pyruvate (15), this means that available pyruvate was used preferably as substrate in the lactate dehydrogenase reaction rather than for transamination in the alanine aminotransferase reaction. This is not surprising because the Michaelis constant of lactate dehydrogenase for pyruvate (23) is substantially lower than that of alanine aminotransferase (24). Pyruvate was therefore preferably converted to lactate rather than to alanine.

In summary, the results of this study suggest that DCA can lower lactate levels by decreasing the availability of pyruvate for synthesis of lactate. This is accomplished by the simultaneous inhibition of rate of glycolysis in some tissue(s) other than skeletal muscle and stimulation of pyruvate oxidation.

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