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Acute Effects of Dichloroacetate in the Depancreatized Dog: Glucose Synthesis and Turnover

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Summary. Blood glucose turnover (entry and removal rates) and the rate of recycling of radiolabelled glucose carbon into newly synthesized blood glucose have been evaluated before and acutely after the administration of dichloroacetate to depancreatized dogs. Blood glucose concentration began to decline immediately after dichloracetate administration and fell to new steady state levels within 1.5–3 h. Analysis of blood glucose kinetics during the decline demonstrated a 52% (average) reduction in the rate of hepatic glucose supply. Glucose supply remained reduced over the duration of these studies (3–4.5 h). Glucose turnover in the steady state following dichloroacetate administration averaged 62% of pretreatment values. Cori cycle activity was depressed by 63% after dichloroacetate administration. The results of these studies are consistent with the hypothesis that a major mechanism underlying the hypoglycaemic action of this drug is the inhibition of glucose synthesis.

Key words: Dichloroacetate, inhibition, glucose synthesis, hypoglycaemic agent.

Investigations with dichloroacetate (DCA) indicate that this rather simple compound has marked effects on glucose metabolism. DCA (di-isopropylammonium salt) was shown to reduce blood glucose concentration in the diabetic but not in the normal rat [1, 2]. An early hypothesis to explain this blood glucose-lowering action was that the drug inhibited the oxidation of non-esterified fatty acids (NEFA). This would lead to a fall in tissue citrate levels, which would lead in turn to the deinhibition of phosphofructokinase and an acceleration of glycolysis [3]. The original observations detailing these actions in rat hemi-diaphragms [2] and in intact rats [3], have been confirmed in other studies [4]. Another biochemical basis for the action of DCA was proposed by Whitehouse and Randle [5] who demonstrated that DCA activated pyruvate dehydrogenase in heart muscle. Studies by Alberti et al. have shown that DCA could ameliorate or reverse the lactic acidosis observed with biguanide therapy [6], a predictable consequence of pyruvate dehydrogenase activation which would shunt pyruvate into the Krebs cycle. A further consequence of such action has been demonstrated in rats starved for 24 h: namely that DCA acutely restricts glucogenic substrate (lactate and alanine) delivery to the liver [7]. Recent studies have shown, however, that glucogenesis in the starved dog is unaffected by DCA [8]. In an effort to learn more about the mechanisms through which DCA leads to the reduction of blood glucose levels in diabetic animals, we have studied the acute effects of DCA on the synthesis and turnover of glucose in the depancreatized dog.

Methods

Preparation of animals

Mongrel female dogs weighing from 10 to 15 kg were depancreatized under sodium pentobarbital anaesthesia and immediately thereafter were maintained on insulin (7 units NPH/day) and antibiotic therapy. A minimum of 1 week was allowed for recovery from surgical stress, when antibiotic therapy was discontinued.

Materials

DCA was obtained from Tokyo Kogyo, Tokyo, Japan. Insulin was obtained from local pharmacy stores. Radio-labelled glucose was obtained from New England Nuclear, Boston, Massachusetts, USA.

Procedures

Studies were begun at 09.00 h on unanaesthetized dogs that had been fasted overnight and had received their last insulin 36 h previously. An intravenous catheter was placed in the saphenous vein of each

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hind limb while the animals were at rest in a Pavlov sling. At the zero time of each study a single intravenous injection of radio-labelled glucose (either 15 µCi of 6-^{14}C glucose given alone, or 15 µCi of 6-^{3}H glucose together with 15 µCi of U-^{14}C glucose) was made through one of the indwelling catheters in order to label the body glucose pool. Blood sampling was begun 15–30 min thereafter. Five ml of blood, drawn without stasis, were transferred rapidly into a measured quantity of perchloric acid to precipitate the blood proteins. In all studies a minimum of four samples were taken over an initial period of 1–2 h. DCA (180 mg/kg in 10 ml saline) or 10 ml saline was then administered IV at 2 ml/min and blood sampling was resumed until the end of study.

Analytical Methods

Glucose, lactate and alanine concentrations were determined with glucose oxidase [11], lactate dehydrogenase [12] and alanine dehydrogenase [13], respectively. The specific activity of glucose in the protein-free filtrates was determined after each filtrate had been passed successively over Dowex 50 × 8 ion-exchange resin to remove alanine and Dowex 1 × 8 to remove lactic acid and pyruvic acid. The ion-exchanged filtrates containing 6-^{3}H glucose were lyophilized to complete dryness in order to remove $^{3}H_2O$, a product of labelled glucose metabolism. These samples were then taken up in small quantities of water for glucose assay and liquid scintillation counting. The ion-exchanged filtrates containing 6-^{14}C glucose were reduced in volume and then subjected to periodate oxidation for the independent determination of the carbon 6 and carbons 1–5 of the glucose molecule [14]. The end product of carbon 6 oxidation, formaldehyde, was then complexed with Dimedon (5,5-dimethyl-1,3-cyclohexanedione) reagent, collected on filter paper, dried, weighed and transferred quantitatively with toluene washing to liquid scintillation vials for counting. CO_2 derived from carbons 1–5 of glucose was collected in NaOH, precipitated as $BaCO_3$, collected on filter papers, weighed and quantitatively transferred to liquid scintillation mixtures containing a thixotrophic gel to suspend $BaCO_3$. The initial specific activity of glucose (obtained by extrapolation of the glucose-specific activity-time curve to zero time), the quantity of isotope injected and the initial glucose concentration were used to estimate the glucose pool size and space [15]. Formulae developed by Medes et al. [16] and later modified by Dunn et al. [17] were used for the calculation of glucose entry and removal from the circulation. During steady state conditions, the equation for glucose turnover is:

$$\text{turnover rate} = (G/t)\, S.\, \ln (Sa_o/Sa_t)$$

where G is the glucose concentration, S is the glucose space and Sa_o and Sa_t are the initial and final glucose specific activities over the period of estimation, *t*.

In the post-DCA non-steady-state period, when the blood glucose levels were declining, glucose entry rate (ER) was calculated from the expression:

$$\text{entry rate} = \frac{(G_o - G_t)\, S.\, \ln (Sa_o/Sa_t)}{t.\, \ln (G_o/G_t)}$$

and glucose removal rate from the blood was calculated from the expression:

$$\text{removal rate} = \frac{(G_o - G_t)\, S + ER}{t}$$

where G_o is the initial glucose concentration and G_t is the glucose concentration at time t.

Studies were carried out, as noted above, with either 6-^{14}C glucose or a combination of ^{3}H glucose and U-^{14}C glucose. The use of glucose-specific activities in the formula above excludes recycling components. The ^{3}H label of the 6-^{3}H glucose is lost in the process of glucogenesis, and the 6-^{14}C label that does recycle in glucogenesis is quantitated and mathematically subtracted following the analytical procedure of periodate oxidation of the glucose molecule.

Quantitation of the extent of recycling of the glucose molecule in these studies was made using the formula of Katz et al. [18].

$$\text{Fraction recycled} = \frac{R_{3_H} - R_{14_C}}{R_{3_H}}$$

where R_{3_H} = the apparent replacement rate of glucose measured with 6-^{3}H glucose and R_{14_C} = the apparent replacement rate of glucose measured with U-^{14}C glucose. For studies carried out with 6-^{14}C glucose, R_{3_H} is derived from the specific activity of 6-^{14}C glucose with the recycling component subtracted, and R_{14_C} is derived from the specific activity of 6-^{14}C glucose with the recycling component included.

Results

Figure 1 illustrates the effect of DCA on the time course of glucose concentration and specific activity (Sa_{3_H}) and the time course of the concentrations of lactic acid and alanine observed in dog 3. Figure 2 illustrates the same data observed in a placebo study of dog 7. Similar data were obtained from the other experiments. Figure 1 shows the changes in the kinetic parameters of glucose and its precursors, alanine and lactic acid, that occurred following the administration of DCA, while Figure 2 documents the continued steady state of these metabolic pools following saline administration. Table 1 shows that glucose concentration fell 15%, while both alanine and lactate concentrations fell approximately 50%. The limited number of observations of alanine concentration preclude significance testing, but the decline in lactate concentration is highly significant ($p < 0.001$). From the data on glucose concentration and specific activity versus time (Figs. 1 and 2), we have made quantitative estimates of glucose turnover (entry and removal) before and after injection of DCA or saline in all studies. After DCA administration glucose concentrations declined and then plateaued between 1.5 and 3 h following DCA. The kinetic parameters of the glucose system, the rates of glucose entry and removal, are listed in Table 2 as 'pre-treatment steady-state turnover rate'. No alterations from the steady state were observed in the placebo studies and accordingly pre- and post-placebo kinetics in these animals are listed as steady-state turnover rates. In the DCA studies, however, there was an almost immediate decline (52%) in the rate of glucose entry into the glucose pool, while at the same time the rate of glucose removal declined by 21%. When a steady state was re-established at the lower glycaemic levels, mean glucose entry and removal rates were 94 $mg \cdot kg^{-1} \cdot h^{-1}$. It is clear that the hypoglycaemic action of DCA is initiated by a decreased hepatic glucose supply, since in no case did the immediate post-DCA removal rate exceed the turnover rate of glucose established before DCA administration.

To evaluate glucose supply more completely, we have estimated the rate of glucose carbon recycling before and after drug administration. Figure 3 illustrates the data used for these calculations and Table 3 lists the rates of glucose recycling calculated from these data. Glucose carbon recycling averaged 32% before DCA versus an average of 20% after drug administration. These data are likely to underestimate recycling (glucose synthesis through lactate) by 50% due to carbon dilution in the oxalacetate pool [19, 20]; allowing for this, we estimate that glucose synthesis from lactate would amount to approximately 99 $mg \cdot kg^{-1} \cdot h^{-1}$ before and approximately 37 $mg \cdot kg^{-1} \cdot h^{-1}$ after DCA. This cal-

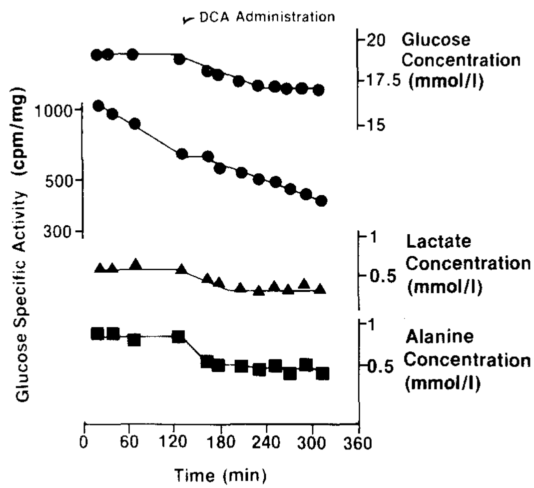

Fig. 1. The effect of DCA on blood glucose (●), lactate (▲) and alanine (■) concentrations and on glucose specific activity (log scale) (●) in the diabetic dog

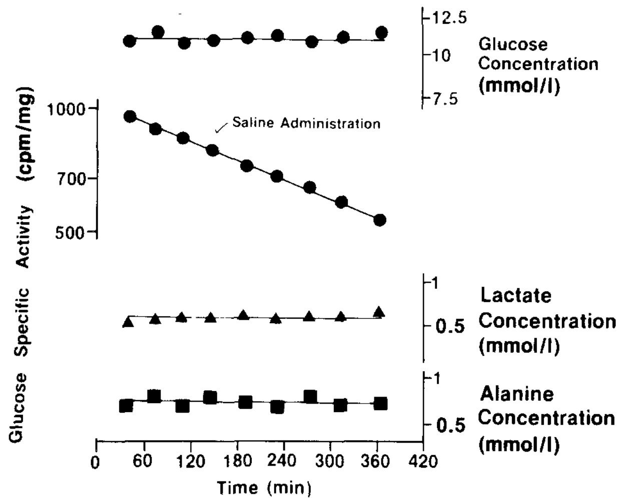

Fig. 2. The effect of saline (placebo) administration on blood glucose (●), lactate (▲) and alanine (■) concentrations and on glucose specific activity (log scale) (●) in the diabetic dog

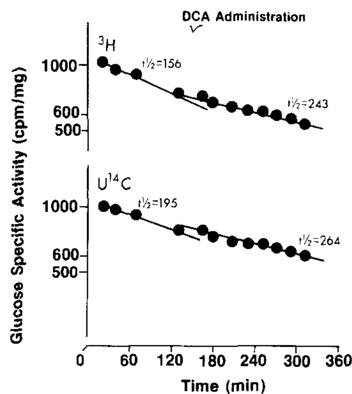

Fig. 3. The effect of DCA on the time course of ^{3}H and ^{14}C glucose specific activities (log scales) in the diabetic dog. t½ = half-time (min) of specific activity decline

Table 1. Acute effects of DCA on glucose and glucogenic substrates in the depancreatized dog

Animal number	Drug	Substrate	Before drug (mmol/l)	After drug (mmol/l)
1	DCA	Glucose	11.8	8.6
2			12.0	9.9
3			12.3	11.5
4			19.0	17.2
5			13.7	11.1
Mean			13.7 $p < 0.05$	11.6
6	Placebo	Glucose	10.9	11.5
7			10.7	10.6
8			12.0	11.4
Mean			11.2	11.1
3	DCA	Alanine	0.86	0.39
4			0.65	0.37
Mean			0.76	0.38
7	Placebo	Alanine	0.79	0.71
1	DCA	Lactate	0.59	0.22
2			0.83	0.43
3			0.92	0.59
4			0.72	0.32
5			0.90	0.43
Mean			0.79 $p < 0.001$	0.40
6	Placebo	Lactate	0.56	0.59
7			0.69	0.56
8			0.71	0.83
Mean			0.65	0.66

Table 2. Glucose kinetics: Effects of dichloroacetate in the depancreatized dog

Treatment group	Animal number	Steady state pre-treatment turnover rate ($mg \cdot kg^{-1} \cdot h^{-1}$)	Non-steady state entry rate/removal rate ($mg \cdot kg^{-1} \cdot h^{-1}$)	Steady state post-treatment turnover rate ($mg \cdot kg^{-1} \cdot h^{-1}$)
DCA	1	161	27/118	86
	2	141	88/115	119
	3	136	92/134	71
	4	151	57/ 81	103
	5	170	103/153	89
	Mean	152	73[a]/120[b]	94[a]
Placebo	6	139		141
	7	164		156
	8	157		144
	Mean	153		147

[a] $p < 0.01$ compared with steady state pre-treatment turnover rate
[b] $p < 0.05$ compared with steady state pre-treatment turnover rate

Table 3. Glucogenesis: effects of dichloroacetate in the depancreatized dog

Treatment group	Animal number	Glucogenesis	
		Pre-treatment ($mg \cdot kg^{-1} \cdot h^{-1}$)	Post-treatment ($mg \cdot kg^{-1} \cdot h^{-1}$)
DCA	2	56	18
	3	53	21
	4	30	8
	5	61	20
	Mean	50 $p < 0.01$	17
Placebo	6	15	16
	7	59	56
	8	50	46
	Mean	41	39

culated decline of 62 $mg \cdot kg^{-1} \cdot h^{-1}$ in glucose synthesis closely approaches the absolute decline observed in glucose supply (58 $mg \cdot kg^{-1} \cdot h^{-1}$). Thus, all of the reduction in glucose supply following DCA could result from inhibition of glucose synthesis from lactate and precursors that cycle through the lactate-pyruvate pool.

Discussion

Acceleration of glucose oxidation, demonstrated in early studies by Stacpoole and Felts [2, 3], was proposed as a basis for the hypoglycaemic action of DCA. The concomitant inhibition of NEFA oxidation together with lowered levels of muscle citrate suggested that the mechanism for the increased glucose oxidation might involve inhibition of NEFA oxidation which, in turn, would lead to a reduction in muscle citrate levels thereby allowing a disinhibition of phosphofructokinase. Observations similar to those cited above were made by McAllister et al. [4] in studies on rat diaphragm and heart in vitro and on dog heart in vivo. Changes in other metabolite concentrations, however, led these investigators to the conclusion that DCA acted by direct stimulation of pyruvate oxidation. Subsequently Whitehouse and Randle [5] demonstrated that DCA directly activated pyruvate dehydrogenase. Later studies by Blackshear et al. [6] demonstrated that DCA lowered blood glucose in the starved rat and these investigators argued that deprivation of glucogenic substrate rather than the acceleration of glucose utilization was the mechanism responsible for the hypoglycaemia. Recent studies in the starved dog, while showing reductions in circulating alanine and lactate following infusion of DCA, did not demonstrate a reduction in glucogenesis or in the concentration of circulating glucose [8]. In the turnover studies reported here, we have shown an acute and significant reduction in the rate of glucose supply to the circulation following DCA administration to diabetic dogs. Both alanine and lactate concentrations were dramatically reduced. ^{14}C tracer data indicated that glucose synthesis from lactate was reduced by 63%. With a correction for the loss of ^{14}C in the oxaloacetate pool [9], we have calculated that the reduction in glucose synthesis from lactate closely matches the quantitative reduction in the rate of glucose supply to the circulation. Although removal of glucose from the circulation declined in these studies following the administration of DCA, we did not quantitate the loss of glucose in the urine before and after DCA and cannot, therefore assess to what extent the actual peripheral utilization of glucose may have been affected by DCA. Nevertheless, our data suggest that the acute hypoglycaemic action of DCA is brought about by a curtailment of glucose synthesis and glucose delivery to the circulation.

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