# Differential effect of dichloroacetate on branched-chain amino acid catabolism in perfused rat hindlimbs

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The effect of 1 mM and 5 mM dichloroacetate on the catabolism of branched-chain amino acids in isolated rat hindlimbs was investigated in perfusions with 0.5 mM 1-$^{14}$C-labeled L-leucine or L-valine. The results demonstrate an increasing effect of dichloroacetate on the flux through skeletal muscle branched-chain 2-oxo acid dehydrogenase. A minor effect was observed with the high dichloroacetate concentration. Evidence is presented that this was essentially due to diminished pyruvate supply.

*Dichloroacetate* *Branched-chain amino acid* *Pyruvate* *Perfused rat hindlimb*

## 1. INTRODUCTION

DCA has been shown to increase the catabolism of BCAA in the perfused rat heart [1]. This seems to be primarily due to the inhibitory effect of the compound on BCOA-DH kinase [2] As in the heart, BCOA-DH in skeletal muscle is regulated by a phosphorylation-dephosphorylation mechanism [3–5]. However, in recirculating perfusion of rat hindquarters, DCA increased BCAA release [6]. In cultured rat skeletal muscle cells, $^{14}CO_2$ production from L-[1-$^{14}$C]leucine was reduced on incubation with DCA [7]. Similar decreasing effects on the catabolism of leucine and valine were observed with incubated rat hemidiaphragms [8,9]. This communication reports on an increasing but differential effect of 1 mM, and 5 mM DCA on BCAA catabolism in isolated rat hindlimbs perfused under steady-state conditions.

*Abbreviations:* BCAA, branched-chain amino acid; BCOA, branched-chain 2-oxo acid; BCOA-DH, branched-chain 2-oxo acid dehydrogenase (EC 1.2.4.4); DCA, dichloroacetate

## 2. EXPERIMENTAL

Non-recirculating perfusion of rat hindlimbs with a medium containing 0.5 mM 1-$^{14}$C-labeled BCAA (0.4–0.9 MBq/mmol) or 0.15 mM [1-$^{14}$C]pyruvate (1.3–2.6 MBq/mmol) as the sole substrate was performed as in [10]. In the effluent, the label incorporated into $^{14}CO_2$ and [1-$^{14}$C]BCOA was determined as in [10]. BCOA concentrations were measured according to [11] and were used to calculate the specific radioactivity and the release rate of BCOA as detailed in [10]. Amino acids were determined on an automatic amino acid analyzer, and lactate was measured as in [12].

The label incorporated into $^{14}CO_2$ and [1-$^{14}$C]BCOA was used to calculate the respective release rates on the basis of the specific radioactivity of the precursor in the influent. To take into account the intracellular dilution of label [5,10], the oxidation rates of BCOA were estimated from the $^{14}CO_2$ production on the basis of the specific radioactivity of 1-$^{14}$C-labeled BCOA in the effluent. The transamination rates were calculated from BCOA release and oxidation rates.

The sources for the radioactive and unlabeled

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material were as in [10]. Results are means ± SD from determinations during the metabolic and isotopic steady state of perfusion ($n$ = 3–8); Student's paired $t$-test was used.

## 3. RESULTS

In perfusions with 1-$^{14}$C-labeled leucine and valine (table 1), addition of 5 mM DCA decreased the release of the corresponding BCOA by about 50 and 80% ($P<0.001$), respectively. The transamination rate of leucine remained unchanged and the transamination of valine was slightly decreased ($P<0.05$). After termination of 5 mM DCA infusion, the BCOA release increased and returned to the control value (fig.1, upper). However, the $^{14}CO_2$ release was transiently enhanced and then gradually declined. In experiments with 1 mM DCA we examined whether this was due to the dilution of DCA during the wash-out period. The increase of BCOA oxidation was more distinct with the low DCA concentration (table 1). No additional increase of $^{14}CO_2$ production occurred after termination of 1 mM DCA infusion.

The effects of 1 mM and 5 mM DCA were directly compared in experiments as shown in fig.1 (lower). Either concentration of DCA decreased the BCOA release to a similar extent (cf. table 1). The portion of the transamination products of leucine and valine undergoing decarboxylation was increased from 40 ± 6% and and 14 ± 5%, respectively, to about 70% by either DCA concentration ($P<0.005$). However, as compared to 1 mM, 5 mM DCA decreased the oxidation rate in perfusions with leucine ($P<0.005$) and valine ($P<0.05$) by about 40%.

It has been argued [9] that decreasing effects of DCA on skeletal muscle BCAA catabolism might be a result of increasing effects on pyruvate catabolism. In fact, in perfusions with leucine, addition of 1 mM and 5 mM DCA decreased the release of lactate (245 ± 35 nmol/min per g muscle) in a concentration-dependent manner by 13 ± 6% ($P<0.05$) and by 36 ± 3% ($P<0.001$), respectively. Release rates of amino acids were not reduced significantly, with the exception of alanine production (45 ± 11 nmol/min per g muscle), which was decreased by 27 ± 3% and by 36 ± 3%

Table 1

Differential effect of dichloroacetate on the catabolism of branched-chain amino acids in isolated perfused rat hindlimbs

| Amino acid added (0.5 mM) | [DCA] (mM) | 2-Oxo acid release | 2-Oxo acid oxidation (nmol/min per g muscle) | Amino acid transamination |
|---|---|---|---|---|
| | 0 | 13.8 ± 1.2 | 10 ± 3 | 23 ± 4 |
| L-Leucine | 1 | 7.6 ± 0.6 | 28 ± 8 | 36 ± 8 |
| | 5 | 7.3 ± 1.2 | 16 ± 4 | 22 ± 5 |
| | 0 | 11.8 ± 0.9 | 2 ± 1 | 14 ± 3 |
| L-Valine | 1 | 4.3 ± 1.1 | 11 ± 2 | 15 ± 3 |
| | 5 | 2.4 ± 1.2 | 8 ± 3 | 9 ± 3 |

Results are means ± SD from determinations before and after addition of DCA. Effects of 1 mM and 5 mM DCA were measured in at least 4 independent experiments with 1-$^{14}$C-labeled BCAA (for experimental protocol see fig.1, upper). 2-Oxo acid refers to the corresponding BCOA. The $^{14}CO_2$ release (6.8 ± 1.5, 12.9 ± 1.2 and 9.2 ± 1.8 nmol/min per g muscle in perfusions with leucine and 1.1 ± 0.2, 5.0 ± 0.7 and 3.2 ± 0.5 nmol/min per g muscle in perfusions with valine at 0, 1 and 5 mM DCA, respectively) was corrected for the intracellular dilution of label to yield BCOA oxidation rates. For further experimental details see section 2

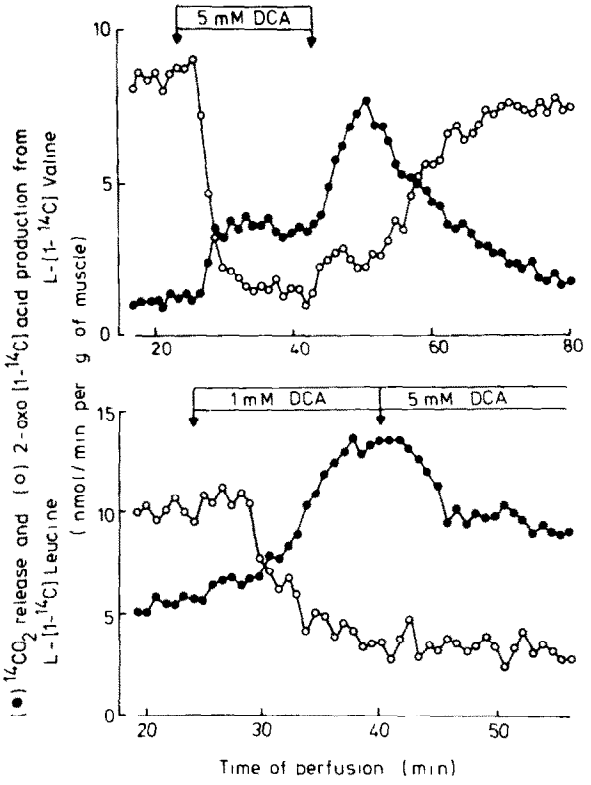


Fig.1. Effect of dichloroacetate on the $^{14}CO_2$ release and 2-oxo 1-$^{14}$C-labeled acid production from 1-$^{14}$C-labeled branched-chain amino acids in isolated perfused rat hindlimbs. Non-recirculating perfusion was carried out with 0.5 mM BCAA as the sole substrate. DCA was added to the influent at the time and concentration as indicated. Representative experiments are shown. Similar effects were observed with either amino acid (cf. table 1). For further details see sections 2 and 3.

($P<0.05$), respectively. These findings are in accordance with the concentration-dependent effect of DCA on $^{14}CO_2$ release from [1-$^{14}$C]pyruvate (fig.2, cf. [7]).

Therefore, the effect of improved pyruvate supply was examined in perfusions with leucine and in the presence of 5 mM DCA (fig.3). After addition of 0.5 mM pyruvate, the BCOA release was increased from 8 ± 2 to 18 ± 6 nmol/min per g muscle ($P<0.05$). The oxidation rate was enhanced from 15 ± 4 to 17 ± 3 nmol/min per g muscle ($P<0.05$). Thus the transamination rate was increased by 52 ± 5% ($P<0.05$).

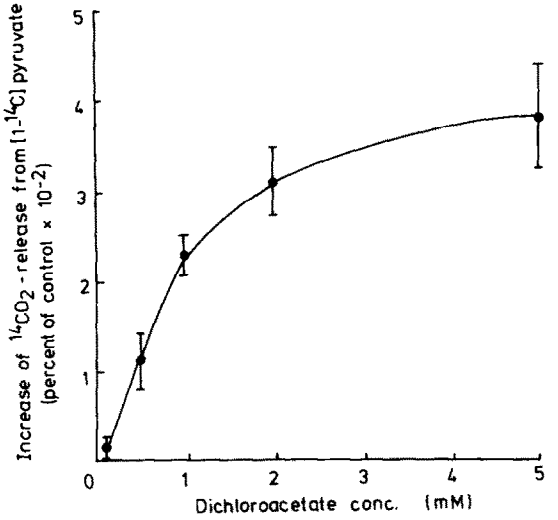


Fig.2. Effect of dichloroacetate on the $^{14}CO_2$ release in non-recirculating perfusion of rat hindlimbs with 0.15 mM [1-$^{14}$C]pyruvate. Results are means ± SD for at least 3 independent experiments (for experimental protocol cf. fig.1, upper). Effects of DCA are expressed as the multiplier of the value during the preceding DCA-free perfusion. The effect of DCA was significant ($P<0.005$) at concentrations $\geq 0.5$ mM. The effect of 5 mM vs 1 mM DCA was significant at $P<0.01$ as determined by Student's non-paired $t$-test. For further details see section 2.

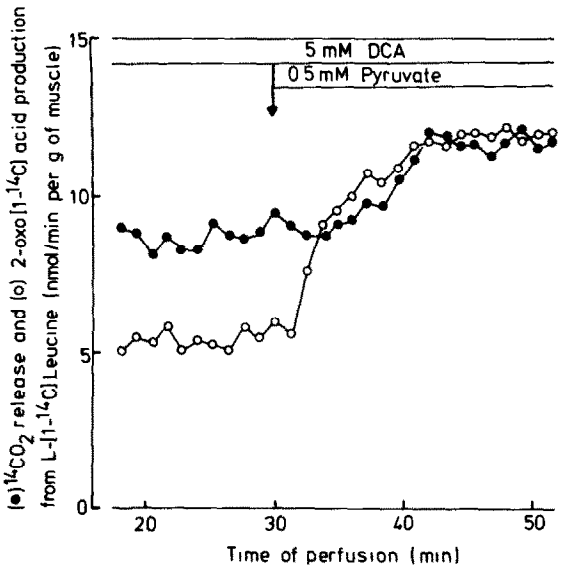


Fig.3. Effect of pyruvate on the $^{14}CO_2$ release and 2-oxo 1-$^{14}$C-labeled acid production from 0.5 mM L-[1-$^{14}$C]leucine in isolated perfused rat hindlimbs in the presence of 5 mM dichloroacetate. Sodium pyruvate was added to the influent at the time and concentration as indicated. For further details see sections 2 and 3.

## 4. DISCUSSION

The present experiments demonstrate an increasing effect of 1 mM and 5 mM DCA on the decarboxylation of leucine and valine in isolated perfused rat hindlimbs. It is rather unlikely that the minor effect of 5 mM DCA was due to an inhibition of the BCOA-DH. This should increase BCOA release, decrease the ratio of oxidation to transamination, and would not explain the differences in the transamination rates. An inhibitory effect on BCAA aminotransferase has been excluded [9]. A reasonable explanation is provided by the hypothesis [9] that in skeletal muscle, BCAA transamination might be coupled to alanine formation by the BCAA aminotransferase (EC 2.6.1.42) and the alanine aminotransferase (EC 2.6.1.2) reaction. Thus the transamination of BCAA could be, at least in part, dependent on pyruvate availability.

Taken together, the concentration-dependent effects of DCA and BCAA transamination rates, the lactate and alanine production, the $^{14}CO_2$ release from [1-$^{14}$C]pyruvate, and the results of pyruvate supplementation experiments suggest that the minor effect of 5 mM DCA on BCOA oxidation was essentially due to a diminished availability of pyruvate, which led to an impaired rate of transamination and to a decreased substrate supply for the BCOA-DH.

With respect to the mechanism it is not likely that DCA greatly affected BCOA-DH flux by changes in tissue BCOA concentrations. In hindlimb perfusions with 0.5 mM leucine, the intracellular BCOA remained low [13] and the concentration seems to be far below an apparent $K_i$ for the BCOA-DH kinase [2,14]. Furthermore, as evidenced by the oxidation/transamination ratios in perfusions with leucine or valine, the DCA-induced degree of increase in BCOA-DH flux was apparently independent from the transamination, i.e. BCOA production rate.

The evidence presented suggests that DCA directly affected the activity state of the BCOA-DH. In the perfused rat heart, the DCA induced-increase of BCAA decarboxylation is presumably due to an inhibition of the BCOA-DH kinase [1,2] with subsequent dephosphorylation and activation of the BCOA-DH. Whether a similar mechanism also applied to the present experiments, is now under investigation.

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