The effect of dichloroacetate and hydroxypyruvate on the entry of ^{14}C from [1-^{14}C]alanine into urea in rat hepatocytes

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Entry of metabolic $^{14}CO_2$ into urea is shown to be decreased by dichloroacetate although the production of $^{14}CO_2$ is stimulated 2-fold. Hydroxypyruvate, a product of dichloroacetate metabolism, increases the incorporation of metabolic $^{14}CO_2$ into urea. It is proposed that these effects result from changes in the cytoplasmic-mitochondrial pH gradient.

Urea synthesis *Equilibration of metabolic CO_2* *Dichloroacetate* *Hydroxypyruvate*
Mitochondrial pH gradient *Hepatocyte*

1. INTRODUCTION

Carbon dioxide formed through the action of pyruvate dehydrogenase enters into urea more readily than it exchanges with the extramitochondrial and extracellular pool of bicarbonate plus CO_2 [1]. This probably occurs because of several factors. The proximity of the site of CO_2 formation (pyruvate dehydrogenase) and its utilisation as bicarbonate (carbamoylphosphate synthetase) within the mitochondria. The rapid conversion of CO_2 to HCO_3^- through the action of the mitochondria carbonic anhydrase [2] and the relative impermeability of the mitochondrial membrane to bicarbonate ions [3] are likely causes of the unexpectedly high specific radioactivity of urea formed from [1-^{14}C]alanine.

The well-known stimulation of pyruvate dehydrogenase by dichloroacetate [6] might be expected to enhance the observed discrepancy between the specific radioactivity of the total bicarbonate pool and that of urea. Experiments are presented which show that dichloroacetate stimulates the formation of $^{14}CO_2$ two-fold from [1-^{14}C]alanine but that the specific activity of urea is significantly lower than in the absence of dichloroacetate. Products of dichloroacetate metabolism include hydroxypyruvate [7] which has the opposite effect to dichloroacetate in that it depresses the formation of $^{14}CO_2$ but increases the specific activity of urea.

2. METHODS

The methods of preparation and incubation of hepatocytes, collection and counting of $^{14}CO_2$ and the assay, and counting of urea were as in [1].

3. RESULTS

3.1. *Effect of dichloroacetate on the specific activity of urea*

The results in table 1 show that, as expected, dichloroacetate increased the formation of $^{14}CO_2$ from [1-^{14}C]alanine by more than 2-fold. This is in keeping with the known effect of dichloroacetate on the activity of pyruvate dehydrogenase [6]. I expected that this increase in $^{14}CO_2$ formation would result in a paralleled increase in the specific activity of urea. Unexpectedly, the specific activity was lower in the presence of dichloroacetate by more than 30% (see experiment 1, table 1 and table 3).

This finding could be explained if dichloroacetate caused an increase in the rate at which

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$^{14}CO_2$, formed in the mitochondria, equilibrated with the extramitochondrial or extracellular CO_2 plus bicarbonate. If this were the case one might expect that dichloroacetate would increase the specific activity of urea in a situation where urea was formed from unlabelled alanine and the label was present as $H^{14}CO_3^-$ added to the incubation medium.

Experiment 2, table 1 shows that this is indeed the case, the increase caused by dichloroacetate being about 24% (see also table 3).

3.2. *Effect of hydroxypyruvate on the specific activity of urea*

Dichloroacetate has been shown to form glucose with hydroxypyruvate as one of the intermediates [7]. Examination of the effect of the intermediates of dichloroacetate metabolism showed that hydroxypyruvate had the opposite effect on urea specific activity to that of dichloroacetate, thus in experiment 1 table 2, hydroxypyruvate (2 mM), lowered the formation of $^{14}CO_2$ from [1-^{14}C]-alanine by 50% but, in the face of this, the specific activity of urea was increased by more than 90% (see also table 3). Conversely, when the origin of the label was the extracellular bicarbonate, hydroxypyruvate caused a decrease in the specific activity of urea (experiment 2 table 2 and table 3).

4. DISCUSSION

The direction in which dichloroacetate modifies the specific activity of urea (positively or negatively) depends on whether the origin of the $H^{14}CO_3^-$ fixed into carbamoylphosphate is intramitochondrial or extracellular. This observation is in keeping with the conclusion that dichloroacetate exerts an effect on the rate of equilibration of intramitochondrial CO_2 with the extracellular pool of bicarbonate.

Authors in [3–5] have shown that bicarbonate cannot enter mitochondria at a significant rate, but that CO_2 enters readily in response to the alkaline-inside pH gradient. The pH of rat liver mitochondria has been measured by several methods, and values from 0.7 to 1.4 pH units have been obtained [8–10]. Entry of CO_2 was blocked by inhibition of carbonic anhydrase with acetazolamide [3] in-

Table 1

Effect of dichloroacetate on the incorporation of ^{14}C into urea in hepatocytes from 48-h starved rats

Substrates added	Exp. 1, [1-^{14}C]alanine		% Change due to dichloroacetate	Exp. 2, alanine + $NaH^{14}CO_3$		% Change due to dichloroacetate
Dichloroacetate addition:	−	+		−	+	
Total radioactivity in flask dpm × 10^{-5}	9.83	9.49		6.28	6.43	
Radioactivity in CO_2 dpm × 10^{-5}	0.61	1.26	+106.6	6.13	6.21	
Radioactivity in urea dpm × 10^{-3}	2.11	1.48	−29.9	7.30	11.8	+61.6
Urea formed μmol/flask	1.13	1.29	+14.2	1.61	2.09	+29.8
Specific activity of urea dpm × 10^{-3}/μmol	1.87	1.14	−39.0	0.45	0.56	+24.4

Duplicate flasks containing 4 ml suspension in Krebs-Henseleit saline were incubated for 30 min at 38°C. Substrates added were alanine (5 mM) plus $NaH^{14}CO_3$ or [1-^{14}C]alanine (5 mM). Sodium dichloroacetate (2 mM) was added where shown. Because of variations in the number of cells, typical experiments are shown. A summary of the percentage effects of dichloroacetate, with statistical significance, is presented in table 3. Values are means of duplicate flasks in 2 experiments as indicated

Table 2

Effect of hydroxypyruvate on the incorporation of ^{14}C into urea in hepatocytes from 48-h starved rats

Substrates added	Exp. 1, [1-^{14}C]alanine		% Change due to hydroxypyruvate	Exp. 2, alanine + $NaH^{14}CO_3$		% Change due to hydroxypyruvate
Hydroxypyruvate addition:	−	+		−	+	
Total radioactivity in flask dpm × 10^{-5}	7.89	7.81		6.61	6.61	
Radioactivity in CO_2 dpm × 10^{-5}	0.73	0.36	−50.7	6.41	6.51	
Radioactivity in urea dpm × 10^{-3}	1.16	1.59	+37.1	12.7	6.0	−52.6
Urea formed μmol/flask	1.53	1.09	−28.8	2.54	1.57	−38.2
Specific activity of urea dpm × 10^{-3}/μmol	0.76	1.46	+92.1	5.01	3.85	−23.2

Duplicate flasks containing 4 ml suspension in Krebs-Henseleit saline were incubated for 30 min at 38°C. Substrates added were alanine (5 mM) plus $NaH^{14}CO_3$ or [1-^{14}C]alanine (5 mM). Sodium hydroxypyruvate (2 mM) was added where shown. Because of variations in the number of cells, typical experiments are shown. A summary of the percentage effect of hydroxypyruvate with statistical significance is presented in table 3. Values are means of duplicate flasks in 2 experiments as indicated

Table 3

Effect of dichloroacetate or hydroxypyruvate and the origin of $^{14}CO_2$ on its incorporation into urea in 48-h starved hepatocytes

Origin of ^{14}C	[1-^{14}C]Alanine	$NaH^{14}CO_3$
% Change due to dichloroacetate	−32.7 ± 12.9 (4)	+13.1 ± 10.2 (4)[a]
% Change due to hydroxypyruvate	+114 ± 39 (4)	−25.6 ± 11.2 (3)[a]

[a] $p = <0.0025$

Values from a number of the typical experiments presented in tables 1 and 2 are summarized as percentage changes due to the various additions and the origin of the incorporated carbon 14. Values are means ± SD with the number of experiments in parentheses; p-values calculated by Student's *t*-test are to compare the effect of origin of the incorporated ^{14}C

dicating that hydration of CO_2 was a necessary step in the uptake of extramitochondrial CO_2.

The metabolism of [2-^{14}C]dichloroacetate has been shown to involve the formation of glyoxalate, glycine, oxalate and $^{14}CO_2$ [7,11,12]. Glycine is formed by the action of alanine–glyoxalate aminotransferase. These reactions necessitate the removal of the chlorine atoms resulting in the production of HCl and consequently a fall in the cellular pH. The presence in high-speed super-

natants of rat liver of a dehalogenase has been described in [14]. The fall in pH in the cytoplasm may be expected to increase the pH gradient between mitochondria and cytoplasm and one can conclude that this lowering of the cytoplasmic pH is related to the observed effect of dichloroacetate.

Dichloroacetamide was also effective in stimulating pyruvate dehydrogenase and depressed the specific activity of urea (not shown). Urea formation was greater in the presence of dichloroacetamide, presumably due to the cleavage of the amide group. Hydroxypyruvate is readily converted to glucose [7]. The formation of a non-ionic product from an ionic precursor would have the effect of increasing the intracellular pH. This shift in pH will be in the cytoplasmic compartment so that the alkaline-inside pH gradient between mitochondria and cytoplasm would be decreased.

These considerations could explain the opposite effects which dichloroacetate and hydroxypyruvate have on the fixation of $^{14}CO_2$ into urea.

Hydroxypyruvate is formed by transamination of serine with pyruvate [14] as a relatively minor reaction (less than 1%) compared to the activity of alanine-glyoxylate aminotransferase [15], hence addition of glycine or serine, intermediates in the formation of hydroxypyruvate from dichloroacetate, had little effect on the incorporation of $^{14}CO_2$ into urea. When this rate-limiting step is bypassed by the addition of substrate amounts of hydroxypyruvate the reverse effect on urea specific activity becomes apparent. The major effect of dichloroacetate, which predominates however, is that which results from the removal of the chlorine atoms.

In experiments where the label is added in the form of $H^{14}CO_3^-$, counts remaining in the acidified extract after removal of the acid volatile counts, represent $^{14}CO_2$ fixed into a number of metabolic intermediates in the various CO_2 fixation reactions. Subtraction of the count found in urea in this acid extract indicates that both dichloroacetate and hydroxypyruvate substantially modify the entry of CO_2 into products other than urea.

It is likely that most, if not all, studies of the rates of CO_2 fixation reactions using ^{14}C-labelled substrates will be influenced by substrates which modify the pH of cell compartments. Entry of $^{14}CO_2$ into glucose through the activity of pyruvate carboxylase is one such reaction which is being investigated.

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