Hydroxypyruvate as a Gluconeogenic Substrate in Rat Hepatocytes*

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At concentrations of 2 mM and above hydroxypyruvate produced no glucose with isolated rat liver cells, although it was rapidly utilized. At a lower concentration of hydroxypyruvate or in the presence of substrates which generate reducing equivalents (ethanol or butyrate), appreciable amounts of glucose were formed from hydroxypyruvate. A possible explanation for this phenomenon is discussed.

Hydroxypyruvate has been implicated as an intermediate in the synthesis of glucose from serine (reactions 1–3) (Lardy *et al.*, 1969; Sallach *et al.*, 1972; Rowsell *et al.*, 1973; Snell, 1974). The enzymes involved in the initial stages are serine-pyruvate aminotransferase (EC 2.6.1.51), glycerate dehydrogenase (EC 1.1.1.29) and glycerate kinase (EC 2.7.1.31) respectively:

Hydroxypyruvate can also be converted into L-glycerate by reduction with lactate dehydrogenase and NADH (Meister, 1952; Stafford *et al.*, 1954).

Early experiments by Dickens & Williamson (1959) with ^{14}C -labelled hydroxypyruvate showed that it was converted into glucose in rat liver (both *in vivo* and *in vitro*) by a route consistent with reactions (2) and (3). From these experiments it was concluded that hydroxypyruvate was an effective gluconeogenic precursor. This view has been contested by Sandoval & Sols (1974), who showed that, in contrast with serine or glycerate, hydroxypyruvate at a concentration of 10 mM yielded virtually no glucose in the isolated perfused rat liver, although it was rapidly removed from the medium. They rightly criticized the studies of Dickens & Williamson (1959) because no quantitative information on the rate of glucose synthesis from hydroxypyruvate was provided. Sandoval & Sols (1974) have interpreted this failure of hydroxypyruvate to form glucose as being part of their evidence in support of the conclusion that the serine dehydratase pathway (EC 4.2.1.13; reaction 4) is the only one of physiological importance in gluconeogenesis:

* Dedicated to Professor Frank Dickens, F.R.S., for his 75th birthday.

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In view of the key role of hydroxypyruvate as an intermediate in the alternative pathway from serine (reactions 1–3) it seemed important to carry out further experiments to resolve the apparent controversy.

Materials and methods

Isolated liver cells were prepared from female rats (starved for 48 h) of the Wistar strain essentially as described by Berry & Friend (1969) with modifications described by Cornell *et al.* (1973) and Krebs *et al.* (1974). The incubation technique was as described by Cornell *et al.* (1973) with about 70–80 mg wet wt. of hepatocytes in each incubation flask (4.0 ml final volume of medium). Incubation times were 20, 40 and 60 min at 37°C in a rapidly moving Dubnoff-type shaker. At the end of the incubation 0.4 ml of 20% (v/v) HClO_4 was added to deproteinize the incubation mixture. The perchlorate ion was removed by neutralization with KOH and subsequent centrifugation. Glucose was determined in the neutralized supernatant by the method of Slein (1963). Hydroxypyruvate was measured with glyoxylate reductase, which does not react with pyruvate (Holzer & Holldorf, 1963); the latter was determined by subsequent addition of lactate dehydrogenase to the cuvette. Lithium hydroxypyruvate was obtained from the Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., and was converted into the sodium salt by passage through a column of Amberlite IR-120 (H^+ form) and neutralization with NaOH. Sodium butyrate was obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Enzymes and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Results and discussion

Experiments with a range of hydroxypyruvate concentrations showed that at initial concentrations

Table 1. *Time-course of gluconeogenesis from hydroxypyruvate in isolated rat liver cells under various conditions*

For experimental conditions see under 'Materials and methods'. In the experiments with ethanol, a further 4 μ mol of hydroxypyruvate was added to the incubation flasks at 20 and 40 min. The results are mean values $\pm$ S.E.M. with the numbers of separate experiments in parentheses. The rate of glucose synthesis is calculated for the incubation period between 20 and 60 min.

Additions	Initial concn. (mM)	Glucose formed (μ mol/g wet wt. of cells)			Rate of glucose synthesis (μ mol/min per g wet wt. of cells)
		0-20 min	0-40 min	0-60 min	
None (9)		3.0 $\pm$ 0.25	4.9 $\pm$ 0.47	6.3 $\pm$ 0.40	0.083 $\pm$ 0.008
Hydroxypyruvate (6)	0.5	6.9 $\pm$ 1.1	12.6 $\pm$ 0.61	16.7 $\pm$ 0.82	0.25 $\pm$ 0.028
Hydroxypyruvate (2)	1	9.4	18.5	23.8	0.36
Hydroxypyruvate (10)	2	2.5 $\pm$ 0.26	3.2 $\pm$ 0.43	4.4 $\pm$ 0.68	0.054 $\pm$ 0.010
Hydroxypyruvate (5)	2	18.0 $\pm$ 0.26	32.6 $\pm$ 3.8	51.4 $\pm$ 2.3	0.83 $\pm$ 0.054
Lactate	5				
Lactate (3)	5	10.7 $\pm$ 1.0	25.6 $\pm$ 2.2	44.3 $\pm$ 1.4	0.83 $\pm$ 0.020
Hydroxypyruvate (4)	2	7.3 $\pm$ 1.1	13.9 $\pm$ 1.7	21.3 $\pm$ 2.4	0.35 $\pm$ 0.035
Butyrate	5				
Hydroxypyruvate (3)	2	18.1 $\pm$ 0.32	31.6 $\pm$ 2.0	42.6 $\pm$ 3.7	0.61 $\pm$ 0.084
Ethanol	10				

Table 2. *Time-course of hydroxypyruvate removal by isolated rat liver cells under various conditions*

For experimental details see under 'Materials and methods'. In the experiments with ethanol a further 4 μ mol of hydroxypyruvate was added to the incubation flasks at 20 and 40 min. The results are mean values $\pm$ S.E.M. with the numbers of separate experiments in parentheses.

Additions	Initial concn. (mM)	Hydroxypyruvate removed (μ mol/g wet wt. of cells)		
		0-20 min	0-40 min	0-60 min
Hydroxypyruvate (5)	0.5	17.0 $\pm$ 1.8	25.3 $\pm$ 1.9	27.7 $\pm$ 2.0
Hydroxypyruvate (10)	2	30.8 $\pm$ 2.3	44.9 $\pm$ 1.9	59.8 $\pm$ 1.7
Hydroxypyruvate (3)	2	62.6 $\pm$ 5.7	90.3 $\pm$ 13.2	107 $\pm$ 12.8
Lactate	5			
Hydroxypyruvate (4)	2	26.2 $\pm$ 3.1	45.7 $\pm$ 3.3	61.0 $\pm$ 2.4
Butyrate	5			
Hydroxypyruvate (3)	2	65.8 $\pm$ 3.0	129 $\pm$ 12.4	184 $\pm$ 22
Ethanol	10			

of 2 mM and above the rate of glucose formation was the same or even lower than that of the control experiments with no added substrate, whereas at 1 mM- and 0.5 mM-hydroxypyruvate the formation of glucose was three- to four-fold higher than in the controls (Table 1). The mean hydroxypyruvate concentration after 60 min incubation was about 0.9 mM when the initial concentration was 2 mM. These findings suggested that high hydroxypyruvate concentrations might inhibit a step in the gluconeogenic pathway.

To test this possibility the effect of hydroxypyruvate (2 mM) on gluconeogenesis from lactate was examined. Hydroxypyruvate had no effect on the rate of gluconeogenesis measured between 20 and 60 min, but it caused an increase in the amount of glucose formed in the first 20 min (Table 1). The time-course for gluconeogenesis showed the lag reported by other workers (Garrison & Haynes, 1973; Cornell *et al.*,

1974), so that one interpretation of the increase in the first 20 min when hydroxypyruvate was also present would be the abolition of this lag. Measurements of lactate removal during the first 20 min of incubation gave a value of 16 μ mol/g wet wt. of cells (mean of three experiments) when lactate was the added substrate, and 23 μ mol/g when lactate plus hydroxypyruvate were present. The corresponding values for pyruvate accumulation in the medium were 14 μ mol/g and 39 μ mol/g respectively. Comparison of these values with the amounts of glucose formed in the same period (Table 1) suggest that hydroxypyruvate contributed to glucose formation. Measurement of hydroxypyruvate removal showed that the presence of lactate caused a 100% increase in the amount removed (Table 2). Thus reducing equivalents generated by the oxidation of lactate were utilized in the reduction of hydroxypyruvate.

These results suggested that under certain conditions conversion of hydroxypyruvate into glucose might be limited by the decreased availability of reducing equivalents at the triose phosphate dehydrogenase step owing to preferential reduction of hydroxypyruvate, a point considered by Sandoval & Sols (1974). Addition of butyrate, which might be expected to increase the generation of mitochondrial reducing equivalents, did not alter the amount of hydroxypyruvate removed (Table 2), but increased (fourfold above control; Table 1) the rate of glucose synthesis. Ethanol, which increases the supply of reducing equivalents in both cytosol and mitochondria, increased the rate of gluconeogenesis even more (sevenfold above control; Table 1) and doubled the initial rate of hydroxypyruvate removal (Table 2).

It can be concluded from these experiments that whether hydroxypyruvate forms glucose or not in a given situation depends on the prevailing hydroxypyruvate concentration and on the rate at which reducing equivalents are generated. At first sight it is difficult to understand why this should be so, because hydroxypyruvate is rapidly removed in all situations. The explanation presumably lies in the two possible products of the enzymic reduction of hydroxypyruvate, L-glycerate with lactate dehydrogenase and D-glycerate with glycerate dehydrogenase, and the competition between these two enzymes for their common substrate. Lactate dehydrogenase is considerably more active (at least two orders of magnitude) than glycerate dehydrogenase in rat liver, though precise information is not available. The relevant point is, of course, the relative activity of the two enzymes on hydroxypyruvate. The K_m for hydroxypyruvate for rat liver L-lactate dehydrogenase is 0.4 mM, whereas the comparable value for D-glycerate dehydrogenase is 0.07 mM (Dawkins & Dickens, 1965). Thus at low hydroxypyruvate concentrations the higher affinity of D-glycerate dehydrogenase for this substrate will tend to counteract the effect of the higher amounts of lactate dehydrogenase and thus favour the formation of D-glycerate. Although L-glycerate can be converted into glucose (Dickens & Williamson, 1960) it is presumed that it must first form D-glycerate via the joint action of lactate dehydrogenase and glycerate dehydrogenase acting as a racemase with NAD as the shuttling coenzyme. Because of the position of the equilibria of the reactions concerned in the oxidation of D- and L-glycerate (Dawkins & Dickens, 1965), conversion of L-glycerate into D-glycerate will not proceed in the presence of high hydroxypyruvate concentrations.

These experiments give no quantitative information on the relative importance of the serine dehydratase versus the serine-pyruvate aminotransferase pathways for conversion of serine into glucose, but they do indicate that under suitable conditions (which approach those *in vivo*) hydroxypyruvate can give rise to appreciable amounts of glucose. In addition, the results indicate a need for studying the metabolism of potential precursor compounds over a wide concentration range that includes that likely to occur *in vivo*.

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