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Acceleration of Gluconeogenesis from Lactate by Lysine

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L-Lysine (2mM) causes an increase (mean 60%) in the rate of gluconeogenesis from lactate in isolated liver cells. The effect is of a catalytic nature. No other amino acid has the same effect, though ornithine is slightly active. The effect is additional to the stimulatory effects of oleate and of dibutyryl cyclic AMP.

This paper reports experimental results that show that lysine at low concentrations can accelerate hepatic gluconeogenesis from lactate. The effect is catalytic. Although the mechanism of action of lysine is still unknown it is probable that lysine plays an important role in the regulation of liver metabolism.

The lysine effect was discovered in the course of experiments on histidine metabolism in the isolated perfused rat liver. Although histidine is a glucogenic amino acid and is readily degraded by the perfused liver it formed no glucose. This was taken to suggest that histidine (like tryptophan) might inhibit gluconeogenesis, and indeed histidine was found to have such an effect, at 2mM, on gluconeogenesis from lactate. These findings were confirmed in experiments with isolated liver cells prepared by the method of Berry & Friend (1969), to which Dr. M. N. Berry had introduced us during a recent stay in this laboratory. Since the isolated cells are a more convenient experimental material than the perfused liver (because many parallel tests can be carried out on a homogeneous suspension of cells from one liver), and having convinced ourselves that the metabolic properties (oxygen consumption, gluconeogenesis from a variety of substrates, urea synthesis, ketogenesis, glycolysis, fructolysis, the redox state of the NAD couple in cytoplasm and mitochondria, selective membrane permeability, i.e. relative impermeability to di- and tricarboxylic acids) of the isolated cells (unlike those of slices; see Krebs, 1970) are quantitatively similar to those of perfused liver, we followed up the investigation of the histidine effect in the first instance in the isolated cells. Ornithine, which causes the NH_3 derived from histidine to be converted into urea, was found to counteract the inhibition of gluconeogenesis from lactate but lysine (tested in order to examine the specificity of the ornithine effect) proved even more effective. Controls revealed that lysine also accelerates the rate of gluconeogenesis from lactate in the absence of histidine, and moreover lysine was found to affect several other metabolic processes involving lactate.

Methods

Major modifications of the method of Berry & Friend (1969) for the preparation of isolated liver cells were as follows. (*a*) Female rats (180–250g) were starved for 48h. (*b*) The apparatus and liver-perfusion technique were as described by Hems *et al.* (1966). (*c*) Lower concentrations of collagenase and hyaluronidase were used (0.03%, w/v, of each enzyme) [both supplied by Boehringer Corp. (London) Ltd., London W.5, U.K.]. (*d*) Ca^{2+}-free Hanks solution (Hanks & Wallace, 1949) for the preparation of the cells was replaced by the physiological saline of Krebs & Henseleit (1932) from which Ca^{2+} was omitted, and Hanks solution for the final incubations was replaced by Krebs–Henseleit saline containing 2.5% (w/v) dialysed albumin (Pentex, supplied by Miles Laboratories, Stoke Poges, Bucks., U.K.). (*e*) EDTA was omitted. (*f*) The gas phase was O_2+CO_2 (95:5) throughout, and no adjustments of pH were necessary.

Minor modifications were: (*a*) a relatively low perfusion rate (15–20ml/min) was used to prevent disruption of the liver by hydrostatic pressure; (*b*) the liver suspension was shaken after perfusion with the enzyme-containing medium used for perfusion and gassed continuously with the O_2+CO_2 mixture; (*c*) the cell suspension was filtered through nylon of mesh size 0.5mm×0.3mm; (*d*) the cells were washed once with about 10vol. of the gassed albumin-containing medium; (*e*) the washed cells were resuspended in 18vol. of medium. The yield of cells is about 50% of the initial weight of the liver. The dry weight of the cell suspension and of the albumin-containing medium was determined for each cell preparation. A factor of 3.7 was used to convert from dry weight into wet weight of cells; about 70mg wet wt. of hepatocytes was used in each incubation flask. Incubations were in 25ml Erlenmeyer flasks containing 2ml of suspension plus 2ml of additions (substrates, effectors and albumin-containing medium). Flasks were gassed with O_2+CO_2 (95:5) and sealed with rubber stoppers. Incubation time was 60min at 37°C in a rapidly moving Dubnoff-type shaker. The cell suspension was inactivated by the addition of 0.14ml of 60% (v/v) $HClO_4$. Glucose was assayed by the method of Slein (1963) on the neutralized supernatants. Histidine and lysine were determined by the manometric decarboxylase method of Gale (1970).

Table 1. *Acceleration by lysine of gluconeogenesis from lactate in isolated liver cells*

Final concentrations added were lactate, 10mM, oleate, 2mM, dibutyryl cyclic AMP, 0.1mM, L-lysine, 2mM. Cells were incubated for 60min at 37°C. Rates are expressed as means±S.E.M. with the number of observations in parentheses. The increase in rate caused by lysine is the mean of the percentage increase calculated for individual experiments.

Addition	Glucose formed (μmol/min per g wet wt.)	Mean percentage increase caused by lysine
Lactate	0.83±0.03 (19)	61 (14)
Lactate+oleate	1.41±0.08 (13)	60 (7)
Lactate+dibutyryl cyclic AMP	1.12±0.12 (5)	57 (3)
Lactate+oleate+dibutyryl cyclic AMP	1.61±0.19 (5)	41 (3)

Results and discussion

The rate of gluconeogenesis from lactate when this was the only added substrate (0.83μmol/min per g; Table 1) was about 80% of the rate reported for the perfused rat liver (1.06μmol/min per g; Hems *et al.*, 1966). As established from experiments on the perfused organ (see Exton & Park, 1968; Ross *et al.*, 1967), addition of oleate or cyclic AMP increased the rate and the effects of these two substances were additive. The presence of lysine caused a further increase of about 60% in the rate of gluconeogenesis under all conditions tested, even when the rate had already been increased by oleate, 6-*N*,2′-*O*-dibutyryl cyclic AMP or a combination of oleate and dibutyryl cyclic AMP. Half-maximal stimulation, according to a Lineweaver–Burk plot, occurred at 0.5mM-lysine. The amounts of lysine removed by the liver under the test conditions were small in relation to the additional glucose formation. Lysine alone gave no glucose. The extra glucose was derived from the lactate; lactate removal was fully accounted for as glucose and pyruvate. Thus the effect of lysine was of a catalytic nature. Ornithine had a similar, but much less marked, effect. Other protein amino acids, or D-lysine, cadaverine, α-aminoadipic acid or ϵ-aminohexanoic acid (at 2mM) had no effects on glucose synthesis from lactate.

NH_4Cl inhibited gluconeogenesis from lactate when added at concentrations below 1mM, but at these low concentrations the stimulating effect of lysine was increased. At 5–10mM, NH_4Cl increased the rate of gluconeogenesis almost as much as did lysine, and addition of lysine had very little effect. As the concentration of NH_3 in the liver tissue is normally controlled within narrow limits at about 0.7mM it is unlikely that NH_3 modulates gluconeogenesis *in vivo*.

The stimulatory effect of lysine on gluconeogenesis was specific for lactate: the rates of glucose synthesis from serine, alanine, pyruvate, glutamine and glycerol were not affected by the addition of lysine. The specificity of the lysine effect indicates a direct or indirect involvement of the lactate dehydrogenase reaction. There is no evidence of a direct effect on this enzyme, which leads to the idea that lysine might affect the intracellular concentration of lactate or of NAD^+, by either promoting the entry of lactate into the cell, or by promoting the reoxidation of NADH to NAD^+. If lysine promoted the reoxidation of NADH, the substrates of other cytoplasmic dehydrogenases should be oxidized at higher rates on addition of lysine. The test, with ethanol as substrate, showed that this is not the case. Lysine had no effect on the activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase or pyruvate kinase in liver extracts (M. Stubbs, personal communication). Thus the mechanism of action of lysine remains to be established.

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