The Hormonal Control of Pyruvate Kinase Activity and of Gluconeogenesis in Isolated Hepatocytes

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Effects of Tryptophan on Gluconeogenesis in the Rat and the Guinea Pig

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The effect of tryptophan on gluconeogenesis in the isolated perfused rat liver was initially demonstrated in Lardy's laboratory (Veneziale et al., 1967). Significant differences in the regulation of hepatic gluconeogenesis exist between the rat and the guinea pig. Addition of fatty acids causes an increase in the rate of glucose production from lactate by the perfused rat liver, whereas a decrease occurs with the guinea pig (Arinze et al., 1973). The intracellular distribution of phosphoenolpyruvatecarboxykinase (EC4.1.1.32) differs between the livers of the two species, being approx. 90% cytosolic and 10% mitochondrial in the rat, whereas in the guinea pig a substantial proportion is mitochondrial.

We have investigated the effects of tryptophan on gluconeogenesis in isolated liver cells prepared from both species by the collagenase perfusion technique (Elliott et al., 1976).

Isolated liver parenchymal cells (60–80mg dry wt.) prepared from 48h-starved animals were incubated in 15ml of Krebs–Henseleit bicarbonate buffer supplemented with 2% bovine serum albumin and containing 10mM-L-lactate. The incubation vessels were shaken at 100cycles/min in Dubnoff-type shaking water baths at 37°C. Cell and incubation-medium fractions were obtained by means of a separating centrifuge tube (Hems et al., 1975) at various times after the addition of 0.5mM-L-tryptophan. Glucose and other metabolites in the cellular and incubation-medium fractions were assayed by standard techniques.

In rat liver cells 0.5mM-L-tryptophan is a potent inhibitor of gluconeogenesis from lactate, decreasing the rate to approx. 10% of that of the control without L-tryptophan. The half-maximal effective concentration is 0.1mM, close to that normally present in plasma. The inhibition is accompanied by a change in the intracellular β-hydroxybutyrate/acetacetate ratio, indicating a shift towards a more reduced mitochondrial redox state. Large increases over the control values of intracellular malate and aspartate (3-fold) occur, together with smaller increases in β-hydroxybutyrate (2-fold), citrate and 2-oxoglutarate (1.5-fold). After 20 min incubation with L-tryptophan the intracellular phosphoenolpyruvate content falls to less than 10% of the control value. There is no change in the phosphorylation state of the adenine nucleotides nor in the cytosolic redox state as indicated by the intracellular lactate/pyruvate ratio.

In the guinea-pig liver cells 0.5mM-L-tryptophan does not inhibit glucose production from 10mM-L-lactate. No significant differences in intracellular metabolites were found between the L-tryptophan-treated and control cells.

Parallel experiments using rat hepatocytes showed that 0.5mM-L-tryptophan also inhibits glucose production from 10mM-pyruvate, -propionate, -l-serine, -l-alanine, -l-proline and -l-glutamine but not from 10mM-glycerol or -D-fructose.