THE METABOLIC CONTROL OF PYRUVATE DEHYDROGENASE AND ITS IMPORTANCE IN THE CONTROL OF THE SYNTHETIC PROCESSES OF LIPOGENESIS AND GLUCONEOGENESIS

by

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ABSTRACT

Pyruvate dehydrogenase is a mitochondrial enzyme which undergoes metabolic interconversion between an active, dephosphorylated state and an inactive, phosphorylated state. The regulatory functions of the interconversion of the enzyme to the processes of hepatic lipogenesis and gluconeogenesis were investigated.

The activity of pyruvate dehydrogenase of liver mitochondria prepared from animals whose livers are actively synthesizing glucose failed to support lipogenesis to the same extent as mitochondria prepared from well-fed animals. In this reconstituted cell-free system dichloroacetate increased the activity of pyruvate dehydrogenase and also the rate of lipogenesis. In isolated hepatocytes prepared from meal-fed rats dichloroacetate and lactate plus pyruvate increased the activity of pyruvate dehydrogenase. With isolated liver cells these incubation conditions have been established to increase lipogenesis. It has been established that N^6,O^2-dibutyryl adenosine-3':5'-monophosphate (dibutyryl-cAMP) and glucagon inhibit lipogenesis in cells from meal-fed rats. However, these compounds do not decrease the activity of pyruvate dehydrogenase. Therefore, it is proposed that pyruvate dehydrogenase can be rate determining to lipogenesis under certain conditions but that glucagon and dibutyryl-cAMP inhibition of lipogenesis is not explained on this basis.

Glucagon and dibutyryl-cAMP are known to inhibit glucose synthesis from pyruvate in isolated liver cells. Pyruvate dehydrogenase has been suggested to be the point of attack of these inhibitors. This study demonstrates, however, that dibutyryl-cAMP does not decrease gluconeogenesis by causing the conversion of the enzyme to the inactive

form. Therefore, this paradoxical inhibition of dibutyryl-cAMP on glucose synthesis was investigated further. Dibutyryl-cAMP was found to stimulate ketogenesis, not to affect the mitochondrial NAD^+ redox state, and to inhibit lactogenesis (reduction of pyruvate to lactate) which is another cytoplasmic NADH requiring process. With an active urea cycle reconstituted, dibutyryl-cAMP was found to stimulate glucose and lactate synthesis from pyruvate. These results suggest that the movement of reducing equivalents and carbon out of the mitochondrion may be involved in the mechanism by which dibutyryl-cAMP inhibits gluconeogenesis from pyruvate. The movement of NADH equivalents from the mitochondrion is made energetically favorable by the urea cycle or, in its absence by the cycling of pyruvate (pyruvate to oxaloacetate to malate in the mitochondrion and malate to oxaloacetate to phosphoenolpyruvate to pyruvate in the cytoplasm). The overall equation for this process is: $NADH_{mito} + NAD^+_{cyto} + ATP \rightarrow NADH_{cyto} + NAD_{mito} + ADP + Pi$. An interruption in this cycle would result in an inhibition of glucose and lactate synthesis for want of NADH and substrate. Inhibition of both processes is caused by 2-n-butylmalonate, an inhibitor of the malate-phosphate antiport. Evidence is discussed which suggests that dibutyryl-cAMP inhibits substrate cycling of pyruvate by decreasing the effectiveness of pyruvate kinase.

In another study it was found that isolated liver cells rapidly oxidize succinate but that succinate is a poor substrate for gluconeogenesis. Succinate oxidation to malate was found to be a property of the damaged cells of isolated liver cell preparations.

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