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SYMPOSIUM ON 'INTEGRATION OF METABOLISM'

Mechanisms for the regulation of ketogenesis

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Under optimum conditions mammalian metabolism is integrated so as to attain the most appropriate 'mixture' of circulating substrates for the prevailing dietary and physiological state of the animal. The regulatory mechanisms for integration are exerted at two levels, within the cell (intracellular integration) and between the various tissues (inter-organ integration). Integration is usually achieved at the intracellular level by key metabolites of one pathway interacting with the enzymes of another related pathway and so altering its rate. Alternatively, hormones acting through 'second messengers' can alter the activity of regulatory enzymes. Although inter-organ integration of metabolism involves intracellular mechanisms the blood-borne 'signals' (metabolites, hormones) are generated in tissues other than their site action.

The aim of this contribution is to discuss the mechanisms for the regulation of ketogenesis in relation to the integration of metabolic fuel supply in omnivorous mammals (man and the rat).

The problem

Ketone bodies (acetoacetate and 3-hydroxybutyrate) are important alternative substrates to glucose for the production of energy in brain and other peripheral tissues. In addition, the acetyl-CoA formed in the metabolism of ketone bodies can be used to synthesize complex lipids, particularly in developing brain and lactating mammary gland (for review see Robinson & Williamson, 1980). The oxidation of ketone bodies brings about metabolic changes which decrease glucose utilization by muscle and other tissues (Randle *et al.* 1966). If ketone bodies are to be effective alternative substrates to glucose their availability in the circulation must increase when that of glucose is restricted, for example, in starvation, on a high fat diet or in diabetes (glucose is available but its entry into insulin-sensitive tissues is depressed). The question to be discussed is how is this integration of substrate supply achieved.

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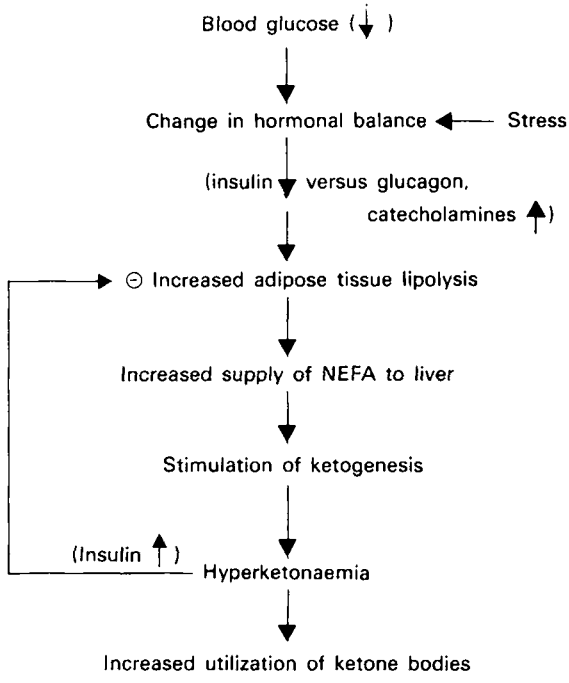

Fig. 1. Sequence of physiological events leading to hyperketonaemia. ⊖ Indicates the inhibitory feed-back effect of ketone bodies.

Extrahepatic regulation

Non-esterified fatty acids (NEFA) stored in adipose tissue as triacylglycerols are the major precursors of ketone bodies and therefore an important determinant of the rate of ketogenesis is the flux of NEFA to the liver which in turn is governed by the rate of lipolysis in adipose tissue. This latter process is not directly affected by glucose, but is exquisitely sensitive to inhibition by insulin and thus the blood glucose via the β-cells of the pancreas can regulate the production of ketone bodies. Adipose tissue lipolysis is stimulated by a number of hormones including catecholamines, thyroxine and glucagon (see Hales *et al.* 1978). In addition, there is evidence that ketone bodies can control their own production by a feed-back mechanism. Ketone bodies stimulate insulin secretion and therefore indirectly regulate adipose tissue lipolysis: they also directly inhibit this process (for review of evidence see Robinson & Williamson, 1980). The extrahepatic regulation of ketogenesis is summarized in Fig. 1.

Intrahepatic regulation

Although there is no doubt that the rate of delivery of NEFA to the liver is the primary determinant of the rate of ketogenesis there is considerable evidence that regulation of this process also occurs at intrahepatic sites. For example, if livers

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from fed and starved rats are perfused with the same load of long-chain fatty acid, the livers from starved rats convert a higher proportion of the fatty acids extracted from the medium to ketone bodies (Mayes & Felts, 1967; McGarry & Foster, 1971*b*; Ontko, 1972; Whitelaw & Williamson, 1977).

Uptake of NEFA by the liver is concentration dependent. The first step in the catabolism of NEFA is their conversion to the fatty acyl-CoA derivatives and these can either enter the mitochondria for oxidation or be converted to esterified products (triacylglycerols, phospholipids) (Fig. 2). Once the fatty acyl-CoA has entered the mitochondria it is committed to oxidation. The disposal of long-chain fatty acyl-CoA between the pathways of esterification and β-oxidation is now considered to be the primary site for the intrahepatic regulation of ketogenesis (for review of the evidence see McGarry & Foster, 1980). In order to enter the mitochondria the fatty acyl-CoA must be converted to the carnitine derivative via the enzyme carnitine acyltransferase I (CAT I) which is located on the outer

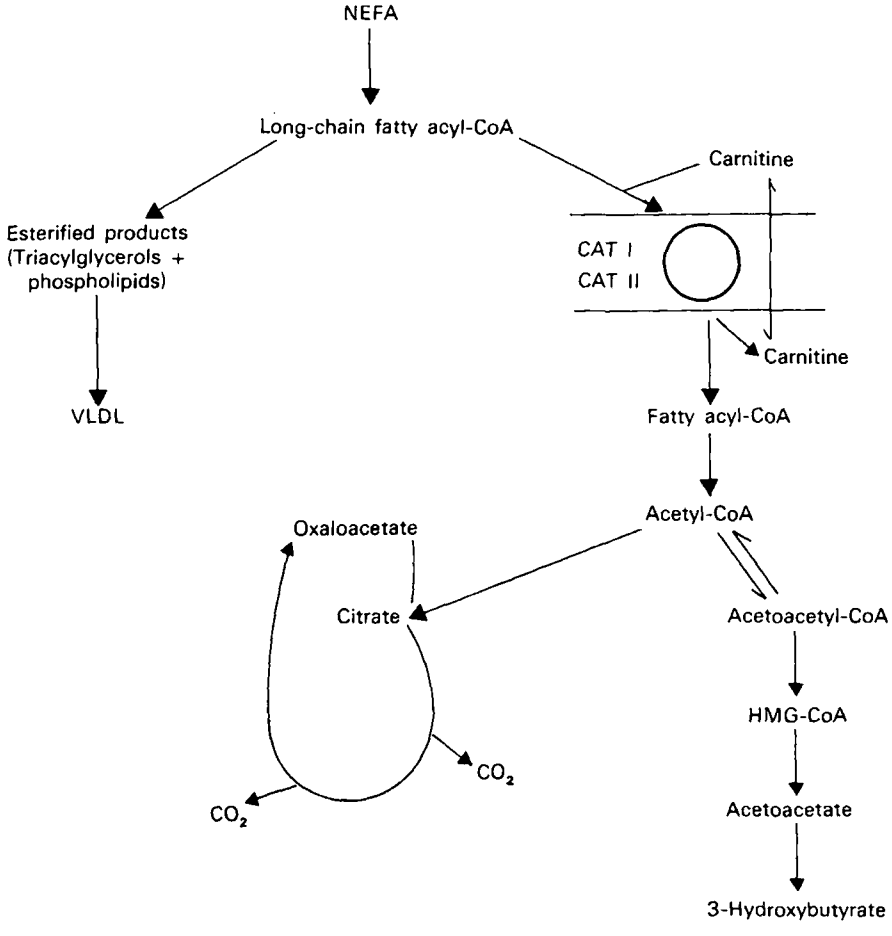

Fig. 2. Simplified scheme of NEFA metabolism in liver.

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surface of the inner membrane of the mitochondria. On the inner surface, carnitine acyltransferase II (CAT II) then reforms fatty acyl-CoA from the acylcarnitine with the liberation of carnitine (Fig. 2). Medium- and short-chain fatty acids which do not require the CAT system for transport into the mitochondria give similar rates of ketogenesis in livers from fed and starved rats (McGarry & Foster, 1971*a*; Whitelaw & Williamson, 1977).

Acetyl-CoA, the end-product of the β-oxidation system, also has two fates; it can either react with oxaloacetate to form citrate and enter the tricarboxylic acid cycle for oxidation or it can be converted via the hydroxymethylglutaryl-CoA pathway to acetoacetate (Fig. 2). Regulation of the fate of acetyl-CoA is the second intrahepatic site for the control of ketogenesis. It should perhaps be emphasized that in most experimental situations the rate of complete oxidation of long-chain fatty acid remains reasonably constant and it is the rates of esterification and ketogenesis which show the largest fluctuations (Mayes & Felts, 1967; McGarry & Foster, 1971*b*; Whitelaw & Williamson, 1977).

Integration of hepatic carbohydrate metabolism and ketogenesis

It has long been appreciated that the carbohydrate status (glycogen content, predominance of glycolysis or gluconeogenesis) has a major influence on the rate of ketogenesis. Clearly, if ample hepatic glycogen is available and this can be mobilized to maintain blood glucose there is no need to produce large amounts of ketone bodies, even if the flux of NEFA to the liver increases. What has not been clear is the nature of the regulatory signal which links the hepatic carbohydrate status and the rate of ketogenesis. A mechanism for integrating the two has recently been elucidated by the elegant work of McGarry and his colleagues (McGarry *et al.* 1977). Malonyl-CoA, an obligatory intermediate in the pathway of lipogenesis, inhibits carnitine acyltransferase I (Fig. 3). This in turn results in greater availability of fatty acyl-CoA for esterification and very low density lipoprotein (VLDL) formation. Glucose derived from glycogen is a major source of carbon for lipogenesis in the liver and the malonyl-CoA concentration is positively related to the rate of lipogenesis and can therefore 'signal' alterations in glycolytic flux and glycogen content. This mechanism of integration predicts a reciprocal relationship between the rates of lipogenesis and ketogenesis, and ketogenesis and esterification, and this has been confirmed experimentally in hepatocytes from fed rats (Benito *et al.* 1979).

Long-chain fatty acyl-CoA is an inhibitor of acetyl-CoA carboxylase (Tubbs & Garland, 1963) and therefore when the supply of NEFA increases the concomitant increase in fatty acyl-CoA will inhibit lipogenesis and lower malonyl-CoA.

Direct hepatic effects of hormones

The importance of hormones, in particular insulin and glucagon, in regulating the flux of NEFA to the liver has already been emphasized. In addition to these extrahepatic effects both insulin and glucagon act directly on hepatic carbohydrate

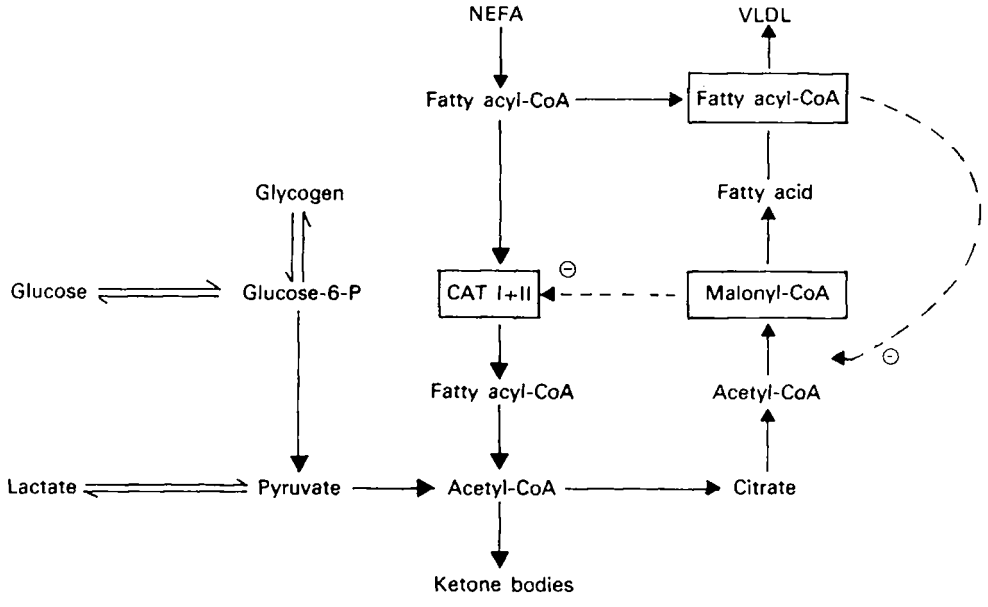

Fig. 3. Simplified scheme of the regulatory link between hepatic carbohydrate status, lipogenesis and ketogenesis. ⊖◂---- Indicates site of inhibition.

metabolism. Insulin promotes glycogen synthesis whereas glucagon activates glycogenolysis (for review see Pilkis *et al.* 1979). In view of these changes in carbohydrate status it is not surprising that these hormones also have opposite effects on the rate of ketogenesis; insulin depresses the rate (Topping & Mayes, 1976), whereas glucagon (or its second messenger–cyclic AMP) stimulates it (Heimberg *et al.* 1969; Benito *et al.* 1979). A key question is; how do these hormones exert their effects? Glucagon can inhibit lipogenesis and therefore lower malonyl-CoA concentrations by at least two mechanisms; (1) inhibition of acetyl-CoA carboxylase (the enzyme responsible for the formation of malonyl-CoA) (Witters *et al.* 1979); (2) inhibition of glycolysis and consequently the supply of substrate for lipogenesis. In the case of insulin the evidence for stimulating effects on lipogenesis is less clear-cut, except in situations where the rate of lipogenesis has already been decreased by glucagon (Beynen *et al.* 1979). Present evidence suggests that insulin and glucagon alter the rate of ketogenesis in hepatocytes from fed rats by modulation of the malonyl-CoA concentration. However, effects of the hormones on other regulatory sites, for example, the esterification pathway, are by no means excluded.

There has been considerable interest recently in the direct hepatic effects of vasopressin (antidiuretic hormone). These include activation of glycogenolysis and glucose output, enhancement of gluconeogenesis, stimulation of phosphatidylinositol turnover, activation of pyruvate dehydrogenase and inhibition

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of lipogenesis (mouse) (for review see Hems, 1979). Although vasopressin does not elevate hepatic cyclic AMP concentrations it exhibits most of the metabolic effects of glucagon, and might therefore be expected to stimulate ketogenesis. Surprisingly, in isolated hepatocytes from fed rats vasopressin inhibits ketogenesis from exogenous oleate (Williamson *et al.* 1980). This antiketogenic effect of the hormone is not due to decreased removal of the fatty acid. It is, however, accompanied by an increase in esterification of the fatty acid and in its complete oxidation to CO_2 (Sugden *et al.* 1980). The increased oxidation of oleate and the decreased rate of ketogenesis in the presence of vasopressin is dependent on the presence of calcium ions in the medium, whereas the increased rate of esterification is independent (Sugden *et al.* 1980). This has led to the conclusion that the stimulation of acetyl-CoA oxidation is the primary reason for the antiketogenic effect of vasopressin.

It is of interest to speculate whether these in vitro findings are of any physiological significance. The plasma concentration of vasopressin rises in stress situations (Hems, 1979) and these are associated with hyperglycaemia and increased concentrations of plasma NEFA. As a consequence of catecholamine action on the pancreatic β-cells the plasma insulin is usually low for the degree of glycaemia, yet blood ketone body concentrations are often within the normal range (Williamson & Smith, 1980). It is possible that vasopressin is in part responsible for the inappropriate ketonaemia in certain stress states.

The author is a member of the External Staff of the Medical Research Council (UK).

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