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N-Acetylglutamate and urea synthesis

Lund & Wiggins (1984) have recently suggested that variations in the intramitochondrial concentration of *N*-acetylglutamate, the essential activator of carbamoyl-phosphate synthetase (EC 6.3.4.16), do not play a role in the regulation of urea synthesis. They claim that carbamoyl-phosphate synthetase is always saturated with *N*-acetylglutamate, independent of the nitrogen supply. This conclusion is based on the fact that a new method for the measurement of intracellular *N*-acetylglutamate yields much higher values for intracellular *N*-acetylglutamate than those published in the literature so far.

Setting aside a discussion of assay methodologies for measuring *N*-acetylglutamate, we wish to discuss the arguments that Lund & Wiggins (1984) put forward in support of their view that carbamoyl-phosphate synthetase is always saturated with *N*-acetylglutamate.

Firstly, we should like to point out that Lund & Wiggins (1984) have not measured the relevant parameter, that is, the intramitochondrial content of *N*-acetylglutamate of the hepatocytes. Instead, they have used literature values on the distribution of *N*-acetylglutamate between the mitochondrial and cytosolic compartments; these indicate that 65% or more of intracellular *N*-acetylglutamate is present in the mitochondria (Shigesada & Tatibana, 1971; Hensgens *et al.*, 1980). However, in both studies this value was obtained with an assay procedure for *N*-acetylglutamate that Lund & Wiggins (1984) consider to be unsatisfactory. In any case, the intramitochondrial *N*-acetylglutamate concentration of freshly isolated hepatocytes calculated in this way was 1 mM or higher. This value does not far exceed the amount of total intramitochondrial *N*-acetylglutamate required for half-maximal activation of carbamoyl-phosphate synthetase in the mitochondrial matrix (0.8 nmol/mg of mitochondrial protein, or 0.8 mM; Stewart & Walser, 1980). The value of 0.1 mM cited by Lund & Wiggins (1984) refers to the K_a for free *N*-acetylglutamate in activating carbamoyl-phosphate synthetase (Lof *et al.*, 1983); the total amount (free plus enzyme-bound) of *N*-acetylglutamate is much higher due to the high concentration of carbamoyl-phosphate synthetase in the mitochondrial matrix (about 0.7 mM; see Charles *et al.*, 1980).

In addition to the direct determination of intramitochondrial *N*-acetylglutamate there is another, independent, way of studying the effect of *N*-acetylglutamate on carbamoyl-phosphate synthetase activity in the mitochondrial matrix. This method is based on measurement of the capacity of isolated liver mitochondria to synthesize citrulline from an excess of ammonia, bicarbonate and ornithine. This capacity is greatly dependent on the nutritional or hormonal status of the animal or of the hepatocytes from which the mitochondria are isolated. There is ample evidence in support of the view that these variations are caused by differences in the intramitochondrial content of *N*-acetylglutamate (see Meijer & Hensgens, 1982, for a review). It is highly unlikely that this phenomenon is an isolation artefact due to a partial loss of *N*-acetylglutamate from the mitochondria during their isolation, because at 0°C (the temperature at which mitochondria are isolated) *N*-acetylglutamate is not transported across the mitochondrial membrane. In our experience, at 0°C the amount of *N*-acetylglutamate in the mitochondrial matrix is completely stable for several hours. Finally, we wish to stress that the measurements of *N*-acetylglutamate in isolated mitochondria have not been questioned by Lund & Wiggins (1984).

From the above we conclude, in contrast to Lund & Wiggins (1984), that the activity of carbamoyl-phosphate synthetase in the mitochondrial matrix of the intact hepatocyte is controlled by variations in the concentration of *N*-acetylglutamate.

What, then, is the implication of this conclusion for the control of urea synthesis? In our opinion, the function of carbamoyl-phosphate synthetase is to regulate the intrahepatic ammonia concentration (cf. Krebs *et al.*, 1973; Westerhoff *et al.*, 1984) rather than to affect urea cycle flux *per se*; *N*-acetylglutamate activation of the enzyme will help to fulfil this task. A change in the intramitochondrial *N*-acetylglutamate concentration will only affect the urea production rate as long as the intracellular concentrations of ammonia and of aspartate, the other substrate for urea synthesis, are kept constant, a condition that is not fulfilled in most experimental systems. In urea synthesis from amino acids, such as glutamine, activation of

carbamoyl-phosphate synthetase by an increase in *N*-acetylglutamate may have little effect on the rate of urea production. However, it is of importance to note that the same flux through the urea cycle can now be maintained at a lower steady state intramitochondrial ammonia concentration than in the absence of such an activation. The advantage of this mechanism is that the increase in urea production rate following an increased supply of amino acids to the liver can be obtained at a relatively constant ammonia concentration in the mitochondria.

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