Apparent Metabolic Regulation of the Coupling between the Potassium Ion Gradient and Methionine Transport in Mouse Ascites-Tumour Cells

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Mouse ascites-tumour cells can concentrate glycine from a Ringer solution by utilizing the energy inherent in the concentration gradients of Na^+ and K^+ acting across the cell membrane (Schultz & Curran, 1970; Christensen, 1970). The sodium pump normally sets up these gradients, but they can be reproduced without the intervention of ATP by soaking the tumour cells in appropriate Ringer solutions containing 2mM-sodium cyanide. Working with 1mM solutions of glycine, Eddy (1968a,b) showed in that way that, whereas the optimum cation gradients established during respiration were associated with about a 25-fold gradient of glycine concentration, the amino acid was concentrated only about eightfold when energy metabolism was prevented and the alkali-metal cation gradients were manipulated in the same optimum range. Typical optimum values for $[\text{Na}^+]_1$, $[\text{Na}^+]_2$, $[\text{K}^+]_1$ and $[\text{K}^+]_2$ respectively were 150, 50, 8 and 140 mequiv./l, where the subscript 1 denotes the extracellular phase and subscript 2 the cellular phase. The lowering of the glycine accumulation ratio in the presence of the metabolic inhibitors is probably not due to cellular damage (Eddy & Hogg, 1969).

To explain the above findings Christensen (1970) has suggested that, in normal physiological circumstances, only part of the energy driving the amino acid pump may be derived from the ionic gradients (hypothesis 1). Indeed Potashner & Johnstone (1971) claim that the latter fraction is relatively small in a certain strain of Ehrlich ascites-tumour cells. They suggest that the major part is provided directly by the hydrolysis of ATP. Of course the contribution of the ionic gradients might be misinterpreted if the effective intracellular concentrations of Na^+ and K^+ were wrongly estimated. This might happen if energy metabolism affected (a) the distribution of the cations between various cellular compartments or (b) the membrane potential. Unfortunately, little is known about the importance of either (a) or (b), though the membrane potential is usually assumed to be relatively small (Eddy, 1968b; Jacquez & Schafer, 1969).

Another possibility, to which little attention has been given hitherto, is that the ionic gradients

provide all the driving force and that the coupling of the spontaneous movements of Na^+ and K^+ to the transfer of the amino acid across the cell membrane varies with the physiological state of the cells (hypothesis 2). Thus Eddy (1968b) found that the gradient of K^+ apparently contributed more energy to the accumulation of glycine taking place during respiration than when respiration was prevented. In the present work we have shown that a similar effect occurs with L-methionine. Our strain of mouse cells accumulates L-methionine almost as well as glycine, both in the presence and in the absence of the inhibitors of energy metabolism.

The absorption of L-[Me- ^{14}C]methionine was studied at 37°C by means of the large-sample technique of Eddy (1968a). The extracellular space associated with each pellet of cells was assayed with tritiated inulin (The Radiochemical Centre, Amersham, Bucks., U.K.) and, in contrast with the earlier work, the tracers were assayed without first drying the samples. A separate series of these was accordingly used to determine the cellular dry weight. Both the cellular and extracellular concentrations of Na^+ and K^+ were manipulated as described by Eddy (1968b). The endogenous amino acid pool probably played no part in the quantitative relationships described below, because similar results were obtained when the endogenous amino acids had been largely removed by subjecting the cells to an osmotic shock (Jacquez & Schafer, 1969) at 37°C.

We shall consider the special case of hypothesis 2 where the coupling to Na^+ is fixed and the coupling to K^+ is regulated by respiration. If L-methionine behaved like glycine, then in the steady state the following relationship would hold between the distributions of Na^+ , K^+ and methionine across the cell membrane (Eddy, 1968b):

$$\frac{[\text{Met}]_2}{[\text{Met}]_1} = \frac{[\text{Na}^+]_1/[\text{Na}^+]_2 \cdot (1 + \theta[\text{K}^+]_2)/(1 + \theta[\text{K}^+]_1)}{\quad} \quad (1)$$

The parameter $\theta = k^*/k^*k_2$ and governs the degree of coupling between the respective flows of methionine and K^+ . It contains two factors, (a) the dissociation constant (k_2) of the carrier complex with

Fig. 1. Application of eqn. (1) to the system containing 2 mM-NaCN and, in most of the assays, 10 mM-2-deoxyglucose as well. The logarithm of the corrected methionine gradient is plotted against the logarithm of the function representing the combined Na^+ and K^+ gradients. $[\text{Met}]_1$ was 1 mM.

K^+ and (b) the ratio (k^k/k^c) of the rates of transfer of the carrier loaded and unloaded respectively with K^+ .

We found, as Potashner & Johnstone (1971) have also indicated, that when $[\text{Na}^+]_1/[\text{Na}^+]_2 = [\text{K}^+]_1/[\text{K}^+]_2 = 1$ the ratio $[\text{Met}]_2/[\text{Met}]_1$ was somewhat larger than 1. In practice the left-hand side of eqn. (1) was therefore divided by a correction factor (f). The best values of f and θ were determined by an iterative procedure based on a least-squares criterion.

Fig. 1 shows that the corrected methionine gradient, observed in the presence of the metabolic inhibitors, varied with the alkali-metal cation gradients in fair agreement with the model ($N = 31$; $r = 0.966$; $P < 0.001$). A satisfactory fit was likewise observed in a series of observations on the respiring tumour cells ($N = 53$; $r = 0.962$; $P < 0.001$). The difference between the value of f in the former case of 1.32 and the value of 1.49 in the latter case was not statistically significant

($P > 0.05$). In contrast, whereas θ was 0.0469 during respiration, it was significantly smaller at 0.0100 in the presence of the metabolic inhibitors ($P < 0.01$). Application of eqn. (1) shows that, when $[\text{K}^+]_1$ is 8 mequiv./l and $[\text{K}^+]_2$ is 140 mequiv./l, the effect of lowering θ to that extent would be to lower $[\text{Met}]_2/[\text{Met}]_1$ by the factor $5.5/2.2 = 2.5$. The component of the methionine gradient due to the Na^+ gradient would be constant at the approximate factor $1.4 \times 150/50$, or 4.2, in either situation.

The behaviour with L-methionine is thus consistent with the special case of hypothesis 2 above, in that the flow of K^+ out of the tumour cells appears to be more efficiently coupled to the methionine pump when respiration is not inhibited. Analogies with the regulatory behaviour of certain enzymes seem relevant in speculation about the mechanism of the effect. We suggest that the magnitude of θ is regulated by a product of energy metabolism, which might indeed be ATP itself. Thus θ would be larger in the presence than in the absence of ATP. The initial rate of entry of glycine (v) was lowered by K^+ to roughly the same extent in the presence and in the absence of cyanide (Eddy & Hogg, 1969). We suggest that, because v depends more on k_2 than on k^k/k^c , the effect of cyanide on the magnitude of θ principally involves the regulation of k^k/k^c . It must be emphasized that, according to hypothesis 2 and in contrast with the version of hypothesis 1 above that Potashner & Johnstone (1971) have proposed, the actual passage of the amino acid across the cell membrane does not directly involve the hydrolysis of ATP.

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- Christensen, H. N. (1970). In *Membranes and Ion Transport*, vol. 1, p. 365. Ed. by Bittar, E. E. London: Wiley-Interscience.
- Eddy, A. A. (1968a). *Biochem. J.* **108**, 195.
- Eddy, A. A. (1968b). *Biochem. J.* **108**, 489.
- Eddy, A. A. & Hogg, M. C. (1969). *Biochem. J.* **114**, 807.
- Jacquez, J. A. & Schafer, J. A. (1969). *Biochim. biophys. Acta*, **193**, 368.
- Potashner, S. J. & Johnstone, R. M. (1971). *Biochim. biophys. Acta*, **233**, 91.
- Schultz, S. G. & Curran, P. F. (1970). *Physiol. Rev.* **50**, 637.