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The degree and the efficiency of coupling between the influxes of Na^+ and α-aminoisobutyrate in Ehrlich cells

P. GECK, E. HEINZ and B. PFEIFFER

Institut für vegetative Physiologie der Universität Frankfurt/Main (Germany)

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SUMMARY

The coupling between the influx of Na^+ and the transport of α-aminoisobutyrate into Ehrlich cells has been investigated as to its degree and efficiency, with and without metabolic inhibition.

For metabolically active cells the maximum degree of coupling q has been measured to be 0.5 from which a maximum efficiency η_{max}, of only 7% can be derived. This efficiency appears to be far too low to be compatible with the claim that the total energy for amino acid transport is supplied by electrochemical potential gradients of alkali ions. The degree of coupling q and hence the maximum efficiency η_{max} were found to increase significantly during metabolic inhibition.

This has been taken as evidence of a direct (chemiosmotic) coupling between amino acid transport and metabolism, acting beside a transport component driven by alkali ion gradients.

The transport of certain amino acids, notably those classified as belonging to the A-group[1] depends in most animal cells strongly on the extracellular $[Na^+]$. Most hypotheses that have been forwarded to explain this dependence seem to belong in one of the following categories:

(1) The *activation hypotheses*, which postulate that the transport of the amino acids is primary active, *i.e.* directly (chemiosmotically) coupled to a metabolic reaction, and that this coupling is activated by Na^+.

(2) The *gradient hypotheses*, which postulate that the transport of amino acids is not directly coupled to metabolism but to the flow of Na^+ into cells (cotransport). Since

this mechanism requires that an adequate electrochemical potential of Na^+ is maintained by the metabolically driven Na^+ pump, the transport is called secondary active.

Meanwhile it has been shown for Ehrlich and other cells that the entry of amino acids is stoichiometrically linked to that of Na^+ (ref. 2) and that with adequate Na^+ gradients, notably during metabolic inhibition, amino acids can be accumulated[2]. Hence there is little doubt that cotransport between Na^+ and the amino acids takes place and that energy available from the electrochemical potential gradient of Na^+ (and possibly from the inverse one of K^+) can be utilized to drive the amino acid uphill.

There are other findings, however, which raise doubts as to whether the alkali ion gradients account for all or even a significant part of the energy required for amino acid transport. (1) The transport of amino acids is not always congruent to the driving forces as resulting from the ion gradients. Thus, in metabolically active cells amino acids may within certain concentration ranges be actively taken up with inverted gradients of Na^+ and K^+ (ref. 3). (2) Even with identical alkali ion gradients, metabolically active cells transport amino acids 2 or 3 times more powerfully than do inhibited cells[2,4]. (3) The energy available from the combined electrochemical potential gradients of the alkali ions, though it has been found to be adequate for the amino acid transport, barely exceeds that required for amino acid transport in metabolizing cells. Therefore it would suffice only if the efficiency of coupling is high, possibly close to 100%[5].

In order to appraise to which extent the 2nd and 3rd observation seriously contradict the gradient hypothesis, we have investigated the efficiency of coupling between the flows of Na^+ and of α-aminoisobutyrate, whose uptake has been shown to depend on Na^+, in Ehrlich cells. In particular we have tried to assess the magnitude of this efficiency as to whether it is of an order compatible with the requirements of the gradient hypothesis, and to test whether and in which way the efficiency depends on the metabolic activity of the cells.

The maximum efficiency of coupling, according to Kedem and Caplan[6], is a function of q, the degree of coupling between two flows:

$$\eta_{max} = \frac{q^2}{(1 + \sqrt{1 - q^2})^2}$$

q is accessable experimentally since it can be evaluated from the mutual dependence between the two flows of α-aminoisobutyrate and of Na^+, expressed as the stoichiometric numbers, *i.e.* the partial derivatives of the flow of α-aminoisobutyrate with respect to that of Na^+, at constant [α-aminoisobutyrate], and of the flux of Na^+ with respect to that of α-aminoisobutyrate (AIB) at constant [Na^+].

$$q^2 = \left(\frac{\partial J_{AIB}}{\partial J_{Na^+}}\right)_{AIB} \cdot \left(\frac{\partial J_{Na^+}}{\partial J_{AIB}}\right)_{Na^+}$$

A possible source of error in determining these differentials may be that the Na^+ pump rapidly extrudes the Na^+ which may have entered the cell with the amino acid. In

order to minimize this error we have applied a newly developed method[7] which allows measuring the flows of Na^+ and α-aminoisobutyrate during incubation times of less than 10 s.

The principle is the following: The cells are incubated while passing through the appropriate medium during sedimentation in a centrifugal field. For this purpose the medium, containing Na^+ and α-aminoisobutyrate both labeled, is placed in a centrifugal tube on top of a silicon layer and covered by a separation medium. The suspension of cells is placed on top of the latter. During centrifugation the cells after having passed the separation medium will enter the incubation medium and, before being sedimented on the bottom of the tube, they will have to leave the incubation medium and cross the silicon layer. In this way the incubation time can be reduced to 10 s or less, depending on the thickness of the incubation layer. The incubation medium dragged along across the silicon layer has been found to be less than 10% of the wet weight of the cellular pellet. The preparation of the cell suspension was described before[3].

Radioactivity of ^{14}C and ^{22}Na was assayed in a liquid scintillation counter (Packard) with a toluol aethanol scintillator. The ATP content of the cells was determined by a luciferase method[8] with some modifications.

Fig.1a shows a typical experiment in which the influx of Na^+ (J_{Na^+}) is plotted against the corresponding influx of α-aminoisobutyrate (J_{AIB}), determined under the condition of constant extracellular [Na^+] and varying extracellular [α-aminoisobutyrate]. Fig.1b shows a typical example of the inverse experiment, the variation of amino acid

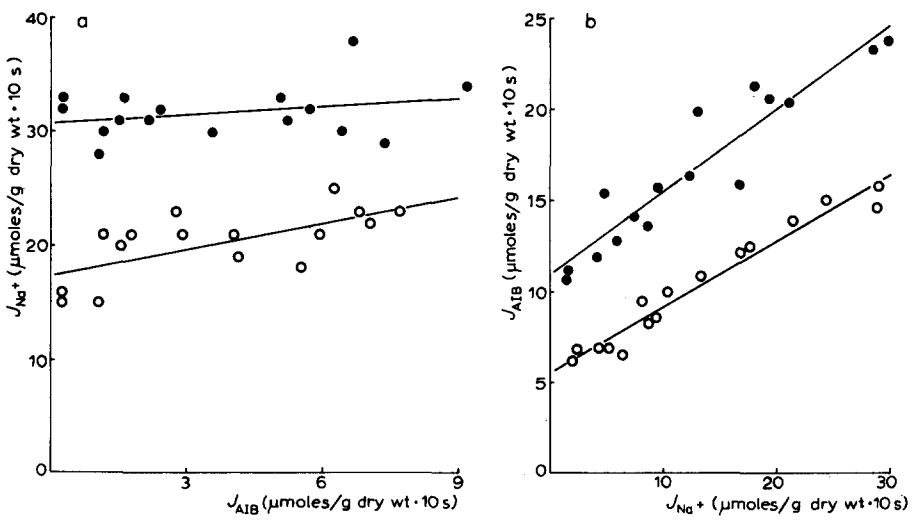

Fig.1. (a) Na^+ influx (J_{Na^+}) as a function of α-aminoisobutyrate influx (J_{AIB}) in Ehrlich cells. Extracellular Na^+: constant at 53 mM. Extracellular α-aminoisobutyrate: varied from 0.1–10 mM. •—•, uninhibited cells; o—o, metabolically inhibited cells. (b) α-Aminoisobutyrate influx (J_{AIB}) as a function of Na^+ influx (J_{Na^+}) in Ehrlich cells. Extracellular α-aminoisobutyrate: constant at 18 mM. Extracellular Na^+: varied from 2–70 mM. •——•, uninhibited cells; o——o, metabolically inhibited cells.

influx (J_{AIB}) as dependent on the Na^+ influx (J_{Na^+}) under the condition of constant extracellular α-aminoisobutyrate and varying Na^+. In both cases we obtained positive and approximately constant slopes from which the stoichiometric numbers can be derived. These stoichiometric numbers, though rather constant for each experiment, rise with the concentration of the nonvaried species, apparently toward a maximum (Table I), as should be expected if cotransport involves the binding of Na^+ and α-aminoisobutyrate to a carrier with saturable sites[9].

In other words, the stoichiometric number of Na^+ flux *versus* α-aminoisobutyrate flux increases with increasing Na^+ concentration, and the stoichiometric number of α-aminoisobutyrate flux *versus* Na^+ flux with increasing α-aminoisobutyrate concentration. The stoichiometric number of the Na^+ flux *versus* α-aminoisobutyrate flux seems always higher than the stoichiometric number of the α-aminoisobutyrate flux *versus* Na^+ flux, as if the two species were intrinsically coupled at a ratio of more than one Na^+ per one α-aminoisobutyrate. This need not be so, however, because of the presence of leakage and other uncoupled flows of these two species the empirical stoichiometric numbers may considerably deviate from the intrinsic ones. Since we do not know the relative magnitudes of such uncoupled pathways, the question of the intrinsic stoichiometric ratio between Na^+ and α-aminoisobutyrate must be left open at the present. The maximum efficiency corresponding to the maximum q of about 0.5 amounts to only 7%, a value clearly far below that to be postulated if the electrochemical potential gradients of Na^+ and K^+ were the only source of energy for the α-aminoisobutyrate transport.

Of special significance in this context would be the dependence of q, and consequently of η_{max}, on the metabolic energy supply of the cell. It has been found that metabolic activity of the cell considerably increases the transport activity for amino acids, even with the same electrochemical potential gradients of Na^+ and K^+ (ref. 4). An increase in the efficiency of coupling between electrolyte flows and amino acid transport caused by metabolic energy supply, *e.g.* of ATP, was postulated to explain this effect[10] If this were true, we should expect that metabolic inhibition should depress the degree of coupling, q. If, on the other hand, part of the amino acid transport were directly coupled to metabolism and not to Na^+ flow, we would expect that metabolic inhibition increases the degree of coupling as the Na^+-linked transport of the amino acid should predominate during metabolic inhibition. To study the dependence of the coupling efficiency on the metabolic state of the cells, the cells were treated with oligomycin and 2-deoxyglucose, which reduce cellular ATP. The results are shown in Table I. The stoichiometric number of the Na^+ *versus* the α-aminoisobutyrate flux at constant Na^+ is strongly increased during metabolic inhibition, whereas the reverse derivative does not seem to change significantly. Accordingly q, and hence η_{max}, strongly increase during metabolic inhibition, an effect which refutes the first of the alternative hypotheses above and supports the second one: namely, that a metabolically driven active pathway for α-aminoisobutyrate entry exists which is largely independent of the Na^+ gradient in the range tested, and which is blocked during metabolic inhibition.

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TABLE I

COUPLING BETWEEN α-AMINOISOBUTYRATE AND Na^+ FLUX IN UNINHIBITED AND METABOLICALLY INHIBITED EHRLICH CELLS

Each particular derivative was determined by regression analysis of 16 experimental points.

Extracellular Na^+ (mM) *	$\left(\frac{\partial J_{Na^+}}{\partial J_{AIB}}\right)_{Na^+}$		*Extracellular α-aminoisobutyrate (mM)*	$\left(\frac{\partial J_{AIB}}{\partial J_{Na^+}}\right)_{AIB}$	
	Uninhibited cells	*Inhibited cells*		*Uninhibited cells*	*Inhibited cells*
2.3	0.15 ± 0.01	–	0.96	0.05 ± 0.002	0.10 ± 0.01
16.6	0.21 ± 0.06	0.47 ± 0.06	2.5	0.08 ± 0.01	0.13 ± 0.01
17	0.25 ± 0.05	–	4.7	0.09 ± 0.03	–
31	0.48 ± 0.10	–	5.0	0.05 ± 0.01	–
35	0.40 ± 0.11	0.54 ± 0.15	6.2	0.06 ± 0.01	0.13 ± 0.01
53	0.23 ± 0.22	0.76 ± 0.23	9.1	0.20 ± 0.05	0.19 ± 0.02
67	0.56 ± 0.19	1.32 ± 0.35	14	0.11 ± 0.01	0.16 ± 0.01
70	0.59 ± 0.19	–	18	0.45 ± 0.04	0.36 ± 0.02
92	−0.17 ± 0.30	1.29 ± 0.35			
126	–	1.03 ± 0.45			
133	0.62 ± 0.55	0.91 ± 0.26			

* The difference between the partial derivatives for inhibited and uninhibited cells is statistically significant ($P < 0.01$) for 16.6, 53, 67 and 92 mM Na^+ and for the combined results of the six Na^+ concentrations tested.

The finding that metabolic inhibition increases only the stoichiometric number of the Na^+ flux *versus* the α-aminoisobutyrate flux, leaving the inverse one almost unchanged, may be also of interest in the present context. Even though a final quantitative explanation cannot yet be given, this finding corresponds well with the assumption of a direct (chemiosmotic) coupling between α-aminoisobutyrate transport and metabolism. If such a coupling exists, not only the Na^+-linked component of α-aminoisobutyrate entry but also the metabolically linked one, provided the latter one has not been saturated, will increase upon a rise of extracellular [α-aminoisobutyrate]. During metabolic inhibition, on the other hand, mainly the Na^+-linked α-aminoisobutyrate influx will so react. It should follow that the stoichiometric number of the Na^+ influx *versus* α-aminoisobutyrate influx must be lower with metabolically active than with inhibited cells. The entry of Na^+, however, having no metabolically driven component, will rise equally with increasing extracellular [Na^+], with and without metabolic inhibition. Hence the stoichiometric number of the α-aminoisobutyrate influx with respect to Na^+ influx should not be affected by metabolic inhibition, as has been observed.

The above results strongly argue against the gradient hypothesis, to the extent that it claims that all the energy required for α-aminoisobutyrate transport derives from the electrolyte gradients, in two important points: (1) The maximum efficiency of coupling is far too low to channel sufficient energy from the electrolyte gradients into amino acid transport. (2) The coupling coefficient increases, instead of decreases, with metabolic inhibition, a finding that is in opposition to the gradient hypothesis.

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