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AMINO ACID TRANSPORT AND CELL VOLUME REGULATION IN EHRLICH ASCITES TUMOUR CELLS

BY ELSE K. HOFFMANN AND IAN H. LAMBERT*
From the Institute of Biological Chemistry A, August Krogh Institute, University of Copenhagen, 13 Universitetsparken, DK-2100 Copenhagen Ø, Denmark

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SUMMARY

1. Cellular and extracellular concentrations of amino acids were measured in Ehrlich ascites cells by amino acid analysis and by distribution of radioactive amino acids between cells and medium. Dilution of the medium results in a reduction in the cellular concentration of non-essential amino acids and taurine and an equivalent increase in the extracellular content of these amino acids.

2. The membrane potential and the electrochemical gradient of sodium were measured. The decrease in the cellular to extracellular gradient of taurine and glycine is not a consequence of a decrease in the sodium gradient but is caused by the changes in osmolarity and cell volume.

3. The unidirectional influx and efflux of glycine and taurine were measured as the initial flux of [^{14}C]glycine and [^{35}S]taurine, using a rapid filter technique. For both amino acids the rate constants for influx are decreased and the rate constants for efflux increased following a reduction in osmolarity.

4. The taurine and glycine fluxes were analysed as a simple pump and leak system. Reduction in osmolarity increases the leak permeability to both taurine and glycine.

5. The results for glycine are also discussed in relation to a Na^+–glycine co-transport model, where reduction in osmolarity increases the leak permeability to glycine.

6. The cellular permeability to taurine and glycine increases as a function of increasing cell volume. The taurine permeability increases 7-fold and the glycine permeability 1·5-fold with an increase in cell volume of 30 %. The absolute increase in permeability is equal for both taurine and glycine.

INTRODUCTION

Ehrlich ascites cells regulate their volume in response to transfer to hypotonic media (Hendil & Hoffmann, 1974). Initially, they swell rapidly as nearly perfect osmometers due to the high permeability to water (see Hoffmann, 1978). This phase is followed by a more prolonged volume regulatory phase where the cell volume is reduced, approaching the original volume. It has been shown that this shrinkage is a consequence of a selective loss of KCl, certain amino acids and taurine (Hoffmann, 1978). The role of amino acids in volume regulatory responses has been demonstrated

* The order of names is alphabetical

in many invertebrates (see Gilles, 1979) and several vertebrates (see Hoffmann, 1977). With respect to mammalian cells, the involvement of amino acids has so far only been demonstrated in Ehrlich ascites tumour cells (Hoffmann & Hendil, 1976) and rat kidney cortex (Sell, Rorive, Pequeux & Gilles, 1980).

In Ehrlich cells, it has been demonstrated that the loss of KCl is a consequence of very selective increases in the permeabilities to K^+ and Cl^- followed by a decrease in the permeability to Na^+ (Hoffmann, 1978). The decrease in amino acids was mainly observed with alanine, glycine and taurine (Hoffmann & Hendil, 1976).

We have shown previously that there is an increase in the oxidative catabolism of both alanine and glycine under hypo-osmotic conditions (Lambert & Hoffmann, 1982). In the case of alanine this increased degradation accounts for one third of the decrease in cellular alanine content, while the degradation of glycine and taurine plays no significant role. Neither could incorporation of glycine into protein account for the observed reduction in the glycine pool (Lambert & Hoffmann, 1982). In the present report, we have therefore studied changes in the parameters of taurine and glycine transport after transfer to hypotonic media. Since it is well established that glycine transport in Ehrlich cells is dependent on the sodium gradient (see Philo & Eddy, 1978*b*), we have in the present report examined the effect of reduced ion concentration and decreased osmolarity separately.

METHODS

Cell suspensions

Ehrlich mouse ascites tumour cells (hyperdiploid strain) were maintained by weekly intraperitoneal transplantation in white female Naval Medical Research Institute (NMRI) mice and 8 days after transplantation were harvested and suspended in a standard Ringer solution containing heparin (2·5 i.u./ml.). This solution had the following composition (mM): Na^+, 150; K^+, 5; Mg^{2+}, 1; Ca^{2+}, 1; Cl^-, 150, sulphate, 1; inorganic phosphate, 1; morpholinopropane sulphonic acid (MOPS), 3·3; *N*-tris-(hydroxymethyl) methyl-2-amino-ethane sulphonic acid (TES), 3·3; and *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES), 5; pH 7·40 (See Eagle, 1971). The cells were washed by centrifugation (45 sec, 700 *g*) with the standard Ringer solution and once with the appropriate experimental Ringer solution (see below). The cytocrit was adjusted to 4 % for pool determination and influx experiments and to 8 % in the efflux experiments (see below).

Experimental Ringer solutions

Three different types of media were used: (*a*) *standard Ringer* solution, 300 m-osmole (see above); (*b*) standard Ringer solutions diluted with distilled water in order to lower both ion concentration and total osmolarity, i.e. *hypotonic 225 m-osmole* and *hypotonic 150 m-osmole*; (*c*) Ringer solutions identical to (*b*) but with addition of sucrose to make the solutions isotonic to (*a*) i.e. *isotonic 225 m-osmole + sucrose* (11·75 g/l.) and *isotonic 150 m-osmole + sucrose* (22·5 g/l.). In the isotonic media the mean cell volume, measured by a Coulter Counter model z_B with a Coulter Channellyzer^R (C-1000), did not differ significantly from the volume in standard Ringer (Lambert & Hoffmann, 1982). The isotonic medium is not isosmotic to the standard Ringer solution. The added amount of sucrose corresponds to half the amount which would make the corresponding hypotonic Ringer isosmotic to the standard Ringer.

Measurements of cellular and extracellular concentrations of amino acids and ions

Na^+, K^+ and Cl^- were determined as described by Hoffmann, Simonsen & Sjøholm (1979).

For determination of the cellular amino acid pool, 1·5 ml. cell suspension was centrifuged (12,000 *g*, 60 sec) in preweighed vials, the supernatant removed and the sample reweighed. The packed cells were lysed in 1 ml. distilled water and deproteinized by addition of 5·0 ml. 1 % (w/v) picric acid. The precipitate was separated from the suspension fluid by centrifugation (12,000 *g*, 10 min). For determination of the extracellular amino acid pool 4·5 ml. cell suspension was

centrifuged (12,000 *g*, 60 sec); 3 ml. of the supernatant was diluted with 1 ml. distilled water, and deproteinized as described above. Excess picric acid was removed on resin beads (Dowex 2 × 8, 200–400 mesh) in a 1 cm × 4 cm column. Amino acid analyses were carried out on an automatic amino acid analyser (Durram D-500).

The cellular concentrations are given as the concentration in cell water after correction for trapped volume ([^{3}H]inulin space) in the cell pellet as described by Hoffmann *et al.* (1979).

Measurements of the membrane potential

Membrane potential was estimated by the valinomycin 'null point' method with 3,3-dipropyloxadicarbocyanine as described by Philo & Eddy (1978*a*). The fluorescence was measured in an Aminco Bowman spectrophotofluorometer (American Instrument Company Inc.). A linear calibration curve, showing the fluorescence intensity at the 'null point' as a function of the corresponding membrane potential, was constructed. The membrane potential was varied from −41·5 mV to −56 mV using ½, 5 and 10 min pre-incubation in the standard Ringer before addition of the dyes, and from −33 mV to −35 mV by 10 min pre-incubation in standard Ringer containing glucose (10 mM) or ouabain (0·3 mM) before addition of the dyes. From this standard curve we derived a membrane potential of −36 mV for Ehrlich cells in standard Ringer at 38 °C.

Amino acid flux experiments

Influx and efflux of amino acids were measured as the initial transport rates. In influx experiments [^{14}C]glycine or [^{35}S]taurine (Radiochemical Centre, Amersham) were added at known concentration and specific activity at time zero. Removal of radioactivity was then followed with time by serially isolating cell-free medium by rapid filtration of the cell suspension as described by Hoffmann *et al.* (1979). The uptake curves were linear within the first 2 min, and we used at least five points taken within this period to estimate the initial rate and the time-zero value by linear regression fit. As a control the time-zero value was also measured in Ringer solution containing the same concentration of radioactivity as the cell suspension. The value was calculated after correction for the cellular volume. By this method it was found in six experiments that the two time-zero values deviated 2·0 ± 0·4 % (S.E. of mean) and we concluded that the measured rate was the initial rate.

In efflux experiments the cell suspensions were equilibrated with [^{14}C]glycine (1·0 μCi/ml.) or [^{35}S]taurine (0·2 μCi/ml.) at the experimental temperature for a period longer than five half-times of cellular amino acid exchange. At the end of the pre-incubation period duplicate samples of the suspension were taken for determination of ion concentration, cell water, dry weight and cellular concentration of [^{14}C]glycine and [^{35}S]taurine (see above). Transfer of labelled cells to efflux medium and isolation of cell-free medium by filtration were carried out as described by Hoffmann *et al.* (1979). At the end of the efflux experiments triplicate samples of the efflux suspension were used for determination of protein using bovine serum albumin as a standard (Lowry, Rosebrough, Farr & Randall, 1951). The amount of protein was converted to amount of dry weight using a protein/dry weight ratio of 0·78 ± 0·02 (S.E. of mean), measured in seven independent experiments.

The ^{14}C, ^{35}S and the ^{3}H activities were measured in a liquid scintillation spectrometer (Packard Tri-Carb^R 460 CD), using a 300 μl. diluted sample in 2·5 ml. Picofluor TM-15 (Packard).

Calculations of rate constants and permeabilities

The unidirectional influx (J^{oi}) was calculated as follows:

$$J^{oi} = k_e \cdot [H_2O]_e \cdot [a.a.]_e \quad (\mu\text{mole/g dry wt.min}) \tag{1}$$

where $[H_2O]_e$ is the extracellular water content (g medium/g cell dry wt.), $[a.a.]_e$ is the extracellular amino acid concentration (μmole/g medium), and k_e is the rate constant for extracellular exchange (min^{-1}) (see Fig. 2).

The unidirectional efflux (J^{io}) was:

$$J^{io} = k_c \cdot [H_2O]_c \cdot [a.a.]_c \quad (\mu\text{mole/g dry wt.min}) \tag{2}$$

where $[H_2O]_c$ is the cell water content (g/g cell dry wt.), $[a.a.]_c$ is the amino acid concentration in the cell water (μmole/g cell water) and k_c is the rate constant for intracellular exchange (min^{-1}) (see Fig. 2).

Since k_e is dependent on the cytocrit of the cell suspension, whereas $k_e \, . \, [H_2O]_e$ is a constant, we use the rate constants:

$$k'_e = k_e \, . \, [H_2O]_e \quad (\text{min}^{-1} \text{ g medium g dry wt.}^{-1}) \tag{3}$$

$$k'_c = k_c \, . \, [H_2O]_c \quad (\text{min}^{-1} \text{ g cell water g dry wt.}^{-1}) \tag{4}$$

These two rate constants are the unidirectional fluxes divided by the concentration in the respective compartments.

The taurine efflux (J^{io}_{tau}) was regarded as a passive leak flux (Kromphardt, 1963). Assuming that 4·4 % of the taurine leaves the cell as an anion and 95·6 % as a neutral molecule (at pH 7·4) the apparent permeability (P_{tau}) was calculated from the measured efflux, according to the equation:

$$J^{io}_{tau} = P_{tau} \, . \, \frac{-EF/RT}{1-e^{EF/RT}} \, . \, 0{\cdot}04 \, . \, [\text{tau}]_c + P_{tau} \, . \, 0{\cdot}96 \, . \, [\text{tau}]_c, \tag{5}$$

where R, F, T, and E are defined as usual and $[\text{tau}]_c$ is the cellular taurine concentration.

The glycine efflux (J^{io}_{gly}) was also treated as a passive leak flux, and the apparent permeability was calculated under the assumption that glycine leaves the cell as a neutral molecule, i.e.:

$$J^{io}_{gly} = P_{gly} \, . \, [\text{gly}]_c, \tag{6}$$

where $[\text{gly}]_c$ is the cellular glycine concentration. In order to express the permeabilities in cm/sec we converted the fluxes from units of μmole/g dry wt. × min to p-mole/cm^2 × sec by multiplication by the factor 0·818 (see Hoffmann *et al.* 1979). This means that the permeabilities are proportional to the rate constants for electroneutral molecules like glycine.

Statistical evaluation

All values are given as the mean ±S.E. of the mean. Student's *t*-test was used to evaluate statistical significance.

RESULTS

Dilution of the medium reduces the intracellular concentrations of non-essential amino acids and taurine.

Table 1 shows the results for cells in a standard Ringer solution and a solution diluted to half the osmolarity and ionic strength. It is seen that there is a significant decrease in the concentration of taurine, aspartic acid, glutamic acid, proline, glycine and alanine in agreement with previous findings, where only osmolarity was changed (Hoffmann & Hendil, 1976). The absolute values at 300 m-osmole are different from the ones reported by Hoffmann & Hendil (1976), caused by differences in solutions and procedure.

The loss of intracellular taurine and amino acids in hypotonic suspensions is reflected by a significant increase in the extracellular concentrations of taurine, aspartic acid, glutamic acid and glycine (Table 1). Results for cells in a solution diluted to three quarters of the osmolarity and ionic strength of the standard Ringer (not shown) confirm these findings.

In three paired experiments (included in Table 1) the loss of cellular amino acids and taurine has been compared to the increase in extracellular amino acids and taurine. The cellular loss amounted to 124, 5·0 and 3·4 μmole/g dry wt., for taurine, aspartic acid and glutamic acid respectively, and the corresponding increases in the medium were 91, 4·9 and 2·4 μmole/g dry wt. Thus, the increase in the amount of these amino acids in the medium was practically equivalent to the loss from the cellular pool. With respect to glycine the increase in the medium exceeded the loss in cellular amount.

TABLE 1. Cellular and extracellular concentrations of amino acids and taurine in Ehrlich ascites cells suspended in standard Ringer solution (300 m-osmol) or hypotonic solution (150 m-osmol). Values are ± S.E. of the mean with the number (n) of independent experiments indicated. The samples were taken 40 min after transfer to the experimental solution, at which time the cells had reached a new steady state. P is the level of significance in Student's t test relative to the 300 m-osmole values

	m-Mole per litre cell water		m-Mole per litre medium	
	300 m-osmole $n = 6$	150 m-osmole $n = 6$	300 m-osmole $n = 3$	150 m-osmole $n = 3$
Taurine	53.0 ± 2.8	6.9 ± 0.2 $P < 0.0005$	0.07 ± 0.05	1.04 ± 0.3 $P < 0.025$
Aspartic acid	1.8 ± 0.2	0.6 ± 0.06 $P < 0.0005$	0.02 ± 0.003	0.09 ± 0.02 $P < 0.025$
Glutamic acid	1.2 ± 0.09	0.3 ± 0.02 $P < 0.0005$	0.01 ± 0.002	0.04 ± 0.005 $P < 0.005$
Proline	2.1 ± 0.03	1.2 ± 0.2 $P < 0.05$	0.03 ± 0.007	0.04 ± 0.01 $P > 0.10$
Glycine	9.1 ± 0.5	6.2 ± 0.9 $P < 0.05$	0.17 ± 0.02	0.45 ± 0.10 $P < 0.025$
Alanine	1.3 ± 0.3	0.4 ± 0.08 $P < 0.01$	< 0.01	< 0.01 $P \gg 0.10$

A change in osmolarity alone, with the ionic concentration constant, gave similar results. If the medium was changed from one with 150 m-osmole ions plus sucrose with total osmolarity 300 m-osmol to one with 150 m-osmole ions without sucrose, the decrease in cellular taurine was 98 ± 15 μmole/g dry wt. and the increase in extracellular taurine was 105 ± 28 μmole/g dry wt. Thus the increase in the medium can account for the total decrease in the cellular pool. It is therefore likely that the decrease in concentration in hypotonic media is achieved by a change in amino acid and taurine transport parameters.

The results obtained for amino acid analysis in Table 1 are confirmed in Fig. 1, which shows the gradients for taurine and glycine in 300, 225 and 150 m-osmole media as measured from the steady-state distribution for [^{35}S]taurine and [^{14}C]glycine. The cellular to extracellular concentration gradient for taurine and glycine therefore have steady-state values which are dependent on the osmolarity and ionic concentration.

The decrease in the cellular to extracellular gradient for taurine and glycine is not secondary to a decrease in the sodium gradient. Table 2 gives the chemical gradient for glycine as well as the electrochemical gradient for taurine and sodium at different osmolarities. Assuming a coupling ratio of 1 : 1 the electrochemical gradient of sodium under the present experimental conditions is just sufficient to account for the glycine gradient if the membrane potential is -36 mV as measured with the fluorescence method, but not if it is equal to the Nernst potential for Cl^-. On the other hand the gradient for taurine is significantly higher than the sodium gradient in isotonic medium. The sodium gradient is unchanged under the three experimental conditions whereas the taurine and glycine gradients are significantly decreased in diluted media.

In order to see whether this decrease is caused by the change in osmolarity and volume or by the decrease in ionic concentration, we have compared hypotonic media with their isotonic controls having the same ionic concentration. Table 3 shows the

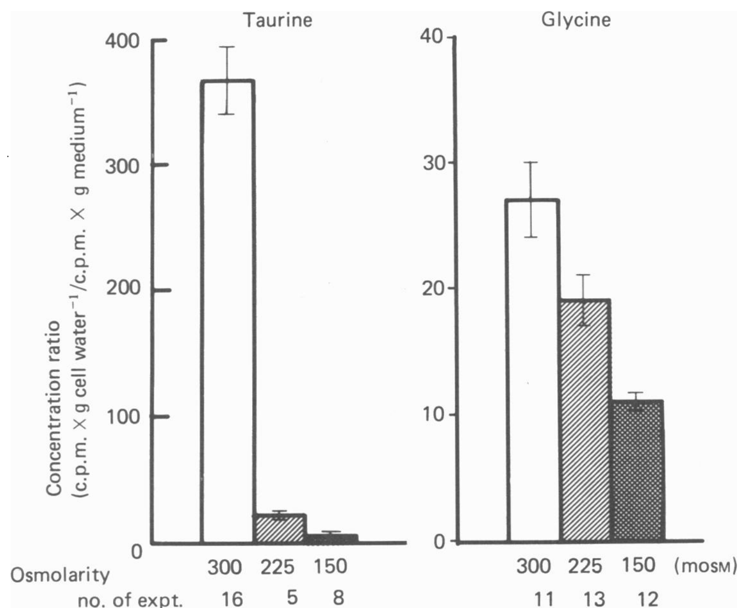

Fig. 1. Taurine and glycine gradients in Ehrlich ascites cells suspended in standard Ringer (300 m-osmole) or hypotonic solutions (225 m-osmole; 150 m-osmole). The gradients are measured as the steady-state gradients for [^{35}S]taurine (2·5 μM; 0·2 μCi/ml.) and [^{14}C]glycine (9·5 μM; 1·0 μCi/ml.) after 30–110 min incubation. Values are mean ±S.E. of mean.

TABLE 2. The chemical gradient of glycine and the electrochemical gradients of taurine and sodium in Ehrlich ascites cells suspended in solutions of different osmolarities. The symbols $\tilde{\mu}$ and μ are the electrochemical and chemical potential respectively. As regards taurine 4·4 % is treated as an anion and 95·6 % as a neutral molecule (pH = 7·4). The gradients are measured as the steady-state gradient of [^{35}S]taurine and [^{14}C]glycine as described in the legend to Fig. 1. Values are mean ±S.E. of the mean; n is the number of independent experiments and P is the level of significance in Student's t test against the 300 m-osmol values

	$(\tilde{\mu}_c - \tilde{\mu}_e)$ tau (kJ/mole)	$(\mu_c - \mu_e)$ gly (kJ/mole)	*$(\tilde{\mu}_c - \tilde{\mu}_e)$ Na^+ (kJ/mole)	†$(\tilde{\mu}_c - \tilde{\mu}_e)$ Na^+ (kJ/mole)
300 m-osmole	15·2 ± 0·2	8·3 ± 0·3	−6·8 ± 0·1	−8·2 ± 0·1
n	16	11	5	5
225 m-osmole	7·9 ± 0·4	7·4 ± 0·3	−7·7 ± 0·2	−8·8 ± 0·2
n	5	13	4	4
P	< 0·0005	< 0·025	< 0·005	> 0·05
150 m-osmol	2·8 ± 0·2	6·1 ± 0·2	−6·8 ± 0·2	−8·6 ± 0·2
n	8	12	4	4
P	< 0·0005	< 0·0005	—	> 0·05

* Membrane potential calculated as the Cl^--Nernst potential.
† Membrane potential measured with the fluorescence method.

gradients of taurine, glycine and sodium in hypotonic media relative to the gradients in their isotonic controls for a series of paired experiments. Taurine and glycine gradients were measured by both isotope distribution and amino acid analysis. There is a significant decrease in taurine and glycine gradients caused by a decrease in osmolarity or increase in volume, while the sodium gradient is unchanged or increased.

TABLE 3. Changes in volume, the taurine and glycine gradients and the sodium electrochemical gradient as a consequence of a decrease in osmolarity. Taurine and glycine gradients are calculated either from isotope distribution (A) or from amino acid analysis (B). Values are relative to the values in the isotonic controls with the same ionic concentration. The media are made isotonic by addition of sucrose. n is the number of paired experiments, P is the value of significance in Student's t test where the values are tested against the hypothetical value 1. Values are given $\pm$S.E. of the mean

	$(\tilde{\mu}_c - \tilde{\mu}_e)$ tau (relative value)		$(\mu_c - \mu_e)$ gly (relative value)		$(\tilde{\mu}_c - \tilde{\mu}_e)$ Na^+ (relative value)	Volume (relative value)
	A	B	A	B		
225 m-osmole	0·58 ± 0·004	0·63 ± 0·02	0·96 ± 0·02	0·91 ± 0·01	1·13 ± 0·06	1·15 ± 0·02
n	6	2	10	2	4	17
P	$\ll$ 0·0005	< 0·025	< 0·01	< 0·025	< 0·10	$\ll$ 0·0005
150 m-osmole	0·29 ± 0·02	0·31 ± 0·05	0·86 ± 0·02	0·84 ± 0·02	1·09 ± 0·01	1·26 ± 0·02
n	8	3	11	3	3	21
P	$\ll$ 0·0005	< 0·005	$\ll$ 0·0005	< 0·01	< 0·025	$\ll$ 0·0005

Effect of osmolarity on taurine and glycine fluxes

The unidirectional influx and the unidirectional efflux of glycine and taurine were measured as the initial flux of [^{14}C]glycine and [^{35}S]taurine as described in the Methods. Fig. 2 shows a typical experiment with glycine in isotonic 225 m-osmole + sucrose (●) and hypotonic 225 m-osmole (○) media. It is seen that hypotonic conditions decrease the rate constant of the glycine influx and increase the rate constant of the glycine efflux. The corresponding k'_e and k'_c values calculated from

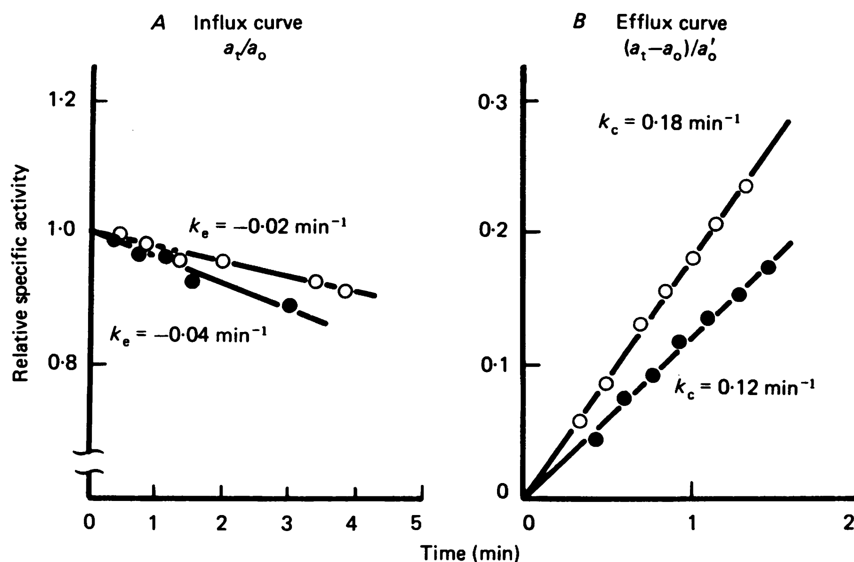

Fig. 2. [^{14}C]glycine influx and efflux curves in a hypotonic solution (○, 225 m-osmole ions) and its isotonic control with the same ionic concentration (●, 225 m-osmole ions plus sucrose). The relative specific activities are followed with time. a_t and a_0 are the specific activities at time t and time zero in the medium and a'_0 is the activity at zero time in the cellular amino acid pool. The slopes k_e and k_c are the rate constants for extracellular and intracellular exchange respectively. The corresponding k'_e and k'_c are 2·7 and 0·7 for the hypotonic solutions and 4·4 and 0·4 for the isotonic solutions respectively.

TABLE 4. Effect of medium ionic concentration and osmolarity on the rate constants k'_e of glycine influx and k'_c of glycine efflux. The media contained 1 mM-glycine in both types of experiments. The unidirectional influx and efflux can be calculated from k'_e and k'_c by multiplying by the extracellular and intracellular glycine concentration, respectively (see Methods). Values are given ±S.E. of the mean, with the number of independent experiments in parentheses

	k'_e (min^{-1} g medium g dry wt.$^{-1}$)	k'_c (min^{-1} g cell water g dry wt.$^{-1}$)
1. Isotonic 300 m-osmole	8·3 ± 1·5 (6)	0·48 ± 0·05 (9)
2. Isotonic 225 m-osmole ions + sucrose	8·1 ± 2·2 (4)	0·43 ± 0·06 (6)
3. Hypotonic 225 m-osmole	6·2 ± 1·3 (4)	0·52 ± 0·07 (7)
4. Isotonic 150 m-osmole ions + sucrose	6·8 ± 0·9 (4)	0·46 ± 0·06 (7)
5. Hypotonic 150 m-osmole	5·8 ± 0·9 (4)	0·71 ± 0·04 (10)

eqns. (3) and (4) are summarized in Table 4 for different concentrations and osmolarities. Dilution of the medium seems to reduce the rate constant of glycine influx (not significantly at the 5 % level), while dilution of the medium to 150 m-osmole increases the rate constant of glycine efflux significantly ($P < 0·005$). In a series of 'zero-trans' efflux experiments i.e. with no glycine in the efflux medium, we found the k'_c values to be 0·26 ± 0·01, 0·38 ± 0·007 and 0·74 ± 0·08 in 300, 225 and 150 m-osmole media, respectively. The increase in rate constants was significant ($P < 0·005$), after dilution to 225 m-osmole as well as to 150 m-osmole.

The change in rate constants in half-diluted media results mainly from a change in osmolarity (compare line 4 and 5 in Table 4) rather than from the change in ionic concentration (compare lines 1 and 4). The rate constant for glycine efflux, with osmolarity decreased to 150 m-osmole was increased significantly ($P < 0·005$) when compared to the isotonic control solution.

The same results are found with taurine. Table 5 summarizes the results for taurine influx and efflux at different ionic concentrations and osmolarities. It is seen that dilution of the medium reduces the rate constant of the taurine influx significantly ($P < 0·01$) and increases the rate constant of the taurine efflux ($P < 0·01$) (compare lines 1, 3 and 5 in Table 5). This change in flux parameters seems to result mainly from the change in osmolarity (compare line 2 with line 3 and line 4 with line 5) rather than from the change in ionic concentration (compare line 1 with lines 2 and 4). The rate constant of the taurine efflux was increased significantly ($P < 0·01$) with decreased osmolarity in both 225 and 150 m-osmole solutions when compared to their isotonic controls.

The apparent permeabilities for glycine and taurine, calculated from the effluxes, are shown as function of the cell volume in Fig. 3. As indicated in the Methods, the apparent permeability for an uncharged molecule like glycine is proportional to the rate constant k'_c in Table 4. The apparent mean permeabilities for glycine in Fig. 3*B* (●) are based on the steady-state efflux with 1 mM-extracellular glycine, while the apparent permeabilities in 'zero-trans' experiments with no extracellular glycine are

TABLE 5. Effect of medium ionic concentration and osmolarity on the rate constants k'_e and k'_c of taurine influx and taurine efflux respectively. 10 μM-taurine was added to the exteral medium in both type of experiments. From k'_e and k'_c the unidirectional influx and efflux can be calculated by multiplying by the extracellular and intracellular taurine concentrations respectively. Values are given ±S.E. of the mean, with the number of independent experiments indicated in parentheses

	k'_e (min^{-1} g medium g dry wt.$^{-1}$)	k'_c (min^{-1} g cell water g dry wt.$^{-1}$)
1. Isotonic 300 mOsm	7·9±0·9 (6)	0·052±0·009 (8)
2. Isotonic 225 mOsm ions + sucrose	6·1±0·5 (3)	0·09±0·01 (5)
3. Hypotonic 225 mOsm	5·6±1·5 (3)	0·28±0·05 (5)
4. Isotonic 150 mOsm + sucrose	5·9±2·2 (3)	0·11±0·03 (5)
5. Hypotonic 150 mOsm	2·3±0·8 (4)	0·38±0·04 (5)

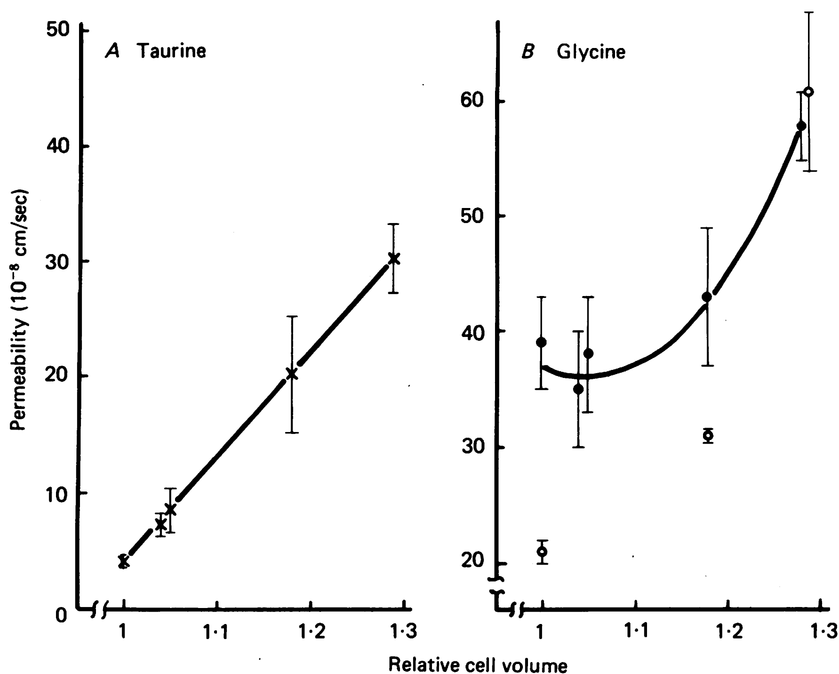

Fig. 3. The apparent permeabilities to taurine and glycine as a function of cell volume. The apparent permeabilities are calculated from [^{35}S]taurine and [^{14}C]glycine efflux as described in the Methods. Values are given ±S.E. of mean. The media contained 10 μM-taurine (×), 1 mM-glycine (●) or 0 glycine (○). Cell volume is given relative to the cell volume measured in a standard Ringer solution.

indicated as (○) in the Figure. It is seen that an increase in cell volume of 30 % causes the apparent permeability for taurine and glycine to increase to 30×10^{-8} and 58×10^{-8} cm/sec respectively, giving a 7-fold increase of the taurine permeability and a 1·5-fold increase of the glycine permeability.

DISCUSSION

The results presented here confirm the previously published finding that volume regulation in Ehrlich ascites tumour cells is accomplished in part by the regulation in the concentration of small non-essential amino acids and taurine (Hoffmann & Hendil, 1976). This is in agreement with findings in invertebrates and vertebrates examined so far (for a review see Gilles, 1979).

Three different possibilities for the decrease in the amino acid pool can be considered: (i) a change in the steady state between synthesis and degradation of the amino acids, (ii) modification of the steady state between the amino acid pool and the proteins, (iii) changes in the parameters of the amino acid transport through the cell membrane. We have previously shown that there is an increase in the oxidative catabolism of both alanine and glycine under hypo-osmotic conditions (Lambert & Hoffmann, 1982). With respect to alanine this increased degradation accounts for 33 % of the decrease in cellular alanine content, while degradation of glycine and taurine plays no significant role. Neither does a change in the protein turnover seem to be involved in the observed reduction in the amino acid pool (Lambert & Hoffmann, 1982).

Table 1 shows that the amino acids are released from the cells into the medium during the volume regulatory decrease. With most of the amino acids the increase in the medium was found to be practically equivalent to the loss from the cellular pool. This indicates that the decrease in the intracellular concentrations caused by transfer to hypotonic conditions is due to a change in amino acid and taurine transport parameters. Hendil & Hoffmann (1974) have previously shown that ninhydrin-positive substances are released during volume regulation from the Ehrlich cells. This has also been found in other systems (see Gilles, 1979). Leakage of taurine into diluted media has been demonstrated in the perfused flounder heart (Vislie, 1980) and in flounder erythrocytes (Fugelli, 1970). As regards taurine, Vislie (1980) has demonstrated that the increase in medium concentration accounted quantitatively for the cellular loss when flounder hearts were exposed to hypotonic media. Measurements of the increase in amounts of individual amino acids in the surrounding medium has been carried out in only a few systems, and an identity between the increase in the medium and the loss from the cellular pool with respect to the individual amino acids has, to our knowledge, not been demonstrated previously.

The cellular to extracellular concentration gradient of taurine and glycine are diminished in diluted media. This is shown both by amino acid analysis (Table 1) and by isotopic distribution (Fig. 1 and Table 2). By comparing Tables 2 and 3 it can be concluded that the reduced osmolarity is responsible for the diminished gradient for glycine and taurine and not the decrease in extracellular ionic concentrations. It is most likely that it is the resulting changes in cell volume which are responsible for

these changes. Fugelli & Rohrs (1980) have presented similar results regarding taurine distribution in flounder erythrocytes.

As shown in Tables 2 and 3 there is no correlation between the change in the electrochemical gradient of sodium and the gradients of glycine and taurine. If the sodium gradient is the driving force for the active transport of glycine and taurine, then the change in osmolarity or volume must have changed some of the other parameters which define the resulting gradient. These could be the leak permeability, the number of or the affinity of the transport sites.

From Table 2 it would appear that the sodium gradient when calculated from the membrane potential measured by the fluorescent method will provide sufficient energy. If however we use the membrane potential estimated from the chloride distribution, the sodium gradients are insufficient. It has been shown previously that the distribution of Cl^-, under many circumstances, is unsuitable for an estimation of the membrane potential (Heinz, Geck, Pietrzyk, Burckhardt & Pfeiffer, 1977). In Ehrlich cells this is mainly because of the anion–cation co-transport system which can lead to an accumulation of chloride in the cells (Geck, Pietrzyk, Burckhardt, Pfeiffer & Heinz, 1980; Hoffmann, Sjøholm & Simonsen, 1981). It is therefore likely that the chloride distribution underestimates the potential. The result in the present work is then in agreement with the generally accepted view that the active glycine pump is driven by the sodium electrochemical gradient (Heinz, Geck & Pfeiffer, 1980; Eddy, 1981).

In the case of taurine the sodium gradient in Table 2 would provide sufficient energy for taurine transport only if we assumed that two sodium ions were coupled to one taurine molecule, but the sodium dependence of taurine transport in Ehrlich ascites cells has not been investigated.

From Tables 4 and 5 we concluded that dilution of the medium reduced the rate constant of glycine and taurine influx and increased the rate constant for glycine and taurine efflux. In the flux experiment it was found that the increase was a result of decreased osmolarity or increased volume and not a result of the change in extracellular ion concentration. We have calculated the apparent permeabilities to glycine and taurine from the efflux data, assuming the fluxes to be pure passive leak fluxes (see Methods). Fig. 3 shows that the permeabilities to glycine and taurine increase with increasing cell volume. It is noted that the apparent permeability to glycine in control media is higher in a steady-state experiment with 1 mM-extracellular glycine than in a 'zero-trans' experiment. This might be caused by an exchange of glycine through the carrier system in the steady-state experiment which does not take place at zero extracellular glycine concentration. The difference between the glycine permeabilities is smaller in the swollen cells where the passive leak dominates.

We have tried to fit our glycine flux data to a model proposed by Philo & Eddy (1978*a*) in which a sodium coupled glycine carrier transport operates in parallel with a diffusional glycine flux. Based on this analysis we obtained a passive leak permeability of 10×10^{-8} cm/sec in standard Ringer and a 4-fold increase with an increase in cell volume of 30 %. The relative increase in the passive leak permeability with cell volume is thus greater than the relative increase in the apparent permeability reported in Fig. 3. This can be explained by the fact that the permeabilities in Fig. 3

also include the flux through the co-transport system which apparently does not increase with volume.

A similar increase in apparent permeability with cell volume has previously been demonstrated for potassium and chloride efflux, whereas sodium permeability has been found to decrease under the same conditions (Hoffmann, 1978).

It is therefore obvious that the outward movement of glycine and taurine during hypo-osmotic stress is partly controlled by an inhibition of the active influx and partly by an increase in leakage. The main factor seems to be the selective increase in the passive permeability to both glycine and taurine.

Changes in passive fluxes appear to play an important role in cell volume regulation. In Ehrlich cells potassium permeability is probably increased by an increase in cytoplasmic free calcium ions, triggered by cell swelling (Hoffmann, 1982). An interaction between taurine and calcium fluxes has been demonstrated in rat brain synaptosomes (Remtulla, Katz & Applegarth, 1979), and it is possible that calcium is also involved in the changes in permeabilities reported here. This question however awaits further investigation.

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