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GLYCINE TRANSPORT BY PIGEON RED CELLS: CALCULATION OF GLYCINE ACCUMULATION RATIOS BY NUMERICAL INTEGRATION OF ENTRY AND EXIT RATE EQUATIONS

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SUMMARY

The rate equations integrated, obtained from previous work, describe Na^+-dependent and independent movement both into and out of hemolysed-and-restored cells, as well as Na^+ entry and exit. The calculated glycine accumulation ratios (both greater and less than unity) were compared with previously measured values.

Active accumulation and expulsion of glycine could be approximately accounted for by a process for which the Na^+ electrochemical gradient furnishes the energy, but discrepancies exist which could be due to an additional energy source or to inexact or inappropriate parameters or assumptions for the rate equations.

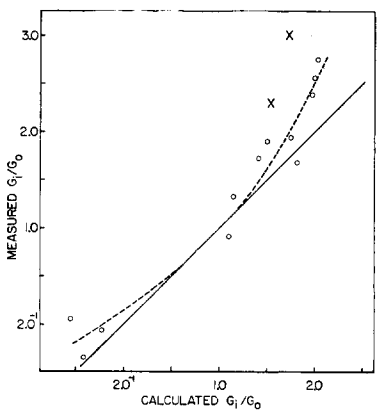
Previous work[1] had yielded rate equations describing Na^+-dependent glycine movement in both directions across membranes of hemolysed and restored pigeon red cells as functions of Na^+ and glycine concentrations on both sides of the membrane. The equations describe a model for which the Na^+ electrochemical activity gradient is the sole energy source for glycine active transport. Earlier, glycine accumulation ratios (G_i/G_o) in hemolysed and restored cells had been determined as functions of Na^+ and glycine concentrations on both sides of the membrane[2]. The rate equations have now been integrated and the calculated accumulation ratios compared with the observed. For reasons discussed elsewhere[1] concentration will be taken to approximate electrochemical activity.

Numerical integration was done with a very small computer (DAC-512, Picker Nuclear). Initial values for internal and external glycine and Na^+ concentrations were iteratively incremented or decremented by amounts due to Na^+-dependent glycine entry (Eqn. 6c of ref. 1), Na^+-dependent glycine exit (Eqn. 5c of ref. 1), Na^+-independent diffusion-like glycine movement ($\delta G/\delta t = k_G[G_o - G_i]$), glycine-dependent Na^+ movement ($2 \times$ [Na^+-dependent glycine movement]), and glycine-independent diffusion-like Na^+ movement ($\delta Na^+/\delta t = k_{Na}\,[Na_o^+ - Na_i^+]$). The summed increments were for 2.5-min time intervals. (Use of 1-min intervals changed calculated ratios by less than 1.5%.) The necessary numerical values were derived from published data and were: $1/E_t k_3 = 0.5888$ ml cell water/μmole (data from refs. 1–3); $K_1K_2K_{G_o} = K_1K_2K_{G_i} = 3407$ mM[3], $K_{G_o} = 2.047$ mM, $K_{G_i} = 1.381$ mM, $\sigma = 0.178$, $\alpha_o = 0.4122$, $\rho_\alpha = 1$, $\gamma_o = \gamma_i = \beta_o = \beta_i = 0$ (from ref. 1); $k_G = 0.019225$/ml

cell water per 2.5 min., $k_{Na} = 0.024275$/ml cell water per 2.5 min. (data from refs. 2 and 4).

Calculated glycine accumulation ratios are shown plotted against the measured values in Fig. 1. (The measured values are from Table III of ref. 2 and 40-min values interpolated from Fig. 1 of ref. 2.) For the best possible agreement between calculated and observed values, the points would be symmetrically scattered around the 45° line. (Since average values for $E_t k_3$, k_G, and k_{Na} were used for calculation, the points could not fall exactly on the line. Values of v_{max} especially, from which $E_t k_3$ is derived, vary from experiment to experiment.) There is a strong correlation between calculated and observed values. On the average the calculated 40-min accumulaton ratios are 85% of the observed ($1/n \Sigma[(G_i/G_o)_{calc}/(G_i/G_o)_{obs}] = 0.85$; range, 0.74–1.08) while, on the average, calculated expulsion ratios (G_o/G_i) are 115% (range, 103–132%) of the observed. However, it is evident that considerable discrepancies exist. The 40-min values (Fig. 1; ○) trend away from the 45° line and the 100 min values (Fig. 1; ×) are still farther away. In Fig. 2 are shown data points from Fig. 1 of ref. 2 for glycine uptake as a function of time together with the calculated curves. The upper curve corresponding to ■ points was for initial external and internal Na^+ concentrations of 40 and 0 mM, respectively, while the next lower curve corresponds to ● points with initial external and internal Na^+ concentrations of 140 and 126 mM, respectively. Because of the known variability of v_{max}, the quantitative discrepancy between the upper curve and points means little, but for the lower curve the discrepancy is clear; some accumulation occurs where a slight loss is calculated. Moreover, reducing the $1/E_t k_3$ value for the calculation does not produce an accumulation stage, *i.e.* the Na^+ gradient should be too small to increase the glycine accumulation ratio above the starting value.

The common feature of the discrepancies is that cells show a greater ability to accumulate glycine and a smaller ability to expel it than the equations predict. This appears most strongly when conditions for accumulation are marginal, as in the



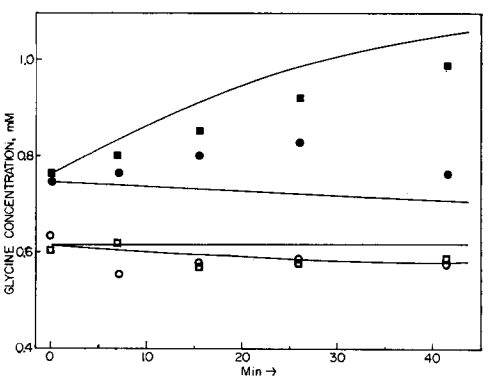


Fig. 1. Observed values of glycine accumulation ratios plotted against calculated values. ○, 40-min incubation points; ×, 100-min incubation points.

Fig. 2. Pellet and medium glycine concentrations plotted against time. Initial external Na^+ = 40 mM, internal Na^+ = 0 mM indicated by: ■, pellets; □, medium. Initial external Na^+ = 140 mM, internal Na^+ = 126 mM indicated by: ●, pellets; ○, medium. Calculated curves in order from the top down correspond to ■, ●, ○, □.

lower curve of Fig. 2 and the 100 min-points (×) of Fig. 1. For the latter the calculated Na^+ gradient is extensively flattened but must maintain a pre-established glycine gradient (*e.g.* for 1 b, Table III, ref. 2: initial $Na_0^+ = 140$ mM, $Na_i^+ = 24$ mM; calculated 100-min $Na_0^+ = 135$ mM, $Na_i^+ = 95$ mM).

Although the discrepancies have been emphasized, the agreement should not be understated. The data points in Fig. 1 cover a wide range of initial conditions with large and small Na^+ gradients at both high and low Na^+ concentrations[2]. The 40-min point discrepancies are around 15%. In the lower curve of Fig. 2 the net uptake at 26.5 min. is 0.084 μmole/ml instead of a calculated net loss of 0.026 μmoles/ml. The discrepancy is roughly 17% of the total calculated glycine entry flux.

There are three kinds of explanations for the discrepancies. As pointed out previously[1], most of the evidence that the Na^+ gradient furnishes energy for glycine active transport by hemolysed and restored pigeon red cells says only that much of the energy comes from the Na^+ gradient, not that all of it does. Only one piece of evidence indicated that all did, the equivalence of entry and exit rates when $Na_0^+ = Na_i^+$ and $G_0 = G_1$. The present report does not change this conclusion. (Note that the curve in Fig. 1 appears to go through the 1:1 intersection.) Recently, there have been several observations[5–7] of amino acid accumulation by Na^+-dependent routes where accumulation apparently could not be wholly accounted for by energy from cation gradients. (For Ehrlich ascites cells, K^+ as well as Na^+ gradients apparently act in glycine[8,9] and α-aminoisobutyric acid[6] accumulation.) In a report on methionine accumulation by Ehrlich ascites cells[7], the lack of effect of trans-Na^+ and the strong effect of cell ATP were opposite to the effects observed on glycine accumulation by pigeon red cells[2,4] and Ehrlich ascites cells[10,11], so this case will be taken as an example of a different system, of interest in its own right, but not directly relevant to the present system. JACQUZ AND SCHAFER[6] on the other hand, studying α-aminoisobutyric acid uptake by Ehrlich ascites cells, which behaves like a cation-energized process, found that the apparent efficiency of utilization of cation gradient energy could reach 100% or perhaps slightly more. This was higher than could be expected. WHEELER AND CHRISTENSEN[5] working with rabbit reticulocytes reported that at an apparently unfavorable Na^+ gradient with low but not zero Na^+ concentrations, significant alanine accumulation occurred. These experiments used intact cells with which there is a problem of cation compartmentalization or binding. However, as discussed before[1], such problems are presumed to be minor in hemolysed and restored pigeon red cells. One possible cause of the discrepancies, then, is a second energy source either cooperating with the cation gradient or acting on a second glycine route parallel to the Na^+ energized route. A second possibility is that one or more of the parameters of the rate equations or one or more of the simplifying assumptions in the derivation are inexact. A third possibility, related to the second, is that hemolysed and restored cells change significantly during the long incubation used for the experiments of ref. 2 so that the rate equations, evaluated with data obtained in 7–9 min incubations, are not entirely appropriate. Since Ca^{2+}, used in the lysing and restoring solutions, inhibits the Na^+/K^+ pump[12] and is largely expelled from lysed and restored mammalian cells within 20 min[13], the larger discrepancies for 100-min incubations than for 40-min ones are consistent with this. In the absence of evidence allowing a choice to be made, lengthy discussion of the possibilities is unwarranted.

The active accumulation and expulsion of glycine by pigeon red cells can be

approximately accounted for by a process for which a Na^+ electrochemical activity gradient furnishes the energy, but discrepancies exist which may be due to an additional energy source, or due to inexact or inappropriate parameters or assumptions for the rate equations.

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