THE EFFECT OF GRAMICIDIN ON THE Na^+-DEPENDENT ACCUMULATION OF GLYCINE BY PIGEON RED BLOOD CELLS*

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Summary: Gramicidin virtually abolishes glycine accumulation by intact pigeon red cells. This effect cannot plausibly be attributed to lack of nucleoside polyphosphate, to gramicidin-induced leakiness of cells to glycine, or to direct inhibition of the Na^+-dependent glycine transport mechanism. This effect of gramicidin implies that virtually all of the energy for glycine accumulation in this system comes from cation gradient(s).

Introduction: In a number of systems amino acids or sugars move against their concentration gradients by Na^+-dependent routes. For several systems, energy appears to come from the co-transport of Na^+ and/or the counter-transport of K^+ down their respective gradients (1). Recently several laboratories (2-6), working with different systems, reported amino acid accumulation greater, and in one case much greater (3,4), than could be supported by the apparent existing cation gradients. In no case however was the activity coefficients nor compartmentation of the internal cations adequately known. Therefore the accumulation that could in theory be supported by cation gradients was also not adequately known.

Gramicidin causes both Na^+ and K^+, with little discrimination, to rapidly cross red cell membranes (7,8) and hence can set both Na^+ and K^+

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electrochemical potential differences to zero. To the extent that an additional or alternate energy source operates, cells should show accumulating ability with gramicidin present.

Intact, rather than hemolysed and restored cells were chosen as being more likely to have additional energy sources. It was found that gramicidin virtually abolishes glycine accumulation.

Materials and Methods: Preparation of red blood cells and incubation in modified Krebs Ringer phosphate were as previously described (9). Gramicidin D, a mixture of gramicidins A, B and C was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Sodium and potassium were determined by flame photometry. Total nucleoside polyphosphate was determined as charcoal absorbable, acid labile phosphate (10,11). Glycine was determined for us with a Spinco amino acid analyser by Dr. Paul J. Mattern by the standard procedure.

Glycine accumulation capacity was determined by incubating aliquots of cells in media with glycine concentrations spanning the initial cell glycine concentration. The external glycine concentration where cells neither gained nor lost glycine, graphically determined from the intersection of the experimental plots with the horizontal dashed line (Fig. 1), divided into the internal glycine concentration is the glycine accumulation ratio the cells can just maintain.

Results and Discussion: In the absence of gramicidin, cells in Na^+-rich medium can maintain a glycine accumulation ratio of 20-40 (Fig. 1 and Table I). With gramicidin added, this ratio drops to near unity and is similar to that of cells in Na^+-free medium with or without gramicidin.

With the gramicidin level used, or one third this level, no further change in cell Na^+ or K^+ (moles/ml cell H_2O) occurs after 3 min at 39° and both Na^+ and K^+ inside/outside concentration ratios are unity (data not shown). Thus internal and external Na^+ and K^+ are at equilibrium throughout the 45 min incubation period.

The lack of accumulation in the presence of gramicidin is not due to

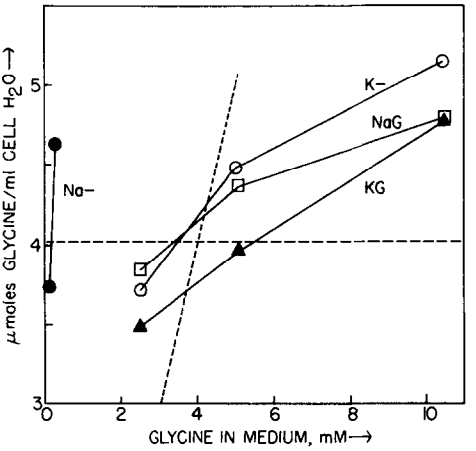

Figure 1. Cell Glycine Concentrations After Incubation in Na^+- and K^+-Rich Media in the Presence and Absence of Gramicidin.

Ten ml of cold cell suspension containing ca. 0.25 gm cells were poured quickly into tubes containing 0.1 ml of 90% ethylene glycol: 10% ethanol containing 7.4 or zero μg/ml gramicidin D, mixed immediately, incubated 45 min 39°, chilled 2 min and centrifuged. Incubation media had either 138.3 mM Na^+ and 7.3 mM K^+ or 145.6 mM K^+ and the concentration of glycine shown on the abscissa. Media also had 2 mM L-threonine and sucrose. For samples "KG", sucrose was 40 mM; for "K-", 23 mM; for "NaG", 31 mM and for "Na-", 7.7 mM. "K" and "Na" specify K^+- or Na^+-rich media respectively and "G" indicates the presence of gramicidin.

The dashed horizontal line shows the initial cell glycine concentration. The dashed slant line is the locus of points where external and internal glycine concentrations are equal.

Pellets and media were extracted with cold trichloroacetic acid (final concentration, 5.5%). To suitably diluted aliquots of extracts, norleucine was added as internal standard and the extracts analysed for glycine.

Extracellular space was determined on duplicate samples representing "KG", "K-", "NaG" and "Na-" which were treated the same as the corresponding samples above except that only one external glycine concentration was used (2.5 mM) and after incubation and chilling, the suspensions were diluted with cold medium containing ^{14}C-glycine and immediately centrifuged. Pellets and media were extracted at 0° with cold methanol (one half saturated with glycine) with 2.00 ml used per pellet and 3.5 ml used per ml of medium. One tenth ml aliquots of extracts were added to 15 ml scintillation solution (2 gm Omnifluor, New England Nuclear, 10 ml Biosolv BBS-3, Beckman, to 500 ml with toluene) and counted. The ratio, DPM/ml pellet H_2O: DPM/ml medium is the fraction of the pellet H_2O which is extracellular space.

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leakiness of the membrane to glycine since with initial external glycine at a much higher or lower concentration than internal glycine, the 45 min incubation is insufficient to equilibrate them (Fig. 1).

The lack of accumulation with gramicidin present is not due to lack of nucleoside polyphosphate (including ATP) since after the incubation with

Table 1. Effect of Gramicidin on Glycine Accumulation Ratios

Expt.	Medium	Incubated	Gramicidin	G_i/G_o	Pellet Weight in gm
1	K^+	+	-	0.58	0.307
	K^+	+	+	--[a]	0.377
	Na^+	+	-	45	0.268
	Na^+	+	+	0.78	0.338
	K^+	-	-	--	0.290
2	K^+	+	-	1.14	0.275
	K^+	+	+	0.73	0.297
	Na^+	+	-	22.4	0.273
	Na^+	+	+	1.17	0.294
	K^+	-	-	--	0.293

The glycine accumulation ratios cells can just maintain (G_i/G_o) were obtained from plots like Fig. 1 as described in "Methods". The experiments were done as for Fig. 1 except that threonine and sucrose were used only in experiment 2. Weights of unincubated, as well as incubated pellets are listed to show swelling.

[a]Some lysis was seen for these samples, preventing meaningful extracellular space estimation.

gramicidin in Na^+-rich medium, cells still have 40-50% of their initial content. (Some loss might be expected from full activation of the Na^+-K^+ dependent ATPase.) The decrease in nucleoside polyphosphate observed is too small to explain the lack of glycine accumulation since the much larger (95%) decrease in going from intact to hemolysed and restored cells leaves some 60% of the glycine transport capacity (12). We assume by analogy with mammalian red cells (13) that ATP, ADP and AMP are in equilibrium. We are currently checking this point however.

The lack of accumulation with gramicidin present is not due to inactiva-

tion of the Na^+-dependent transport system since Na^+-dependent ^{14}C glycine entry was found after 35 min incubation with gramicidin.

Small differences may remain between accumulation from Na^+- and K^+-rich media even in the presence of gramicidin (Table I). To evaluate these, the effects of unequal H_2O uptakes and of exchange between internal amino acids and external glycine will need to be accounted for. In experiment 2 of Table I (also shown in Fig. 1), 2 mM L-threonine was present in the medium to suppress exchange via the ASC route (14). In this experiment sucrose also was present to control swelling. However, from other experiments initial volumes would still be below final volumes so H_2O movements would still affect the glycine accumulation ratios. More data is needed to evaluate the apparent residual Na^+-dependent glycine accumulation.

Gramicidin should be generally useful for assessing the contribution of energy from cation gradients to non-electrolyte "active transport".

References:

(1) Schultz, S. G., and Curran, P. F., Physiol. Revs. 50 637 (1970).
(2) Jacquez, J. A., and Schafer, J. A., Biochim. Biophys. Acta. 193 368 (1969).
(3) Potashner, S., and Johnstone, R. M., Biochim. Biophys. Acta 203 445 (1970).
(4) Lin, K. T., and Johnstone, R. M., Biochim. Biophys. Acta 249 144 (1971).
(5) Vidaver, G. A., Biochim. Biophys. Acta. 233 231 (1971).
(6) Schafer, J. A., and Heinz, E., Biochim. Biophys. Acta 249 15 (1971).
(7) Chappell, J. B., and Crofts, A. R., in B. B. A. Library, "Regulation of Metabolic Processes in Mitochondria", Ed. by Tager, J. M., Papa, S., Quagliarello, E., and Slater, E. C., Elsevier Publishing Co., Amsterdam, Vol. 7, pg 293 (1966).
(8) Henderson, P. J. F., McGivan, J. D., and Chappell, J. B., Bioch. J. (London) 111 521 (1969).
(9) Vidaver, G. A., Biochemistry 3 662 (1964).
(10) Crane, R. K., and Lipmann, F., J. Biol. Chem. 201 235 (1953).
(11) Gomori, G. L., J. Lab. Clin. Med. 27 3 (1942).
(12) Vidaver, G. A., Biochemistry 3 803 (1964).
(13) Rose, I. A., Proc. Natl. Acad. Sci. U.S. 61 1079 (1968).
(14) Eavensen, E., and Christensen, H. N., J. Biol. Chem. 242 5386 (1967).