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ROLE OF SYSTEM *Gly* IN GLYCINE TRANSPORT IN MONOLAYER CULTURES OF LIVER CELLS

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Summary

The high-affinity component of glycine uptake by the hepatoma cell line HTC and by the ordinary rat hepatocyte corresponds to System *Gly*, the agency serving for glycine uptake by pigeon red blood cells and rabbit reticulocytes, and at most to only a minor extent to System *ASC*. This component was identified in HTC by its sensitivity to inhibition by sarcosine but scarcely by 2-(methylamino)isobutyric acid, by its insensitivity to lowering of the pH, and by the unique relation of its rate to the square of the Na^+ concentration. The identity of the low-affinity component with System *A* was confirmed by opposite properties, and by its stimulation by insulin or amino acid starvation. Both components differed sharply from the System *ASC* uptake as measured with threonine.

Introduction

Reichberg and Gelehrter recently concluded that the two systems they observed to participate in the uptake of glycine in the hepatoma cell line HTC* are Systems *A*, at low affinity, and *ASC*, at high affinity (1). In a study of the partition of the Na^+-dependent uptake of 15 neutral amino acids by isolated rat hepatocytes, as inhibitable and not inhibitable by 25 mM 2-(methylamino)-isobutyric acid (MeAIB*), we had just found that over half of the uptake of 0.1 mM glycine escaped inhibition (2). For most of the amino acids tested we believe on the basis of other tests (2), that MeAIB used in this way partitions their Na^+-dependent uptake between Systems *A* and *ASC*. Glutamine had already proved an exception, however, because it migrates by a fairly specific Na^+-dependent route designated System *N* (3). Despite the new conclusion for HTC (1), we suspected that glycine might represent another exception, and not migrate by *ASC* at high affinity either in HTC or hepatocytes, because in pigeon red blood cells and rabbit reticulocytes glycine is a poor *ASC* substrate (4-7). Furthermore we expected System *Gly* largely to escape inhibition by MeAIB: It does tolerate N-methylation of its substrate, transporting sarcosine nearly as well as glycine, but AIB and alanine inhibit *Gly* only weakly (6), indicating

*The abbreviations used are: HTC = hepatoma cell line derived from the Buffalo rat, supplied by Dr. Thomas Gelehrter; MeAIB = 2-(methylamino)isobutyric acid.

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poor acceptance of a third carbon atom on the amino acid chain. Hence we were led to test closely whether the high-affinity component of glycine uptake in both cells types really is *ASC*. We conclude that it occurs instead by System *Gly*, although a minor uptake by *ASC* is not excluded. Low-affinity uptake by System *A* as in Ehrlich cells (8) was confirmed.

Materials and Methods

The HTC cell line, obtained from Dr. T. D. Gelehrter of this University, was grown at 37°C in Medium 199 (Gibco) containing 26 mM $NaHCO_3$, 62.5 µg/ml penicillin, 5.8 µg/ml streptomycin, 31.2 µg/ml gentamycin, and 8 to 10% fetal bovine serum. Cells were seeded in 24-well trays (obtained from Costar) 3 or 4 days before the transport assay. Cells were then transferred 20 to 24 hours before the experiment to medium in which 0.1% bovine serum albumin replaced the serum. When amino-acid-free conditions were needed for several hours, a sterile Krebs-Ringer bicarbonate medium was used. Amino acid transport was measured during 30 s from Krebs-Ringer phosphate medium, following a 1 hour depletion in amino-acid-free medium. Details for culturing and measuring transport in Costar trays with rapid addition and removal of media from the monolayers will be published shortly by White and Gazzola. Sarcosine was purchased from Sigma Chemical or Schwarz-Mann Co.

Experimental Results

Fig. 1 shows the inhibitor sensitivity of the uptake of glycine (A), sarcosine (B), and MeAIB (C) at 0.05 mM, and of glycine (D) at 7.5 mM. These concentrations were selected to emphasize in turn the high-affinity and the low-affinity routes of glycine uptake. Panels A and B show that glycine and sarcosine uptake is readily and virtually completely inhibited (saturated) by glycine or sarcosine in excess; but that MeAIB inhibits their uptake only weakly. At 7.5 mM glycine (panel D), the three inhibitors have, however, more nearly similar effects, consistent with a large increase in the proportion of glycine entering by System *A*. If glycine had entered exclusively by System *A* in panel D, the results should resemble those of panel C, where MeAIB was the substrate. Extending the curves to 100 mM inhibitor levels in a repetition of the experiment of panel D eliminated most of the Na^+-dependent uptake without modifying the interpretation, except to indicate that the difference in the inhibitor sensitivities of the main routes of glycine and sarcosine entry is a relative rather than an absolute one.

Fig. 2 reports that glycine at 7.5 mM (curve in the middle) shows a pH sensitivity of the uptake rate entirely different from that of glycine and sarcosine at 0.05 mM (upper curves), and one quite different from that for 0.05 mM MeAIB. Threonine uptake at 0.05 mM shows pH sensitivity totally different from that applying either to MeAIB or to glycine or sarcosine at low concentrations. The rate rises about 20% between pH 7.4 and 6.5, and then steadily falls to a very low rate at pH 4.5 (our unpublished result). Accordingly, neither the high-affinity nor the low-affinity component for glycine uptake appears to in-

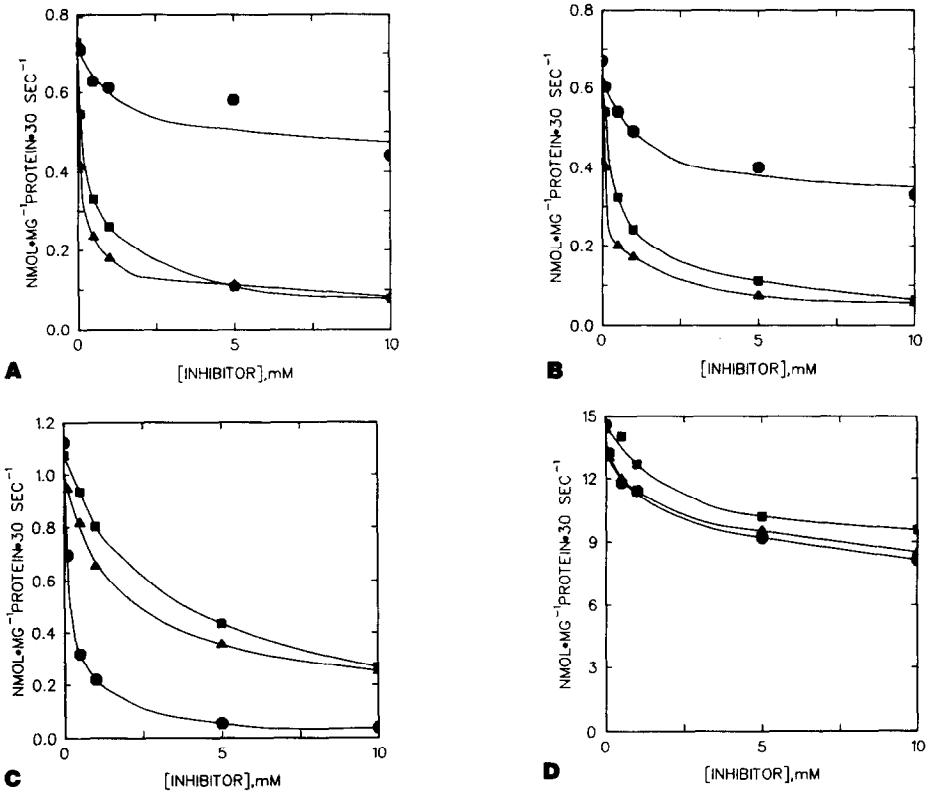

Fig. 1. Inhibitor sensitivity of the uptake rate for glycine (panel A), sarcosine (panel B), and MeAIB (panel C) at 0.05 mM, and of glycine at 7.5 mM (panel D). Inhibitors were glycine (■), sarcosine (▲), and MeAIB (●). The HTC cells used for panel C had been starved with regard to amino acids 6 hr to enhance uptake by System *A*.

clude much uptake by the route defining the pH profile for threonine, which we identify in our unpublished study as the most specific natural substrate for System *ASC* in HTC, in contrast to the similar service of cysteine in the ordinary rat hepatocyte (9).

Fig. 3, panel at left, shows that the rates for glycine and sarcosine uptake at 0.05 mM bear nearly linear relations to $v/[Na^+]^2$, corresponding to an observation by Vidaver for glycine uptake by the pigeon red blood cell (10, cf. 6). For MeAIB uptake (System *A*) the more usual linear relation to $v/[Na^+]$ was seen (right). The relation for 7.5 mM glycine (not shown) was about equally good for $v/[Na^+]^2$ and $v/[Na^+]$, although crude in both cases.

Glycine showed a K_i of 3.1 ± 0.25 mM in inhibiting the uptake of MeAIB at 0.05, 0.1, and 0.2 mM, consistent with its transport by System *A* indicated by our other evidence (Fig. 4, left). In this test MeAIB showed a K_m of

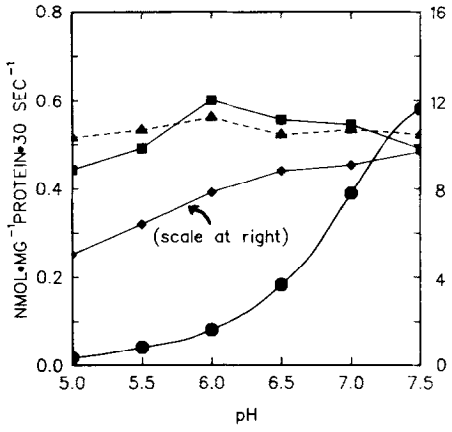

Fig. 2. Sensitivity to pH of the uptake rates for glycine (■), sarcosine (▲), and MeAIB (●) at 0.05 mM, and of glycine (◆) at 7.5 mM. The ordinate scale at the left refers to the lower amino acid concentrations, the one at the right to the higher concentration. The cells for MeAIB uptake had been starved with regard to amino acids 6 hr.

0.31 ± 0.05 mM, V_{max} = 8.7 ± 1.0 nmol·mg^{-1}protein·min^{-1}. When the substrate for transport was 0.05 mM threonine to focus attention instead on System *ASC*, inhibition by MeAIB (at 10 mM, 7%), sarcosine (at 10 mM, 37%), and glycine (at 10 mM, 57%) increased in that order, all three kinetic curves indicating high K_i values. For glycine the K_i by computer program was 7.9 ± 1.0 mM (Fig. 4, right). We take this result to support a distinct although not high-affinity inhibition of System *ASC* by glycine. Note however, that by no means all amino acids that inhibit System *ASC* undergo clearly detectable transport by that system (2,11). Threonine was in turn only a weak inhibitor of glycine uptake (data not shown).

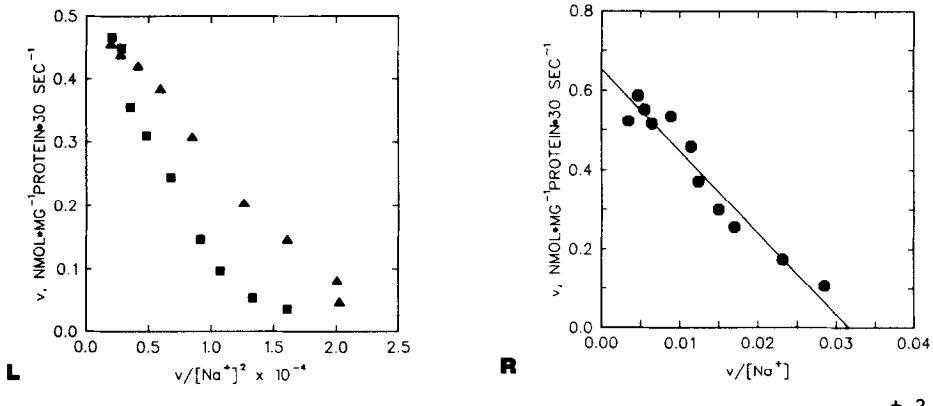

Fig. 3. Relation of uptake rates for glycine (■) and sarcosine (▲) to $v/[Na^+]^2$ (left) and for MeAIB (●) to $v/[Na^+]$ (right). The amino acids were 50 μM, $[Na^+]$ was from 0 to 150 mM, and the pH 7.4. Cells starved 6 hr with regard to amino acids were used at the right.

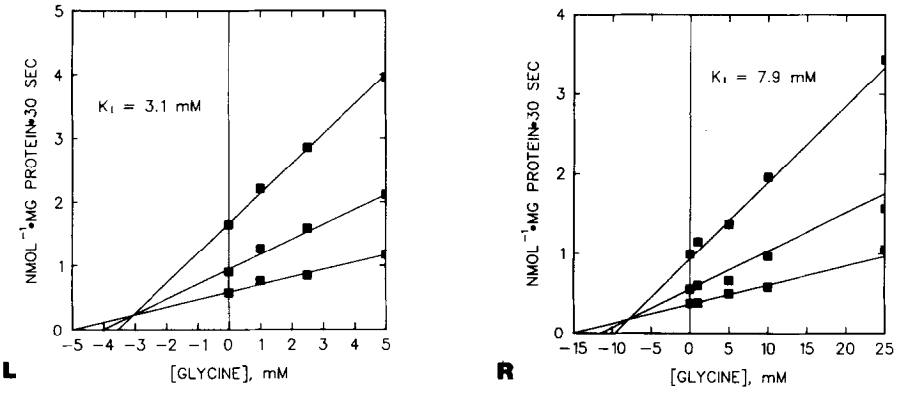

Fig. 4. Glycine inhibition of uptake by Systems *A* and *ASC*. Left, inhibition of the uptake of (1-^{14}C)-MeAIB at 0.05, 0.10, and 0.20 mM at pH 7.4 by HTC cells starved 6 hr with regard to amino acids to stimulate System *A* uptake. Right, uptake of (G-^{3}H)-L-threonine at the same levels, and at pH 5.5, HTC cells depleted of amino acids for only the usual hour. The studied covered the inhibitor ranges 0 to 25 mM (left) and 0 to 50 mM (right), and the lines drawn for best fit by computer program over those ranges, even though the lines in these figures are truncated.

In experiments in which MeAIB uptake by HTC was clearly but not strongly stimulated by 4 to 16 hr exposure to insulin (compare with ref. 12) and inhibited by the same interval of exposure to dexamethasone, the uptake of 0.05 mM glycine or sarcosine was increased by only 8 to 26% by insulin, and decreased by only 20 or 30% by dexamethasone. These small effects were not eliminated by making the uptake test at pH 5.5 rather than 7.4. These results neither establish nor exclude minor effects of the hormones. Glucagon failed to stimulate even System *A* uptake in HTC (cf. 12). The stimulation by up to 24 hr of amino acid starvation was similarly weak and equivocal for uptake of 0.05 mM glycine, whether measured at pH 7.4 or 5.5 (data not shown).

In ordinary rat hepatocytes in monolayers (3), glycine tested at 25 μM showed a component of Na^+-dependent uptake of 0.044 nmol per mg protein per min which we assign to System *Gly* on the following basis: It is saturable by glycine or sarcosine, but not appreciably by MeAIB, cysteine, threonine, or homoserine. Hence at that glycine level this is the predominant component. Furthermore this uptake is relatively insensitive to lowering of the pH.

Discussion

Sarcosine showed a normal V_{max} (see Fig. 13 in ref. 13) and a K_m of about 5 mM for uptake by the Ehrlich cell via System *A*, values rather similar to those for glycine. Glycine reacted only weakly with System *ASC* in the pigeon red blood cell, the K_i of glycine in inhibiting the uptake of serine, proline and alanine being about 3 mM, or about 18 times the K_m glycine showed for up-

take (5). Although as expected sarcosine readily inhibits the System *A* uptake of serine in the Ehrlich cell (i.e. the component inhibited by MeAIB), its action on the *ASC* (MeAIB-insensitive) component was weak, K_i = 21 mM (ref. 14, documented in text p. 5242). From these results we may note that although two routes of glycine uptake were previously detected, study has so far been relatively simple because in each of three cells, the Ehrlich cell, the pigeon red blood cell, and the rabbit reticulocyte (15), uptake occurred by only a single route. We could not expect this convenient situation to continue for all cell types.

Acknowledgment

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