Biochimica et Biophysica Acta, 598 (1980) 588—594

BBA 78734

GLYCINE TRANSPORT IN RAT BRAIN AND LIVER MITOCHONDRIA

JESUS BENAVIDES, MARIA LUISA GARCIA, JOSEFA LOPEZ-LAHOYA, MAGDALENA UGARTE and FERNANDO VALDIVIESO

Departamento de Bioquímica y Biología Molecular, Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid-34 (Spain)

(Received November 28th, 1979)

Key words: Glycine transport; Amino acid uptake; Swelling; (Mitochondria)

Summary

Transport of glycine by rat brain and liver mitochondria has been investigated by both [^{14}C]glycine uptake and swelling experiments. Glycine enters mitochondria passively down its concentration gradient by a respiratory-independent carrier-mediated process. This view is supported by the following observations: (a) glycine inside the mitochondria reaches the incubation medium concentration; (b) mitochondria swell in the presence of isoosmotic solutions of glycine in a concentration-dependent fashion; (c) the uptake of glycine is not influenced by respiratory inhibitors such as KCN or by uncouplers such as carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; (d) initial rates of uptake approach saturation kinetics, the apparent K_m of the rat brain mitochondria for glycine being 1.7 mM and that of the liver mitochondria being 5.7 mM; (e) the rate of swelling is inhibited by methylmalonate, propionate and, at pH 6.5, by mersalyl, and (f) uptake is inhibited by phosphoserine, methylmalonate and propionate, but not by alanine or proline.

Introduction

Glycine, an amino acid with numerous important metabolic functions [1], has also been reported as a inhibitory transmitter [2]. Glycine synthase (EC 2.1.2.10) the main enzyme catalyzing glycine degradation in liver [3] and, most likely, in brain [4], and the other enzymes of glycine catabolism, i.e. glycine aminotransferase (EC 2.6.1.4) and serine hydroxymethyltransferase (EC 2.1.2.1) are localized in the mitochondrial fraction [4—6]. Thus, glycine

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

must penetrate the mitochondria to be metabolized.

In spite of the several studies on the permeability of the mitochondrial membrane to neutral amino acids, the mechanism of entry of glycine is open to debate. Although Garfinkel [7] suggested a glycine carrier and Gamble and Lehninger [8] proposed the existence of a general carrier for neutral amino acids in liver mitochondria, Halling et al. [9] suggested that neutral amino acids, in a ring form, cross the mitochondrial membrane requiring no specific transport mechanism. However, recently Meyer [10] has suggested that proline transport in rat liver mitochondria is mediated by a specific carrier.

The present paper reports experiments showing that glycine enters rat brain and liver mitochondria passively by a respiratory-independent but carrier-mediated process.

Methods

Mitochondria were isolated from brains and livers of adult male rats of the Wistar strain weighing 150—200 g. Brain mitochondria were prepared by the method of Clark and Nicklas [11]. Liver mitochondria were prepared according to Schneider [12]. Protein was determined by the fluorimetric method of Resch et al. [13], calibration being made by the method of Lowry et al. [14] with bovine serum albumin as standard.

The uptake of [^{14}C]glycine by brain and liver mitochondria was determined by the Millipore filtration technique described by Wrinkler et al. [15]. Aliquots of 20 μl of mitochondrial suspension (about 0.5 mg mitochondria protein) were incubated at 27°C with 100 μl of a solution containing [U-^{14}C]glycine (1 mM unless otherwise stated) in 120 mM KCl, 20 mM Tris-HCl and 10 μM rotenone, at pH 7.5. The uptake was terminated by diluting with 5 ml of ice-cold incubation medium and immediately filtering with moistened Sartorius filter (0.45 μm pore size). The filters were rinsed twice with 5 ml of ice-cold incubation medium. The dilution, filtration and washing processes were conducted within 15 s. The filters were dried at 60°C, placed in microvials and their radioactivity content measured by liquid scintillation after addition of 2 ml of the following medium: 5.5 g of 2,5-diphenyloxazole and 68.6 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene in 1 l of toluene/Triton X-100 (2 : 1, v/v). All the experiments were corrected for a control obtained by diluting the mitochondria suspension before adding the radioactive substrate solution. $^{14}CO_2$ was not produced at all during the uptake experiments.

The mitochondria matrix space as determined as the difference between ^3H_2O and [^{14}C]sucrose space.

In swelling studies, mitochondria (2—3 mg protein) were suspended in 2 ml of a solution containing 250 mM glycine, 3 mM Hepes/Tris (3 mM Hepes adjusted with Tris hydroxide to pH 7.5 or 6.5), 0.5 mM EGTA and 4 μM rotenone at pH 7.5 or 6.5. The change in absorbance of mitochondrial suspension was recorded at 620 nm. Typical single experiments of swelling are presented. However, all experiments were performed at least five times with similar results.

[U-^{14}C]Glycine, [U-^{14}C]sucrose and ^3H_2O were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Glycine, rotenone, carbonylcyanide

p-trifluoromethoxyphenylhydrazone, mersalyl, *N*-ethylmaleimide, succinate and all compounds used as inhibitors were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals were reagent grade and were obtained from commercial sources.

Results

The time course of the glycine uptake by brain and liver mitochondria is shown in Fig. 1. In both cases uptake is linear for at least 30 s, however, glycine is apparently taken up faster by brain mitochondria than by liver mitochondria. After 5 min of incubation with 1 mM [^{14}C]glycine the maximum uptake was reached in both brain and liver mitochondria. The internal concentrations of glycine achieved in the matrix of the brain and liver mitochondria were of the same order of magnitude and about 1 mM since under our experimental conditions the volumes of the matrix water were 0.90 and 1.02 μl/mg protein for brain and liver mitochondria, respectively. When KCl was isoosmotically replaced by NaCl or D-mannitol in the incubation medium no significant difference was observed in the glycine uptake (data not shown).

When swelling experiments were carried out with brain and liver mitochondria in isoosmotic solutions (250 mosM) (Fig. 2), the initial rate of swelling, as an indication of the rate of permeation of the amino acid, was dependent of the glycine concentration. The experiments in Fig. 2 also show that the rate of swelling in isoosmotic glycine solution was faster in brain than in liver mitochondria at all the different glycine concentrations.

In order to know the energy dependence of the glycine uptake by mitochondria, the effects of mitochondrial substrates, a respiratory inhibitor and an uncoupler were investigated. Table I shows that neither KCN nor carbonylcyanide *p*-trifluoromethoxyphenylhydrazone affect the initial rate of the uptake of glycine when mitochondria were energized by succinate. However, addition of succinate alone had a small non-significant inhibitory effect on the initial rate of glycine uptake.

The effect of increasing concentration of glycine on the initial rate of uptake by brain and liver mitochondria is illustrated in Figs. 3 and 4. Initial rates of

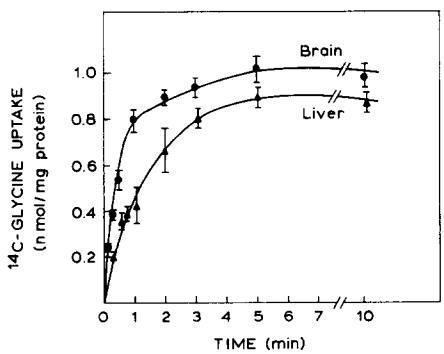

Fig. 1. Time course of glycine uptake by brain (•) and liver (▲) mitochondria. Mitochondria were incubated in the presence of 1 mM [^{14}C]glycine. Each point represents the mean ± S.E. of at least three triplicate experiments.

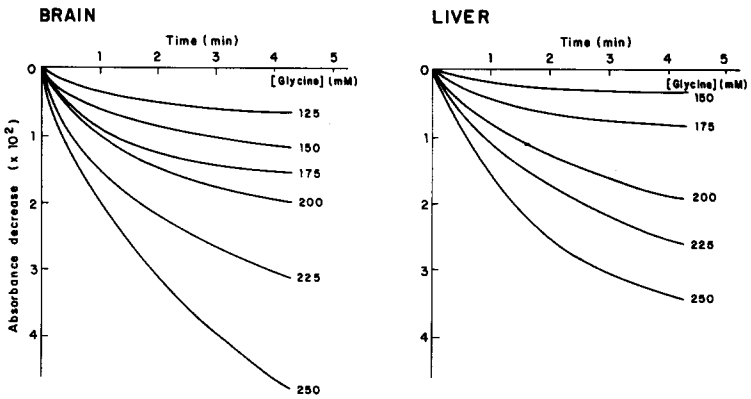

Fig. 2. Swelling of brain and liver mitochondria in isoosmotic glycine solutions. Mitochondria (2—3 mg protein) were suspended in 2.0 ml of a solution containing 3 mM Hepes/Tris, 0.5 mM EGTA, 4 μM rotenone at pH 7.5 and different concentrations of glycine made isoosmotic (250 mM) with sucrose. The absorbance was recorded at 620 nm.

TABLE I

EFFECT OF THE MITOCHONDRIAL ENERGETIC STATE OF THE GLYCINE UPTAKE

20 μl of mitochondrial suspension (about 0.5 mg of protein) were preincubated for 1 min at 27°C in 100 μl of a medium containing 120 mM KCl, 20 mM Tris-HCl (pH 7.5), 10 μM rotenone and the additions at the following concentrations: 3 mM succinate, 1 mM KCN and 50 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone. [^{14}C]Glycine was added (final concentration 1 mM) and after 30 s uptake was stopped as described. Each value is the mean ± S.E. of three experiments each carried out in triplicate.

Additions	Initial rate of glycine uptake (pmol/min per mg protein)	
	Brain	Liver
None	564 ± 40	240 ± 32
Succinate	456 ± 28	216 ± 10
Succinate + KCN	480 ± 32	220 ± 32
Succinate + carbonylcyanide *p*-trifluoromethoxyphenylhydrazone	470 ± 44	204 ± 14

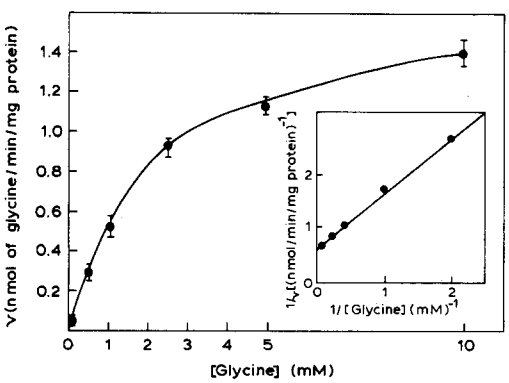

Fig. 3. Initial rates of glycine uptake by brain mitochondria in the presence of increasing concentrations of glycine. Mitochondria were incubated for 30 s in media containing varying concentrations of [^{14}C]-glycine. Inset presents double-reciprocal plot of glycine uptake against glycine concentration. Each point represents the mean ± S.E. of at least three triplicate experiments.

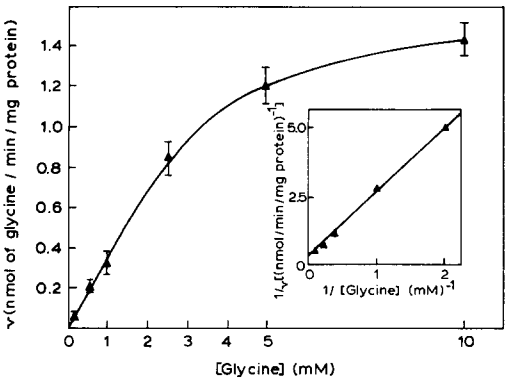

Fig. 4. Initial rates of glycine uptake by liver mitochondria in the presence of increasing concentrations of glycine. Mitochondria were incubated for 30 s in media containing varying concentrations of [^{14}C]-glycine. Inset presents double-reciprocal plot of glycine uptake against glycine concentration. Each point represents the mean ± S.E. of at least three triplicate experiments.

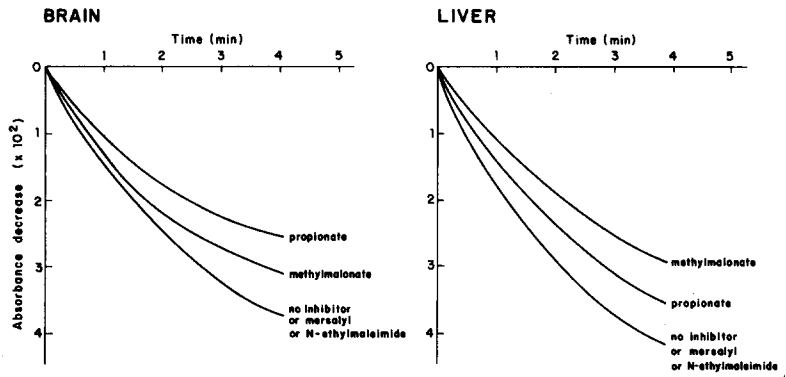

Fig. 5. Effect of different compounds on the swelling of brain and liver mitochondria at pH 7.5. Mitochondria were suspended in 2.0 ml of a 250 mM solution of glycine containing 3 mM Hepes/Tris, 0.5 mM EGTA and 4 μM rotenone at pH 7.5. All added compound were present in the medium before the addition of mitochondria at the following concentrations: propionate, 5 mM; methylmalonate, 5 mM; mersalyl, 40 nmol/mg protein, and *N*-ethylmaleimide, 40 nmol/mg protein.

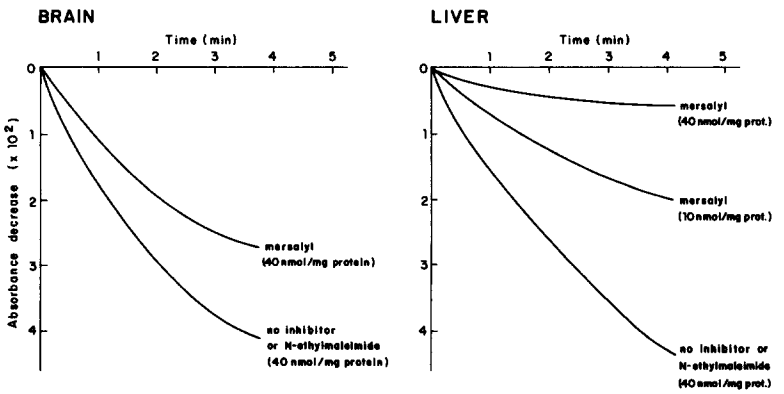

Fig. 6. Effect of thiol reagents on the swelling of brain and liver mitochondria at pH 6.5. Mitochondria were suspended in 2.0 ml of a 250 mM solution of glycine containing 3 mM Hepes/Tris, 0.5 mM EGTA and 4 μM rotenone at pH 6.5. All added compound were present in the medium before the addition of mitochondria.

TABLE II

EFFECT OF SEVERAL COMPOUNDS ON THE RATE OF GLYCINE UPTAKE BY BRAIN AND LIVER MITOCHONDRIA

Mitochondria were preincubated as described in Table I. All added compounds were at a concentration of 1 mM. The values are the mean ± S.E. of three experiments, taking the control value to be 100%.

Compounds	Initial rate of uptake	
	Brain	Liver
None	100	100
α-Alanine	117 ± 10	116 ± 6
β-Alanine	128 ± 14	—
Aminoethanol	128 ± 11	—
Phosphoserine	39 ± 12	42 ± 14
Sarcosine	70 ± 9	—
Glyoxylate	80 ± 8	82 ± 10
Proline	118 ± 4	123 ± 14
Methylmalonate	46 ± 9	27 ± 6
Propionate	23 ± 12	47 ± 10

entry of glycine approach saturation kinetics. Double-reciprocal plots (insets in Figs. 3 and 4) of the initial rate of uptake against glycine concentration were linear and gave K_m values of 1.7 and 5.7 mM for brain and liver mitochondria, respectively.

Glycine uptake by mitochondria at pH 7.5, as indicated by swelling experiment, was inhibited by methylmalonate and propionate but not by mersalyl, *N*-ethylmaleimide, KCN or carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, as shown in Fig. 5. At pH 6.5, however, swelling was inhibited by mersalyl (Fig. 6).

Table II shows the effect of several compounds on the initial rate of glycine uptake by brain and liver mitochondria. Of these compound, phosphoserine, methylmalonate and propionate inhibited the glycine uptake at concentration of 1 mM in both brain and liver mitochondria. However, it is noteworthy that neither alanine nor proline had any inhibitory effect.

Discussion

Even though Garfinkel [7] reported that glycine and some other amino acids penetrate mitochondria and suggested a glycine carrier, and Gamble and Lehninger [8] proposed a general carrier for neutral amino acids, the mechanism of transport of glycine across the mitochondrial membrane has been in dispute. The data presented in this report demonstrate that glycine is taken up by brain and liver mitochondria by a carrier-mediated process without requiring energy. That the uptake by mitochondria reflects transport rather than free diffusion is supported by several lines of evidence: (a) the existence of saturation kinetics, with apparent values of K_m of about 2 and 6 mM; (b) the inhibition by compounds such as methylmalonate and propionate, and (c) the inhibition, although only at pH 6.5, by the hydrophilic thiol reagent mersalyl.

The no sodium dependence of the glycine uptake by brain mitochondria is a clear indication of the little contamination of the preparation by synaptosomes.

This is in full agreement with the high purity of the mitochondrial fraction demonstrated by the marker enzyme distribution (data not shown) which is similar to that reported for the original preparation method of brain mitochondria [11].

The fact that both brain and liver mitochondria at equilibrium reach a glycine concentration equal to that in the suspending medium together with the inability of KCN and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone to inhibit glycine uptake suggests that this is not an energy-dependent process.

Neither mersalyl at pH 7.5 nor *N*-ethylmaleimide inhibited glycine uptake. These observations indicate that glycine does not share its carrier with proline, because as reported by Meyer [10] proline uptake is inhibited by mercurials at pH 7.5. The possibility exists, that glycine shares its transport system with the other neutral amino acids which were also unaffected by mersalyl and *N*-ethylmaleimide. However, the fact that neutral amino acids such as α-alanine, β-alanine and proline did not affect glycine uptake indicates that different carriers are responsible for transport of these amino acids.

The observation that mersalyl at pH 6.5 inhibits swelling but to a greater extent in liver than in brain together with the different values of K_m for glycine uptake raises the possibility of a different transport system in liver than in brain mitochondria.

Finally, the observations reported here on the effect of methylmalonate and propionate on glycine uptake by brain and liver mitochondria, as well as being to interest in themselves, may have important metabolic implications in relation to the pathogenesis of methylmalonic acidemia and propionic acidemia [16].

Acknowledgements

We wish to thank Prof. F. Mayor for fruitful discussion and criticism of the manuscript. This work was supported by a grant from Comisón Administradora des Descuento Complementario.

References

1 Meister, A. (1975) in Biochemistry of the Amino Acids, Vol. 2, pp. 636—673, Academic Press, New York
2 Aprinson, M.H., Daly, E.C., Shank, R.P. and McBride, W.J. (1975) in Metabolic Compartmentation and Neurotransmission (Berl, S., Clarke, O.D. and Schneider, D., eds.), pp. 37—63, Plenum Press, New York
3 Yoshida, T. and Kikuchi, G. (1970) Arch. Biochem. Biophys. 139, 380—392
4 Bruin, W.J., Frantz, B.M. and Sallach, H.J. (1973) J. Neurochem. 20, 1649—1658
5 Daly, E.C. and Aprinson, M.H. (1974) J. Neurochem. 22, 877—885
6 Johnston, S.A.R. and Vitali, M.U. (1969) Brain Res. 12, 471—473
7 Garfinkel, D. (1963) J. Biol. Chem. 238, 2440—2444
8 Gamble, J.G. and Lehninger, A.L. (1973) J. Biol. Chem. 248, 610—618
9 Halling, P.J., Brand, M.D. and Chapell, J.B. (1973) FEBS Lett. 34, 169—171
10 Meyer, J. (1977) Arch. Biochem. Biophys. 178, 387—395
11 Clark, J.B. and Nicklas, W.J. (1970) J. Biol. Chem. 245, 4724—4731
12 Schneider, W.C. (1948) J. Biol. Chem. 176, 259—266
13 Resch, K., Imm, W., Ferber, E., Wallach, D.H.F. and Fischer, H. (1971) Naturwissenschaften 58, 220
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Wrinkler, H.H., Bygrave, F.L. and Lehninger, A.L. (1968) J. Biol. Chem. 243, 20—28
16 Ugarte, M., López-Lahoya, J., García, M.L. Benavides, J. and Valdivieso, F. (1979) J. Inherited Metab. Dis. 2, 93—96