Biochimica et Biophysica Acta 903 (1987) 1–10
Elsevier

BBA 73676

Chloride dependence of the sodium-dependent glycine transport in pig kidney cortex brush-border membrane vesicles

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(Received 20 February 1987)

Key words: Brush-border membrane; Proximal tubule; Glycine transport; Chloride dependence; Sodium dependence; (Porcine kidney)

The Na^+-dependent glycine uptake in pig kidney cortex brush-border membrane vesicles is specifically enhanced by the presence of Cl^-. The Na^+-independent glycine uptake is not affected by Cl^-. Various anions tested could not substitute Cl^- in the activation of the Na^+-dependent glycine transport. Cl^- is specifically required on the outer membrane side. The Na^+-dependent glycine uptake is higher in the presence of an inwardly directed Cl^- gradient than the one measured in the presence of equilibrated Cl^-. The Na^+-dependent glycine uptake depends on, and is saturable at increasing Cl^- concentrations. By studying the activation of glycine uptake by Na^+ in the presence and in the absence of Cl^-, evidence was found that two different Na^+-dependent glycine transport pathways are present in pig kidney cortex brush-border membrane vesicles. The kinetics of the glycine uptake measured in the presence of an inwardly directed NaCl gradient show the presence of two glycine transport systems, a low-affinity, high-capacity one and a high-affinity, low capacity one. In the absence of Cl^- the high-affinity, low-capacity transport is almost suppressed, thus indicating the presence of a high-affinity glycine transport system simultaneously dependent on both Na^+ and Cl^- ions.

Introduction

The existence of genetically determined aminoaciduria and the presence of the neonatal aminoaciduria have been correlated with possible defects in kidney glycine reabsorption, thus giving rise to a certain interest in the study of transport mechanisms of glycine in kidney proximal tubule [1–9]. Various independent observations have indicated that the transport of glycine across the renal brush-border membrane could occur by means of more than one carrier-mediated transport system [2,4–7]. The role of the Na^+ electrochemical gradient in the glycine transport process by using isolated brush-border membrane vesicles (BBMV) has been stressed by different research groups [5,9].

Recently it has become evident that, besides Na^+, ions such as K^+ and/or Cl^- are involved in the transport of some amino acids and amino acid derivatives in epithelial and non-epithelial cell membranes [10–15]. In particular, the Na^+-dependent glycine transport in red blood cells [10], intestinal brush-border membrane vesicles [16] and brain synaptosomes [17], has proven to be Cl^- dependent.

In this paper, we present data indicating that part of Na^+-linked glycine uptake in brush-border membrane vesicles isolated from pig kidney cortex

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is Cl^- dependent. Preliminary results of the present paper have been reported elsewhere [18].

Materials and Methods

Chemicals. [2-^{3}H]Glycine chloride free, was obtained from Amersham International (U.K.); all chemicals used were of analytical grade purity.

Preparation of membrane vesicles. Pig kidneys were obtained fresh from the city slaughterhouse. Brush-border membrane vesicles were prepared within 4 h after the death of the animal. 10 g of cortex slices, 1 mm thick, were first suspended in ice-cold buffer containing 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5), then cut into small pieces with scissors and decanted twice with the same buffer. Cortex slices were suspended in 60 ml of the previous buffer, diluted 1 : 5 (v/v) with distilled water and homogenized with a mixer for 3 min at the maximum speed (Braun Melsungen, F.R.G.). Subsequently the brush-border membrane vesicles were prepared according to the Mg^{2+}/EGTA method described by Biber et al. [19], using a Sorvall RC 5B centrifuge equipped with the SS 34 rotor.

Protein determination. Protein content was determined by the method of Bradford [20], using the Bio-Rad kit (Bio-Rad, Richmond, CA, U.S.A.) and γ-globulin as a standard.

Enzyme assays. The purity of the brush-border membrane vesicle preparations was monitored by measuring the specific activities of alkaline phosphatase (EC 3.1.3.1) and aminopeptidase M (EC 3.4.11.2), according to Berner and Kinne [21] and Haase et al. [22], respectively.

The specific activities of alkaline phosphatase and aminopeptidase M measured in the homogenate were: 107 ± 23 and 27 ± 4 nmoles of substrate consumed/min per mg protein, measured at 37 °C, while in the final brush-border membrane vesicle pellet the specific activities were 10.3 ± 1.6 and 19.6 ± 3.7 times over that measured in the homogenate, respectively (results are the mean ± S.D. of eight individual experiments).

Uptake studies. For uptake studies the brush-border membrane vesicles were suspended in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) (Buffer A) or 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5) (Buffer B).

Membrane vesicles were preloaded with different salts to obtain the experimental conditions described in the legends. Preloading of the membrane was started by mixing an aliquot of membrane vesicles prepared in buffer A or B with an aliquot of the same buffer containing 2 M salts. 1 h at room temperature was considered sufficient to equilibrate them across the membranes, since preincubation for longer times did not lead to significantly different results; however, under these conditions, it is possible that very low permeable salts (e.g., potassium gluconate) are not equilibrating during the 1 h preincubation, but this would affect only the glycine equilibrium uptake, and it would not significantly influence the glycine initial uptake rate.

While either buffer A or B were present on the inside of the membrane vesicles, as indicated in the legend of the figures, 100 mM mannitol and 20 mM Hepes-Tris (pH 7.5) were present in all incubation media (outside the membrane vesicles) of the experiments reported.

Uptake studies were carried out using the rapid filtration technique as described elsewhere [15]. Briefly, 10 μl of membrane vesicles were mixed with 90 μl of incubation medium. At the time indicated, 20 μl of the mixture (equivalent to 30 μg of membrane protein) were directly pipetted onto the Millipore filter (45 μm pore size). The filter was immediately rinsed with 5 ml of ice-cold stop solution of the following composition: 100 mM mannitol, 100 mM NaCl, 5 mM Hepes-Tris (pH 7.5).

For kinetic studies the procedure was modified as follows. 10 μl of membrane protein (40 μg) were added to 20 μl of incubation medium. At the time indicated 1 ml of ice-cold stop solution was added to the incubation test tube, mixed on a vortex mixer and pipetted onto the filter.

The radioactivity trapped on the filters was measured by standard liquid scintillation techniques. Membrane free incubation media were used as blanks: counts of the samples were at least 3-times higher than those of the blanks.

Each experiment was performed at least 4 times, only the results of a typical experiment are shown.

Experiments were always performed in triplicate, data are generally expressed as pmol/mg protein ± S.D. of the mean. Unless indicated, S.D.

values do not exceed the size of the symbols. In the kinetic studies S.D. did not exceed 10% of the mean. The kinetics of the glycine uptake was analyzed with the aid of an Olivetti M 20 personal computer (Olivetti SPA, Ivrea, Italy). Where indicated valinomycin in 95% ethanol was added to the incubation media at 10 μg/mg protein. Ethanol did not exceed 1–2% in the final incubation media.

Results

Time course

The time course of glycine uptake in pig kidney cortex brush-border membrane vesicles (0.1 mM final concentration in the incubation medium) is shown in Fig. 1. It was performed both in the absence and in the presence of 100 mM Na^+ outside the membrane (Na^+ gradient), as well as with 100 mM Na^+ equilibrated across the membrane.

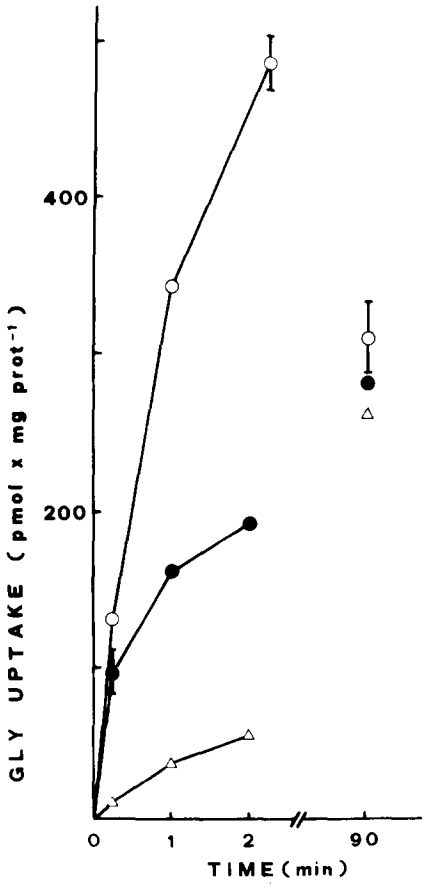

TABLE I

SPECIFICITY OF Cl^- REQUIREMENT: ANION DEPENDENCE

Membrane vesicles, suspended in buffer A were preloaded with a medium containing different sodium and potassium salts. At the start of the uptake, being X− each anion listed in the table, 100 mM NaX, 20 mm KX, were present on both sides of the membrane, 0.35 mM [^{3}H]glycine and valinomycin outside the membrane. The glycine uptake measured after 15 s of incubation is shown in the second column. The third column refers to the data of the second column expressed as percentage of the uptake measured in the presence of chloride.

Anion	Glycine uptake (pmol·(mg prot)$^{-1}$)	%
Cl^-	259.5 ± 2.6	100
Br^-	86.8 ± 2.4	33.4
I^-	109.8 ± 1.4	42.3
SCN^-	95.3 ± 1.5	36.7
Sulfate	142.2 ± 1.4	54.8
Gluconate	67.8 ± 1.5	26.1

The data show that, compared to the uptake values obtained in the absence of Na^+, equilibration with 100 mM Na^+ stimulated the uptake of glycine. Moreover, in the presence of the Na^+ gradient, glycine was accumulated into the vesicles against its own chemical gradient, thus indicating the presence of a sodium-driven glycine uptake.

Specificity of chloride requirements

The influence of various anions on glycine uptake has also been studied. Table I shows the glycine uptake (0.35 mM) in the presence of different anions: Cl^-, Br^-, I^-, SCN^-, sulfate and gluconate. Glycine uptake was measured in the absence of a salt gradient. 100 mM Na^+ and 20 mM K^+ salts of the different anions were present on both sides of the membrane; valinomycin was also present so as to minimize the influence of the diffusion potential. Under these experimental conditions glycine uptake could not be influenced by the electrical potential created by the membrane's

Fig. 1. Time course. Membrane vesicles suspended in buffer A were preloaded with a medium containing choline chloride or NaCl (100 mM). Membranes preloaded with choline chloride were incubated in media containing 100 mM KCl (△) or NaCl (○). Membranes preloaded with NaCl were incubated in media containing 100 mM NaCl (●). The external incubation media contained 0.1 mM [^{3}H]glycine under all the experimental conditions.

different permeability to the tested anions.

As can be observed, the maximal rate of uptake was found in the presence of Cl^-; whereas with either Br^- or I^- a drastic reduction of the glycine uptake was observed. Similar results were obtained when Cl^- was substituted either by SCN^- or by sulfate or gluconate.

Other anions tested (e.g. cyclamate, toluensulfonate; not shown) caused a decrease of the glycine uptake to a similar extent.

These results clearly indicate that, in order to obtain maximal rates of uptake, glycine transport has a specific requirement for Cl^-.

Thus the following questions arise: (i) Is Cl^- required on one or on both membrane sides? (ii) Is Cl^- gradient an additional driving force for glycine uptake?. (iii) Is Cl^- itself able to stimulate or energize the glycine uptake in the absence of Na^+?

To answer these questions the time course of glycine uptake was measured with Na^+ and Cl^- equilibrated across the vesicle membrane or substituting Cl^- by gluconate either only on the inner membrane side or on the outer side, or on both sides (Fig. 2).

Fig. 2A shows that the glycine uptake measured in the presence of Cl^- equilibrated across the membrane vesicles is higher than that measured in its absence. When Cl^- was present only on the outer side (in the presence of an inwardly directed Cl^- gradient) an increase in glycine uptake was obtained compared to the uptake values measured in the presence of Cl^- on either sides of the membrane. When Cl^- was substituted by gluconate on the outer side of the membrane, a drastic reduction of glycine uptake similar to that obtained in the complete absence of Cl^- was observed. Data in Fig. 2B, show that Cl^- was unable to enhance glycine uptake in the absence of Na^+, thus indicating that the activation by Cl^- of the uptake is strictly Na^+ dependent.

From these experiments it can be concluded that Cl^- is necessary to fully activate the Na^+ dependent glycine transport and that it exerts this action from the outer side of the membrane.

Chloride concentration dependence

Further insight into the mechanism of the stimulation by Cl^- of the Na^+-dependent glycine

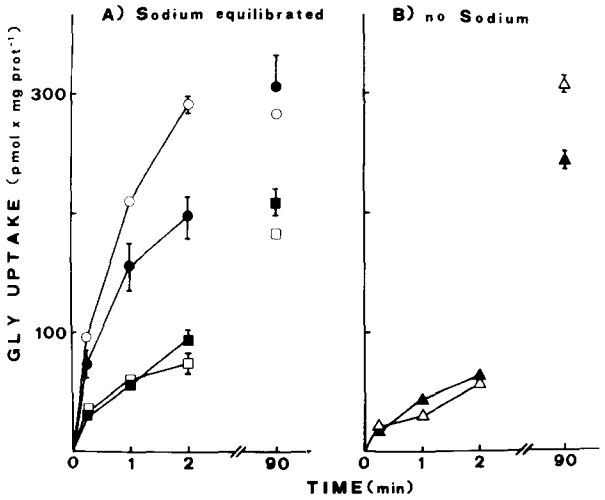

Fig. 2. Specificity of Cl^- requirement: side requirement of chloride. Membrane vesicles suspended in buffer A were preloaded with different sodium and potassium salts. Being $X^- = Cl^-$ or gluconate. (A) Membrane preloaded with medium containing 100 mM NaX, 20 mM KX were incubated in media containing 100 mM NaX, 20 mM KX. Chloride was present either on the outer or on the inner or on both sides of the membrane, or totally substituted by gluconate, to obtain the following experimental conditions: $[Cl^-]_{in} = [Cl^-]_{out}$ (●); $[gluconate]_{in} = [Cl^-]_{out}$ (○); $[Cl^-]_{in} = [gluconate]_{out}$ (□); $[gluconate]_{in} = [gluconate]_{out}$ (■). (B) Membrane vesicles suspended in buffer A and preloaded with medium containing 120 mM potassium gluconate, were incubated in media containing 120 mM KCl (△) or 120 mM potassium gluconate (▲). 0.1 mM [^{3}H]glycine and valinomycin were present in the external incubation media in all the experimental conditions.

uptake was obtained by studying glycine uptake as a function of increasing Cl^- concentrations (Fig. 3A).

The data reported in Fig. 3A show that the initial rate of glycine uptake (10 s of incubation; 0.1 mM glycine) depends on the Cl^- concentration and is saturable, thus indicating the presence of Cl^- activating site(s) on the glycine transporter.

These data were analyzed according to Fukuhara and Turner [23] after subtraction of the uptake value at abscissa zero which represents the Cl^--independent uptake. Briefly, the following Hill-type equation

$$V = V_{max}[A]^n/(K^n_{0.5} + [A]^n)$$

assumes the existence of n essential cooperative site(s) for the activator A, on the glycine transporter. Plotting $V/[Cl^-]^n$ against V, the correct

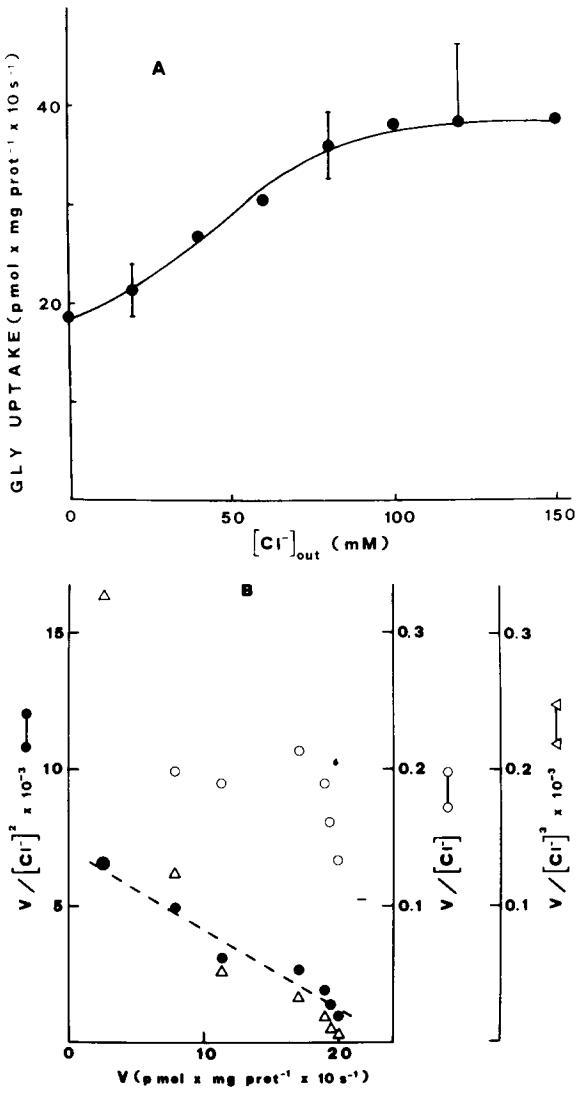

Fig. 3. Chloride concentration dependence. Membrane prepared in buffer A were preloaded with medium containing 100 mM potassium gluconate and incubated for 10 s in media containing 100 mM sodium at varying chloride concentrations as indicated in A. Chloride was isotonically replaced with gluconate to reach the concentrations indicated. In order to obtain 120 and 150 mM Cl^-, 20 mM and 50 mM choline chloride were added, respectively, in incubation media containing 100 mM NaCl. The incubation time was 10 s. All the incubation media contained 0.1 mM [^{3}H]glycine and valinomycin. (A) Plot of V (glycine uptake) vs. [Cl^-]. (B) Plots of $V/[Cl^-]^n$ vs. V: for $n = 1$ (○), 2 (●), 3 (△).

value of n will yield a straight line with slope $1/K_{0.5}^n$. As can be seen in Fig. 3B the best fit was obtained for $n = 2$ ($r = 0.98$) and the half maximal activation of glycine uptake is 58 mM Cl^-.

Sodium concentration dependence

The activation of glycine uptake (0.1 mM) by Na^+ was studied in the presence of fixed concentrations of Cl^- or gluconate (100 mM), as shown in Fig. 4A (10 s of incubation; 0.1 mM glycine).

The initial glycine uptake rate measured in the presence of Cl^- proves saturable when the Na^+ concentration was increased. In the presence of gluconate instead of Cl^-, glycine uptake shows saturable kinetics, but its uptake values were lower for each tested Na^+ concentrations. At abscissa zero ($[Na^+]_{out} = 0$) the uptake values of the two curves coincided, thus indicating that Cl^- did not influence glycine uptake in the absence of Na^+. The uptake value at abscissa zero represents the Na^+-independent uptake. As can be seen, the value of V_{max} found in the absence of Cl^- corresponds well to the uptake value at abscissa zero in Fig. 3A. This suggests that the Na^+-dependent Cl^--independent glycine uptake is due to two different components: an Na^+-independent and an Na^+-dependent Cl^--independent transport pathway. Cl^- markedly increases the V_{max} of the Na^+-activated uptake, suggesting the presence of a third component of glycine uptake, simultaneously dependent on the presence of both Na^+ and Cl^- ions.

The data relative to the curve obtained in the presence of gluconate have been analyzed as previously indicated, after subtraction of the value for the Na^+-independent glycine uptake (Fig. 4B). The best fit was obtained for $n = 2$ ($r = 0.96$) and the glycine half-maximal activation as calculated from the slope is 38 mM Na^+. On the basis of this result we have fitted the theoretical activation curve previously described as follows: $V = (10{:}9[Na^+]^2)/(38^2 + [Na^+]^2) + 9.55$; where the kinetics parameters, $V_{max} = 10.9$ (pmol/mg protein per 10 s) and $K_{0.5} = 38$ (mM) were calculated from Fig. 4B (dashed line) and 9.55 represents the Na^+-independent uptake. This curve is the dashed line in Fig. 4A and the squares represent the experimental data. It can be seen that: (a) the experimental data fit to the theoretical curve well; (b) the sigmoidicity of the curve would not be immediately revealed by simply looking at the experimental points, because the inflection point is too close to the origin.

As for the activation curve obtained in the

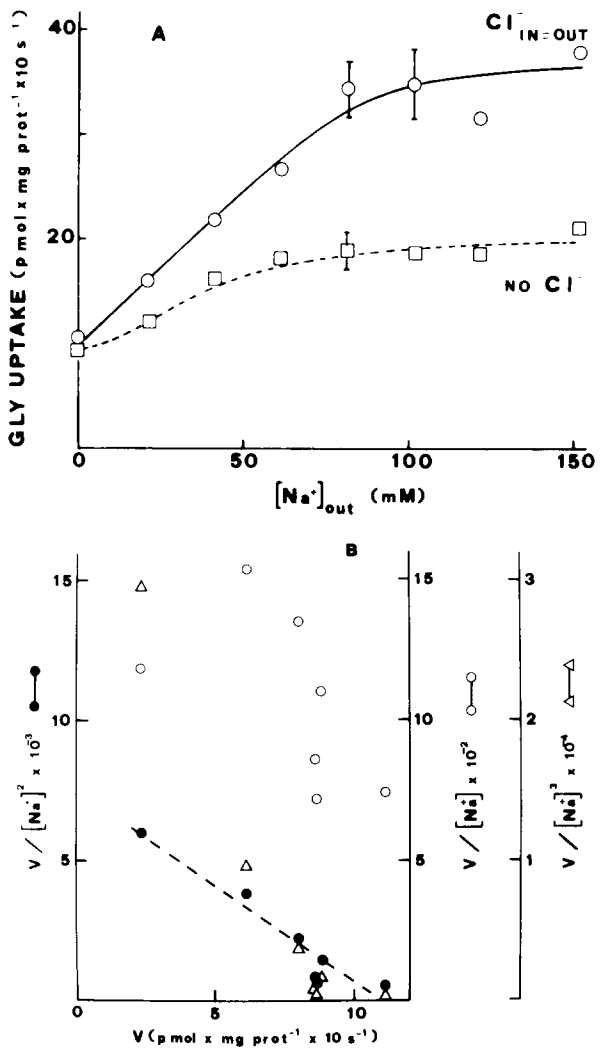

Fig. 4. Sodium concentration dependence. Membranes prepared in buffer A were preloaded with KCl or potassium gluconate (100 mM). Membranes preloaded with KCl were used of the measurements in the presence of 100 mM Cl^- on both sides of the membrane at varying concentrations of Na^+ outside the vesicles, upper curve (○). In order to keep the Cl^- concentration constant, NaCl was isotonically replaced with potassium gluconate in the incubation media. Membranes preloaded with potassium gluconate were used for the measurements in the absence of Cl^-. Sodium gluconate was present in the external incubation media at varying concentrations, lower curve (□). Sodium gluconate was isotonically replaced with potassium gluconate in the incubation media. Incubation time was 10 s. Membrane vesicles were incubated in media containing 0.1 mM [^{3}H]glycine and valinomycin in all conditions. (A) Plots of V (glycine uptake) vs. [Na^+]. (B) Plot of $V/[Na^+]^n$ vs. V; for $n=1$ (○), 2 (●), 3 (△). (B) In the presence of chloride (upper curve in A).

presence of Cl^-, this curve is the result of the activation of two Na^+-dependent mediated components of the glycine transport, and it is either based on the experimental observations previously discussed, or on others which are discussed later in this paper. Thus, the same analysis was applied to the values obtained by substracting the curve in the presence of gluconate from the curve in the presence of Cl^-. However, no good fit could be obtained for values of n between 1 and 4.

Glycine concentration dependence

The glycine uptake rate is reported as a function of increasing glycine concentrations in Fig. 5A.

The initial rate of glycine uptake was studied over the concentration range from 0.054 mM to 20 mM under different experimental conditions, in the presence of either NaCl or sodium gluconate or choline chloride (100 mM), outside the membrane vesicles. Incubation time was 7 s, sufficiently short to ensure measurements during the linear part of the uptake (the glycine uptake measured in the presence of NaCl was linear up to 20 s of incubation throughout the glycine concentrations used; not shown).

The glycine uptake measured in the absence of Na^+ appears to be a linear function of glycine concentrations. The slope of the straight line thus obtained represents the glycine permeability coefficient ($P = 7.14$ nl · (mg protein)$^{-1}$ · s^{-1}).

In the presence of NaCl, glycine uptake approaches saturation kinetics. Under the same experimental conditions, when Cl^- is substituted completely by gluconate in the incubation media, the uptake rate decreases throughout the glycine concentration range tested.

Fig. 5B shows the Eadie-Hofstee plot of the data after subtraction of the values for the estimated diffusion.

The data obtained in the presence of NaCl are clearly distributed in an hyperbole which indicates the presence of more than one transport system for glycine uptake. The data have been analyzed by computer, fitting a two-site Michaelis-Menten equation to the data

$$V = (V_{max1}[S])/(K_{m1}+[S]) + (V_{max2}[S])/(K_{m2}+[S])$$

The computer analysis used is based on the

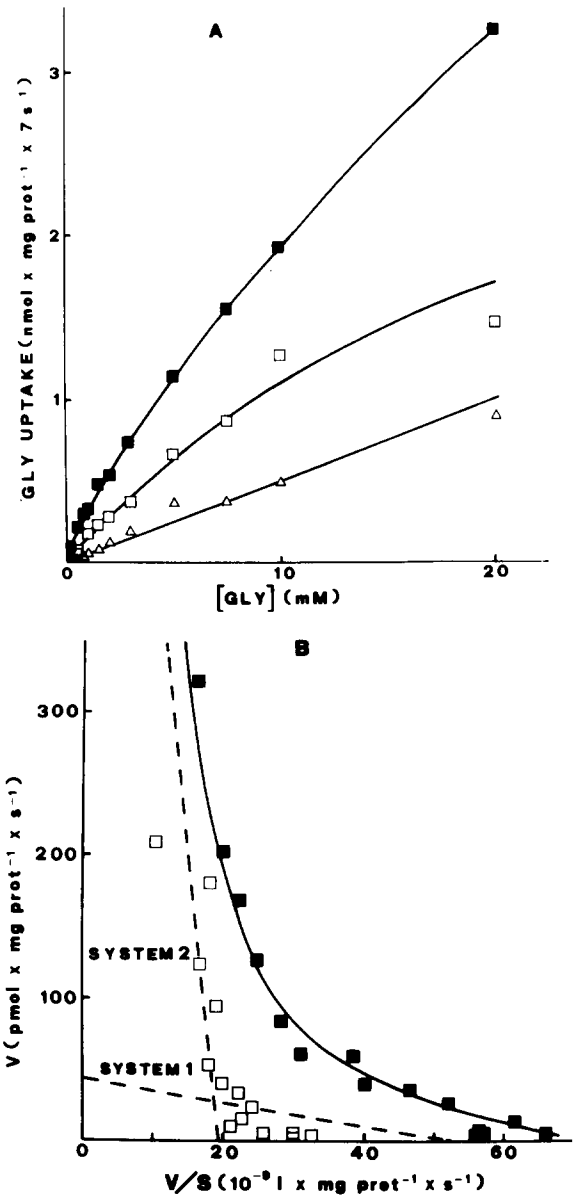

Fig. 5. Glycine concentration dependence. (A) Membrane vesicles suspended in buffer B were in part preloaded with buffer containing 20 mM KCl and then incubated in media containing 100 mM NaCl and 20 mM KCl (■) or 100 mM choline chloride 20 mM KCl (△). Another portion of the vesicles was preloaded with buffer containing 20 mM potassium gluconate and incubated in media containing 100 mM sodium gluconate and 20 mM potassium gluconate (□). All the incubation media contained [^{3}H]glycine at the concentrations indicated, in the range from 0.054 to 20 mM. The incubation time was 7 s. Valinomycin was present in all the incubation media. (B) Eadie-Hofstee plot: V (glycine uptake) vs. V/S (glycine uptake/glycine concentration). Uptake rate measured in the presence of NaCl (■) and sodium gluconate (□) were corrected for the diffusional component. Analysis of the data determined in the presence of NaCl (see results) led to the description of two glycine transport systems, indicated in the figure by the dotted lines. The full line represents the theoretical curve resulting from the sum of systems 1 and 2.

iterative stripping method of Spears et al. [24]. This method describes two glycine transport systems with the following kinetic constants: System 1, high-affinity, low-capacity ($K_{m1} = 0.84$ mM, $V_{max1} = 43.6$ pmol · (mg protein)$^{-1}$ · s^{-1}) and System 2, low-affinity, high-capacity ($K_{m2} = 46$ mM, $V_{max2} = 879$ pmol · (mg protein)$^{-1}$ · s^{-1}); both indicated in Fig. 5B by the dotted lines. The theoretical curve, sum of the two systems, indicated by the full line, suggests that this is a reasonable description of the empirical data.

The same analysis made for the data obtained in the presence of sodium gluconate did not give a satisfactory result. However, the empirical values seem to be reasonably distributed around the theoretical fitting of System 2 (see Fig. 5B). Infact few experimental points at low glycine concentrations do not fit on System 2. Thus System 2 does not seem to be affected either by the presence or by the absence of Cl^- in the incubation media, whereas System 1 is almost unable to transport glycine in the absence of Cl^-, thus clearly indicating that only System 1 depends on both Na^+ and Cl^- in the incubation media.

Rheogenicity

The rheogenicity of the system(s) was tested both in the presence and in the absence of Cl^- in the incubation media (Figs. 6A and 6B).

The inside-negative potassium-diffusion potential induced by valinomycin enhances glycine transport in the presence as well as in the absence of Cl^- in the incubation media, thus indicating the presence of a rheogenic glycine transport under either conditions.

This experiment was performed in the absence of a chemical Na^+ gradient in both experimental conditions.

The absolute values of the valinomycin-induced uptake are similar under the two different experimental conditions.

The absence of any additional effect when Cl^- was also present suggests that the Na^+-dependent,

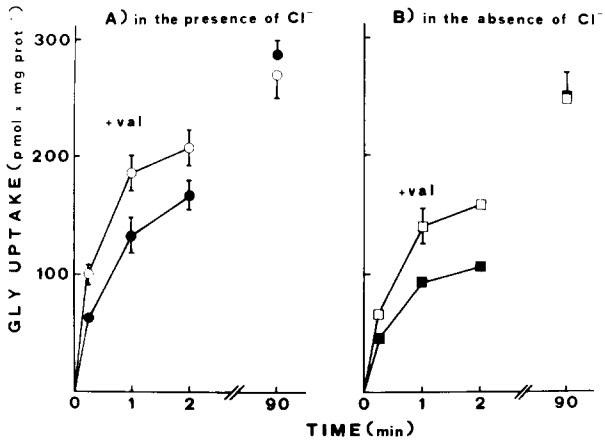

Fig. 6. Rheogenicity. (A) In the presence of Cl^-. Membrane vesicles suspended in buffer A, were preloaded with buffer containing 50 mM NaCl, 50 mM KCl and incubated in buffer containing 50 mM NaCl, 5 mM KCl in the absence (●) and in the presence of valinomycin (○). (B) In the absence of Cl^-. Membrane vesicles, suspended in buffer A, were preloaded with buffer containing 50 mM sodium gluconate, 50 mM potassium gluconate and incubated in buffer containing 50 mM sodium gluconate, 5 mM potassium gluconate both in the absence (■) or in the presence of valinomycin (□). 0.1 mM [^{3}H]glycine was present in all the incubation media.

Cl^--dependent component, which is the major component of glycine uptake was not affected by the change in the electrical membrane potential.

Discussion and Conclusions

The present work provides experimental evidence that Cl^- is a specific activator of the Na^+-dependent glycine transport in pig kidney cortex brush-border membrane vesicles and that its effect is strictly linked to the presence of Na^+ (Figs. 2B, 4, 5). Our data indicate that the effect of Cl^- on the stimulation of the Na^+-dependent glycine uptake is highly specific, with respect to other anions tested and that Cl^- is specifically required by the outer membrane side (Cl^- ions had no effect when present only on the inner face of the membrane, Fig. 2A).

The Cl^- activation is a saturable phenomenon, thus indicating a direct interaction of Cl^- with specific binding sites (Fig. 3).

By studying the Na^+ concentration dependence of the initial uptake rate, we found that V_{max} is strongly influenced by Cl^-. The strong reduction of the V_{max} observed in the absence of Cl^- indicates that part of the Na^+-dependent glycine uptake is also Cl^- dependent. The presence of a Cl^--dependent component of glycine uptake was confirmed by studying the dependence of the uptake on the amino acid concentration.

From these data (Fig. 5) it appears that the transport of glycine is sustained by three different transport mechanisms: (1) diffusion, (2) Na^+-dependent, Cl^--independent mediated transport, (3) Na^+-dependent, Cl^--dependent mediated transport.

The diffusional component is quite small (smaller than the one found for alanine in intestinal brush-border membrane vesicles [25]); it contributes very little to the total uptake at physiological concentration.

The Na^+-dependent, Cl^--independent transport is a low-affinity, high-capacity transport system; it significantly contributes to the amino acid uptake. At 0.1 mM glycine concentration, about 30% of the mediated uptake occurs by this mechanism. The system is clearly electrogenic (Fig. 6B), indicating the existence of a sodium glycine cotransport mechanism possibly involving two sodium ions per glycine molecule (Fig. 4).

The major component of the glycine uptake, at physiological glycine concentration, is due to the high-affinity, low-capacity transport system.

Two glycine transport systems in kidney cortex of different animal species have been described by various authors [4–7,26]. The kinetic constants of the systems described in our study are similar to those found for glycine transport in human kidney cortex [6]. The pig low-affinity glycine transport system might be similar to the so called iminoglycine transport system (shared by glycine, proline, hydroxiproline) [4]. In isolated perfused tubule, Barfuss et al. [7] have shown that glycine transport occurs via two different transport systems located at different length of the proximal tubule (proximal convoluted and proximal straight tubules). It cannot be excluded that the two transport components found in our study reflect two different membrane vesicle populations isolated from the proximal convoluted and proximal straight tubules.

The high-affinity, low-capacity glycine transport system (system 1 in Fig. 5B) is simulta-

neously dependent on the presence of both Na^+ and Cl^- ions.

To explain the effect of Cl^- on glycine transport, two hypotheses could be advanced: (1) Cl^- interacts with specific binding sites on the Na^+/amino acid cotransporter at the external side of the membrane, without being transported; (2) Cl^- is one of the substrates of an Na^+/Cl^-/amino acid cotransport mechanism.

The first hypothesis was advanced by Vidaver [10] to interpret the Cl^- stimulating effect on glycine transport in red blood cells. The chloride ion was supposed to be a cofactor of the Na^+/glycine cotransport; the requirement of Cl^- by this transport system is scarcely specific. This interpretation has also been proposed to explain the Cl^- stimulatory effect on glutamate transport in rat intestinal brush-border membrane vesicles [15]. On the other hand in spite of much indirect evidence, the only available data in favour of direct coupling and cotransport of the Cl^- ions are those by Gerencser [27]. These data obtained by short-circuit current measurements, demonstrate that glycine specifically increases the Cl transepithelial fluxes.

Recently, in renal brush-border membrane vesicles a direct coupling of the β-alanine transport with both sodium and chloride ions has been demonstrated [28].

As regards the effect of Cl^- on the high-affinity Na^+-dependent glycine transport system located in pig kidney cortex brush-border membrane, two Cl^- ions seem to interact with system 1 (Fig. 3). Furthermore the observation that this system appears to be electroneutral (Fig. 6) would fit with a model of a cotransport mechanism involving Na^+, Cl^- and glycine, with a stoichiometry ratio 2 : 2 : 1.

The existence of a Na^+/Cl^-/amino acid cotransport would also explain the stronger stimulating effect of the Cl^- gradient on the Na^+-dependent glycine uptake (Fig. 2A).

Cassola et al. [29] have shown that Cl^- is accumulated against its electrochemical gradient in rat kidney proximal tubule cells and thus the existence of a Cl^- active transport has been proposed. In the case that the Cl^- electrochemical gradient in pig proximal tubular cells is similar to that of rat kidney the high-affinity glycine transport system using the Na^+ electrochemical gradient would permit the absorption of both the amino acid and Cl^- ions.

Acknowledgements

We thank Dr. G. Prezioso for reading the manuscript, and Dr. B. Tarantino for revising the English text.

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