

Stoichiometry of sodium- and chloride-coupled glycine transport in synaptic plasma membrane vesicles derived from rat brain

M. Carmen Aragón, Cecilio Giménez and Federico Mayor

Departamento de Biología Molecular, Centro de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

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The stoichiometric properties of the glycine transporter were studied in synaptic plasma membrane vesicles from rat brain. The present results, together with previous data from our laboratory, allow us to suggest a stoichiometry of 2 Na⁺ and 1 Cl[−] per glycine zwitterion for the translocation cycle catalyzed by the glycine carrier. We propose a kinetic model with an ordered mechanism for the binding/debinding of solutes.

Stoichiometry; Glycine transport; Membrane vesicle; (Rat brain)

1. INTRODUCTION

It is now well established that the active step in the vectorial transport of many solutes across mammalian plasmatic membranes occurs via cotransport of the substrate with Na⁺ at one side of the membrane [1]. Using synaptic plasma membrane vesicles, it has been shown that this general concept also applies to neurotransmitters in the brain. Thus, the electrochemical potential gradient of Na⁺ serves as a direct driving force for the transport of GABA [2], glutamate [3], glycine [4], and aspartate [5]. These high affinity, sodium-dependent transport systems are directly involved in the termination of the neurotransmitters' action, and may probably also function in the release process by providing carrier-mediated efflux of cytoplasmic neurotransmitter amino acids [6,7]. Glycine has been shown to be an inhibitory

neurotransmitter in the central nervous system of vertebrates, mainly in the spinal cord and in some areas of the brain [8,9]. We have previously reported [4] that the transport of glycine in membrane vesicles derived from rat brain synaptosomes is carried out by two systems with different affinities for the substrate, the high-affinity one being an electrogenic process strictly dependent on the simultaneous presence of Na⁺ and Cl[−] in the medium. Recent experiments from our laboratory strongly suggested that the glycine carrier catalyzes the influx of glycine coupled with the influx of both Na⁺ and Cl[−] [10]. Direct measurements of the stoichiometric coupling of Na⁺ and Cl[−] to the transport of this neurotransmitter are important from a more fundamental point of view since they provide information essential for the determination of the mechanistic aspects of the transport process.

Here, we describe a kinetic estimation in order to study the stoichiometry of the process in synaptic plasma membrane vesicles. Evidence is provided indicating that 2 Na⁺ and 1 Cl[−] are involved in the translocation cycle catalyzed by the glycine transporter.

Correspondence address: M.C. Aragón, Departamento de Biología Molecular, Centro de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

2. MATERIALS AND METHODS

Membrane vesicles from adult male rats were prepared and stored as in [2,11]. The vesicles were preloaded, prior to the transport assays, with a 406 mosM medium, pH 7.4, and an ionic composition depending on each particular experiment. Portions (10 μ l) of the suspension of membrane vesicles derived from rat brain (~0.1 mg protein), preloaded with the desired ionic medium, were preincubated for 1 min at 25°C. Uptake was started by adding 40 μ l of a solution containing [14 C]glycine (20 μ M final concentration, spec. act. 118 Ci/mol). The stopping of the reactions, filtration, washing and counting were performed as described for glycine transport [4]. Membrane protein was determined by the method of Resch et al. [12]. Experimental points were determined in triplicate. The error bars shown in the figures are the standard deviations on the points. The results of representative experiments are shown.

The equation for each kinetic model (those of Garay and Garrahan [13] and Hill – see [14]) was fitted to the experimental data for membrane vesicle contents (calculated from the label contents) by means of a conventional computer program with a weighted least-squares iterative algorithm.

3. RESULTS AND DISCUSSION

Direct measurements of the coupling of Na^+ and Cl^- to the high-affinity transport of glycine were performed, with the aim of obtaining ion/solute stoichiometries. We used a kinetic approach involving direct measurements of the initial rate of glycine upon imposition of an ion gradient across the membrane [15]. The sigmoid relationship between the initial rate of glycine uptake and the increase in Na^+ concentrations (iso-osmolarity maintained with choline chloride) observed in fig.1 suggests that more than 1 Na^+ is associated with the process. On the other hand, as shown in fig.2, an increasing Cl^- concentration in the extravesicular medium (iso-osmolarity maintained with sodium gluconate) produces a hyperbolic stimulation in the rate of glycine transport. The kinetic characteristics of the interaction of Na^+ and Cl^- with the glycine carrier were analyzed by using two models designed to describe the kinetics of multiple substrate/activator reactions. Similar

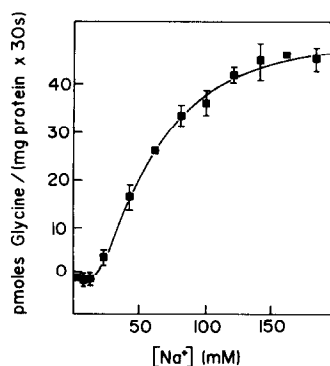


Fig.1. Effect of increasing Na^+ concentrations on the transport of glycine. Membrane vesicles were preloaded with 180 mM choline chloride, 22 mM K^+ phosphate, 1 mM MgSO_4 , pH 7.4. The vesicles were diluted into a medium containing labelled glycine, 22 mM K^+ phosphate, 1 mM MgSO_4 and increasing concentrations of NaCl , with choline replacing sodium iso-osmotically. $V_{\max}$ and K' values from the Hill model were 49.57 pmol/mg protein per 30 s and 3376.03 mM, respectively for an h value of 2. $V_{\max}$ and K' parameters were obtained by fitting the experimental data with a non-linear least-squares algorithm, assuming constant integer values for the parameter h .

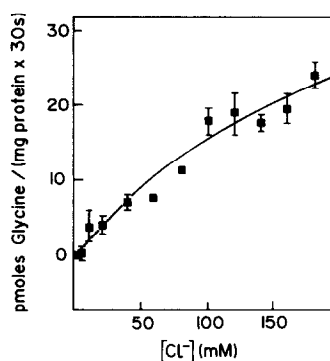


Fig.2. Effect of increasing Cl^- concentrations on the transport of glycine. Membrane vesicles were preloaded with 180 mM Na^+ gluconate, 22 mM K^+ phosphate, 1 mM MgSO_4 , pH 7.4. The vesicles were diluted into a medium containing labelled glycine, 22 mM K^+ phosphate, 1 mM MgSO_4 and increasing concentrations of NaCl ; with gluconate replacing chloride iso-osmotically. $V_{\max}$ and K' values from the Hill model correspond to 58.66 pmol/mg protein per 30 s and 278.02 mM, respectively for an h value of 1. These parameters were calculated as indicated in fig.1.

results were obtained for Cl^- with either the Garay and Garrahan model and/or the Hill model, the best fit being attained when the number of Cl^- involved in the process is 1. In both cases (Garay-Garrahan and Hill models) a Michaelis-Menten type of dependence on chloride concentration is obtained. This suggests that the chloride sites are non-interacting and could mean that 1 Cl^- interacts with the carrier for each glycine molecule. However, for Na^+ , the optimum fit is achieved when the experimental data are analyzed by the Hill equation, corresponding to an h (Hill index) value of 2.

The fact that the process is electrogenic (positive charge moving inward) imposes restrictions on the possible values for the stoichiometry of the process. Assuming that glycine is transported in its predominant form at neutral pH the zwitterion, a relationship for the carrier like $2 \text{Na}^+ : \text{Cl}^- : \text{glycine}$ suggested from our results is adequate to explain the electrogenicity of the process and the kinetic characteristics of the interaction of Na^+ and Cl^- with the glycine transporter. On the other hand, it is interesting to recall some of the classical studies by Imler and Vidaver [16] on the glycine carrier from pigeon erythrocytes. This system transports two Na^+ per glycine molecule, and also displays an absolute requirement for Cl^- . It is noteworthy to emphasize the potential importance of coupling multiple Na^+ to the transport of any substrate. Coupling coefficients greater than 1 impart a thermodynamic advantage for uphill transport; the potential energy in the Na^+ electrochemical gradient is a power function based on the coupling coefficient [17].

In fig.3, we present a kinetic model of the Na^+/Cl^- -glycine transport system, that appears to be consistent with the present experimental observations and those reported in previous studies [4,10].

Similar models have been proposed for GABA, L-glutamic acid and β -alanine transport systems [18–22]. This model implies that only fully loaded carriers ($\text{TNa}_2\text{-SCl}$) can transport glycine, and suggests that binding and debinding sequences occur through an ordered mechanism having the same order and stoichiometry, according to a symmetrical property of the influx and efflux processes. Both occur via the same route, but in opposite directions.

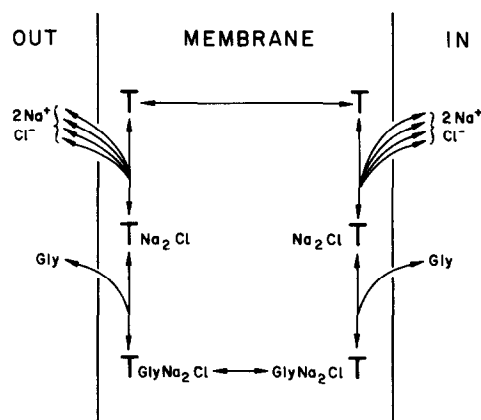


Fig.3. Proposed model for the mechanism of glycine translocation cycle. As discussed in the text the binding and debinding orders of Na^+ , Cl^- and glycine, indicated here for the inside and the outside of the membrane, fit the experimental data, however other models are not ruled out.

One interesting observation is the apparent independence of the homoexchange from the external Cl^- and Na^+ , although the effect is exerted via the carrier [10]. This fact suggests that, upon translocation from the inside, radioactive glycine is released on the outside and unlabelled glycine rebinds, whereas Cl^- and Na^+ would remain bound to their sites on the carrier and would not need to reassociate from the outside.

ACKNOWLEDGEMENT

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