Choline transport by synaptosomal membrane vesicles isolated from insect nervous tissue

Heinz Breer

Universität Osnabrück, Arbeitsgruppe Zoophysiologie, Seminarstraße 20, D-4500 Osnabrück, FRG

Received 13 December 1982; revision received 17 January 1983

Membrane vesicles derived from insect nervous tissue are capable of accumulating choline via a high affinity, carrier-mediated process with ion gradients as the sole driving force. The transport is strictly dependent on the presence of Na^+ and Cl^- in the medium and is stimulated by a membrane potential.

Choline uptake, high affinity *Insect synaptosome* *Membrane vesicle* *Ion gradient*

1. INTRODUCTION

The basic mechanism of the high affinity choline uptake, a carrier-mediated process which seems to be restricted to cholinergic nerve endings [1] and is supposed to be a rate-limiting step in the synthesis of acetylcholine [2], is still poorly understood; partly because adequate studies have been impeded by intracellular sequestration and metabolic activities. Experiments using membrane vesicles derived from synaptosomal preparations have greatly contributed to evaluation of the basic requirements for the transport of neuroactive amino acids [3–5]. These vesicles lack the complex energetics, metabolism and compartmentation of intact synaptosomes and thus provide a suitable system for studying mechanisms of transport. Attempts to analyse the transport of choline, the essential precursor of acetylcholine, in membrane vesicles derived from rat cortical synaptosomes were not successful [6]. Therefore, membrane vesicles were used here which derived from synaptosomal preparations of insect central nervous tissue being much more cholinergic than vertebrate brain tissue [7].

2. MATERIALS AND METHODS

2.1. *Materials*

[*N-Me*-^{3}H]Choline chloride (spec. act. 80 Ci/mmol) was obtained from New England Nuclear. Hemicholinium-3, choline bromide, valinomycin and gramicidin, was received from Sigma. Monensin was purchased from Calbiochem.

2.2. *Preparation of membrane vesicles*

From the nervous tissue of locust crude synaptosomal fractions were prepared as in [8] and membrane vesicles were produced according to [3] as modified for insect nervous tissue [9]. Membrane vesicles were suspended in loading solution (0.1 mM potassium phosphate buffer (pH 6.8), 1 mM $MgSO_4$) frozen in liquid nitrogen and stored at −70°C.

2.3. *Transport assay*

For transport assays vesicles were thawed and incubated for 10 min at 25°C, then the vesicles were centrifuged (10 min, 27000 × *g*) and resuspended in loading solution. Aliquots of the preloaded vesicles were incubated in a medium of 150 mM NaCl, 1 mM $MgSO_4$ buffered with 5 mM Hepes (pH 7.0) and 0.5 μM choline (spec. act. 1.05 Ci/mmol) 25°C for 2 min. The experiment was terminated by diluting with 5 ml ice-cold NaCl (0.8 M) and immediately filtering through a moistened cellulose acetate filter (0.45 μm pore size; Sartorius, Göttingen) attached to a vacuum assembly. The filters were rinsed twice with 2 ml ice-cold NaCl. The dilution, filtration and washing

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00 © Federation of European Biochemical Societies

procedures were performed within 15 s. After drying, the radioactivity on the filter was measured by liquid scintillation spectrometry using Triton-Lipoluma (Baker) as scintillant with an efficiency of 35%. All the experiments were corrected for a control obtained by dilution of the external medium before addition of the membrane suspension. Protein was determined as in [10].

When the filtered membranes were washed with distilled water instead of the isotonic NaCl solution, little or no radioactivity was retained on the filters, indicating that choline was accumulated within membrane fractions susceptible to osmotic lysis.

3. RESULTS

Membrane vesicles derived from synaptosomal preparations of insect ganglia, loaded with potassium phosphate and incubated in NaCl accumulated choline with a time course illustrated in fig.1. The transport was hemicholinium- and temperature-sensitive and characterized by an initial rate of 75 pmol.min^{-1}.mg protein^{-1} and a maximal extent of ~160 pmol/mg after 5 min. This uptake was apparently transport, since the accumulated material was found to be sensitive to the osmolarity of the medium. If one considers a maximum intravesicular volume of 5.6 μl/mg protein [9] the accumulated solute represented internal [choline] of ~30 μM. Since the external [choline] was 0.5 μM this represents a concentration gradient of ~60-fold. These values represent a minimum degree of concentration, since it is presumed that not all the vesicles in the preparations which contribute to the osmotic space are capable of accumulating choline via a high affinity transport system.

The transport was essentially dependent on the presence of Na^+ as well as the existence of a sodium gradient (out > in). Similar gradients of other monovalent cations ($Tris^+$, Li^+) were ineffective (table 1). The importance of a sodium gradient was further emphasized by results using ionophores. Gramicidin D as well as the more specific monensin drastically reduced both the initial rate of uptake and the overall accumulation of choline (table 1).

The replacement of Cl^- in the incubation solution with phosphate ions, achieved by substituting sodium phosphate buffer for NaCl almost completely abolished the transport. Chloride could also not be replaced by other anions like sulphate and thiocyanate. However, a chloride gradient in the absence of Na^+ was not able to drive the choline transport (fig.1); thus maximal accumulation was achieved when both ion gradients were present. In experiments to determine the specific requirements of the intravesicular ions it could be demonstrated that only membrane vesicles containing K^+ were able to accumulate choline (table 1); sodium, $Tris^+$ as well as glycine (non-electrolyte) failed to promote choline transport. The potassium diffusion potential mainly contributes to the membrane electrical potential, therefore the effect of valinomycin, an ionophore which selectively enhances the membrane potential

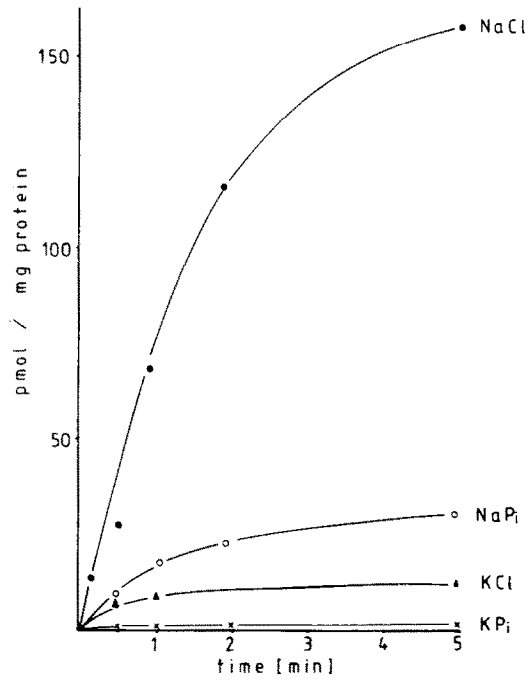


Fig.1. Choline uptake by synaptosomal membrane vesicles of insect. Aliquots of membrane vesicles preloaded with 0.1 M potassium phosphate (pH 6.8), 1 mM Mg_2SO_4, were incubated as in section 2 in the following media containing 1 mM $MgSO_4$: (●) NaCl (150 mM), Na^+ and Cl^- gradient; (▲) KCl (150 mM), Cl^- gradient in the absence of Na^+; (○) Na^+-phosphate (150 mM), Na^+ gradient in the absence of Cl^-; (×) K^+-phosphate (150 mM), no gradient.

Table 1

The effect of ions and ionophores on choline transport in membrane vesicles

Internal	External	Addition	Uptake 5 min (% control)
K-phosphate	NaCl	–	100
	LiCl	–	9.84
	KCl	–	7.46
	Tris–HCl	–	7.12
	NaCl	Gramicidin	19.05
	NaCl	Monensin	22.80
	Na-phosphate	–	15.79
	Na-acetate	–	13.52
	NaSCN	–	3.56
Na-phosphate	NaCl	–	7.75
Tris-phosphate	NaCl	–	3.51
Glycine	NaCl	–	1.72

and produces a more negative intravesicular electrochemical force, was studied. Valinomycin significantly stimulated transport of choline (fig.2); however, valinomycin had no effect in the absence of a potassium gradient (in > out). Furthermore the proton ionophore carbonyl cyamide *m*-chlorophenylhydrazone (CCCP) substantially inhibited choline transport. These data are consistent with the concept that choline transport is electrogenic.

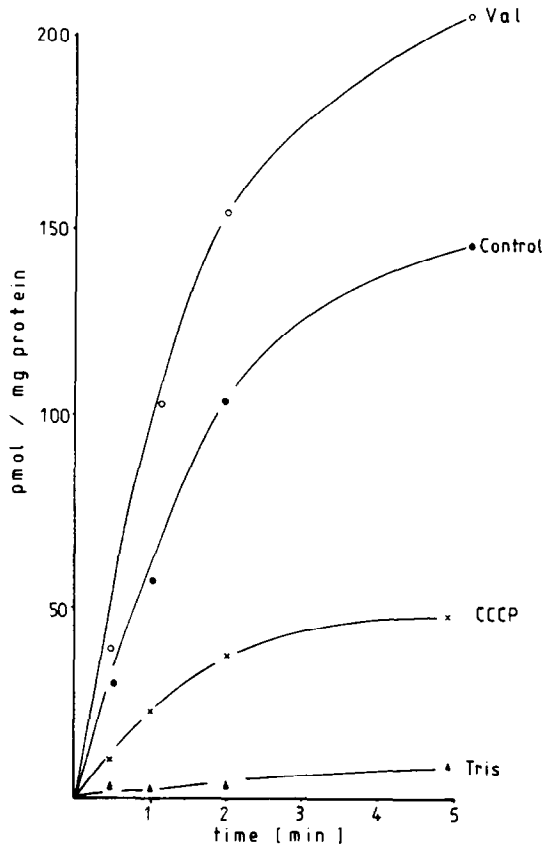


Fig.2. Choline transport into membrane vesicles under different energy states. Vesicles were loaded with 0.1 M potassium phosphate, 1 mM $MgSO_4$, 5 mM Tris (pH 6.8). Incubation media: (●) NaCl, control; (○) NaCl + valinomycin, Na^+ gradient + membrane potential; (×) NaCl + CCCP, Na^+ gradient, no membrane potential; (▲) Tris–HCl, no Na^+ gradient + membrane potential.

4. DISCUSSION

Membrane vesicles prepared from insect synaptosomes displayed concentrative transport of choline in the absence of any exogenous energy source other than that imposed by artificially created ion gradients. Choline transport appears to be essentially dependent on the presence of external Na^+ and Cl^-; and, as with the translocation of amino acids and glucose in membrane vesicles of various origin [11–13], the sodium electrochemical potential is obviously a main driving force for high-affinity choline uptake. The effects of valinomycin and CCCP indicate that the transport of choline is an electrogenic process which is stimulated by a membrane potential. With respect to substrate affinity and Na^+ dependence, choline transport resembled the uptake of choline by insect synaptosome [14]. In intact synaptosomes, the ion gradients are presumably created by $(Na^+ + K^+)$-ATPase, which may account for the ouabain-sensitivity of choline transport in synaptosomes [14]. Experiments in progress may help to evaluate the specific contributions of the various ions in the process of choline translocation (cotransport, carrier activation, membrane potential) and to get a more quantitative insight in the stoichiometry of the respective solutes. To get more detailed knowledge on the molecular aspects of the high affinity choline transport in nerve endings isolation

and reconstitution of the choline transporter in proteoliposomes [6,15] appear to be an appropriate approach.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the excellent technical assistance of Mrs Düvel and is indebted to Professor W. Lueken and Dr G. Jeserich for encouragement and valuable discussion. This investigation was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Kuhar, M.J. and Murrin, L.C. (1978) J. Neurochem. 30, 15–21.
[2] Barker, L.A. and Mittag, T.W. (1975) J. Pharm. Exp. Ther. 192, 86–94.
[3] Kanner, B.I. (1978) Biochemistry 17, 1207–1211.
[4] Roskoski, R., Rauch, N. and Roskoski, L.M. (1981) Arch. Biochem. Biophys. 207, 407–415.
[5] Mayor, F. jr, Marvizon, J.G., Aragòn, M.C., Gimènez, C. and Valdivieso, F. (1981) Biochem. J. 198, 535–541.
[6] Meyer, E.M. and Cooper, J.R. (1982) Science 217, 843–845.
[7] Breer, H. (1981) J. Comp. Physiol. 141, 271–276.
[8] Breer, H. (1981) Neurochem. Int. 3, 155–163.
[9] Gordon, D., Zlotkin, E. and Kanner, B.I. (1982) Biochim. Biophys. Acta 688, 229–236.
[10] Bradford, M.M. (1976) Anal. Biochem. 65, 248–254.
[11] Rudnick, G. (1977) J. Biol. Chem. 252, 2170–2174.
[12] Sacktor, B., Rosenblom, I.L., Liang, C.T. and Chang, L. (1981) J. Membr. Biol. 60, 63–72.
[13] Goto, K. and Mizushima, S. (1978) J. Biochem. 84, 251–259.
[14] Breer, H. (1982) J. Neurobiol. 13, 107–117.
[15] King, R.G. and Marchbanks, R.M. (1982) Biochem. J. 204, 565–576.