Ca TRANSPORT AND ATPase ACTIVITY OF SYNAPTOSOMAL VESICLES FROM RAT BRAIN

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1. Introduction

Nerve endings are characterized morphologically by the presence of a large number of small vesicles. These synaptic vesicles are generally accepted as the site of neurotransmitter storage [1,2]. Recently, evidence from several laboratories indicated [3,4] that beside their function in storing and releasing neurotransmitters, synaptic vesicles exhibit an ATP-dependent calcium uptake. To date, direct evidence does not exist that these two functions, neurotransmitter storage and calcium transport, reside in the same or two separate populations of vesicles. For this reason we refer to the organelle that transports calcium as synaptosomal vesicle [4].

Synaptic vesicles from *Torpedo marmorata* [5] and coated synaptic vesicles from calf brain [3] were shown to possess Ca-Mg ATPase. ATPase from synaptic vesicles of guinea pig brain was activated [6] by magnesium and partially by calcium. We report here that rat brain synaptosomal vesicles possess a Mg-ATPase activity which is associated with the calcium transport of rat brain synaptosomal vesicles.

2. Methods

Synaptosomal vesicles were prepared by centrifuging rat brain synaptosomal lysates at 100 000 X *g* for 60 min as in [4]. Calcium transport studies were performed with $^{45}CaCl_2$ (The Radiochemical Centre, Amersham) using the millipore filtration technique. Following incubation at 22°C the reactions were stopped by quick dilution with ice cold 0.15 M NaCl and rapidly filtered through 0.45 μm millipore filters. The filters were washed twice with 2 ml 0.15 M NaCl, dried and counted in liquid scintillation counter. ATPase activity was determined by measuring the amount of phosphate liberated following a 10–20 min incubation period at 22°C by the method in [7]. Phosphate was not liberated either by the vesicles in the absence of added ATP, or by ATP itself when vesicles were absent. Protein was determined by a micro-modification of the Lowry method [8]. The composition of incubation media is given in the appropriate legends to tables or figures.

All reagents used were of analytical grade.

3. Results

The ATP-dependent calcium transport in synaptosomal vesicles is completely dependent on the presence of magnesium. This is illustrated in fig.1. In the absence of magnesium, calcium was not transported. Addition of magnesium to synaptosomal vesicles in the presence of 2 mM ATP led to calcium transport which increased with increasing magnesium concentrations till a maximal value was observed about 0.5 mM $MgCl_2$.

Magnesium activated an ATPase located in the synaptosomal vesicle preparation. This ATPase could also be activated by manganese. Table 1 shows the activation of the ATPase with divalent ions and the calcium transport resulting under identical conditions.

EGTA, which is well known to chelate calcium ions was tested for its effect on ATPase activity. This was done in order to test for endogeneous-bound cal-

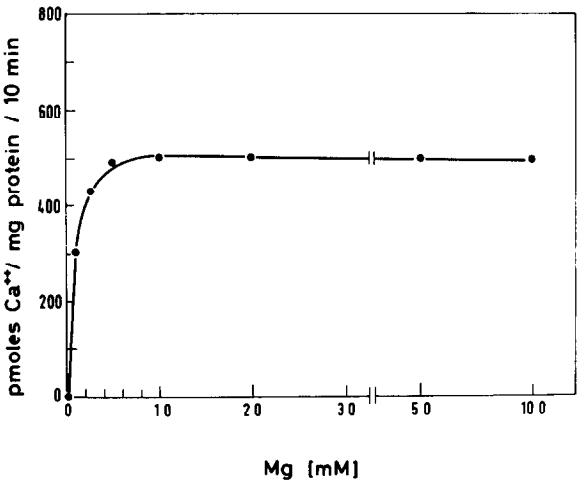

Fig.1. Magnesium dependence of calcium transport. The reaction mixture contained in final vol. 0.2 ml: 2 mM ATP, Tris, pH 7; 0.58 μM $^{45}CACl_2$; 10 mM Tris–HCl, pH 7.4; 0.3 M mannitol; $MgCl_2$ as stated 50 μg vesicle protein. The ATP-independent calcium transport was subtracted.

cium ions in the synaptosomal vesicle preparation. As seen in table 1, addition of 0.1 mM EGTA to 0.1 mM $MgCl_2$ resulted in the same ATPase activity as obtained in the presence of 0.1 mM magnesium only.

Magnesium and manganese could both activate the ATPase under similar experimental conditions to approximately the same extent (table 1). But calcium transport in the presence of magnesium showed a plateau between 0.5 mM and 5.0 mM, while increasing the concentration of manganese ions resulted in a biphasic curve (fig.2) and above 1 mM manganese, inhibition of calcium transport ensued.

Calcium could also activate the ATPase. Figure 3 shows the ATPase activity obtained in the presence of calcium, magnesium and a mixture of these two ions. It can be seen that although calcium could substitute for magnesium in ATPase activation, the maximal activity achieved with Mg^{2+} was about 2.5-times higher than that with calcium. Addition of both divalent ions resulted in a lesser activity compared to magnesium only.

Table 1
The effect of divalent cations on the ATP-dependent calcium transport and ATPase activity

Additions (mM)	ATP hydrolysis (μmol/mg protein/10 min)	Ca transport (pmol/mg protein/10 min)
Mg		
0.1	1.051	92.28
0.5	2.38	148.3
5.0	2.68	144.7
Mg 0.1, EGTA$^+$ 0.1	0.889	–
Mg 0.5, EGTA$^+$ 0.1	2.42	–
Mn		
0.1	0.889	52.6
0.5	2.58	152.7
5.0	2.15	17.1

Reaction mixture contained, in 0.2 ml: 10 mM Tris–HCl pH 7.4; 0.3 M mannitol, 2 mM ATP, Tris, pH 7.0; 0.48 μM $^{45}CaCl_2$; synaptosomal vesicle protein about 50 μg and divalent cations as stated

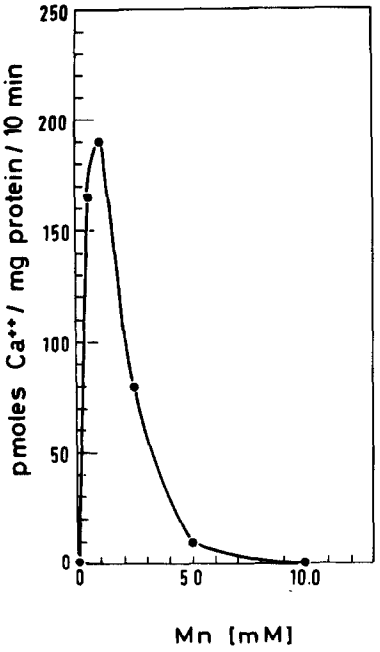

Fig.2. The effect of manganese on calcium transport. Reaction mixture contained in 0.2 ml: 10 mM Tris–HCl, pH 7.4; 2 mM ATP, Tris, pH 7; 0.58 μM $^{45}CaCl_2$; 0.3 M mannitol; $MnCl_2$ as stated. The ATP-independent calcium transport was subtracted.

ATP-dependent calcium transport and Mg-ATPase activity are strongly linked together. Inactivation of the ATPase with DCCD (dicyclohexyl carbodiimide) an agent well known to inhibit ATPases [9] led to comparable inhibition of the calcium transport (table 2).

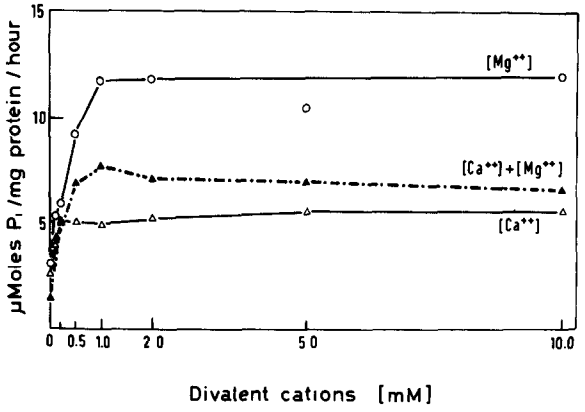

Fig.3. The effect of magnesium, calcium and magnesium + calcium on the ATPase activity of synaptosomal vesicles. The reaction mixture contained in final vol. 0.2 ml: 10 mM Tris–HCl, pH 7.4; 2 mM ATP Tris, pH 7; 0.3 M mannitol; divalent ions were added as stated.

4. Discussion

Regulation of intracellular calcium ion concentration plays a major role in most secretory and release processes [10]. Two main parameters contribute to the amount of $[Ca]_{in}$:

1. The entry and exit of calcium through the cell plasma membrane.
2. Its sequestration and release by intracellular organelles and proteins [11–13].

Since membranes have very low permeability to calcium ions under resting physiological conditions, special transport mechanisms exist to control the

Table 2
The effect of DCCD on calcium transport and Mg-ATPase activity in synaptosomal vesicles

DCCD (μM)	Calcium transport (pmol/mg protein/10 min)	Mg-ATPase activity (μmol/mg protein/10 min)
0	238.77	1.182
50	184.4	0.538
100	111.2	Q.28

Composition of incubation medium: 10 mM Tris–HCl, pH 7.4; 0.3 M mannitol; 2 mM ATP; 5 mM $MgCl_2$, 0.9 μM $^{45}CaCl_2$, synaptosomal vesicles about 50 μg in final vol. 200 μl. DCCD was added to the vesicles for 10 min preincubation and then the reaction was started by addition of Ca^{2+} and ATP

level of intracellular calcium. In most cases where isolation of the relevant membraneous structures was possible, special ATPases were found to be directly involved in energy-dependent transport process of calcium. The best studied examples are the Ca-Mg-ATPase of the sarcoplasmic reticulum [14], the calcium transport system of mitochondria [15] and the Ca-Mg ATPase of erythrocyte plasma membrane [16]. Except in the case of the mitochondrial calcium transport system where transport of calcium can take place by using the energy supplied by the electron transport [17]; in all other cases the ATPase activity could not be dissociated from the calcium transport [14].

In this work, the nature of the ATPase involved in Ca transport into synaptosomal vesicles has been investigated. The results suggest that this ATPase is magnesium dependent. This conclusion is based on the following:

(i) Mg as the sole ion in the presence of EGTA (to sequester endogeneous calcium in the vesicle preparation) activates the ATPase.
(ii) Maximal activation of the ATPase was obtained in the presence of magnesium as a sole divalent ion.
(iii) Calcium could substitute for magnesium but did not activate the enzyme to the same extent.
(iv) Addition of calcium to the magnesium did not further activate the ATPase and even inhibited it.

Immunological evidence [3] pointed to the similarity between sarcoplasmic reticulum and coated synaptic vesicles possessing ATPase activity and taking up calcium in an energy-dependent fashion. The residual ATP-dependent calcium uptake found in shocked synaptosomes may originate [18] in sarcoplasmic reticulum type membrane vesicles. Synaptosomal vesicles transporting calcium possess glucose-6-phosphatase activity [4] indicative of endoplasmic reticulum type membraneous structures. Some properties of the ATPase of the synaptosomal vesicles are markedly different from those of the sarcoplasmic reticulum. The main difference lies in the ability of synaptosomal vesicle ATPase to hydrolyze ATP to the same extent in the presence and in the absence of calcium (reached by addition of 0.1 mM EGTA) provided 0.1 mM Mg was present. Sarcoplasmic reticulum Ca-Mg ATPase was completely inhibited under these conditions even in the presence of Mg as high as 5 mM [19]. Sensitivity to Mersalyl was much more pronounced in the sarcoplasmic reticulum ATPase which was completely inhibited by 10^{-6} M Mersalyl, while synaptosomal vesicle Mg-ATPase and calcium transport were inhibited only to about 50% by 10^{-4} M Mersalyl.

Contrary to mitochondrial calcium transport which is very sensitive to Ruthenium Red [14] we could not observe any inhibition of the synaptosomal vesicle calcium transport with Ruthenium Red up to 1 mM.

Manganese could substitute for Mg in the activation of the ATPase very efficiently. The inhibition of the calcium transport was probably due to competition with calcium for the uptake process, since in many calcium transporting organelles Mn^{2+} transport can take place [14,15].

A calcium-dependent ATPase activity in a subfraction from the 14 000 g pellet of synaptosomal lysates was found [20]. In this crude fraction, however, very high Ca-independent background ATPase activity was reported [20] which complicated the interpretation.

In view of all these properties, it seems that the calcium transport synaptosomal vesicles is an independent and newly discovered process within the nerve terminals and might well play a major role in the regulation of $[Ca]_{in}$ and neurotransmitter liberation.

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