CALCIUM TRANSPORT IN A VESICULAR MEMBRANE PREPARATION FROM RAT BRAIN SYNAPTOSOMES

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1. Introduction

Intracellular calcium ion concentration plays a major role in neurotransmitter release [1]. Nerve cells at rest are almost impermeable to calcium ions. The arrival of the action potential at the nerve terminal leads to an increase in calcium conductance of the presynaptic membrane. Since $[Ca]_{in}$, as measured directly in the squid axon [2], is 10^{-7} M or less and $[Ca]_{out}$ is at least 3 orders of magnitude higher, the increase in calcium permeability leads to an influx of calcium, a rise in $[Ca]_{in}$ and consequently to a transmitter release.

Several processes take part in lowering the transiently higher $[Ca]_{in}$: calcium uptake by intraterminal mitochondria [3], calcium extrusion through the presynaptic membrane in a sodium-dependent process [4], calcium binding to cytoplasmic proteins and small molecules [5] and calcium diffusion from the terminal area.

Recently the involvement of reticulum type membrane [6] in calcium metabolism of nerve terminals was suggested. Isolated coated synaptic vesicles were shown to take up calcium in an ATP-dependent fashion [7]. In the present work we show that an isolated vesicular fraction from rat brain nerve endings is capable of transporting calcium against a concentration gradient. The calcium transporting properties of these vesicles are such that they could participate in the regulation of $[Ca]_{in}$ of nerve endings.

2. Experimental

Fractionation of day 14 rat brain homogenates was performed essentially as in [8]. Synaptosomes were collected from the 12%/8%. Ficoll interphase washed in 0.3 M Mannitol and lysed for 40 min in 5 mM Tris–HCl buffer, pH 7.4, 1 mM EDTA (K). The 20 000 × *g* pellet of the synaptosomal lysate was discarded and the vesicular membrane preparation was obtained by centrifuging for 60 min at 100 000 × *g*. This vesicular fraction is named synaptosomal vesicles. Transport studies were performed with $^{45}CaCl_2$ (The Radiochemical Center, Amersham) using the Millipore filtration technique. Millipore filters, 0.45 μm, were used. The calcium concentration in the solutions was measured in a Perkin Elmer atomic absorption spectrophotometer and the values taken into account calculating calcium concentrations. The calcium ionophore A23187 was a generous gift from Dr R. J. Hosley of Ely Lilly, Indianapolis, IN. Protein was determined by a micromodification of the procedure in [9] and phosphate was determined as in [10]. ATPase was assayed as in [11], 5′-AMP nucleotidase as in [12] and glucose-6-phosphatase as in [13]. All reagents were of analytical grade.

3. Results

The uptake of calcium by synaptosomal vesicles is shown in table 1. At 1.6 μM [Ca], addition of ATP increases calcium uptake by about 6-fold, when compared with the uptake obtained in its absence.

In order to show that the calcium taken up by the synaptosomal vesicles is due to net transport of calcium from the medium into the vesicles, 2 types of experiments were performed: The average internal volume of the vesicles was calculated, from the distribution

Table 1
Calcium uptake by synaptosomal vesicles

	Medium[a]	Medium[a] + 2 mM ATP
Mean calcium uptake (pmol/mg protein/10 min)	311.28	1855.3
Standard deviation	100.44	225.18
No. expts[b]	8	8
SEM	35.4	78.16
Mean ATP-dep. uptake / Mean ATP-indep. uptake	5.96	

[a] Medium composition was the following: 80 mM KCl, 10 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, 1.62 μM $^{45}CaCl_2$. Final vol. 200 μl, containing approx. 100 μg vesicular protein

[b] Each experiment is a separate preparation from 30 g rat brain

of ^3H_2O and [^{14}C]inulin in the supernatant and in the vesicular pellet [14]. The average internal volume of the vesicle preparation is 3.04 μl/mg protein (table 2).

Table 2
Internal volume of the synaptosomal vesicles

	μl/mg protein
Internal volume	3.04
Standard deviation	0.709
No. expts	5
SEM	0.317

Table 4
Enzyme activities in the vesicles

	μmol P_i formed/mg protein/h
ATPase	12.05
5′-AMP nucleotidase	0.87
Glucose-6-phosphatase	1.81

Since the mean calcium taken up by the vesicles is 1855 pmol/mg protein/10 min, the mean concentration of calcium within the vesicles is 618 μM. Therefore, the intravesicular [Ca] is 400-fold higher than the calcium concentration of the medium (see section 4).

The second type of experiment involved addition of the calcium ionophore A23187 to the synaptosomal vesicles. From table 3 one can learn that the addition of the calcium ionophore A23187 to the synaptosomal vesicle preparation causes a 40% decrease in the calcium content of the vesicles. This decrease occurs since the ionophore presumably causes a dissipation of the [Ca] gradient formed by the ATP-dependent transport process across the vesicular membrane.

3.1. *Enzyme activities located in the vesicular membrane fraction*

In order to trace the morphological origin of the synaptosomal vesicle preparation the following enzyme activities were tested: ATPase, 5′-AMP nucleotidase and glucose-6-phosphatase. Their average activities are given in table 4.

Table 3
The effect of the calcium ionophore A23187 on the calcium taken up by synaptosomal vesicles

	Medium[a]	Medium[a] + 1.5 μM A23187
Mean ATP-dep. calcium transport (pmol/mg protein/10 min)	1440	380
Standard deviation	450	200
SEM	200	89
No. expts[b]	5	

[a] The medium contained: 80 mM KCl, 10 mM $MgCl_2$, 10 mM Tris–HCl buffer, pH 7.4, 2 mM ATP, 1.62 μM $^{45}CaCl_2$, approx. 100 μg vesicular protein in 200 μl vol.

[b] Each experiment is a separate preparation from about 30 g rat brain

4. Discussion

In the present work we have isolated a vesicular membrane fraction from rat brain nerve terminals capable of concentrating calcium. Our results show that synaptosomal vesicles exposed to 1.6 μM [Ca] for 10 min and supplied with ATP can concentrate calcium at least 400-fold. This number is most probably a low estimate since it assumes that all the vesicles in the preparation are active in calcium transport.

Nerve terminals are characterized morphologically by the abundance of vesicular structures. Their known function is storage of neurotransmitters. Evidence exists that following a nerve impulse and rise in $[Ca]_{in}$ they fuse with the presynaptic membrane, release their contents into the synaptic cleft and are presumably reformed by membrane retrieval [15]. Thus, synaptic vesicles can be expected to share common membrane components with the presynaptic plasma membrane. 5'-AMP nucleotidase is an enzyme thought to be localized in plasma membranes from rat brain [16]. Indeed, the synaptosomal vesicle preparation exhibited 5'-AMP nucleotidase activity of 0.87 μmol AMP hydrolyzed/mg protein/h. Most ATPases found in plasma membranes are of the Na^+/K^+ ouabain-sensitive type. In the synaptosomal vesicle preparation ouabain inhibited calcium transport and ATPase activity only partially (about 25%) and at rather high concentration of ouabain (4 mM). However, if synaptosomal vesicles are formed from plasma membranes by an endocytotic process the ouabain-sensitive site is expected to be on the inside of the vesicles and not easily accessibile to added ouabain.

We found, however, strong dependence on added Mg^{2+} and Ca^{2+} (to be published). Ca, Mg-ATPase activity was found also in pure preparations of *Torpedo* synaptic vesicles containing acetylcholine [17]. The possibility that the synaptosomal vesicles transporting calcium are identical to neurotransmitter storage vesicles is suggested by our finding (E.A., M.Sc. thesis) that acetylcholine formed in intact synaptosomes from labeled choline is recovered within the same fraction. In addition, synaptic vesicles at the frog neuromuscular junction were shown to posses calcium-binding sites [18]. The alternative possibility that the calcium-transporting synaptosomal vesicles originate from endoplasmic reticulum type membrane is, of course, not ruled out since the enzyme marker glucose-6-phosphatase for brain endoplasmic reticulum [19] is present in every synaptosomal vesicles preparation tested. Synaptosomal vesicles are not an artifact formed from submitochondrial particles due to exposure of the nerve endings to hypo-osmotic media since their calcium transport is insensitive to uncouplers of oxidative phosphorylation or added atractyloside. (H.R., in preparation). Whatever the origin of these synaptosomal vesicles, their ability to concentrate calcium is presumably of physiological importance in regulating $[Ca]_{in}$ and thus transmitter release.

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