ATP DEPLETION INCREASES Ca^{2+} UPTAKE BY SYNAPTOSOMES

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1. Introduction

The role of an increase in the cytosolic Ca^{2+} activity in the initiation of synaptic transmitter release is well known [1,2]. From work done on squid axons, and isolated synaptosomes it seems that the main mechanism for the initiation of transmission occurs through an opening of verapamil-sensitive Ca^{2+} channels [3–5] leading to an increased Ca^{2+} influx and hence an increased cytosolic Ca^{2+} activity.

At rest, the cytosolic Ca^{2+} activity in squid axons is maintained near 10^{-7} M [1,6,7] and indirect estimates suggest that it is far below 10^{-6} M in synaptosomes [5]. Much controversy exists concerning the mechanisms involved in Ca^{2+} regulation in nervous tissue. Blaustein [8] has claimed that the cytosolic Ca^{2+} activity in squid axons and synaptosomes [9,10] is regulated by Ca^{2+} extrusion mediated through a Na^+/Ca^{2+} exchange mechanism with a stoichiometry of 3 or even 4 Na^+/Ca^{2+} [8]. Such a mechanism would be very dependent upon the Na^+ electrochemical potential across the plasma membrane. In isolated synaptosomes Ca^{2+} distribution appears, however, to be independent of the Na^+ electrochemical potential if the Ca^{2+} channels are blocked with verapamil [11]. Dipolo et al. have suggested that a Ca^{2+}-ATPase, which extrudes Ca^{2+} across the plasma membrane is a main regulator of the cytosolic Ca^{2+} activity in squid axons [12–14] as in non-excitable tissues [15]. Furthermore, Na^+/Ca^{2+} exchange can only be demonstrated when the cytosolic $[Ca^{2+}]$ is extremely high [11,12].

Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; EGTA, ethyleneglycol bis-(α-aminoether)-*N,N'*-tetraacetic acid; Tes, 2-[(2-hydroxy-1,1-bis(hydroxymethyl) ethyl)-amino] ethanesulphonate

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The aim of this study was to obtain evidence for the existence of a Ca^{2+}-ATPase linked Ca^{2+} extrusion mechanism in synaptosomes. We show that ATP depletion causes an increased Ca^{2+} uptake by synaptosomes as compared to otherwise similar control conditions where ATP is largely maintained.

2. Methods and materials

Synaptosomes from guinea pigs of the Duncan-Hartley strain (aged 4–8 weeks) were isolated according to [16] as modified in [17]. The synaptosomes were preincubated for 15 min in a medium containing 122 mM NaCl, 3.1 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM KH_2PO_4, 5 mM $NaHCO_3$, 20 mM Tes and 10 mM D-glucose (pH 7.4) at 30°C before the initiation of Ca^{2+} uptake by the addition of 1.3 mM $CaCl_2$ together with $^{45}Ca^{2+}$ [5]. The distribution of various other isotopes were measured as in [5,11] and briefly in the figure legends. The ATP content was measured using the luciferin–luciferase assay [18]. All the isotopes were from the Radiochemical Centre (Amersham, Bucks) and all the other reagents were of the highest available purity [5,11].

3. Results

ATP depletion was achieved by incubating synaptosomes in the presence of iodoacetic acid to inhibit glycolytic ATP production and FCCP to prevent oxidative phosphorylation. The relative control contained FCCP and oligomycin to prevent ATP hydrolysis by activation of the mitochondrial ATPase by the uncoupler. The ATP content of synaptosomes falls below 10% of its initial value within 5 min after ini-

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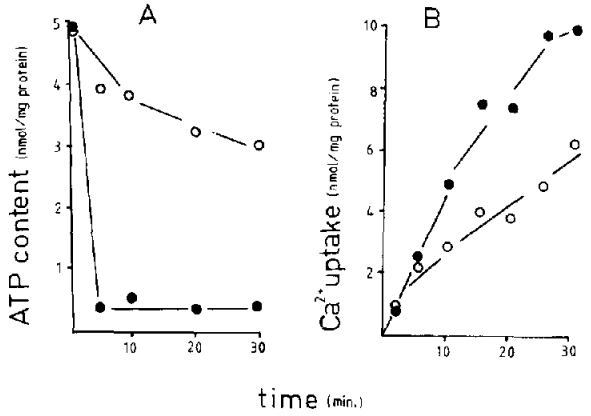

Fig.1. Effect of ATP depletion on synaptosomal Ca^{2+}-uptake. Synaptosomes (1.5 mg protein/ml) were preincubated in the basal experimental medium containing 4 μCi/ml [^{3}H]sucrose as external marker. At time = 0, 1.3 mM $CaCl_2$, 1 μCi/ml $^{45}Ca^{2+}$, 0.3 mM ouabain, 1 μM FCCP with 1 mM iodoacetate (○) or 4 μg/ml oligomycin (●) were added. (A) At the times indicated, 100 μl suspension was withdrawn into 25 μl ice-cold perchloric acid. The samples were centrifuged in an Eppendorf 3412 bench centrifuge, the supernatant neutralised using 3 M K_2CO_3/2 M Tris-base and the ATP content measured from the supernatant after recentrifugation. (B) Samples of 0.5 ml were withdrawn and pipetted onto the top of 50% (v/v) silicone oil (Dow-Corning 550)/dinonylphthalate containing 200 μl of a mixture of EGTA and ruthenium red in the basal medium to give of 2 mM and 5 μM final conc., respectively, whereafter the samples were centrifuged for 1 min. The pellets were extracted with 12% (w/v) perchloric acid for ≥2 h, neutralised with 1.8 M Tris-base/50 mM EGTA and counted.

tiation of ATP depletion, whereas in control conditions 60% of the initial ATP content is still maintained at 30 min of incubation (fig.1). Ouabain was present in both conditions to exclude any effects of ATP depletion upon the Na^+ electrochemical potential. In both conditions, uptake of Ca^{2+} by intrasynaptosomal mitochondria is totally prevented due to abolition of the mitochondrial potential by the uncoupler, i.e., abolition of the driving force of Ca^{2+} uptake (reviews [19–21]).

Fig.1 shows that ATP depletion significantly enhances Ca^{2+} uptake into the synaptosomes.

To exclude that ATP depletion affects Ca^{2+} distribution indirectly through a mechanism not directly linked to the ATP content other functional parameters were measured. There was no difference in the Na^+ content, cytosolic pH or $^{86}Rb^+$ equilibrium potential between the 2 comparable conditions (table 1).

4. Discussion

Endoplasmic reticulum is a main regulator of the cytosolic [Ca^{2+}] of synaptosomes. However, if the major part of intrasynaptosomal Ca^{2+} would be sequestered within the endoplasmic reticulum one would observe a decrease in the Ca^{2+} content of synaptosomes upon ATP depletion rather than the increase observed here, especially since the Na^+ electrochemical potential is decreased to the same extent

Table 1
Effect of ATP depletion and ouabain on the Ca^{2+} content, Na^+ content, cytosolic pH and ^{86}Rb equilibrium potential in synaptosomes at 20 min of incubation ± SEM (n = 4)

Condition	Ca^{2+} uptake (nmol/mg protein)	Na^+ uptake (nmol/mg protein)	$\frac{[MeNH_2\ in]}{[MeNH_2\ out]}$	$\frac{[^{86}Rb\ in]}{[^{86}Rb\ out]}$
Control	3.7 ± 0.4	49 ± 5	1.92 ± 0.03	9.8 ± 0.6
FCCP/oligomycin/ ouabain	4.8 ± 0.1	106 ± 6	1.37 ± 0.08	2.7 ± 0.13
FCCP/iodoacetate/ ouabain	6.3 ± 0.2	109 ± 8	1.2 ± 0.4	2.8 ± 0.05

The conditions of incubation were identical to those in fig.1. Na^+ uptake was measured using $^{22}Na^+$, which was present at the beginning of the preincubation period at 1 μCi/ml and 100 μl samples were diluted 9-fold into a medium containing: 125 mM LiCl, 1.2 mM $MgSO_4$, 0.4 mM KH_2PO_4, 2.5 mM Li_2CO_3, 20 mM Tes (Li-salt), 10 mM D-glucose (pH 7.4) and spun through oil within 10 s. The cytosolic pH was determined from the methylamine distribution ratio ($[MeNH_{2\ in}]/[MeNH_{2\ out}]$) using 1 μCi/ml (8 μM) [^{14}C]methylamine and the $^{86}Rb^+$ distribution ratio in the presence of 0.5 μCi/ml $^{86}Rb^+$. In both cases, 0.5 ml samples were directly spun through oil and the pellet were treated as in fig.1

in both conditions (table 1). The same would be the case if intrasynaptosomal Ca^{2+} would be mainly bound to ATP.

We had obtained evidence suggesting that Ca^{2+} distribution across the plasma membrane of synaptosomes in resting conditions is the result of 2 independent transport mechanisms; a kinetically slow continuous Ca^{2+} uptake and an active Ca^{2+} extrusion [11] when these 2 transport mechanisms balance a steady-state is achieved which can be altered by altering the kinetic properties of either mechanism. If the efflux mechanism is linked to ATP hydrolysis it is thus conceivable that an increase in Ca^{2+} uptake by the synaptosomes would occur as a response to ATP depletion.

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