Myosin II roles in vesicle transport and fusion in Chromaffin Cells

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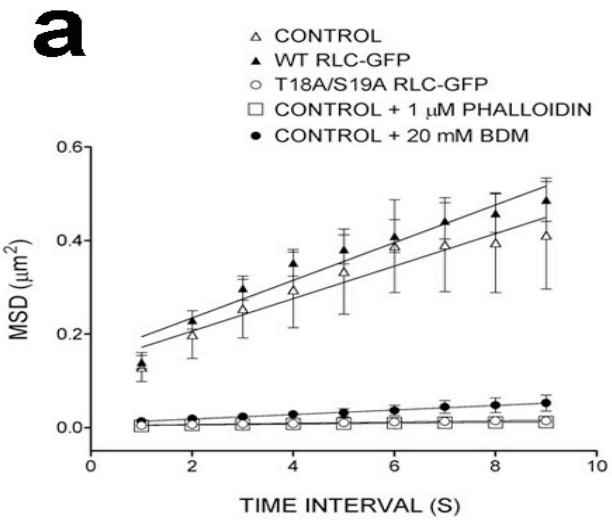
Cytoskeletal proteins play an essential role among the cytosolic elements organizing the transport of vesicles from the internal regions where chromaffin granule biogenesis takes place towards the subcortical region where they are stored to form a reserve pool to sustain continuous stimulation[1,2]. In these sense different myosins have been suggested to play a motor role in such transport and therefore they could modulate vesicle supply during secretion[3,4,5]. Here we studied whether the overexpression of myosin II regulatory light chain (RLC) and an inactive form affect different stages of the secretory process. Our results suggest the implication of myosin II in the transport of vesicles, and, surprisingly, in the final phases of exocytosis involving transitions affecting the activity of docked granules, and therefore uncovering a new role for this cytoskeletal element.

RESULTS AND DISCUSSION

Dynamic confocal microscopy has been used to study vesicle movements in chromaffin cells overexpressing wild type RLC-GFP or its inactive unphosphorylatable form (T18A/S19A RLC-GFP) and loaded with quinacrine. Using this technique we have observed a marked decrease in vesicle mobility in cells expressing such unphosphorylatable form when compared with control non-treated cells of cells expressing the wild type form. In effect, calculation of the coefficient of diffusion through mean square displacements (MSD) vs time plots (Figure 1a) yielded values of $10.1\pm1 \times 10^{-3}$ $\mu m^2/s$ for RLC-GFP moving granules and $0.32\pm0.02 \times 10^{-3}$ $\mu m^2/s$ for vesicles in cells expressing such unphosphorylatable form. This reduction in vesicle mobility was similar to the caused by agents affecting F-actin (phalloidin) or myosin ATPase activity (BDM) as shown in Figure 1a. The study of individual fusion kinetics is potentially useful when looking for the mechanisms underlying the different effects of RLC-GFP constructs and can be achieved by analyzing the shape of single amperometric events[6] using carbon fiber electrodes. The analysis was performed in $BaCl_2$ stimulated cells, in order

to search for well-separated spikes with amplitudes over 2.5 pA. We measured spike amplitude (Imax), event charge (Q), half-width ($t_{1/2}$) and time-to-the-peak (Tp). Non-infected cells or cells expressing wild-type RLC-GFP were characterized by a very similar pattern of distribution, showing mean amplitudes of 68 and 73 pA respectively. However, the mean amplitude obtained with mutants T18A/S19A RLC-GFP (Figure 1b) was clearly reduced to half that of the control value (32±5 pA). The observed alteration in mean amplitude did not represent a subsequent change in the amount of catecholamines released per event, since charge remained relatively unaltered. It is clear from the modifications in single vesicle fusion parameters, that the secretory events found in cells expressing the T18A/S19A RLC-GFP construct were considerably slower than control or wild type RLC-GFP expressing cells, whilst maintaining the charge released per event (Figure 1 b). These data demonstrate that myosin II activity influences the very final events linked to the exocytotic fusion of membranes and the release of catecholamines in this neuroendocrine cell model.

This might imply that myosin II activity influenced either the kinetics of exocytotic pore expansion or the degree of neurotransmitter dissociation from the vesicular matrix. It is unlikely that myosin II could influence processes taking place in the interior of the vesicular matrix, therefore we favor the first possibility. In conclusion, the present work demonstrates that cytoskeletal elements such as conventional myosin II affect different stages of the exocytotic process and not merely the transport of vesicles. Orienting research toward these newly proposed interactions may help to clarify and unravel some of the complex aspects of the exocytotic cycle.


a
CONTROL
WT RLC-GFP
T18A/S19A RLC-GFP
CONTROL + 1 μM PHALLOIDIN
CONTROL + 20 mM BDM
MSD (μm²)
0.6
0.4
0.2
0.0
0
2
4
6
8
10
TIME INTERVAL (S)

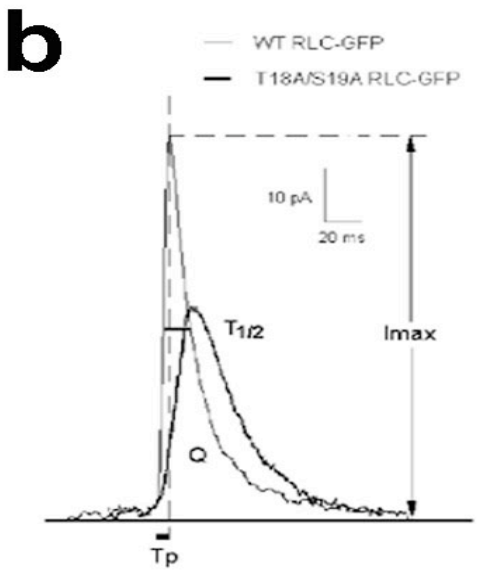

b
WT RLC-GFP
T18A/S19A RLC-GFP
10 pA
20 ms
T1/2
Imax
Q
Tp

Figure 1. a. Average of square of the mean distance moved (MSD) during the time intervals expressed in the abscissa, for vesicles in wild type RLC-GFP expressing cells, and cells expressing T18A/S19A RLC-GFP. In addition, were given MSD vs time plots for control cells treated with 1 μM phalloidin or 20 mM BDM. Also plotted were the best linear fits for the different curves, used to estimate the diffusion coefficient. **b.** This panel shows spikes representing the average properties of events in cells expressing wild type RLC-GFP and T18A/S19A RLC-GFP constructs, in addition to describe the parameters analysed.

A long version of this work can be found in reference 7.

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