EVALUATION OF SYNAPTIC VESICLES CYCLE DURING POSTNATAL DEVELOPMENT OF NEUROMUSCULAR JUNCTION IN MICE

Rodrigues, H. A.[1]; Amaral, E. A.[1]; Guatimosim, C.[1]

[1] Department of Morphology, Cell Biology Program – Federal University of Minas Gerais, Brazil – hermannfisio@yahoo.com.br, ernaniaa@gmail.com, cguati@icb.ufmg.br

Introduction

The neuromuscular junction forms in a series of steps that involve the exchange of signal among its three cellular components: nerve terminal, muscle fiber and Schwann cell. The formation of the neuromuscular synapse and the differentiation in mature synapse is dependent upon neurotransmission [1]. The spontaneous and evoked neuromuscular transmissions begin within minutes after the establishment of contact between nerve and muscle [2]. The maintenance of the synaptic transmission relies on the availability of release-competent synaptic vesicles filled with a high concentration of neurotransmitter [3]. All presynaptic functions, directly or indirectly, involve local synaptic vesicles recycling, which can be described in two great events: exocytosis and endocytosis [4]. In this work, we followed synaptic vesicles exo-endocytosis cycles during postnatal development.

Objective

To investigate if synaptic vesicles undergo cycles of exo-endocytosis during the postnatal development of the neuromuscular junction in mice.

Materials and Methods

In this work, we used fluorescence microscopy to visualize synaptic vesicles recycling at mouse motor nerve terminal. Briefly, diaphragm muscle associated with the corresponding nerve was isolated and bathed in mouse ringer (135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 1 mM NaH_2PO_4, 11 mM D-glucose, pH 7.4) bubbled with a misture of 5%CO_2/ 95%O_2. The preparations were then stained with the vital dye FM1-43 (4 µM) in different time-points of the postnatal life: 0 (newborn), 5, 10, 14 days and adult. The styryl dye FM1-43 is a powerful tool to track exocytosis, endocytosis and recycling of synaptic vesicles. Due to its unique structure, dye molecules reversibly partition into the outer leaflet of surface membrane without permeating due to two cationic charges located in their headgroup. When a nerve is stimulated to evoke exocytosis, FM1-43 molecules that were inserted in the membrane are internalized during compensatory endocytosis, and newly formed synaptic vesicles become stained with dye (staining/endocytosis). If stained secretory granules or vesicles undergo exocytosis in dye-free medium, due to concentration gradient, FM1-43 molecules dissociate from the membrane and lose fluorescence (destaining/ exocytosis) [5]. Using a fluorescence microscope (Leica DM2500) attached to a CCD camera, we followed FM1-43 staining and destaining at the neuromuscular junction during post-natal development.

Results

- The synaptic vesicles are capable to suffer cycles of exocytosis and endocytosis since early postnatal life (newborn) until the adult age (figure 1);
- The presynaptic terminals stain weakly in the initial phases of the postnatal development (figure 2);
- The morphologic aspect and distribution of the presynaptic terminals on the muscle modify during the postnatal development (figure 3).

Conclusion

Our results may indicate that there is less vesicles during the initial phases of the development than in the adult age. However, we cannot discard the idea that high frequency of spontaneous neurotransmitter release might occur in early postnatal life. In addition, we observed that the morphological aspect and distribution of the motor terminals change during development.

References

[1] T. Misgeld, R.W. Burgess, R.M. Lewis, J.M. Cunningham, J.W. Lichtman and J.R. Sanes, Neuron 36 (2002) 635-648.
[2] J. Evers, M. Laser, Y.A. Sun, Z.P. Xie and M.M. Poo, The Journal of neuroscience 9 (1989) 1523-1539.
[3] F.E. Schweizer and T.A. Ryan, Current Opinion in Neurobiology 16 (2006) 298-304.
[4] T.C. Südhof, Annual Review of Neuroscience 27 (2004) 509-547.
[5] W.J. Betz and G.S. Bewick, Science 255 (1992) 200-203.

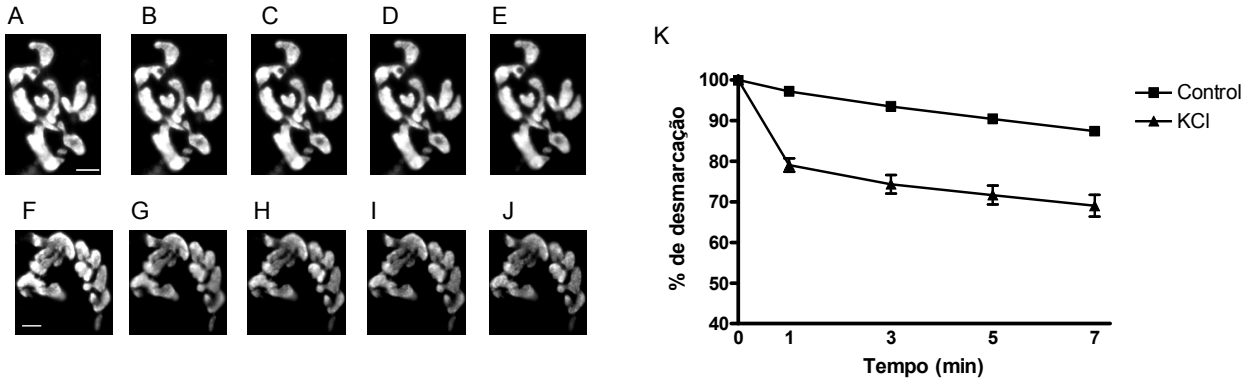

Figure 1. Animal in adult age. Vesicular release evoked by depolarizing stimulation. (A – E, control) Representative mouse neuromuscular junctions stained with FM 1-43, in normal Ringer, at the beginning of a control experiment (A) and after 1 (B), 3 (C), 5 (D) and 7 minutes (E). The reduction of the fluorescent signal was due to the photobleaching. (F – J, KCl) Motor terminals stained with FM 1-43, in normal Ringer, at the beginning of an experiment (F) and after 1 (G), 3 (H), 5 (I) and 7 minutes (J) in Ringer with high potassium concentration. Destaining indicates exocytosis of synaptic vesicles. (K) Time-course of experiments similar to that described in (A – J). (Scale bars: 10 µm. Curves in (K) represent the mean of at least three independent experiments. At the end of 7 min, $p < 0.05$ for KCl curve. Error bars: ± S.E.M.).

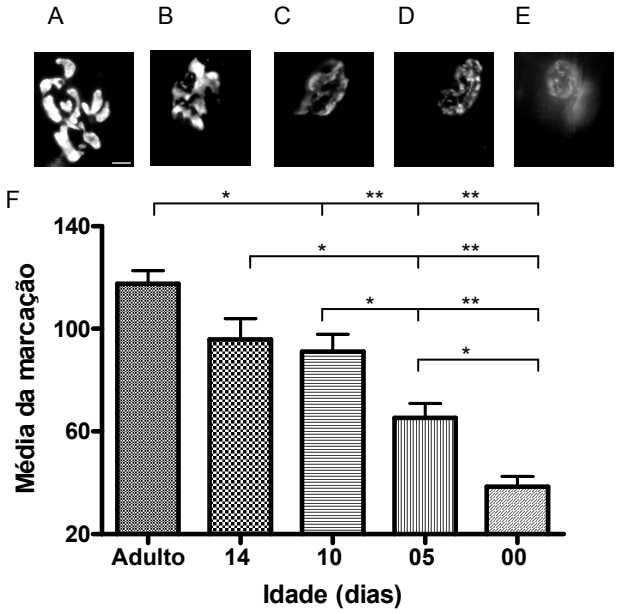

Figure 2. Motor terminals staining with FM1-43 during synaptic vesicles endocytosis. (A – E) Representative mouse neuromuscular junctions stained with FM 1-43, in normal Ringer, in the adult age (A), 14 (B), 10(C), 05 (D) and 00 (E) days (newborn). (F) Graph comparing the staining intensity of the motor terminals in respective ages described in (A – E). Note that the staining intensity is higher in old ages animals. (Scale bar: 10 µm. Bar graph in (F) represent the mean of at least three experiments for each age. Error bars: ± S.E.M. * p<0,05 e ** p<0,001).

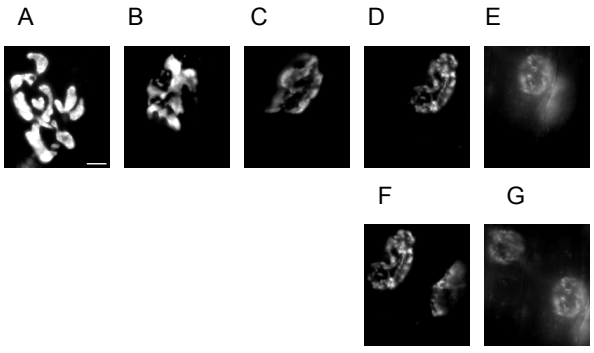

Figure 3. Morphological aspect and distribution of the motor terminals change during development. (A – E) Representative mouse neuromuscular junctions stained with FM 1-43, in the (A) adult age, (B) 14, (C) 10, (D) 05 and (E) 00 days (newborn). (F) and (G) show the proximity of the motor terminals in the 05 and 00 days (newborn), respectively. (Scale bar: 10 µm).