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Calcium Transient and Quantal Release in Mouse Neuromuscular Junction Under Extracellular Calcium Concentration Change

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Abstract
In mouse neuromuscular junction, the amplitude of the presynaptic calcium (Ca^{2+}) transient was measured and correlated with mediator release at different extracellular Ca^{2+} concentrations. Fluorescent calcium-sensitive dye Oregon Green 488 BAPTA 1 hexapotassium salt was used for Ca^{2+} transient registration. The quantal content of release was assessed by the amplitude of the endplate potentials (EPPs) and was measured using intracellular microelectrodes. The amplitude of the EPPs changed more significantly than the amplitude of the Ca^{2+} transient when the extracellular calcium concentration was changed. Linear approximation of the dependence of the quantal content on the amplitude of the Ca^{2+} transient on double logarithmic scale gave a slope showing that the biochemical cooperativity was 2.86. The obtained value is comparable with the data calculated earlier in the neuromuscular junction of the rat and other synapses using electrophysiological measurements. Our data suggest that the change of the Ca^{2+} transients recorded from the whole volume of the nerve terminal properly reflects the variation of calcium concentration responsible for the neurotransmitter release in active zone. Thus, analysis of the bulk Ca^{2+} transient can be used to evaluate the calcium entry into the nerve endings and compare it with the number of quanta released under different conditions.

Keywords Neuromuscular junction · Mediator release · Optical imaging · Calcium transient · Fluorescence dyes · Quantal content

1 Introduction

Action potential triggers the Ca^{2+} entry through voltage-gated calcium channels which subsequently activates synaptic vesicle release [1]. The presynaptic calcium sensor is the synaptotagmin composed of a transmembrane domain, a linker sequence, and two C-terminal domains which are universal Ca^{2+}-binding modules [2, 3]. From previous studies, it was calculated that in the peripheral neuromuscular junction of the frog, as well as in the giant squid synapse, release of one synaptic vesicle requires binding of at least 4 Ca^{2+} to the calcium sensor [4–6]. This value numerically characterizes the biochemical cooperativity of this process [4, 7]. It can be calculated from the fitting of the dependence of the synaptic release (measured by the amplitude of the postsynaptic response) on extracellular Ca^{2+} concentration by power law function [4]. However, it was not clear how alteration of extracellular calcium concentration is translated into changes of intracellular Ca^{2+} transients and, subsequently, of the quantal content of the endplate potentials (EPPs). Optical methods for the Ca^{2+} entry measurements using specific fluorescent Ca^{2+}-sensitive dyes allow to estimate the change in the intracellular Ca^{2+} concentration (Ca^{2+} transient) in response to the presynaptic action potential [8] and correlate it with the number of released quanta. In peripheral nerve endings, the Ca^{2+} transient reflects the integral calcium influx into the presynaptic cell terminal during the action potential, its diffusion and interaction with intracellular Ca^{2+} buffer systems. Vesicle fusion occurs in so-called microdomains, presynaptic substructures functionally connecting voltage-gated calcium channels and release machinery [9, 10]. For proper correlation of quantal

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