Spatio-temporal morphology of calcium sparks recorded on intact amphibian skeletal muscle fibres

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Intact skeletal muscle fibres were isolated enzymatically from the toe muscles of the frog. To this end frogs were first anaesthetized and killed then the muscles were removed. Isolated cells were allowed to spontaneously attach to the bottom of glass Petri dishes coated with extracellular matrix (EMG gel; Growth Factor reduced from Engelbreth-Holm-Swarm murine sarcoma). Fibres were loaded with the AM form of the calcium sensitive fluorescent dye Fluo-4. Calcium sparks were measured at high time resolution using the Ziess-Live laser scanning confocal microscope on both *x-y* (30 frames/s) and line-scan (*x-t*) images (65 lines/ms). Calcium release events detected at rest (control condition) or elicited either by the addition of 1 mmol/L caffeine or by a depolarization to −60 mV were identified by a locally developed computer program that applied the method of wavelet-transform (Szabo *et al.*, 2010). The program, apart from identifying an event on either *x-y* or *x-t* images of fluorescence, automatically calculated the corresponding background and determined the characteristic event parameters. While under control conditions a typical spark appeared in one frame only, 17.3 and 26.0% of spark positions overlapped on consecutive frames following the treatment with caffeine or the depolarization, respectively. Both interventions increased the frequency of sparks, as estimated from *x-y* images. Importantly, the morphology of sparks was different if elicited by caffeine or by the depolarization. Both the amplitude (in $\Delta F/F_0$; 0.49±0.025 *vs* 0.29±0.001; n = 22426 *vs* 23714; mean±SEM, $p<0.05$) and the full width at half maximum (in μm; parallel with fibre axis: 2.33±0.002 *vs* 2.21±0.005; perpendicular to fibre axis: 2.07±0.003 *vs* 1.88±0.004) of sparks was significantly greater after caffeine treatment than on depolarized cells. These observations were confirmed on sparks identified in line-scan images. The distribution of FWHM values revealed a more excentric spatial profile of spark elicited by depolarization. On *x-t* images calcium sparks had significantly slower rising phase under both conditions as compared to the control. On the other hand, while the rate of rise of signal mass was decreased after depolarization, it increased in the presence of caffeine.

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