## Effect of protease-activated receptor stimulation on electrically evoked calcium transients elicited in cultured skeletal C2C12 myotubes

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Inflammation accompanies muscle injury. Protease-Activated Receptors (PARs) are a novel class of G-protein coupled receptors activated by serine proteases that play a prominent role in the inflammatory response. PAR expression has been demonstrated in skeletal myotubes, and PARs predominantly signal *via* the phospholipase C (PLC) pathway, which is also found in skeletal myotubes. In this study, we examined the effect of PAR stimulation on electrically evoked $Ca^{2+}$ transients elicited in skeletal C2C12 myotubes. C2C12 myoblasts were grown on collagen-coated cover slips and incubated in Dulbeccos modified Eagles medium with 20% foetal calf serum at 37°C and 5% $CO_2$. Cytosolic $Ca^{2+}$ was measured with fura-2 using a Cairn spectrophotometer connected to an inverted epifluorescence microscope. The myotubes were stimulated electrically *via* platinum electrodes connected to a pulse stimulator (0.3 ms pulse duration at 0.1 Hz). The serine protease thrombin caused a brief transient increase in the resting [$Ca^{2+}$], and a sustained decrease in the peak of electrically evoked SR $Ca^{2+}$ transients in C2C12 myotubes. $Ca^{2+}$ transient peaks dropped to 51.4 ± 9.8% (n=15) of initial values after thrombin exposure, compared to a drop to 97.1 ± 1.7% of initial values with no thrombin exposure (n=15, $p<0.001$). The effect on the $Ca^{2+}$ transients continued for up to 60 minutes. Synthetic peptides that specifically activate PAR receptors without proteolytic cleavage revealed PAR-1 as the receptor involved. The effect of thrombin on the $Ca^{2+}$ transients was prevented by the PLC blocker U37122, implicating involvement of the PLC pathway in the thrombin-mediated effect. These results show that thrombin can decrease sarcoplasmic reticulum $Ca^{2+}$ release in skeletal muscle cells, and could play a role in the muscle weakness observed during injury-induced inflammation.