Role of protease-activated receptors (PARs) in muscle inflammation and cytokine release

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Protease-Activated Receptors (PARs) are a newly identified family of G protein-coupled receptors that mediate a diverse range of cellular activities, particularly during inflammation. PARs are activated by serine proteases such as thrombin and tryptase. These proteases cleave the extracellular N-terminus of the receptor, resulting in a new N-terminus that acts as a tethered ligand, binding to and activating the receptor. Four PAR subtypes have been identified (PAR 1, 2, 3 & 4). PAR activation commonly activates Ca^{2+} signalling pathways within target cells, and has been shown to play a vital role in orchestrating both inflammatory and proliferative responses to tissue damage. Activation of PARs and can result in production of the inflammatory cytokine interleukin 6 (IL-6) in many tissues, including airway epithelia, oral mucosa, blood vessels and connective tissue (Steinhoff *et al.*, 2005). During skeletal muscle development and regeneration, satellite cells proliferate and resulting myoblasts fuse into multinucleate myotubes, which eventually mature into adult muscle fibres. PARs are reported to play an important role in skeletal muscle development by enhancing myoblast proliferation and inhibiting myoblast apoptosis (Chinni *et al.*, 2000). However, little is known about the role of PARS in the function of myotubes and skeletal muscle. Skeletal muscle has recently been shown to be a major producer of IL-6 especially during exercise where it plays a role in glucose and lipid metabolism (Pedersen, 2007). IL-6 may also play a role in promoting the differentiation and maturation of myoblasts (Okazaki *et al.*, 1996; Baeza-Raja & Munoz-Canoves, 2004). In this study we examined the effects of PAR activation on IL-6 production in cultured C_2C_{12} myotubes.

Experiments were performed on cultured C_2C_{12} myoblasts and myotubes grown in Dulbecco's modified Eagles medium with 20% and 2% foetal calf serum, respectively. PARs were activated with thrombin (activates PAR-1, 3 & 4 isoforms) trypsin (activates PAR-2) or specific PAR activating peptides mimicking the tethered activating ligand of the cleaved PAR receptor. Ca^{2+} was measured using the fluorescent Ca^{2+} indicator fura-2. Cytokine levels were detected by ELISA. IL-6 levels was determined by ELIZA.

In untreated myotubes, exposure to thrombin resulted in a rise in intracellular Ca^{2+} (mean amplitude; 0.38 $\pm$ 0.03 μM) in only 14% of cells tested (n=14). This success rate was increased by pre-exposure of myotubes to inflammatory mediators such as TNFα (10 nM) (success rate 57%, n=12) and thrombin (5 U/ml) (success rate 92%, n=12). An investigation into the effect of serine protease exposure on myokine production showed that exposure to thrombin for 24 hours increased the production of IL-6 by 40% in untreated myotubes compared to controls (n=8, $p<0.01$). Thrombin increased IL-6 production by 80% compared to controls after thrombin pre-exposure (1 hr exposure to 10U/ml thrombin 12 hours before experimentation) (n=8, $p<0.001$). Exposure to trypsin also increased IL-6 secretion by 20% compared to controls in untreated myotubes (n=8, $p<0.001$). This secretion was not enhanced by pre-exposure to trypsin.

These results suggest that inflammatory mediators may up-regulate PAR receptor expression in skeletal muscle. Increased IL-6 production induced by PAR activation could play a role in skeletal muscle development and regeneration by increasing myoblast differentiation into myotubes during skeletal muscle development and/or regeneration.

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