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Unique patterns of calcium transients associate to hormone response in skeletal muscle cells

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Both K+ depolarization and tetanic electrical stimulation have been shown to produce slow Ca2+ signals, unrelated to contraction and associated to regulation in gene expression in muscle cells (Carrasco et al. 2003; Eltit et al. 2004). Several hormones are known to produce anabolic effects in skeletal muscle. We studied the effect of IGF-I, insulin and testosterone on intracellular Ca2+ in primary cultured myotubes and cell lines. The fluorescent dye Fluo-3 AM was used for temporal and spatial characterization of Ca2+ signals. Insulin produced a fast (<1 s) and transient Ca2+ increase lasting less than 10 s. IGF-I induced a transient Ca2+ increase, reaching a fluorescence peak 6 s after stimulus, to return to basal values after 60 s (Espinosa et al. 2004). Testosterone induced delayed (35 s) and long lasting (100-200 s) signals, frequently associated to oscillations (Estrada et al. 2003).

We studied the role of capacitative calcium entry (CCE) on intracellular Ca2+ oscillations induced by testosterone at the single cell level in primary myotubes. Testosterone (100 nM) rapidly induced an intracellular Ca2+ rise, accompanied by Ca2+ oscillations with a periodicity of 20.3 ± 1.8 s (mean ± SD) in the majority (76%) of myotubes. In Ca2+-free medium, an increase in intracellular Ca2+ was still observed, but no oscillations. Neither nifedipine nor ryanodine affected the testosterone-induced Ca2+ response. Intracellular Ca2+ store depletion in Ca2+-free medium, using a SERCA-pump inhibitor, followed by re-addition of extracellular Ca2+ gave a fast rise in intracellular Ca2+, indicating that CCE was present in these myotubes. Application of either testosterone or albumin-bound testosterone induced Ca2+ release and led to CCE after re-addition of Ca2+ to Ca2+-free extracellular medium. The CCE blockers 2-APB and La3+, inhibited testosterone-induced Ca2+ oscillations and CCE. The steady increase in Ca2+ induced by testosterone was not, however, affected by La3+. These results demonstrate testosterone-induced Ca2+ oscillations in myotubes, mediated by the interplay of IP3-sensitive Ca2+ stores and Ca2+ influx through CCE. G protein inhibitors, PTX and GDPbS, only slightly modified the response to IGF-1, but both testosterone and insulin-induced Ca2+ increase was blocked. The different intracellular Ca2+ patterns produced by testosterone, IGF-1 and insulin, may help to understand the role of intracellular calcium kinetics in the regulation of gene expression by hormones in skeletal muscle cells.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.