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Basic Science

Abstract Title
Extracellular vesicle release with acute electrical pulse stimulation in skeletal muscle is AMPK-dependent

Background
Extracellular vesicles (EVs) are membrane-bound nanoparticles secreted by all cells, and carry bioactive cargo that can alter cell function. We previously demonstrated that chronic electric pulse stimulation (EPS) of C2C12 myotubes enhanced EV release after 21hrs of recovery, and that these EVs evoked mitochondrial biogenesis in other cells. Chronic EPS activates several signalling kinases, including AMP-activated protein kinase (AMPK).

Objective
Although acute EPS has been reported to increase EV secretion, the underlying signalling mechanisms remain unclear. We hypothesize that acute EPS will increase EV secretion in an AMPK-dependent manner.

Methods
Mouse muscle cells were differentiated into myotubes (N=7), and subjected to acute EPS (3hrs @14V, C-PACE EM, IonOptix). A separate set of EPS experiments were done with, or without AMPK inhibitor, Compound C (0, 5, 10μM; N=4). Unstimulated myotubes were used as controls. EVs were isolated from conditioned media, and characterized using single-particle analysis. Cells were examined under a microscope for morphological changes, assessed for changes in protein concentration/yield using BCA assay, and protein expression (AMPK phosphorylation, cellular stress markers such as HMGB1, AIF, and HSP70) using immunoblotting. Data were analysed using t-tests, RM one-way, or two-way ANOVAs.

Results
Acute EPS led to a 2.2-fold increase in EV concentration ($p=0.0045$), and size distribution analysis showed higher EV concentration at all sizes (~50-200nm, $p=0.0006$) vs. control. Average EV size (~110nm) was unaffected with EPS. Treatment with 10μM of Compound C ameliorated the EPS-induced ~2-fold increase in EV secretion ($p=0.0197$) to control levels. No changes in cell morphology, and total protein yield between treatments were observed. Likewise, protein expression levels of HMGB1, AIF, and HSP70 remained unchanged. AMPK phosphorylation validation of results is in progress.

Conclusion
Our data demonstrate that acute EPS enhances EV secretion in an AMPK-dependent manner. Overall, our work highlights a novel putative regulatory mechanism controlling EV secretion with EPS.

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