Phosphoproteome of continuously and interval-paced HL-1 cardiomyocytes as determined by the SILAC approach

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Purpose:
Atrial fibrillation (AF), the most common arrhythmia in clinical practice, is characterized by electrical, contractile and structural remodeling of the atria. Many of the adverse atrial remodeling processes during AF are associated with or caused by reversible alterations of protein-phosphorylation. However, aberrant cellular signaling in AF remains to be elucidated fully. Here, we report the systematic characterization of AF-dependent alterations in signal transduction / phosphoproteome in a model of AF using the murine atrial cardiomyocyte cell line HL-1.
Methods: HL-1 cells were subjected to 24 h (i) continuous (cRAP) and (ii) interval rapid pacing (iRAP) (Fig. 1). The phosphoproteomes of RAP HL-1 cells were analyzed in the presence of an internal SILAC-standard by phosphopeptide enrichment and high-accuracy mass spectrometry (HPLC-MS/MS). Altered protein phosphorylation at both tyrosine and serine/threonine phosphorylation sites was identified and quantified by analyzing the meta-data using bioinformatic approaches.
Results: For the first time, the phosphoproteomes of HL-1 cells exposed to 24 hours continuous or interval RAP, respectively, were systematically determined. After 24 h of cRAP or iRAP, 5,166 or 4,460 phosphorylation sites were detected in 1,825 or 1,638 proteins, respectively. Applying a cut-off of 2.0 and higher, 60 (in 57 proteins) phospho-sites were increasingly phosphorylated in response to cRAP, whereas 85 (in 74 proteins) were increased by iRAP. Furthermore, 75 phospho-sites (in 64 proteins) were found to be less phosphorylated by cRAP, while 75 phospho-sites (in 66 proteins) showed decreased phosphorylation in response to iRAP. Only a small fraction of phospho-sites (7 in 6 proteins) were equally regulated by cRAP and iRAP (cut-off ≥ 2.0). Phosphoproteins showing differential phosphorylation in response to RAP were categorized according to protein families, signaling pathways, and cellular functions and include e.g. ion channels (ERG-1, KVLQT1), kinases (MEKKK 4 and 5, striated muscle-specific serine/threonine-protein kinase (Speg).
Conclusions: Highly divergent phosphorylation patterns as observed in response to either continuous or interval RAP supports the view that atrial remodeling due to short episodes of RAP or AF could be attenuated by existing recovery mechanisms. These may help to limit AF progression and add to our basic understanding of mechanisms underlying reversibility of early atrial remodeling during AF.

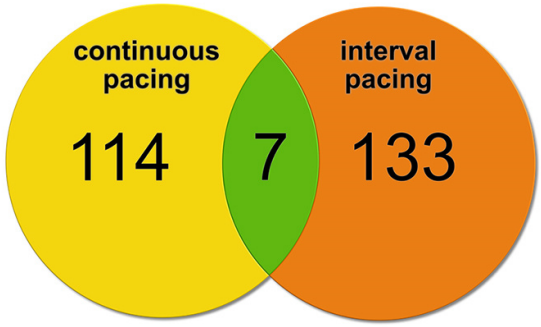

Fig.1: Venn diagramm demonstrating the ≥ 2fold changed phosphorylation of phospho-sites in response to 24 h continious or interval rapid-pacing of HL-1 cardiomyocytes *in vitro*.