Abnormal calcium handling in transgenic mice over-expressing calumenin abnormal calcium handling in transgenic mice overexpressing calumenin
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Recently, we have shown that calumenin, a 6 EF hand Ca2+ binding protein is highly expressed both in rabbit cardiac and skeletal muscles. To elucidate the functional role of calumenin in mammalian heart, transgenic (TG) mice overexpressing mouse calumenin (25 fold) was generated. The primary phenotype shows no hypertrophy and normal survival rate. Major excitation-contraction coupling proteins show no changes in mRNA and protein levels. However, the catalytic subunit of PKA is up-regulated. PKA activation leads to increased phosphorylated phospholamban as the downstream effect. This phosphorylation will lead to increased calcium uptake into the SR and altered contractility. According to echocardiography, in many cases TG mice show sustained bradycardia (WT: 494+/−46.9 beats/min, n=11, vs. TG: 381+/−40.9 beats/min, n=25) combined with arrhythmia and enlargement of ventricles (LVM/BW, WT: 3.01+/−0.41 mg/g vs. TG: 4.33+/−1.60 mg/g). This study shows interesting cardiac symptoms caused by overexpression of calumenin in heart.

Actin cytoskeleton dynamics by Akt/PKB
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Actin cytoskeleton is dynamically regulated by various signaling molecules in response to growth factor stimulation, and finally evokes cellular responses such as membrane ruffling and chemotaxis. Here we show that stimulation of cells derived from mouse embryonic fibroblast (MEF) with platelet-derived growth factor (PDGF) formation of membrane ruffling was transient response and completely blocked by PI3K inhibitor such as LY294002 and Wortmannin. Genetic loss of either Akt1/PKBα or Akt2/PKBβ resulted in detect of PDGF-induce membrane ruffling formation. On the other hand, loss of Akt2/PKBβ augmented PDGF-induced ruffling formation. Effect of Akt/PKB isoforms on actin cytoskeleton rearrangement by PDGF stimulation was further verified by using cells add-backed with corresponding Akt/PKB isoforms. Add back of Akt1/PKBα into Akt1/PKBα-null cells restored PDGF-induced membrane ruffling. Also, add back of Akt2/PKBβ restored unusual activation of membrane ruffling formation. Finally, null mutation of Akt/PKB isoforms showed defect in PDGF-derived chemotactic cell movement. Given these results we suggest here that Akt/PKB modulates PDGF-induced cell motility by regulating actin cytoskeletal rearrangement.

Activation of HGF/c-Met signaling induces delayed STAT3 phosphorylation via increase in interleukin 6 expression
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c-Met receptor tyrosine kinase mediates pleiotropic physiological or pathological cellular responses following activation by HGF. Activation of STAT3 has been known to be involved in HGF/c-Met induced liver regeneration and tumorigenesis. Interestingly, when we add HGF to NIH3T3 fibroblasts and Chang liver cells, phosphorylation of Tyr705 of STAT3 was observable not from several minutes but from 2 h and 6h, respectively. That phenomenon of delayed phosphorylation of STAT3 was blocked by pretreatment with cycloheximide and Actinomycin D, and the conditioned media from the cells at the time of STAT3 activation induced STAT3 phosphorylation within 15 minutes when applied to a new dish of cells, strongly suggesting that a newly synthesized secretory protein was responsible for the delayed STAT3 phosphorylation. Among the known mediators inducing STAT3 phosphorylation, interleukin 6(IL-6) mRNA and protein was observed to be induced by HGF. Furthermore, the neutralizing IL-6 antibody abolished STAT3 phosphorylation, evidencing that IL-6 was the responsible protein. Induction of IL-6 was abolished by LY294002, a PI3 kinase inhibitor, but not MAPK inhibitors. In conclusion, HGF induces delayed STAT3 phosphorylation via expression of IL-6 mRNA and protein, which might be mediated by PI3 kinase pathway, but not NF-kB.

Aldolase potentiates reversible DIDS-activation of ryanodine receptor 1
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The disulfonic stilben derivate, DIDS is known to triggers Ca2+ release from skeletal muscle sarcoplasmic reticulum (SR). The present study characterized the effects of DIDS on rabbit skeletal Ca2+ release channel/ryanodine receptor type 1 (RyR1) incorporated into planar lipid bilayer. When junctional SR vesicles were used for channel incorporation (native RyR1), 10 µM DIDS induced long-lived open events. However, when purified RyR1 was examined in the same condition, DIDS became considerably less potent, suggesting that some protein(s) is required for potentiation of the DIDS effects in the native RyR1. By DIDS-affinity column chromatography using solubilized junctional SR and by MALDI-TOF analysis of the affinity-column associated proteins, we found four major DIDS binding proteins. Among them, aldolase greatly potentiated the DIDS effects on purified RyR1 by single channel study. The association between RyR1 and aldolase was confirmed by co-immunoprecipitation and aldolase-affinity batch column chromatography. Taken together, aldolase associated with RyR1 could confer the remarkable potentiation of DIDS effects on RyR1.