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# Protein kinase G modulates human myocardial passive stiffness by phosphorylation of the titin springs

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## Background

The sarcomeric titin springs influence myocardial distensibility and passive stiffness. Titin-isoform composition and protein kinase-A (PKA) dependent titin phosphorylation are variables contributing to diastolic heart function. However, diastolic tone, relaxation speed, and left-ventricular extensibility are also altered by protein kinase-G (PKG) activation. We used back-phosphorylation assays to determine whether PKG can phosphorylate titin and affect titin-based stiffness in skinned myofibers and isolated myofibrils.

## Results

PKG in the presence of 8-pCPT-cGMP (cGMP) phosphorylated the two main cardiac-titin isoforms, N2BA and N2B, in human and canine left ventricle. In human myofibers/myofibrils dephosphorylated prior to mechanical analysis, passive stiffness dropped 10–20% upon application of cGMP-PKG. Autoradiography and anti-phosphoserine blotting of recombinant human I-band-titin domains established that PKG phosphorylates titin's N2-B and N2-A domains. Using site-directed mutagenesis, serine residue S469 near the COOH-terminus of the cardiac N2-B-unique sequence (N2-Bus) was identified as a PKG and PKA phosphorylation site. To address the mechanism of the PKG-effect on titin stiffness, single-molecule AFM force-extension experiments were performed on engineered N2-Bus-containing constructs. The presence of cGMP-PKG increased the bending rigidity of the N2-Bus to a degree that explained the overall PKG-mediated decrease in cardiomyofibrillar stiffness. Thus, the mechanically relevant site of PKG-induced titin phosphorylation is most likely in the N2-Bus; phosphorylation of other titin sites could affect protein-protein interactions.

## Conclusion

The results suggest that reducing titin stiffness by PKG-dependent phosphorylation of the N2-Bus can benefit diastolic function. Failing human hearts revealed a deficit for basal titin phosphorylation compared to donor hearts, which may contribute to diastolic dysfunction in heart failure.