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EDITORIAL

Can stress make you relax?

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This editorial refers to 'Stretch-induced compliance: a novel adaptive biological mechanism following acute cardiac load' by A.M. Leite-Moreira *et al.*, pp. 656–667.

Cardiac force adaptations to increased preload are well-known, with a biphasic increase in contractility, i.e. a rapid phase that involves enhanced calcium sensitivity of cardiac myofilaments (the basis of the Frank–Starling mechanism)[1] and a delayed phase due to an increased Calcium transient (the basis of the classical Anrep effect).[2] Concurrent adaptations of ventricular compliance and diastolic pressure are much less defined. Increased compliance with ensuing fibre and sarcomere stretch (within physiological range of sarcomere length) would allow further recruitment of diastolic reserve to optimize ventricular filling and the adaptation of contractility (according to the earlier mechanisms) in the face of increased venous return (e.g. during exercise). Such phenomena have been proposed in response to paracrine release of myocardial 'relaxants' such as natriuretic peptides (NPs) and nitric oxide (NO).[3] In this issue of *Cardiovascular Research*[4], Leite-Moreira (father and son) and colleagues now systematically examine the occurrence of stretch-induced compliance adaptation in several animal and human experimental preparations and propose an underlying mechanism.

They observed that not only stretching rabbit papillary muscle, but also human right atrial and left ventricular strips resulted in a sustained decrease in passive tension over 15 min, that was reproduced in intact rat left ventricles with reduced end-diastolic pressure. Notably, skinned cardiac myocytes isolated from slowly stretched ventricles exhibited a decreased passive tension-sarcomere length relationship over a physiological range of sarcomere lengths but this was not observed in similar preparations from non-stretched ventricles. This means that the phenomenon is not a consequence of mere skinning of the myocytes but involves a perturbation of sarcomeric proteins that persists *ex vivo*. Conversely, stretching skinned myocytes did not reproduce the phenomenon, showing that signalling in the intact ventricle is needed for the sarcomere modulation to take place. The authors point that these observations argue against stress relaxation and creep due to passive viscoelastic properties[5] but instead suggest a durable modulation of the properties of sarcomeric proteins.

What then is the underlying biochemical mechanism? Here, the authors make an 'educated guess' and focus on post-translational regulation of the sarcomeric protein, titin, by phosphorylation, particularly in response to cGMP-activated protein kinase (PKG). The giant protein titin is well-known as a major determinant of viscoelastic and compliant properties of the sarcomere.[6] Cardiac titin comes in two isoforms, a shorter and 'stiffer' N2B and a longer, more 'compliant' N2BA. Both isoforms can be phosphorylated by PKG, specifically on the conserved Ser4099 residue in all species but also Ser4185 in the cardiac-specific N2-Bus domain of human titin.[7,8] In both cases, phosphorylation results in decreased titin-based passive stiffness.[9] Although the authors did not examine phosphorylation on these specific sites, they found increased total titin phosphorylation (by ProQ Diamond staining) in extracts of stretched rabbit and human cardiac muscle. The functional relevance of this modification in their models is suggested from the abrogation of stretch-induced passive tension changes upon incubation with protein phosphatase. Moreover, a relatively specific inhibitor of PKG also inhibited the increased compliance induced by stretch; and conversely, incubation with PKG increased compliance of skinned myocytes from non-stretched hearts only.

What is (are) the upstream activator(s) of cGMP/PKG mediating this effect of stretch *in vivo*? Two obvious candidates are NO and NPs, as activators of the cytosolic and membrane-bound guanylyl cyclases, respectively.[10,11] These mediators are also known to be released upon cardiac muscle stretch and to increase cGMP levels in cardiac myocytes.[10,11] Indeed, cGMP content was increased in stretched hearts and combined pharmacologic inhibition of NO signalling and NPR-A (the receptor for ANP and BNP) reduced the amplitude of the passive tension decay to the same extent as PKG inhibition. However, these interventions did not fully abrogate the stretch-induced compliance in isolated muscles, leaving the possibility for other mediators. Other kinases are known to phosphorylate the N2-Bus domain of titin.[12] Alternatively, stretch may activate additional mechanisms (independent of titin) to modulate compliance. One, as pointed out by the authors, is the production of reactive oxygen species,[13] which can directly activate PKG through oxidation at Cys42.[14] PKG, in turn, was shown to phosphorylate cardiac phospholamban on Ser16 and activate relaxation. Notably, this pathway would be entirely confined in cardiac myocytes and it would have been interesting (albeit technically challenging) to examine, in this study by Leite-Moreira,[4] whether the passive tension adaptation to stretch could be recapitulated in intact, isolated cardiac myocytes stretched *ex vivo*. Other mechanisms may be at play but have not been tested here. For example, the potentiation of the EC coupling gain and increased calcium transient by stretch, in addition to contributing the Anrep effect, may also activate the mitochondrial production of ATP and decrease cytosolic ADP, a key modulator of

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myofilament calcium sensitivity.[15] Decreased ADP and myofilament sensitivity would, in turn, favour relaxation and increase compliance.

Regardless, the authors must be commended for trying to verify their paradigm in normal volunteers as well as patients with cardiac disease. Despite the obvious limitations due to small numbers and potential confounders (e.g. autonomic adaptations, concurrent medications in patients, anaesthesia during surgery), they found qualitatively similar trends for increased ventricular compliance upon volume load in normal volunteers (by non-invasive measurement of E/E' upon leg elevation) and open-chest patients (by applying single-beat assessment of end-diastolic pressure–volume relationship from haemodynamic measurements, avoiding changes in volume loads by *vena cava* obstruction). Next, using the classical TAC model in rats, they examined whether the stretch-induced increase in ventricular compliance was preserved in hypertrophic hearts; it was not.

Beside the clear identification of an adaptive increase in compliance in response to cardiac muscle stretch across different animal and human species, the study also suggests that loss of this response (together with remodelling of extracellular matrix and fibrosis) in hypertrophic hearts may participate to the inability to cope with increased preload and to exercise intolerance in patients with cardiac remodelling and defective relaxation, typically seen in HFpEF.[16] Although the study falls short from nailing down titin phosphorylation as the sole mechanism, the tantalizing data on PKG signalling would highlight the interest for therapeutic strategies aiming at reinforcing upstream inducers of cGMP elevation.[11,17,18]

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