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INVITED EDITORIAL

Heart spotting

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Abstract Cardiac function depends upon several factors, including adequate cellular mass, intact contractile machinery, and adequate production of ATP. An appropriate homeostasis on all these levels is crucial for the daunting life-long task the myocardium faces. Not surprisingly, many alterations in the above factors have been spotted when the heart fails and hypothesized to play a causal role in the genesis of the failing heart. Indeed, development of cardiac hypertrophy and failure is associated with chamber remodeling as well as with changes of the phenotype at the level of the individual myocyte. Disturbed energy metabolism with impaired fatty acid oxidation and lower expression of proteins involved in ATP synthesis occurs during myocardial hypertrophy and heart failure. The altered expression of proteins from metabolic pathways may reflect mitochondrial dysfunction as a feature of the transition from compensated myocardial hypertrophy with preserved fatty acid metabolism to impaired energy metabolism in heart failure.

Key words calcineurin – heart failure – energy metabolism

Spot |spät| *noun.*
A small round or roundish mark, differing in color or texture from the surface around it.
verb. (**spot•ted, spot•ting**).
See, notice, or recognize (someone or something) that is difficult to detect or that one is searching for.

Cardiac function depends upon several factors, including adequate cellular mass, intact contractile machinery, and adequate production of ATP. An appropriate homeostasis on all these levels is crucial for the daunting life-long task the myocardium faces. Not surprisingly, many alterations in the above factors have been spotted when the heart fails and hypothesized to play a causal role in the genesis of the failing heart. Indeed, development of cardiac hypertrophy and failure is associated with chamber remodeling as well as with changes of the phenotype at the level of the individual myocyte [5, 11]. Disturbed energy metabolism with impaired fatty acid oxidation and lower expression of proteins involved in ATP synthesis occurs during myocardial hypertrophy and heart failure [3]. The altered expression of proteins from metabolic pathways may reflect mitochondrial dysfunction as a feature of the transition from compensated myocardial hypertrophy with preserved fatty acid metabolism to impaired energy metabolism in heart failure.

Calcineurin and "compensatory" cardiac remodeling

Cardiac hypertrophy, typically the resultant of elevated preload (as would occur in situations of in-

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creased volume loading such as athletic training, pregnancy or myocardial infarction) or increased afterload situations (typically resulting from aortic stenosis or chronic hypertension), was originally postulated to be a compensatory, beneficial response to normalize wall stress. Yet, in patients with aortic stenosis, higher left ventricular mass predicted left ventricular dysfunction more strongly than wall stress, and this persisted in patients in whom wall stress was normalized by left ventricular hypertrophy [7, 10]. In addition, regression of concentric left ventricular hypertrophy upon treatment with the ACE-I lisinopril was associated with an improvement of midwall fractional shortening, which was more strongly dependent on left ventricular mass reduction than on the reduction of circumferential end-systolic wall stress [15]. Moreover, experimental evidence now provides us with sufficient examples that normalization of wall stress may play a secondary effect of cardiac hypertrophy inhibition. Several studies using genetically engineered mice with markedly blunted growth responses to increase pre- or afterload appear to be protected from adverse effects of stress signaling and heart failure progression [4, 18]. Thus, clinical and experimental evidence have spotted a more nuanced interpretation of this phenomenon of "compensatory hypertrophy".

Calcineurin signaling is recognized to contribute to the progression of disease in a number of models, as its inhibition has been recognized to prevent cardiac hypertrophy [2]. This pathway is activated by prolonged increases in cytosolic Ca^{2+}, which leads calcineurin to dephosphorylate the transcription factor nuclear factor of activated T cells (NFAT), allowing it to translocate to the nucleus and engage in transcriptional activation of a hypertrophic gene program [13]. Given its ability to provoke a pathological form of hypertrophy, fibrotic myocardial remodeling and dramatic changes in the cardiac transcriptome [6], general consensus now holds that calcineurin/NFAT signaling cannot be viewed as part of a "compensatory" response of the myocardium to increased loading conditions.

Calcineurin metabolomics

In the current issue of *basic research of cardiology*, Schott and coworkers may have spotted a flaw in this simplified model where calcineurin signaling solely acts to provoke maladaptive responses in the myocardium [17]. The authors used rabbit right ventricular isolated papillary muscles cultivated in a muscle chamber system under physiological conditions and either maintained them completely unloaded or stretched to mimic elevated preload, while performing isotonic contractions (zero afterload). After 6 h, proteome changes were spotted by two-dimensional gel electrophoresis and ESI-MS/MS. Using this impressive proteomic approach, they were able to identify 28 proteins (spots) that were upregulated by preload compared to the unloaded group. Specifically, mechanical load upregulated a variety of enzymes involved in energy metabolism, including aconitase, pyruvate kinase, fructose bisphosphate aldolase, ATP synthase alpha chain, acetyl-CoA acetyltransferase, NADH ubiquinone oxidoreductase, ubiquinol cytochrome c reductase, and hydroxyacyl-CoA dehydrogenase. Most remarkably, cyclosporine A treatment (1 μmol/L), an experimental calcineurin inhibitor often utilized to study calcineurin biology, abolished the preload induced upregulation of these proteins.

In parallel, muscle strip contractile function was assessed after 6 h of culture with and without the calcineurin inhibitor cyclosporine. Raising the stimulation rate resulted in a significant frequency-dependent increase in developed twitch tension (positive force–frequency relationship, FFR). A positive FFR is a typical finding in myocardium exhibiting competent Ca^{2+} homeostasis. In preloaded preparations, the steepness of the frequency-dependent increase in developed twitch tension was significantly higher compared to unloaded preparations. In contrast, after culturing the strips in the presence of the calcineurin inhibitor, muscle strips still exhibited a positive FFR, but the preload-induced increase in steepness of the FFR was abolished by CsA treatment.

Thus, this study is the first to uncover and combine two new findings on the function of calcineurin activity in myocardium: its necessity to mediate the preload-induced optimization in Ca^{2+} homeostasis (and associated improvement in mechanical performance), and acute upregulation of a variety of enzymes involved in energy metabolism. The same group recently spotted in the same experimental model that acutely elevated myocardial preload triggers a short-term response characterized by enhanced expression of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) and improved Ca^{2+} homeostasis [9], prompting the authors now to formulate the hypothesis that calcineurin activity may actually be part of an acute, compensatory response to elevated preload to improve energy metabolism and Ca^{2+} homeostasis.

Making sense of it all

How should we interpret these conflicting results with what is currently accepted on calcineurin biology?

Supporters of the hypothesis formulated by Schott and coworkers would point to the concept that diverse triggers ignite relatively consistent changes in heart cells, including defective SR calcium loading, the successes obtained so far by phospholamban antagonism or SERCA2 overexpression in a variety of model systems, and the attractive prospect that one simple genetic rescue approach (correcting Ca^{2+} handling) could overcome a highly complex disease as heart failure. Additionally, one may argue that all positive reports on calcineurin function in maladaptive hypertrophy were obtained in mice, while the current study was performed in a species that seems closer to the human physiology, even if it were just for its size.

Notwithstanding these valid arguments, the mere contention that heart failure remains unchanged in the face of normalized calcium transients [8], remains a sobering biological fact, demanding a fundamental shift in our perception of the (patho)physiology of cardiac calcium cycling, the players involved, and the importance for calcium in cardiac adaptation to increased workload. Also, the direct link between calcineurin activity and SERCA2 expression is based upon observational studies without clear, mechanistic proof whether or not SERCA2 is a direct NFAT or MEF2 gene target [14].

With respect to the beneficial metabolic enzyme profile observed in this study, it is perplexing to observe that a recent study describes quite the opposite metabolic phenotype in hearts from transgenic mice expressing a constitutively active calcineurin in the myocardium [16]. Cardiac mitochondria isolated from these mice exhibit a maximal rate of electron transfer in heart mitochondria that was reduced by ~50% without loss of respiratory control. These mice revealed a 20%–30% reduction in subunit 3 of complex I (ND3) and subunits I and IV of cytochrome oxidase. Since calcineurin could not be spotted in the mitochondrial fraction, the authors of this work suggest that calcineurin can regulate metabolic enzymes and mitochondrial function through an indirect effect, possibly through transcriptional pathways. Hence, the metabolic phenomenon instigated by calcineurin has clearly negative effects on energy metabolism and mitochondrial function in the intact myocardium.

Another point of critique involves the model system employed in this paper. The most frequently employed animal models for pressure- or volume overload are more complex than the simplified system described in this paper. Of course it is difficult to study load dependence of gene expression in vivo, because load cannot be experimentally modified without concomitant induction of neurohumoral factors. Nevertheless, the simplification of the current model calls for a cautionary extrapolation to the in vivo situation. It would therefore be of interest to test the current findings in an acute intact myocardial situation to observe whether the findings in multicellular preparations would still hold in a relevant in vivo situation.

The more final, but maybe most important caveat of the current study, is the use of the pharmacological calcineurin inhibitor. Studies on cyclosporine are difficult to interpret, since these agents display systemic toxicity and significant non-calcineurin dependent effects [1]. Cyclosporin (Sandimmune) and FK506 or tacrolimus (Prograf) are used in organ transplantation and in the treatment of various immune disorders. Cyclosporin is a neutral, lipophylic and very hydrophobic, cyclic polypeptide of 11 amino acids extracted from *Tolypocladium Inflatum Gams*. The mechanism of action involves binding to ubiquitous cytosolic peptidyl-propyl isomerase cyclophylin. One welcome side effect of this complex is its ability to reduce calcineurin enzymatic activity. However, it has been shown that cyclosporine provokes immunosuppressive effects through induction of TGFβ1 in a mechanism likely to be independent from calcineurin. Cyclosporine was also shown to inhibit the activation of some members of the mitogen-activated protein kinases (MAPK) family, although one could now argue that this may be an indirect response to calcineurin inhibition [12]. More importantly for this paper, chronic cyclosporine stimulation induces changes in the properties of SERCA2 and in the kinetics of L-type Ca^{2+} channels [1], suggesting that what the authors observed may be parallel to and possibly independent of calcineurin activity. Accordingly, it is becoming increasingly clear that cyclosporine has calcineurin-independent effects in multiple organs, and does not constitute the optimal tool to test for a potential role of calcineurin in the setting of myocardial biology. To circumvent this particular caveat the authors could use the increasing number of genetic mouse models with dominant negative or null mutations for calcineurin/NFAT signaling. Alternatively, or in parallel, given the low accessibility of the rabbit genome for genetic manipulation, it would be of interest to perform these studies using siRNA technology to create rabbit papillary muscle preparations with genetic null mutations in the calcineurin/NFAT signaling cascade.

Notwithstanding latter critical notes, the current study utilized a remarkably impressive proteomic approach to demonstrate for the first time that an acute increase of myocardial preload causes upregulation of enzymes involved in fatty acid oxidation and glucose metabolism, thereby increasing the capacity

of the myocardium to generate ATP production. As such, this study puts the spot on novel insights into the proteome changes that seem to be part of a short-term adaptation to acutely enhance preload of the myocardium.

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