
Despite several major advances in the management of congestive heart failure such as the introduction of angiotensin-converting enzyme inhibitors and beta-blockers, the prognosis of CHF remains poor and a major public health issue in westernised countries [1]. Accordingly, improved long-term pharmacological management of cardiac diseases will require new research characterised by basic work on the pathophysiology of the diseased cardiomyocyte.

Heart failure often results from long-term consequences of haemodynamic overload, which creates a mechanical stress on the cardiomyocytes. One of the earliest responses of the latter to this insult is reactivation of the foetal genes program, preceded by activation of transcription factors [2]. In most of these instances, reactivation of the foetal genes is transient and with no obvious link with the progressive deterioration of cardiac function. Nevertheless, some other genes are activated for long periods of time [3]. Follow-up of the expression profile of these genes could serve as a dynamic marker of the transition from hypertrophy to cardiac failure and help to identify potential pharmacological targets.

Among these newly discovered genes, the nuclear-specific transcription factor CARP (‘‘cardiac adriamycin-responsive protein’’ [4] or ‘‘cardiac ankyrin repeat protein’’ [5]) could be such a candidate. In fact, in the search for the molecular pathways guiding ventricular chamber specification and morphogenesis in rodents, Jeyaseelan and co-workers [4], simultaneously with Zou et al. [5], identified a gene coding for the nuclear protein CARP, whose expression was stimulated by the homeobox gene product Csx/Nkx2-5. These works also demonstrated that CARP activation was accompanied by repressed expression of the ventricular isoform of myosin light chain 2, atrial natriuretic factor and cardiac troponin C genes. Since that initial discovery, several groups have confirmed the expression of CARP in foetal and adult hearts [6,7]. By analogy with re-expression of foetal genes in several cardiac pathologies, it was logical to assess CARP expression in similar situations. Thus, Eschenhagen’s group [8] demonstrated that CARP expression, at the mRNA and protein levels, was increased in failing ventricular muscle from dogs submitted to rapid ventricular pacing as well as in explanted ventricular muscle from patients suffering from various causes of heart failure. Increased CARP mRNA was also reported in hypertrophied mouse hearts overexpressing calsequestrin [9] or submitted to stimuli promoting cardiac hypertrophy [10].

Data clearly converge to point out the existence of a link between CARP re-expression and various cardiac insults. The next logical question was then to know whether CARP re-activation was only a marker of cardiac damage or whether it had functional consequences, knowing the repressor role of CARP on the expression of several contractile proteins (ventricular isoform of myosin light chain 2 or cardiac troponin C), as demonstrated in rodents.

In this issue of Cardiovascular Research, the report from Zolk et al. [11] shows, for the first time, short-term contractile effects of CARP overexpression, in addition to a new pathway of CARP gene expression mediated by beta-adrenoceptors. These results and conclusions have been gained from several approaches combining in vivo (adult rats made hypertensive by osmotic pump-driven infusion of low-dose isoprenaline for 4 days) and in vitro investigations such as adenovirally-mediated overexpression of CARP into neonatal rat cardiomyocytes cultured from 2 to 12 days. Functional consequences of CARP overexpression were particularly elegantly investigated as it involved incorporation of CARP-overexpressing neonatal rat cardiomyocytes into a collagen gel, leading to an engineered cardiac tissue with measurable contractile properties. These approaches led to two key issues. Firstly, CARP gene expression was controlled in part by beta-adrenergic stimulation. Thus chronically isoprenaline-infused rats presented an increase expression (although of variable extent) of ventricular CARP protein (relative to calsequestrin) and mRNA (relative to GADPH). These results are a first indication that beta-adrenergic receptors
could indeed play a role in the control of CARP expression but changes in haemodynamic load and/or and cardiac rhythm (tachycardia) could not be underestimated. Demonstration of the direct involvement of cardiac beta-adrenoceptors in controlling CARP expression was obtained in cultured neonatal rat cardiomyocytes exposed to 1 μM isoprenaline for 24 hours. Myocytes thus displayed increased relative expression of CARP mRNA. This effect was blocked by selective inhibition of the beta1- and/or beta2-adrenoceptors, as well as by inhibitors of protein kinase A and calmodulin kinase. Furthermore, in actinomycin-treated cultured neonatal rat cardiomyocytes, isoprenaline increased mRNA levels by slowing its degradation. A role for adrenergic stimulation in the control of CARP expression was previously demonstrated Maeda et al. [12], who reported that alpha1-adrenergic stimulation led to a prazosin-sensitive increase in CARP mRNA in cultured neonatal rat cardiomyocytes transfected with the mouse CARP gene. Finally, in cultured neonatal cardiomyocytes, the CARP promoter was activated by p38 kinase, which is one distal mediator of alpha1-adrenergic stimulation [10]. These results collectively suggest that several adrenoceptor-mediated pathways control the expression of CARP.

Secondly, adenovirally-mediated overexpression of CARP in 12-day-cultured neonatal rat cardiomyocytes subsequently embedded in a collagen-based matrix (engineered 3-dimensional cardiac tissue) promoted decreased contractile responsiveness to externally applied isoprenaline or calcium but unchanged basal force when compared to green fluorescent protein-overexpressing (control) cardiomyocytes. These data illustrate that increased CARP expression, as reported in several models of cardiac diseases, could partly be responsible for the depressed contractile responsiveness of failing hearts to catecholamines, although other mechanisms such as decreased density of beta1-adrenoceptors cannot be excluded.

The novelty of Zolk and co-workers’ work is to provide evidence that increased CARP expression in diseased hearts is not merely an indicator of reactivation of the foetal gene program but could also play a pathophysiological role in the contractile responsiveness of diseased cardiac muscle. Nevertheless this report deserves a note of caution as well as raises new questions. Thus the relevance of the results obtained in neonatal rat cardiomyocytes (especially those cultured for 12 days and used for contractile experiments) to the adult failing heart is questionable. Further demonstration of the functional (contractile and electrophysiological) effects of CARP overexpression in adult cardiomyocytes will require adenovirally-mediated CARP overexpression of short-term-cultured isolated myocytes or ventricular trabeculae [13], both preparations in which excitation-contraction coupling can be investigated.

An intriguing issue raised by the work from Zolk et al. is the link between overexpression of CARP and modified responsiveness to isoprenaline or calcium despite unchanged basal contractile amplitude. In fact, this seems contradictory because CARP is known to repress the expression of several contractile proteins (at least in neonatal rodents cardiomyocytes) which, by itself, should modify basal contractile characteristics (although Zolk and co-workers did not document the duration of the different phases of contraction, which could have been relevant). Because the contractile responses following exposure to either isoprenaline or external calcium share proper expression/function of calcium-regulating proteins, the expression profile of the latter is worth investigating in CARP-overexpressing cardiac preparations. These results do not exclude however that CARP overexpression also decreases the expression/function of proteins involved in the beta-adrenergic pathway. The functional consequences of CARP overexpression raise an obvious question: is it possible that CARP directly regulates cardiac contractility?

This hypothesis seems at odd with the nuclear localisation of CARP (see for instance [4,5]). However this exclusive nuclear localisation has been challenged by reports of cytoplasmic expression CARP in cardiomyocytes and at the I-band level in adult skeletal muscle cells [14,15]. It is therefore possible that CARP could directly modulate cardiac excitation-contraction coupling by associating with, for instance, calcium-regulating proteins.

Finally, the sensitivity of CARP expression to both alpha1- and beta-adrenergic stimulation is intriguing. However, alpha1-adrenergic stimulation is a well-known hypertrophic stimulus [16], which is relevant with the increased expression of CARP in several models of hypertrophy in the adult heart [10]. By contrast, long-term beta-adrenergic stimulation of adult cardiomyocytes is known to decrease the membrane density of beta1-adrenoceptors and to increase the expression of G_{i} proteins [17]. The overall functional consequence is that beta-adrenergic responsiveness is decreased in failing heart, as also reported here by Zolk and co-workers.

In conclusion, the present report from Zolk et al. supports the idea that the increased CARP expression, described in several models of cardiopathies, does not only indicate foetal gene program re-activation, during which CARP would merely behave as a marker, but can also promote functional (contractile) disturbances in failing hearts, such as decreased responsiveness to elevated concentration of catecholamines. The consequences of increased expression of CARP on excitation-contraction coupling in adult normal and failing hearts is definitely worth investigating, as this protein could represent a potentially new pharmacological target.

References


