Calcineurin, myocardial hypertrophy, and electrical remodeling

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See article by Dong et al. [7] (pages 320–332) in this issue.

The pathogenesis of cardiac ventricular hypertrophy (CVH), both compensated and that associated with cardiac dysfunction, has attracted a lot of attention and this field still continues to expand. This huge clinical and scientific interest is related to the fact that CVH has been identified as a powerful risk factor for total and cardiovascular mortality. Interestingly, the Framingham study also demonstrated that CVH significantly increases the risk of sudden cardiac death, both in men and in women [1]. Subsequent studies provided evidence that cardiac arrhythmias are an important mechanism contributing to the high mortality and sudden death in patients with CVH [2–4]. Not surprisingly, a lot of effort has been expended to characterize the electrophysiological phenotype and to elucidate the mechanisms of arrhythmias in CVH.

New research tools have been provided with the advent of modern molecular biology techniques. Thus it is now possible to up- or down-regulate cardiac expression of various elements of intracellular signaling pathways in order to investigate their specific role in the process of myocardial normal growth as well as pathological remodeling. One such signaling pathway (although not the only one) is calcineurin-dependent activation of intracellular transcription factors.

Calcineurin is an intracellular protein phosphatase regulated by Ca²⁺/calmodulin and activated by micromolar calcium concentrations [5]. Calcineurin and its downstream transcriptional effector NFAT (nuclear factor of activated T-cell) mediate cardiac hypertrophic responses initiated by increases in cytoplasmic calcium. Overexpression of calcineurin in mice has been previously shown to lead to extensive CVH, heart failure, and sudden death [6]. In the present issue of the Journal, Dong et al. [7] present new experimental data that shed some new light on the mechanisms of sudden death and cardiac electrophysiological remodeling in mice with CVH induced by calcineurin overexpression.

1. Calcineurin—a common pathway for electrical remodeling?

It is noteworthy that, in spite of many different experimental models of CVH, hypertrophy-induced changes in cellular electrophysiology are qualitatively very similar. The most consistent phenotypic abnormality is a prolongation of action potential duration, which is seen in various species, both in isolated cells and in whole heart preparations [8,9]. Prolongation of the duration of cellular action potential is due to an increase in depolarizing currents or a decrease in repolarizing currents. The exact molecular mechanisms that trigger these electrical changes are not fully understood. On the one hand, electrical remodeling (i.e. changes in the properties or density of various transmembrane ion currents) may be secondary to the hypertrophy process itself. On the other hand, it is possible that alterations in ion currents are directly and independently linked to the same intracellular signaling pathways as myocardial hypertrophy. In many (although not all) studies and different experimental models, pharmacological or non-pharmacological inhibition of calcineurin has been shown to attenuate or prevent hypertrophy (both in vivo and in culture) induced by a variety of stimuli, such as phenylephrine, angiotensin II, endothelin-1, aortic banding, Dahl-salt sensitive or renovascular hypertension, exercise, myocardial infarction, etc. [10,11]. Thus, it seems that many different models of CVH may share this intracellular signaling pathway. Because many depolariz-
ing and repolarizing currents are regulated by ion channel phosphorylation/dephosphorylation [12,13], experimental models involving changes in intracellular calcineurin activity might address more directly the hypothesis that electrical remodeling and myocardial hypertrophy are independently linked to the same intracellular signal transduction pathway. This might explain some electrophysiological phenotypic similarities between various models of CVH.

1.1. Repolarizing currents

In this issue of the Journal, Dong et al. [7] report that CVH due to calcineurin overexpression in the mouse is associated with a decrease in the density of three outward currents: \( I_{to,f} \) (a rapidly activating and inactivating, fast transient outward current), \( I_{to,s} \) (a rapidly activating and slowly inactivating, slow transient outward current), and \( I_{k,slow} \) (a rapidly activating and very slowly inactivating current). Treatment with cyclosporin A (a calcineurin inhibitor) not only prevented the development of CVH, but also prevented the decrease in \( I_{to,f} \) density. However, the decrease in \( I_{to,s} \) and \( I_{k,slow} \) seen in transgenic animals was not reversed by cyclosporin A treatment. This latter observation was explained by the fact that cyclosporin A also reduced \( I_{to,s} \) and \( I_{k,slow} \) (but not \( I_{to,f} \)) in non-transgenic animals, suggesting that the effects of cyclosporin A on these currents are independent of changes in ventricular mass. Calcineurin activity was not measured in this study, so that it is impossible to make any direct inference about a quantitative or qualitative correlation between the degree of hypertrophy, electrical remodeling and intracellular calcineurin activity. Importantly, although cyclosporin A reverses hypertrophy in this model, it does not reverse mechanical dysfunction or cardiac fibrosis, and, in fact, increases the incidence of sudden death [14,15]. Therefore, because animals treated with cyclosporin A display features of cardiac dysfunction (failure), the cardiac electrophysiological phenotype in these animals cannot be simply ascribed to reversal of myocardial hypertrophy. In fact, this is a new phenotype of cardiac failure, associated by itself with some degree of electrical remodeling. Unfortunately, Dong et al. do not report the effects of cyclosporin A treatment on action potential duration or QT interval.

In another model of CVH secondary to myocardial infarction (characterized by increased calcineurin activity, depressed \( I_{to,f} \) density and decreased Kv4.2 and Kv4.3 mRNA levels), Deng et al. [16] found that cyclosporin A treatment resulted in a decrease in calcineurin activity and in a lesser degree of \( I_{to,f} \) and Kv4.2/4.3 mRNA depression. Interestingly, in the sham group, in the absence of heart failure, cyclosporin A markedly decreased calcineurin activity, but had no effect on Kv4.2/4.3 mRNA levels (changes in \( I_{to,f} \) in this group were not reported). These observations, together with those reported by Dong et al. [7], suggest that calcineurin is unlikely to exert any significant direct effect on \( I_{to,f} \) at a transcriptional level, and possibly also at a cell membrane level. This conclusion is further supported by the observations in isolated myocytes that pharmacological inhibition of calcineurin does not affect potassium currents responsible for repolarization in the mouse (\( I_{to} \) and \( I_{sus} \)) [17] or in the rat (\( I_{to} \) and \( I_{k} \)) [18]. However, recent preliminary results from mice overexpressing calcineurin (i.e. with increased phosphatase activity) and those overexpressing NFAT (which is distal to calcineurin in the signal transduction pathway and has no phosphatase activity) challenge the earlier pharmacological data. Namely, it has been shown that although both calcineurin and NFAT overexpression cause myocardial hypertrophy, calcineurin down-regulates \( I_{to,f} \), \( I_{to,s} \) and \( I_{k,slow} \), whereas NFAT up-regulates these currents [19]. These observations suggest that calcineurin might directly affect electrical remodeling due to its phosphatase activity. Furthermore, the ultimate electrophysiological phenotype of calcineurin-induced hypertrophy is probably a net result of the direct effects of calcineurin on membrane ion channels and of the hypertrophy process itself.

Intriguingly, the results reported in this issue of the Journal by Dong et al. [7] (i.e. a decrease in the density of \( I_{to,f} \), \( I_{to,s} \), and \( I_{k,slow} \) in CVH induced by calcineurin overexpression) disagree with a previous report in the same CVH model, in which action potential duration was prolonged in association with an increase in the density of the \( I_{sus} \) current (reflecting the sum of the steady state, \( I_{s} \), and \( I_{k,slow} \) currents) and transient outward peak current \( (I_{to,peak}) \) [20]. When the \( I_{to} \) current was calculated (as \( I_{to,peak} - I_{sus} \)), it also tended to increase. Certain methodological differences (such as a much slower pacing rate used by Dong et al.) cannot fully explain this discrepancy. It should be noted however that, in spite of a similar age of animals used in both studies, the degree of myocardial hypertrophy (expressed as heart/body weight ratio) was markedly greater in the study by Dong et al. [7]. Interestingly, Petrashevskaya et al. [20] reported that, in transgenic mice at a heart failure stage, \( I_{to} \) was significantly decreased. It suggests that electrical remodeling in this model is a dynamic process and perhaps depends on the degree of hypertrophy and the state of functional compensation. The reason why, in two separate studies, transgenic mice of a similar age and obtained using similar methodology [6] should develop different degrees of CVH is another puzzle. Could that be a gender effect? All animals used by Dong et al. were females [7]. Petrashevskaya et al. [20] do not mention the gender of their study animals, so I assume that they used both male and female mice. It is very interesting to speculate that gender may modify the degree of hypertrophic response and electrical remodeling secondary to calcineurin overexpression. This hypothesis requires further studies.

1.2. Depolarizing currents

Although not investigated by Dong et al. [7], previous studies have reported that the density of the L-type calcium
current ($I_{Ca}$) is increased in hypertrophied myocytes from transgenic animals with calcineurin overexpression (without any changes in unitary single-channel conductance) [20,21]. The kinetics of $I_{Ca}$ inactivation was also faster in transgenic animals due to altered calcium release from the sarcoplasmic reticulum. Furthermore, enhanced $I_{Ca}$ was not due to altered channel protein levels, but rather due to an increase in functionally active channels. Chronic treatment with cyclosporin A prevented the development of CVH and changes in $I_{Ca}$. The effects of chronic treatment with cyclosporin A on $I_{Ca}$ in non-transgenic animals were not directly reported. However, acute administration of cyclosporin A to myocytes from normal or transgenic hearts had no significant effect on $I_{Ca}$. Also, $I_{Ca}$ was not affected by overexpression of calcineurin inhibitory domain from proteins Cain or AKAP79. These results seem to suggest that intracellular calcineurin does not directly regulate $I_{Ca}$, and electrical remodeling in this model is mediated by other mechanisms related to the hypertrophy process. Nevertheless, it appears that this issue is still far from being settled. Wang et al. [22] reported that chronic administration of cyclosporin A to normal mice decreases ventricular peak $I_{Ca}$. And yet, another group reported an increase in $I_{Ca}$ in isolated mouse myocytes treated with cyclosporin A for 10–30 min and suggested that, in its effects on $I_{Ca}$, calcineurin opposes the actions of protein kinase A [17].

New experimental results may provide some important insights into the mechanisms of interaction between calcineurin and $I_{Ca}$ in CVH. In a recent study, hypertrophy was induced by angiotensin II in cultured myocytes and was associated with a substantial increase in $I_{Ca}$, accompanied by increased $\alpha_{1c}$ subunit transcript and protein levels (cyclosporin A abolished both the increase in cell size and $I_{Ca}$) [23]. Exposure of cultured myocytes to adenovirus encoding constitutively active calcineurin caused a similar (or even greater) increase in cell size, and a relatively smaller increase in $I_{Ca}$ density. Interestingly, compared with control cells, myocytes hypertrophied by angiotensin II demonstrated significantly decreased radioactive phosphate incorporation within a 240-kDa band (consistent with the $\alpha_{1c}$ subunit) detected by autoradiography. These preliminary results suggest that the modulation of $I_{Ca}$ in hypertrophy is a complex process, which might involve both changes in $I_{Ca}$ channel expression and phosphorylation. It is plausible that calcineurin may play an important role in both of these processes, although its net effect will depend on the model and stage of myocardial hypertrophy and on the contribution of other intracellular signaling pathways.

2. Electrical heterogeneity in the heart

The electrophysiological properties of healthy ventricular myocardium are very heterogeneous [24]. Among others, there are intrinsic electrical differences between the myocytes from different regions of the heart, which are the result of different contributions of ionic currents to the transmembrane action potential. Mouse ventricular myocardium is also heterogeneous in that various ion currents are differentially distributed [25,26]. For example, it has been demonstrated that $I_{K,\text{slow}}$ is present in all left ventricular cells isolated from the apex and in most (but not all) left ventricular septum cells (with $I_{K,\text{slow}}$ density being significantly lower in the septum), whereas $I_{N\alpha,S}$ is found only in left ventricular septum cells (and not in the apex) [26]. The densities of $I_{K,\text{slow}}$ and $I_{N\alpha,S}$ are lower in the septum than in the apex [26]. Significant differences in potassium currents also exist between the base and apex of the mouse heart [27].

The electrophysiological effects of myocardial hypertrophy are also heterogeneous, with qualitative and quantitative regional differences in ion currents [8,9,24,28,29]. It implies that it is impossible to assess fully the character and magnitude of hypertrophy-induced electrical remodeling without taking these regional differences into account. Similarly, different hypertrophy models or experimental results cannot be reliably compared with one another unless these regional differences are considered.

The mouse myocytes studied by Dong et al. [7] in this issue of the Journal were isolated from the apex of the left ventricle. The differences between the results of this study and those reported by Petrashevskaya et al. [20] (as discussed earlier in this editorial) may, in part, be related to regional electrical heterogeneity, because Petrashevskaya et al. studied myocytes dissociated from the whole ventricle. On the other hand, the presence of the $I_{K,\text{sub}}$ current in apical myocytes in the study by Dong et al. is in contrast with the previous report that $I_{K,\text{sub}}$ is found only in the septum (but not in the apex) [26] and suggests that the cells studied by Dong et al. might have been ‘contaminated’ by cells from non-apical areas of the left ventricle. It should be noted that Petrashevskaya et al. [20] demonstrated that the duration of action potentials and, in particular, the action potential shape may differ among myocytes isolated from transgenic hearts with myocardial hypertrophy. This observation suggests the possibility of regional heterogeneity in electrical remodeling induced by calcineurin overexpression. It raises several interesting questions. For example, is calcineurin uniformly expressed in this and other models of CVH? If so, are there any regional differences in calcineurin-related intracellular signaling pathways and the phenotypic consequences thereof?

Another interesting consideration regarding the problem of electrical heterogeneity is related to the fact that, in the study by Dong et al. [7], the QT interval was significantly prolonged in transgenic animals, whereas at a cellular level the myocytes from transgenic hearts had significantly longer APD50, but not APD90. It is notable that, in the study by Petrashevskaya et al. [20], both APD50 and APD90 were markedly prolonged in transgenic animals. Therefore, it is possible that QT prolongation in the study...
by Dong et al. may be accounted for by significant APD_{90} prolongation in cells from other regions of the heart, which were not studied by the authors. Another intriguing possibility is that QT prolongation in the mouse heart (in the absence of differences in APD_{90}) is caused by delayed depolarization in some areas of the heart due to slowing of conduction. This explanation is consistent with the presence of atrio-ventricular conduction block observed by Dong et al. and suggests that intraventricular conduction should be a subject of further studies in this model of calcineurin-induced myocardial hypertrophy.

3. Arrhythmogenic mechanisms

It has been previously reported that calcineurin over-expression in mice leads not only to myocardial hypertrophy and heart failure, but also to sudden death [6]. The exact mechanisms of sudden death in these animals are not known. Dong et al. [7], in this issue of the Journal, suggest that at least in some mice with calcineurin-induced hypertrophy, sudden death may be associated with polymorphic ventricular tachycardia and/or high degree atrio-ventricular block. These findings are of interest, especially that the authors also report an age-dependent increase in the proportion of mice with non-sustained ventricular tachycardia and Wenckebach block. Nevertheless, these observations need to be confirmed in a larger number of animals.

The mechanisms of bradyarrhythmias seen in this model are poorly understood. Similarly, the actual electrophysiological mechanisms of tachyarrhythmias are unclear. The significance of action potential prolongation is somewhat questionable, because the magnitude of this effect, as well as the magnitude of dispersion of repolarization, are probably very small at physiological heart rates. However, at slower heart rates (which are also characteristic of this model) it cannot be excluded that non-uniform prolongation of repolarization may underlie ventricular tachyarhythmias.

It should be pointed out that arrhythmias and sudden death reported by Dong et al. [7] were seen in mice which were much older than those used in the patch-clamp studies. In the original description of this hypertrophy model [6], dilatation of the ventricular chambers was observed with increasing age in transgenic mice. The mice, which died suddenly, were characterized not only by myocardial hypertrophy, but also by myocardial fibrosis, biventricular dilatation and lung edema (consistent with heart failure). It is likely therefore that mechanisms other than prolonged action potential duration (such as myocardial fibrosis, increased electrical anisotropy, changes in intracellular calcium handling, etc.) may play an important role in arrhythmogenesis in this model. In support of this conclusion is the observation that treatment with cyclosporin A (which reverses hypertrophy, but not fibrosis in this model) does not decrease the incidence of sudden death [14, 15].

Another interesting finding in the study by Dong et al. [7] (unfortunately, also limited by a small number of observations) is that dofetilide (an I_{Kr} blocker) may be proarrhythmic in transgenic animals with hypertrophy-induced electrical remodeling. This is somewhat puzzling, because the I_{Kr} current does not play a prominent role in the process of repolarization in normal adult mouse myocytes [30], especially at physiological heart rates and temperature. The proarrhythmic effect of dofetilide may therefore reflect decreased repolarization reserve in the transgenic mice (with a greater relative contribution of I_{Kr} to repolarization) or/and may possibly be related to ‘compensatory’ up-regulation of the I_{Kr} current. This latter hypothesis remains to be tested. Also, since repolarization is known to be influenced by sex hormones and female hearts are generally more prone to drug-induced tachyarrhythmias, it remains to be seen whether this observation reported by Dong et al. in female hearts can also be extrapolated to male mice.

4. Conclusions

The model of calcineurin-induced myocardial hypertrophy and sudden death can provide important new insights into the mechanisms of cellular hypertrophy, electrical remodeling and cardiac arrhythmias. A particularly interesting and as yet unresolved question is whether the process of electrophysiological remodeling can be dissociated, at least in part, from the process of hypertrophy itself. Also more studies are needed to address the issue of regional electrophysiological effects of calcineurin overexpression. Whether and how this knowledge will be translated into human pathophysiology and therapy is, at this stage, unknown. It is interesting however that many existing therapies have the potential to decrease calcineurin activity [31, 32].

References


