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Commentary

It has long been noted that while patients with familial hypertrophic cardiomyopathy due to cardiac troponin T (cTnT) mutations often suffer sudden cardiac death, they do not develop significant ventricular hypertrophy, suggesting that a distinct cellular mechanism apart from alterations in myocardial contractility is responsible. A new study has revealed that a single missense mutation in cTnT causes a striking disruption to energy metabolism, leading to cardiomyopathy.

Cardiac troponin T and familial hypertrophic cardiomyopathy: an energetic affair

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It has long been noted that while patients with familial hypertrophic cardiomyopathy due to cardiac troponin T (cTnT) mutations often suffer sudden cardiac death, they do not develop significant ventricular hypertrophy, suggesting that a distinct cellular mechanism apart from alterations in myocardial contractility is responsible. A new study (see the related article beginning on page 768) has revealed that a single missense mutation in cTnT causes a striking disruption to energy metabolism, leading to cardiomyopathy.

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The hallmarks of familial hypertrophic cardiomyopathy (FHC) are unexplained hypertrophy of the left ventricle and of the interventricular septum without dilatation of the ventricular chamber, and cardiac myocyte disarray (reviewed in refs. 1–5). A prominent feature in nearly all patients with FHC is abnormal diastolic function due to impaired relaxation and reduced compliance (6). FHC is a frequent cause of sudden cardiac death (SCD), particularly in young individuals and competitive athletes (7). The disease is phenotypically heterogeneous. Some individuals remain asymptomatic throughout life, and others develop progressive symptoms with or without heart failure or experience SCD. In some families, FHC is a malignant disease with a high incidence of SCD. Eleven genes are involved in the pure form of the disease, all encoding components of the contractile apparatus of the cardiac myocyte (reviewed in ref. 8). Genotype-phenotype analyses revealed some significant differences in prognosis between the different FHC genes and between different mutations in the same gene (reviewed in refs. 3, 9). Cardiac troponin T (cTnT) mutations in particular predispose the affected patients to SCD, and one of the most malignant mutations in FHC is a missense mutation that results in an exchange of a glutamine for an arginine at residue 92 (R92Q), in the tail portion of cTnT (10). Individuals bearing this mutation are at high risk for SCD, despite the absence of overt ventricular hypertrophy. The work of Javadpour et al., reported in this issue of the *JCI* (11), describes sound and consistent energetic and mechanical data that provide insight into the mechanism by which patients develop defective energy metabolism and cardiomyopathy due to an FHC-causing mutation in cTnT.

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Nonstandard abbreviations used: familial hypertrophic cardiomyopathy (FHC); sudden cardiac death (SCD); troponin T (TnT); cardiac troponin T (cTnT); maximum rate of left ventricular pressure decay (–dP/dt).

Troponin T is a key regulator of cardiac contraction

In striated muscles, troponin T (TnT), together with troponin C (TnC), troponin I (TnI), and tropomyosin, belongs to the group of so-called regulatory proteins of the sarcomere. Early studies have established a role for each of them. TnI prevents the interaction of actin with myosin heads, an inhibition relieved by calcium binding to TnC. TnT binds the two other troponins to tropomyosin, which itself binds the whole protein complex to the actin thin filament. In this model, TnT behaves simply as a passive link between other more functionally implicated proteins. This simplistic scheme has been questioned recently by a number of elegant biochemical and biophysical studies (12–17). TnT now appears to be the most important regulatory protein of the sarcomere, which could explain why it exists as so many alternatively spliced isoforms. Experiments using peptides expressed in *Escherichia coli* have shown that a portion of the TnT tail (residues 70–170) is crucial for TnT binding to tropomyosin, tropomyosin binding to actin, and stabilization of the complex with overlapping tropomyosin extremities. FHC mutations localized between residues 92 and 110 alter these TnT functions, whereas those outside the region are neutral (12). More importantly, the tail portion of the TnT molecule also seems to be essential for regulating the function of the whole troponin-tropomyosin complex. Indeed, it regulates the position of the complex on the thin filament vis-à-vis the myosin heads by stabilizing either a blocked B-state of the filament — in which, in the absence of calcium, tropomyosin binds to the actin outer domain in a position where it prevents muscle contraction (13) — or a closed C-state in which actin-myosin interaction is still weak (16). A number of FHC mutations in this area would impair TnT function through alterations in TnT tail flexibility (17).

Functional and energetic consequences of the R92Q mutation

The R92Q missense mutation examined by Javadpour and colleagues in the present study (11) is located in a TnT domain that interacts with

tropomyosin and that therefore is critical for cooperative actin binding and regulatory function. The functional consequences of this mutation have been extensively characterized in isolated myofibrils (reviewed in ref. 12) and transgenic mouse models (18, 19), but the results have varied according to the model system employed. For example, in cultured quail myotubes (20) and rat ventricular myocytes (21), the mutant TnT decreases the calcium sensitivity of force production, whereas in permeabilized rabbit cardiac fibers, the free-calcium concentration required for tension generation is significantly lower (22). These differences are not very surprising, considering the difficulties of working with a structure as complex as the myofibril. Recently, the R92Q mutation has been compared with the ΔLys-210 deletion, known to cause dilated cardiomyopathy in in vitro assays of ATPase activity and filament motility (23). Whereas the ΔLys-210 deletion gave a significantly reduced sliding speed as compared with wild-type TnT and produced less enhancement of ATPase at activating calcium concentrations, the inverse occurred with the R92Q mutation. At activating calcium concentrations, it enhanced thin-filament sliding velocity and activation of ATPase activity. Even more interestingly, at low calcium concentrations, it induced less inhibition of filament sliding velocity and of ATPase activity than did wild-type TnT, suggesting a global shift toward an increased function, regardless of the calcium concentration and therefore of the functional stage in the contraction-relaxation cycle.

Consistent with these observations, Javadpour et al. (11) show that the R92Q mutation, when expressed in the mouse heart (67% replacement of the wild-type protein), decreases the energetic driving force within cardiac myocytes ($|\Delta G_{\sim ATP}|$ = free energy of ATP hydrolysis) by increasing ATP utilization, most likely because of a mutation-induced boosting of myofibrillar ATPase activity. Very interestingly, all mechanical parameters at the base-line calcium concentration of 2.5 mM were normal, except the maximum rate of left ventricular pressure decay (–dP/dt), which was decreased by 26%, indicative of diastolic dysfunction. This can be explained by the fact that the decrease in free energy released from ATP in R92Q brings the value of $|\Delta G_{\sim ATP}|$ to 51.3 kJ/mol, below that required for the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) to function optimally (24). Therefore, it could be expected that left ventricular mechanical function would worsen when hearts are submitted to an increase in workload. Indeed, when hearts were perfused with 4 mM calcium, only a very small fall in free energy of ATP hydrolysis was observed, which could not even be transformed into an increase in mechanical work.

Is a mismatch in energy utilization a novel pathogenic mechanism in FHC?

A critical feature of this study is that measurements were taken in the intact heart, where the impact of small perturbations at the protein level can be summed over a large number of myofibrils (11). The results are important for a number of reasons. They extend in vitro data indicating that the R92Q TnT mutation enhances the activation of myofibrillar ATPase activity. However, it remains now to understand how mutations in the TnT tail, a portion of the TnT molecule that normally prevents ATPase activation, transforms this peptide into an activating molecule that finally results in a boost in overall myofibrillar ATPase activity. Another intriguing finding of the study is the inability of the mutated TnT to transduce the increase in calcium to increased contractile performance, which contrasts markedly with the effect of the R403Q α-myosin heavy chain mutation. In a more speculative way, one may wonder why adaptive mechanisms do not take place in the R92Q TnT mouse heart to reset $|\Delta G_{\sim ATP}|$ at a normal level. This question may be related to that of why no overt ventricular hypertrophy is observed with this mutation and it can be hypothesized that both resetting of $|\Delta G_{\sim ATP}|$ at a normal level and development of ventricular hypertrophy would further deteriorate the energetic cost for a given workload.

Another important finding of the study by Javadpour et al. (11) that could only be demonstrated using an in vivo model is that the deleterious effect of the mutation occurs particularly during increases in workload. An important next step will be to determine the incidence of SCD in these mice and to elucidate how it occurs. Confirmation that the energetic alterations are responsible for SCD would invite systematic investigation of whether genes that encode proteins involved in maintaining the energetic homeostasis of cardiac myocytes are modifier genes. The work of Javadpour et al. also provides a new framework for our understanding of a number of unresolved cases of diastolic dysfunction, although some findings may need some clarification, such as the unexpectedly small improvement of –dP/dt and the absence of an increase in end-diastolic pressure when hearts of R92Q mice are submitted to an increase in workload.

That such a pathophysiological process develops almost exclusively during an increase in workload in the absence of detectable cardiac hypertrophy points to the need to determine patients at high risk for SCD by performing investigations not only at rest but also during exercise. Such a need may be relevant not only to the screening of probands within an affected family but also to all individuals who play sports, and not only high-level athletes.

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Hypertension: β testing

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A new study (see the related article beginning on page 717) demonstrates that angiotensin-induced hypertension results in a marked decrease in expression of the β subunit of the BK channel, suggesting a role for this critical subunit in the regulation of vascular tone.

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Hypertension, a silent killer, affects more than 40 million Americans, approximately a third of whom are not aware of their condition, resulting in an increased risk of heart attack, stroke, and kidney disease. The prevalence and severity of hypertension increases markedly with age, and some estimates suggest that as many as 90% of adults will suffer from systolic hypertension by the age of 80. Despite these daunting statistics, the root causes of progressive hypertension remain elusive. One important component of the regulation of vascular tone, a major determinant of blood pressure, has been identified within the last several years, however, and several findings now suggest that this signaling system may play an important role in systemic hypertension.

Increases in arterial vascular smooth muscle tone narrow the arteries and lead to chronic increases in systemic blood pressure. Evidence suggests that a major signaling system within myocytes serves to hyperpolarize and relax arterial smooth muscle. This system consists of intracellular Ca^{2+} channels (ryanodine receptor) expressed on the sarcoplasmic reticulum, and large conductance Ca^{2+}-activated K^+ (BK) channels. As shown in Figure 1, the gating of ryanodine receptors releases Ca^{2+} ions close to the myocyte membrane, resulting in the activation of a few BK channels, and a small hyperpolarizing current, producing (1). These events occur in single arterial myocytes at a frequency of approximately 1 Hz, contributing a tonic hyperpolarization throughout the electrically coupled arterial smooth muscle. Thus, alterations in the activity of this signaling pathway could have important consequences on arterial tone and systemic blood pressure.

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Nonstandard abbreviations used: Ca^{2+}-activated K^+ (BK); angiotensin II type 1 (AT1); sarcoplasmic reticulum (SR).

BK channels regulate vascular tone

BK channels are made up of pore-forming α and regulatory β subunits. While a single gene encodes the α subunit, which is expressed ubiquitously, there are four distinct β subunits that show marked tissue specific expression and account for much of the functional diversity of the channel complexes observed in different cell lineages (for review see ref. 2). The β1 subunit (3), which is selectively expressed in smooth muscle and which markedly increases the Ca^{2+} sensitivity of the channel complex, has received substantial attention recently regarding its role in the regulation of vascular tone. Binding of steroid hormones such as estradiol to the β subunit activates BK channels and relaxes smooth muscle (4), thereby providing a non-genomic mechanism for vasorelaxant actions of these hormones. Further, two recent studies of β1 knockout mice have documented that loss of expression of this subunit results in disrupted coupling between Ca^{2+} release and the activation of hyperpolarizing BK currents, result-