Calcineurin signalling mechanisms in myocardial hypertrophy

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Abstract Calcineurin dephosphorylates multiple serine residues near the N terminus of NFAT proteins enabling them to translocate from cytoplasm to nucleus, where they activate a subset of hypertrophic response genes. Transgenic mice over-expressing a constitutively active form of calcineurin or NFAT3, developed obviously hypertrophy and heart failure or sudden death proving its pathogenic role. Here we used literatures on MEDLINE (2000-2011), systematically reviewed the new development of calcineurin signaling pathway in myocardial hypertrophy (J Geriatr Cardiol 2010; 7:189-192).

Key words Calcineurin; signal transduction; hypertrophy

Introduction

Numerous pathophysiological stimuli have been identified that can induce cardiac hypertrophy, for example mechanical stress, G-protein coupled receptor (GPCR) agonists (including endothelin-1, human urotensin II, and angiotensin II), cytokines, and growth factors. Many intracellular signaling pathways have also been implicated in this process, such as mitogen-activated protein kinase cascades (MAPKs), phosphatidyl inositol 3-kinase (PI3 K), protein kinase C (PKC), Janus kinase - signal transducers and activators of transcription (JAK-STAT), interleukin 6 - interleukin 6 receptor - glycoprotein 130 (IL6-IL6R-gp130), and calcineurin. The signaling pathways are relatively independent, but cross-talk with each other in some specific conditions. In this review, we focus on the calcineurin pathway in myocardial hypertrophy.

The general characters of calcineurin

Calcineurin, a Ca\(^{2+}\)/Calmodulin (CaM) - dependent serine/threonine protein phosphatase, is composed of two distinct subunits. The catalytic subunit A (CnA), a 58 KDa protein, contains an N-terminal catalytic domain, central calcineurin B (CnB) and CaM binding domains, and a C-terminal autoinhibitory domain. Regulatory subunit B (CnB), a small 19 KDa protein, has two global Ca\(^{2+}\) binding domains, each containing two Ca\(^{2+}\) binding sites that are aligned along the hydrophobic surface of the CnB-binding helix of CnA (EF1, EF2, EF3, and EF4). Recent evidence suggested that CnB was able to bind CnA and regulate its phosphatase activity even in the absence of calcium. Calcineurin is encoded by three genes designated α, β and γ, which share 81% sequence identity in the catalytic site. Interestingly, CnA α and A β genes share about 99% sequence identity between mouse and human species. Both CnA α and β are present in cardiac myocytes, whereas CnA γ protein represents a testis-restricted expression pattern. Calcineurin is sensitive to inhibition by the immunosuppressants cyclosporin A (CsA) and FK-506, and is insensitive to okadaic acid and calyculin A. These characteristics are useful in distinguishing PP2B from the more abundant protein phosphatases, PP1 and PP2A.

Although physiological substrates for calcineurin have not been firmly established, under in vitro conditions it dephosphorylates not only phosphoserine and phosphothreonine but also phosphotyrosine, suggesting a wide physiological role. A role for calcineurin has been involved in lymphocyte activation, skeletal muscle growth and neuronal development. It also plays a fundamental role in normal cardiac development through phosphorylation of the transcription factor, nuclear factor of activated T-cells (NFAT). Calcineurin is widely distributed in various mammalian tissues, and is highly abundant in the central nervous system. Ritter et al. found that the expression of a calcineurin fragment containing the catalytic domain was increased in the ventricle of heart failure patients while a fragment containing the autoinhibitory domain was reduced compared to ventricle from healthy donor heart.

The role of calcineurin in cardiac hypertrophy and heart failure

NFATs are a family of Rel homology transcription factors.
It has been shown that they are of critical importance in regulating the growth response of cardiac myocytes. In the inactive state, NFATs are phosphorylated and are retained in the cytoplasm. When T-lymphocytes bind to antigen-presenting cells through T-lymphocyte receptors, cytoplasmic Ca\(^{2+}\) concentrations increase and activate calcineurin, leading to NFAT dephosphorylation. This allows NFAT to migrate into the nucleus, where it regulates the transcription of a number of genes. Bourajjaj et al. reported that calcineurin-induced cardiac growth was abrogated in transgenic nfatc2-null mice with activated calcineurin in the postnatal myocardium. The absence of NFAT2 protected the myocardium from geometrical, functional, and molecular deterioration resulting from biomechanical stress. These findings not only confirmed the hypothesis that calcineurin mediates myocyte hypertrophy through activation of NFAT, but indicated an important role of NFAT2 in the downstream of calcineurin. The disruption of both nfat3 and nfat4 genes in mice is associated with embryonic lethality, thin ventricles, pericardial effusion, and reduced ventricular myocyte proliferation, suggesting a pivotal role of NFAT3 and NFAT4 in mitochondrial energy metabolism required for cardiac morphogenesis and function. NFAT5 is a novel member of the NFAT family and regulates the cardiomyocyte survival positively. Experiments of cardiomyocyte culture indicated that inhibition of NFAT5 could decrease cell viability and increase creatine kinase leakage. Consistently, small interfering RNA targeting nfat5 gene enhanced myocyte hypertrophy.

Li et al. ligated the infrarenal aortic artery in rats, which did not increase blood pressure or stimulate myocardial hypertrophy, and found that calcineurin activity was increased in cardiac myocytes. In comparison with age-matched healthy volunteers, the serum calcineurin activity was significantly enhanced by 2-fold in patients with hypertensive hypertrophy, 3-fold in hypertrophic cardiomyopathy, and 4-fold in hypertensive hypertrophy. Serum calcineurin activity was significantly increased by 2-fold in patients with hypertensive hypertrophy, 3-fold in hypertrophic cardiomyopathy, and 4-fold in hypertensive hypertrophy. Serum calcineurin activity was significantly increased by 2-fold in patients with hypertensive hypertrophy, 3-fold in hypertrophic cardiomyopathy, and 4-fold in hypertensive hypertrophy. Serum calcineurin activity was significantly increased by 2-fold in patients with hypertensive hypertrophy, 3-fold in hypertrophic cardiomyopathy, and 4-fold in hypertensive hypertrophy.

The data of calcineurin in heart failure were inconsistent. Choudhary et al. reported that the calcineurin activity was not increased in dilated cardiomyopathy, but Diedrichs et al. revealed that the calcineurin enzymatic activity was elevated by 80% in such pathological state compared with non-failing human hearts, which was in line with the protein levels of calcineurin B and NFAT-3. In addition, Yang et al. also found that calcineurin was activated in the failing ventricular tissue, associated with a marked upregulation of angiotensin II and calpains. These disparate results might be caused by experimental variables, such as patient characteristics and tissue sample for calcineurin detection, which suggest a complex activation pathway possibly involving release of neural mediators.

Factors that contribute to calcineurin activation

Ca\(^{2+}\) and CaM

Ca\(^{2+}\) is critical in both physiological and pathological functioning of the heart. Many studies have identified alterations in Ca\(^{2+}\) handling in the failing heart where intracellular basal Ca\(^{2+}\) concentration (diastolic Ca\(^{2+}\)) is increased, the transient amplitude of systolic Ca\(^{2+}\) is decreased, and sequestration of Ca\(^{2+}\) in the sarcoplasmic reticulum during diastole is defective, resulting in prolongation of the intracellular Ca\(^{2+}\) transient. Calcineurin is unique in its specific responsiveness to sustained increases in diastolic Ca\(^{2+}\), suggesting that alterations in intracellular Ca\(^{2+}\) handling progressively exacerbate hypertrophy through sustained activation of the calcineurin pathway.

Calcineurin activity is regulated by two structurally similar (35% sequence homology) but functionally different Ca\(^{2+}\)-binding proteins, CnB and CaM. EF hands, EF3 and EF4, which are in the C-terminus of CnB, are also called high affinity Ca\(^{2+}\) binding sites of CnB. Although these sites bind tightly to Ca\(^{2+}\), even when the Ca\(^{2+}\) concentration is below 0.1 M, calcineurin remains inactive. Increasing the Ca\(^{2+}\) concentration in the absence of CaM ensures that the low affinity sites (EF1, EF2) become occupied, although this leads to only a small activation of calcineurin. However in the presence of CaM, highly cooperative activation of calcineurin will occur. Kinetic evidence suggests that Ca\(^{2+}\) binding to the low affinity sites of CnB is required for CaM activation. Thus, Ca\(^{2+}\) binding to the low-affinity sites of CnB changes the structure of CnB and induces a conformational change in the regulatory domain of CnA, resulting in the exposure of the CaM-binding domain. This structural alteration is necessary for the partial activation of calcineurin in the absence of CaM and its full activation by CaM. Calcineurin activity is uniquely regulated by displacement of the C-terminal autoinhibitory domain within the catalytic subunit in response to CaM binding to an adjacent domain.
Neurohormonal factors

Previous studies have investigated the role of endogenous Gq-coupled receptor agonists in patients with chronic heart failure. The activation of receptors by the agonists may contribute to the activation of calcineurin. 26-28 Zhu et al 29 investigated whether ET-1 could stimulate calcineurin activity in rat neonatal ventricular myocytes. Results showed that ET-1 stimulated calcineurin activity within 30 sec, with activity peaking at 1 min, and returning to basal levels after 15 min, suggesting that ET-1 can rapidly but transiently increase calcineurin activity in rat neonatal ventricular myocytes.

This raised the question "can GPCR agonists increase calcineurin activity in human heart?" Our previous study 30 used tissue bath methodology to investigate the role of GPCR agonists on calcineurin activity in human heart. Right ventricular trabeculae from patients with end stage heart failure were stimulated to contract at 1 Hz and tissues were incubated with or without ET-1, Ang II, and hUII for 30 min at 37°C in physiological solution. Calcineurin activity was measured and results showed that ET-1, Ang II, and hUII all significantly increased calcineurin activity, suggesting that GPCR agonists can increase calcineurin activity in human heart. Recent experiments in rat cardiomyocytes demonstrated that ET-1 upregulated the transcription of CaN subunit partially through calcium/calmodulin-dependent protein kinase II hypertrophic signaling pathway, 31 and such cardiac hypertrophy could be prevented by rosiglitazone, an agonist of peroxisome proliferator-activated receptor. 32

Calpain

Calcineurin can be reversibly activated and inactivated in response to diastolic Ca++ concentrations. However, some in vitro studies found that calcineurin can be irreversibly activated by limited proteolysis. 33 After proteolysis calcineurin becomes constitutively active, no longer requiring Ca++ or CaM for full activity. Calpain is a Ca++-activated neutral cysteine-protease and is a strong candidate for the enzymatic post-translational modification of calcineurin. Calpain was up-regulated in pathophysiological states including stroke, myocardial ischemia, neuromuscular degeneration, and cataract formation, conditions in which Ca++ served as a trigger for cellular injury.

Two major calpain isozymes have been well characterized: calpain I (m-calpain) and calpain II (m-calpain), both of which are ubiquitously expressed in mammalian cell cytoplasm. Both isoforms consist of an 80-KDa large subunit (from the genes Capn1 and Capn2, respectively) and a common 28-KDa small subunit (Capn4). The large subunit contains a distinct cysteine protease domain and a Ca++-binding domain with four helix (EF-hand) structures. The small subunit is made up of a glycine-rich region responsible for membrane-interactions and another set of four EF-hand structures.

Calpain is a neutral cysteine protease requiring a free thiol group and Ca++ for activity. 35 The calpain isozymes differ in their sensitivity to Ca++-concentration; calpain I being activated at low micromolar Ca++ concentrations while calpain II being activated at higher micromolar Ca++ concentrations. Both isozymes undergo Ca++-dependent autolysis, which greatly increases their sensitivity to Ca++ concentration and increases their activation; the Ca++ requirement of calpain is reduced in the presence of phospholipids. Since the intracellular Ca++ concentration normally fluctuates at sub-micromolar levels, calpain I is more likely to function in cells under physiological and pathological conditions.

Calcineurin is an important target for calpain, which can convert it to the active form via hydrolysis. Previous studies have shown that removal of the autoinhibitory domain from CaN results in full Ca++/CaM-independent activity in vitro. 36, 37 Wu et al 38 investigated calpain-mediated cleavage of calcineurin in glutamate and kainate-treated cultures of primary cortical neurons. They found that calpain cleaved CaN into three fragments, two of which (45-KDa and 48-KDa proteins) were constitutively active. Over-expression of the 45-KDa or 48-KDa calcineurin fragments induced neuronal cell death. Calpain inhibitors blocked not only calcineurin cleavage but also glutamate- and kainite-induced excitotoxic neuronal cell death. Calpain inhibitors blocked not only calcineurin cleavage but also glutamate- and kainite-induced excitotoxic neuronal cell death. The absence of CaM, calpain rapidly transformed the 60KDa catalytic subunit into a transient 57KDa fragment and thereafter a 43KDa fragment. The proteolyzed forms of calcineurin were not only insensitive to Ca++ or Ca++/CaM, but also had a higher activity than the Ca++/CaM-stimulated full-length enzyme. The rate of calpain-dependent proteolysis of calcineurin was increased in the presence of CaM. Proteolyzed calcineurin retained a small degree of sensitivity to Ca++/CaM, being further stimulated by 15%-20%.

Calpain has been associated with ventricular hypertrophy 39, 40 although it is not yet clear whether this was attributed to its effects on the calcineurin-pathway. In addition to its effect on calcineurin, calpain also cleaved the endogenous calcineurin inhibitor, cain/cabin1. 41, 42

Cardiac Aetiology

Studies implicate calcineurin in the pathogenesis of myocardial hypertrophy. Ritter et al 9 showed a significant elevation of calcineurin activity in hearts obtained from patients with hypertrophic obstructive cardiomyopathy and from patients with aortic stenosis, compared with normal hearts. These findings were supported by another study that showed increased expression of calcineurin protein levels in hearts obtained from patients with idiopathic dilated cardiomyopathy compared to control hearts. A concomitant redistribution of NFAT-3 from the cytosol to the nucleus, and an increased expression of GATA-4 was also reported in the failing hearts. 18

Further study

Although great progress has been made in calcineurin
mechanism in myocardial hypertrophy and heart failure, many questions still unanswered. For example, the relative contribution of the Ca$^{2+}$/CaM stimulated-, and the calpain-mediated proteolytic pathways in calcineurin-dependent hypertrophy remain to be determined. While evidence suggests a role for calpain in the activation of calcineurin, this has not been investigated in human heart. Calpain-dependent calcineurin activation is independent of Ca$^{2+}$ concentration and presumably dependent upon degradation for inactivation. Whether this provides a mechanism for prolonged activation of calcineurin. The answers of these questions may provide new methods for preventing myocardial hypertrophy and heart failure.

Reference