Relationship between CaMKII-CREB and Myocardial Hypertrophy in Rats Exposed to Alcohol

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Abstract: Myocardial hypertrophy is an adaptive response of myocardium to various stimuli, but long-term myocardial hypertrophy is recognized as an independent risk factor for multiple cardiovascular complications. Calmodulin kinase II (CaMK II) activity and expression were significantly increased in hypertrophic myocardium induced by alcohol. Ca^{2+}/CaM-dependent CaMK II signal transduction pathway plays a key role in the formation of hypertrophy and the occurrence of ventricular arrhythmia in hypertrophic myocardium. Abnormal phosphorylation of nuclear transcription factor CREB also plays an important role in transcriptional regulation of myocardial hypertrophy gene. However, it is unclear whether CaMII mediates alcohol-induced cardiac hypertrophy through CREB phosphorylation. Persistent cardiac hypertrophy due to long-term stress can eventually lead to dilated cardiomyopathy, heart failure, and sudden death. The CaMKII signal transduction pathway is expected to be a new target for clinical cardiac hypertrophy and arrhythmia prevention. It is used to further explore CaMKII and provide a basis for better application in the future.

1. Introduction

Myocardial hypertrophy is the most common complication of hypertension. It is not only the result of compensatory growth of myocardial tissue caused by cardiac overload in hypertension, but also the process of pathological remodeling of heart [1]. Myocardial hypertrophy is an adaptive response of myocardium to various stimuli. The early manifestation was increased myocardial contractility, which could relieve ventricular wall pressure. However, long-term cardiac hypertrophy is recognized as an independent risk factor for cardiovascular complications [2]. In addition to hypertrophic factors, the inhibition of cardiac hypertrophic factors in the body is also a hot spot for people to seek and study. Myocardial hypertrophy is a common pathological process in many cardiovascular diseases. It is an adaptive pathological change caused by long-term overload of myocardium [3]. The activity and expression of calmodulin kinase II (CaMKII) was significantly increased in hypertrophic myocardium induced by alcohol. The Ca^{2+}/CaM-dependent CaMKII signal transduction pathway plays a key role in the development of cardiac hypertrophy and hypertrophic ventricular arrhythmia [4]. Changes in Ca^{2+} concentration can conduct different signals through a variety of calcium-regulated enzymes. Calmodulin-dependent protein kinases in the calcium/calmodulin-dependent protein kinase family (Ca^{2+}/CaMK) play an important role.

The mechanism of alcohol-induced cardiac hypertrophy is still not fully defined, and mechanical, neurohumoral and other factors can stimulate a series of second messengers. Calcium as a second messenger plays a key role in the development of cardiac hypertrophy, and CaMII is one of the most important calcium-binding proteins [5]. It is generally believed that it forms a Ca^{2+}-CaMII complex with Ca^{2+} and leads to cardiac hypertrophy via calcium phosphatase-NFAT, calcineurin-MEF2 and other signal transduction pathways. Although initial cardiac hypertrophy is a beneficial compensatory response in order to balance the increase in myocardial stress. However, persistent cardiac hypertrophy caused by long-term stress can eventually lead to dilated cardiomyopathy, heart failure and sudden death. Abnormal phosphorylation of nuclear transcription factor CREB also plays an important role in transcriptional regulation of cardiac hypertrophy gene [6]. However, it is not clear whether CaM II mediates alcohol-induced cardiac hypertrophy through CREB phosphorylation. Signal transduction pathways play an important role in the formation of

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myocardial hypertrophy [7]. It is helpful to elucidate the molecular mechanism of myocardial hypertrophy. It may also open up a new way of thinking for drug intervention to prevent and treat myocardial hypertrophy.

2. Establishment of Myocardial Hypertrophy Model

Although the causes of myocardial hypertrophy are different, there is abundant evidence that there are two basic links in alcohol-induced hypertrophic myocardium: abnormal cytoplasmic signal transduction and abnormal nuclear reaction. CREB is activated and has transcriptional activity, which is further combined with transcriptional co-activator CBP. CBP interacts with basic transcription complexes to initiate target gene transcription. Previous studies on hypertrophy of hypertensive myocardium mainly focused on hypertrophic factors. The synergistic effect of self-inhibitory region and calmodulin binding region in regulatory domain determines the active response of Ca MK II to intracellular calcium concentration change [8]. Sciatic nerve ligation can increase the sensitivity of nerve fibers at the ligation, resulting in abnormal ectopic discharge of the afferent nerve. Activation of nuclear transcription factors regulates target gene expression induced by noxious stimuli and mediates long-term plasticity changes in spinal cord neurons caused by noxious stimuli. Ca^{2+} plays an important role in many different cellular functions, including gene transcription, which is the basis of cardiac remodeling. The occurrence of disease is often the result of a balance between the damage factor and the protective factor in the body.

There are four subtypes of CaMKII in the body: α, β, γ, δ, which are encoded by different genes, each of which has a plurality of splices. The catalytic domain and regulatory domain sequences between the various subtypes are highly homologous and conserved. The alpha and beta subtypes of CaMKII are primarily restricted to expression in neural tissue, while the gamma and delta isoforms are present in a variety of tissues, including the heart. In cardiovascular diseases such as hypertension, myocardial infarction, heart failure, and congenital heart disease. The different spliceosome of CaMKIIδ in the heart mainly includes δB and δC, of which δB has a Nuclear localization sequence. It is mainly distributed in the nucleus and may be closely related to the transcription of regulatory genes under pathological conditions.

Myocardial hypertrophy is a common pathological process and a basic compensatory reaction. Delta subtype is the most important expression form of CaMKII in the heart. During normal cardiac growth and development and various cardiac diseases, there are changes in the relative expression levels of different spliceosome of δ subtypes. Myocardial nuclei were isolated by differential centrifugation and sucrose density gradient centrifugation. The extraction medium contains inhibitors of various proteases and EGTA. So as to prevent the active components in the nucleus from leaking out of the stone hole while ensuring the integrity and high purity of the extracted nucleus. The recovery of myocardial nuclei is shown in Table 1.

Table 1 Recovery of Cardiomyocytes

	Protein yield (mg/g)	DNA yield (mg/g)	Protein/DNA
Homogenate	89.27	0.46	217.48
Nuclei	0.75	0.13	5.95

There should be some factors in the body that prevent or reverse the occurrence and development of cardiac hypertrophy. The relative or absolute lack of these factors may also be an important aspect of the occurrence and development of alcohol-induced cardiac hypertrophy. Persistent cardiac hypertrophy eventually leads to heart failure due to cardiac dysfunction. Cardiac hypertrophy has been recognized as a powerful independent risk factor for cardiovascular events such as sudden death and heart failure. δC has no nuclear localization sequence and is mainly localized in the cytoplasm, concentrated in the cytoplasmic surface of the Z-band T-tubule of cardiomyocytes. Adjacent to the ryanodine receptor and L-type calcium channel, it participates in Ca^{2+}-dependent signal transduction pathway [9]. At the same time, phosphorylation regulates Ca^{2+} regulatory proteins in the cytoplasm, affecting intracellular calcium homeostasis. It plays an important role in arrhythmia, myocardial hypertrophy and heart failure. Recent sequencing of

phosphorylation sites of many CaMK II substrates indicates that the core sequence of the minimum phosphorylation induced by kinase is Arg-X-X-Ser/Thr. In addition to phosphorylated Ser/Thr sites, all substrates also need more Glu, Gln and Asp bases. It is easier to be recognized by CaMK II to produce phosphorylation reaction.

3. Changes of CaMKII-CREB in Hypertrophic Myocardium of Rats

Intracellular Ca^{2+} is a signal of myocardial hypertrophy. Current studies suggest that calcium overload occurs in the plasma of cardiac myocytes during cardiac hypertrophy, and the contractility of cardiac myocytes recovers significantly after inhibiting calcium overload. Previous studies have focused on the role of CaM II in the cytoplasm of myocardial hypertrophy. Different subcellular localization of calcium signals may encode foreign information through different frequency and amplitude changes. However, how the signal of cytoplasmic calcium overload transduces into the nucleus and the mechanism through which it causes abnormal gene expression is not fully clear. Substrates related to CaMK II include receptors, cytoskeleton proteins, enzymatic proteins, ion channels and transcription factors. When cardiomyocytes are stimulated by mechanical stretch, increased load, or neurohumoral factors, intracellular Ca^{2+} concentrations increase in reactivity. The high degree of structural conservation is important to maintain interaction with a wide variety of CaM binding protein families. Changes in Ca^{2+} concentration can conduct different signals through a variety of calcium-regulated enzymes, of which CaMK plays an important role.

CaMKII specific inhibitor KN93 has no effect on normal cardiomyocyte growth, but inhibits TNF-α-induced cardiomyocyte protein synthesis, protein content and cell volume increase. It is suggested that CaMKII is involved in TNF-α-induced cardiomyocyte hypertrophy. It was observed that the expression of eNOS and the decrease of NO production in hypertrophic myocardium of rats with renal hypertension were observed. CaM or CaM-like polypeptides are also present in prokaryotic cells, and heat-resistant small molecule acidic proteins are found in protozoa and bacteria. It has typical CaM-like characteristics and is cross-reactive with anti-CaM antibodies [10]. The phosphorylated CREB protein increased in myocardial hypertrophy, but there was no significant change in CREB protein. Phosphorylation of CaMK II acts on the major Ca^{2+} regulatory proteins related to intracellular calcium homeostasis, which is similar to that of protein kinase A, suggesting that hypertrophic signals can stimulate the kinase-inducing domain of CREB in cardiac hypertrophy. CREB is phosphorylated and has transcriptional activity, which regulates the transcription of downstream genes. CaM II staining in the nucleus and cytoplasm of myocardial hypertrophy group was enhanced, which indicated that the expression of CaM II in the nucleus was up-regulated, but the reason remained to be further studied.

Activated intracellular signal transduction pathways, on the one hand, alter the excitability of neurons by phosphorylating ion channels or receptors of neuron membranes. CaMK II is a multifunctional protein kinase, which exists in many important organs in vivo, especially in nerve tissue. The activity of subcellular marker enzymes in the purified nucleus of cardiac myocytes was less than 6%, and the activity of calcium-ATPase was about three times higher than that of homogenate. The detection of subcellular marker enzymes is shown in Table 2.

Table 2 Detection of subcellular component marker enzymes

	G-6-P	5-NT	Na^+-K^+-ATPase	SDH
Homogenate	98.16	138.43	243.12	4738.97
Nuclei	3.75	12.59	4.36	62.48

CREB is a member of the transcription factor CREB/ATF family and exists in the nucleus. When Ca^{2+} is deficient, the self-inhibitory domain binds to the catalytic domain to maintain the conformation of kinase inactive. When the intracellular Ca^{2+} level increased, Ca^{2+} could bind to CaM. They further bind to the calmodulin binding region, resulting in changes in the conformation of the kinase and inactivation of the self-inhibitory region. In recent years, it has been found that it is also expressed in cardiac myocytes. From in vitro experiments to animal models and human

experiments, CaMK II in cardiac myocytes is an important receptor of Ca^{2+} signal changes. It is also an important effector of Ca^{2+} regulatory proteins and transcriptional responses. CaMKII is widely involved in gene transcription regulation, calcium-sensitive protein phosphorylation and other processes. Purified CaMKII is almost inactive at the time of Ca^{2+}-CaMII deletion. CaMKIIδB has a nuclear localization signal, which can lead to the expression of hypertrophy-related genes, and is an important link in the pathogenesis of cardiac hypertrophy caused by alcohol.

4. Conclusion

Ca^{2+}/CaM-dependent CaMKII signal transduction pathway plays an important role in the formation of cardiac hypertrophy, regulation of gene transcription level, and on the occurrence of hypertrophic myocardial excitation-contraction coupling and arrhythmia. By inhibiting the activity and expression of CaMKII, it can reduce the formation of cardiac hypertrophy, improve the heart function and reduce the occurrence of hypertrophic myocardial malignant arrhythmia, and even sudden death. TNF-α may induce cardiac hypertrophy by regulating the intracellular calcium concentration and then regulating the expression of CaMKIIδB, which is closely related to calcium ions in cardiac myocytes. The content of pCREB protein in hypertrophic myocardium was significantly higher than that in normal myocardium, but there was no significant change in CREB protein compared with normal myocardium, indicating that the phosphorylation of CREB in hypertrophic myocardium was increased. The expression of CaM II protein in the nucleus of myocardial hypertrophy increased, which may further up-regulate the expression of c-fos gene by activating CREB. CaMKII signal transduction pathway may become a new target for the prevention and treatment of myocardial hypertrophy and arrhythmia in clinic. New and more specific inhibitors of CaMK II are also needed. It can be used to further explore CaMK II and provide a basis for better clinical application in the future.

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