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Mini Review

Multiple functions of junctin and junctate, two distinct isoforms of aspartyl beta-hydroxylase

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Abstract

The single genomic locus, AβH-J-J, encodes three functionally distinct proteins aspartyl beta-hydroxylase, junctin and junctate by alternative splicing. Among these three proteins, junctin and junctate could play important roles in the regulation of intracellular Ca^{2+} by regulating either Ca^{2+} release from intracellular Ca^{2+} stores or Ca^{2+} influx in various biological processes. Here we review recent findings concerning the expressional regulations and the proposed functions of junctin and junctate.

Keywords: Ryanodine receptor; Calsequestrin; Triadin; Striated muscle; Excitation–contraction coupling; Calcium signaling

AβH-J-J isoforms

Aspartyl beta-hydroxylase (AβH), junctin and junctate are three functionally distinct isoforms encoded from a single genomic locus, AβH-J-J, by alternative splicing [1]. AβH was first identified in the bovine liver as an enzyme to hydroxylate Asp and Asn within certain epidermal growth factor (EGF)-like domains of a number of proteins [2,3]. Junctin was first identified as a 26-kDa calsequestrin binding protein in cardiac sarcoplasimic reticulum (SR) [4] and a subsequent study showed that junctin could interact with ryanodine receptor (RyR), calsequestrin and triadin to form a Ca^{2+} release complex at the junctional SR of striated muscle cells [5]. Junctate was identified as a novel Ca^{2+}-binding protein located at endoplasmic reticulum (ER)/SR membranes [6–8].

Gene structure, tissue distribution and expressional regulation of AβH-J-J locus

Gene structure and tissue distribution of AβH, junctin and junctate

AβH was universally found in a variety of tissues [9], whereas junctin was found only in striated muscles [10]. The gene structure of AβH consists of the AβH type N-terminal cytoplasmic region, the universal transmembrane region, the highly charged acidic luminal region and the AβH catalytic domain, whereas, that of junctin consists of the junctin type N-terminal region, the universal transmembrane region, a short highly charged acidic luminal region and the junctin-specific basic luminal region (Fig. 1). So far, two major junctate isoforms have been identified. The junctin-type junctate has the junctin-type N-terminal region, the universal transmembrane region and the highly charged acidic luminal region, whereas AβH-type junctate has the AβH-type N-terminal region (Fig. 1) [6–8]. The junctin-type junctate was found only in the heart, whereas AβH-type junctate was universally detected [7].

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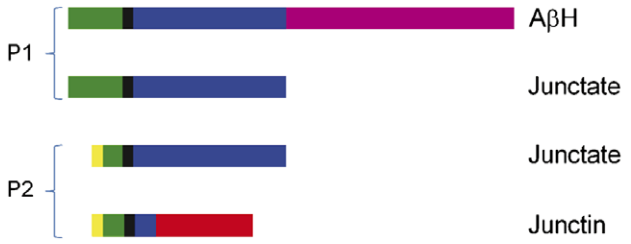

Fig. 1. Schematic diagrams of the major alternatively spliced products from the AβH-J-J locus. AβH- and junctin-type N-terminal domains are encoded from two different promoters, P1 and P2, respectively. Each region is presented in different color: green, AβH-type N-terminal cytoplasmic regions; black, transmembrane region; blue, highly charged acidic luminal regions; purple, AβH catalytic domain; yellow, junctin type N-terminal region; red, junctin-specific basic luminal region.

Expressional regulation of AβH-J-J locus

The existence of two junctate isoforms containing different N-terminal domains with unique expression patterns implies two alternative promoters in AβH-J-J locus [1,7,11]. Two different promoters (P1 and P2) have been identified in the human AβH-J-J locus [1,11]. The P1 promoter is similar to many housekeeping gene promoters, whereas the P2 promoter is activated by MEF-2 to induce transcription of junctin and junctate in excitable tissues [1,11]. The isoforms having AβH-type N-terminal domains are encoded from the P1 promoter in most tissues, whereas the transcription mediated by the P2 promoter is specific to excitable tissues such as striated muscles [1,11].

Functional roles of junctin in striated muscles

Basic properties of junctin

Junctin is an integral membrane protein with a single transmembrane domain, a cytoplasmic short N-terminal domain and an SR luminal C-terminal tail [4,12]. Its SR luminal tail is highly basic and mediates a physical interaction with SR Ca^{2+} release complex composed of RyR, calsequestrin and triadin [4,5]. Junctin and triadin may transmit Ca^{2+} sensing activity of calsequestrin to RyR [13].

Gain-of-function or loss-of function studies for junctin

A number of transgenic (TG) and knockout (KO) mouse studies were performed to elucidate the function of junctin in the heart (Table 1) [14–19]. In junctin TG heart, junctional SR showed increased and extended interaction to surface membranes with the dense matrix of calsequestrin in the lumen [14,15]. Overexpression of junctin resulted in reduced expression of triadin and RyR and up-regulation of dihydropyridine receptor (DHPR) [15,16]. The increased L-type Ca^{2+} current prolonged action potential duration, then caused bradycardia. This abnormal Ca^{2+} handling probably led to impaired cardiac function and enlargement of the heart [15]. Delayed relaxation and reduced Ca^{2+} release with diminished SR Ca^{2+} load were also reported [16,18]. An age-dependent study further suggested that junctin overexpression could result

Table 1
Functional analyses of junctin, triadin and calsequestrin in TG or KO mice hearts

Animal model	Protein level	Ca^{2+} cycling and contractility	Phenotypes	References
Junctin TG (adult)	Triadin 1 ↓	SR Ca^{2+} load ↓	Heart enlargement	[14]
	RyR ↓ DHPR ↑ NCX ↓	L-type Ca^{2+} current ↑ Action potential duration ↑ Prolonged Ca^{2+} transient Ca^{2+} spark ↓ Prolonged relaxation contractility ↓	Bradycardia Atrial fibrillation Fibrosis	[15] [16] [18]
Junctin TG (young)	Triadin 1 ↓	SR Ca^{2+} load ↑		[17]
Junctin KO	NCX ↓	SR Ca^{2+} load ↑ Ca^{2+} spark ↑ NCX current ↑ Contractility ↑	Arrhythmias (ISO)	[19]
Triadin1 TG	Junctin ↓ RyR ↓	Prolonged Ca^{2+} transient Prolonged relaxation SR Ca^{2+} load ↑ Ca^{2+} spark ↑ contractility ↓	Hypertrophy	[24] [25]
Calsequestrin TG	Triadin 1 ↓ Junctin ↓ RyR ↓ FKBP12 ↓ SR Ca ATPase ↑ PLB ↑ Calreticulin ↑	SR Ca^{2+} load ↑ Ca^{2+} induced Ca^{2+} release ↑ Ca^{2+} spark ↓ NCX current ↑ Contractility ↓ L-type Ca^{2+} current ↓	Hypertrophy	[22] [23]
Calsequestrin KO	Junctin ↓ Triadin 1 ↓	Normal basal Ca^{2+} release Normal basal contractility Diastolic SR Ca^{2+} leak ↑ (iso) Premature Ca^{2+} spark (ISO)	Ventricular arrhythmias (ISO)	[28]

TG, transgenic; KO, knockout; ISO, isoproterenol treated.

in the compensated down-regulation of RyR as a consequence of reduced triadin expression and increased SR Ca^{2+} load [17].

To examine the acute effect of junctin knockdown (KD) and overexpression, an adenoviral gene transfer system was employed to adult cardiomyocyte. The overexpression of junctin caused impaired Ca^{2+} kinetics and contractile characteristics, while its down-regulation resulted in augmented and faster Ca^{2+} kinetics with accelerated contractile parameters [20,21]. Consistent with the *in vitro* result, the junctin KO heart showed increased contractility and Ca^{2+}-cycling parameters along with SR Ca^{2+} overload [19]. The augmented SR Ca^{2+} load was compensated by increased Na^{+}–Ca^{2+} exchanger expression. Interestingly, in the failing human heart with dilated cardiomyopathy, the expression of junctin was undetectable probably due to a compensatory down-regulation to improve SR Ca^{2+} release activity.

Taken together, the genetic perturbation studies suggest that junctin may not just be a simple structural protein, but rather it could play a critical role in keeping Ca^{2+} cycling going on in the SR.

Functions of junctin interacting proteins

The functional roles of calsequestrin and triadin have been extensively studied by various genetically modified mouse models [22–28] (Table 1). TG mice overexpressing cardiac calsequestrin showed cardiac hypertrophy with enhanced SR Ca^{2+} loading and decreased expression of the interacting proteins [22,23]. Deletion of calsequestrin was functionally compensated by dramatically increased SR volume and decreased junctin and triadin expression to maintain near-normal Ca^{2+} release and cardiac contractility [28]. The studies employing the adenoviral gene transfer system to manipulate the expression level of calsequestrin in isolated adult cardiomyocytes showed that the overexpression of calsequestrin induced increased SR Ca^{2+} content and prolonged duration of SR Ca^{2+} release without affecting the expression levels of other Ca^{2+}-cycling proteins, while KD of calsequestrin resulted in reduced SR Ca^{2+} load [29,30]. A study using triadin TG showed cardiac hypertrophy, impaired relaxation and contractility and reduced expression of RyR and junctin [24]. The TG mice also showed an increased SR Ca^{2+} load unlike the junctin TG study [25]. In spite of their structural similarity, junctin and triadin may lead to differential effects on SR Ca^{2+} handling by dynamically changing their expression levels in response to various pathophysiological conditions.

Proposed functional roles of junctate

Junctate has a single transmembrane domain, a short N-terminal cytoplasmic segment and a long and highly acidic C-terminal tail that protrudes into the SR lumen [6,8]. Evidence has shown that junctate could be involved in the peripheral coupling of ER and plasma membrane contributing to Ca^{2+} homeostasis in eukaryotic cells by forming a multimolecular complex with inositol 1,4,5-trisphosphate receptor (IP3R) and canonical transient receptor potential protein (TRPC) 3 Ca^{2+} entry channel. Junctate could modulate both IP3 induced Ca^{2+} release from the ER and Ca^{2+} entry through TRPC3 [31]. In the case of rodent sperm, junctate could interact with TRPC which mediates Ca^{2+} influx to induce the acrosomal reaction, the beginning step of fertilization [32]. The function of junctate was further investigated using a junctate overexpressing TG mouse model in skeletal muscle. The overexpression of junctate resulted in enhanced SR Ca^{2+} storing capacity and Ca^{2+} release activity, but had no effect on SR Ca^{2+} uptake. This altered function of SR was not mediated by any changes of expression levels of SR Ca^{2+} binding proteins [33].

Conclusions

Junctin and junctate are two isoforms generated by alternative splicing from a single locus of AβH-J-J. Both proteins have the same membrane topology with single transmembrane domain at SR/ER. In spite of their opposite charges in the C-terminal, both proteins are involved in the regulation of intracellular Ca^{2+} concentration. The positively charged C-terminal of junctin mediates the interaction of RyR and calsequestrin, while the negatively charged C-terminus of junctate binds to Ca^{2+}. The alternative splicing is an important mechanism which economically increases the biological complexity from the restricted pool of the genome. The case of junctin and junctate is one of the best examples that demonstrates the functional diversity derived by alternative splicing. Recent studies indicate that junctin and junctate could play distinctly different physiological roles. It was suggested that altered expression of junctin or junctate can modify the cellular Ca^{2+} handling and perturb the balanced activity of other Ca^{2+} regulatory proteins. Great efforts are still needed to elucidate the precise roles of junctin and junctate in the maintenance of proper Ca^{2+} transport and homeostasis in the normal physiological condition. Identification of the signaling and transcriptional factors regulating the expression of these proteins under different perturbations will enhance our understanding of the system-level genetic controlling mechanisms.

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