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Distribution and function of sodium channel subtypes in human atrial myocardium

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ABSTRACT

Voltage-gated sodium channels composed of a pore-forming α subunit and auxiliary β subunits are responsible for the upstroke of the action potential in cardiac muscle. However, their localization and expression patterns in human myocardium have not yet been clearly defined. We used immunohistochemical methods to define the level of expression and the subcellular localization of sodium channel α and β subunits in human atrial myocytes. $Na_v1.2$ channels are located in highest density at intercalated disks where β1 and β3 subunits are also expressed. $Na_v1.4$ and the predominant $Na_v1.5$ channels are located in a striated pattern on the cell surface at the z-lines together with β2 subunits. $Na_v1.1$, $Na_v1.3$, and $Na_v1.6$ channels are located in scattered puncta on the cell surface in a pattern similar to β3 and β4 subunits. $Na_v1.5$ comprised approximately 88% of the total sodium channel staining, as assessed by quantitative immunohistochemistry. Functional studies using whole cell patch-clamp recording and measurements of contractility in human atrial cells and tissue showed that TTX-sensitive (non-$Na_v1.5$) α subunit isoforms account for up to 27% of total sodium current in human atrium and are required for maximal contractility. Overall, our results show that multiple sodium channel α and β subunits are differentially localized in subcellular compartments in human atrial myocytes, suggesting that they play distinct roles in initiation and conduction of the action potential and in excitation–contraction coupling. TTX-sensitive sodium channel isoforms, even though expressed at low levels relative to TTX-sensitive $Na_v1.5$, contribute substantially to total cardiac sodium current and are required for normal contractility. This article is part of a Special Issue entitled "Na^+ Regulation in Cardiac Myocytes".

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1. Introduction

Cardiac action potentials are generated and propagated through the coordinated activity of multiple types of ion channels. Voltage-gated sodium channels generate the upstroke of the action potential, and their activation and inactivation set the conduction velocity through cardiac tissue and the refractory period between conducted action potentials. Mutations in genes encoding voltage-gated sodium channels are known to cause arrhythmias [1] and to be involved in cardiomyopathy [2–4]. Differential expression and localization of sodium channel subunits are likely to be important determinants of electric excitability of cardiac myocytes. This study defines the subcellular localization of sodium channel subunits in human atrial myocardium.

Voltage-gated sodium channels are composed of a pore-forming α subunit with one or two auxiliary β subunits [5]. Ten different genes encoding sodium channel α subunits have been identified and nine have been functionally expressed [5,6]. The different α subunit isoforms have distinct patterns of development and localization in the nervous system, skeletal and cardiac muscle, and they have different pharmacological properties. Isoforms preferentially expressed in the central nervous system ($Na_v1.1$, 1.2, 1.3, 1.6) are inhibited by nanomolar concentrations of the puffer fish toxin, tetrodotoxin (TTX), a highly specific sodium channel blocker. The isoform present in adult skeletal muscle ($Na_v1.4$) is also blocked by nanomolar TTX-concentrations. In

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contrast, the primary cardiac isoform ($Na_v1.5$) requires micromolar concentrations of TTX for inhibition due to substitution of a cysteine for an aromatic residue in the pore region [6,7].

Four genes encoding different β-subunits—β1, β2, β3 and β4—have been identified [8,9]. β1 and β3 are noncovalently associated with α subunits, whereas β2 and β4 are disulfide-linked to α. The β subunits modulate channel gating, interact with extracellular matrix, cytoskeleton, and cell adhesion molecules, play a role in adhesive interactions, and influence cell surface localization of sodium channels [10]. Our previous work showed that sodium channel β subunits are differentially localized in the transverse tubules, surface membrane, and intercalated disks of mouse ventricular myocytes [11].

$Na_v1.5$ has often been termed the "cardiac" sodium channel. However, we showed previously that the "brain" sodium channels $Na_v1.1$ and $Na_v1.3$ are also expressed in mouse heart and have distinct distributions and functions from $Na_v1.5$ [11–13], and other groups have extended these findings [14–18]. Multiple sodium channel isoforms are also expressed in human atrial myocytes [19].

2. Materials and methods

2.1. Tissue samples

Samples of human atrial tissue were obtained from patients undergoing elective cardiac surgery for multiple indications. Tissue from patients with congestive heart failure or atrial rhythm disorders including atrial fibrillation was excluded to avoid structural and/or electrophysiological alterations in the right atrial myocardium. A detailed description of tissue isolation and preparation is provided in Supplemental Material. All procedures conformed to the principles outlined in the Declaration of Helsinki and were in agreement with the policies of the Ethics Committee of the University of Würzburg.

2.2. Immunohistochemistry of human atrial tissue

A full description of the antibodies and immunohistochemical methods is given in Supplemental Material. Patches of atrial muscle were removed during surgery, immediately frozen in liquid nitrogen and sectioned (10 μm) on a cryostat. After mounting, fixation and antibody treatment, sections were viewed using Bio-Rad MRC 600 or Leica TCS SL confocal microscopes. More than 20 tissue sections from 14 patients were analyzed for each antibody.

2.3. Quantification of immunohistochemistry

For these studies we used saturating concentrations of antibodies recognizing each sodium channel α subunit [20]. All images in a single experiment were collected using the same laser gain and offset settings so that the intensity of staining of different antibodies could be compared. Staining intensity for each antibody was normalized to staining of $Na_v1.5$ from the same experiment. For quantification, three to five images from 5 different atrial samples of the same patients as mentioned above were analyzed for each antibody.

2.4. Electrophysiology

Single human cardiac myocytes were prepared from right atrial tissue according to the modified protocol of Bustamante et al. [21,22]. Whole-cell sodium current (I_{Na}) was recorded at room temperature from rod-shaped, striated and Ca^{2+}-tolerant cells within 8 h of isolation.

2.5. Contractility measurements in human atrial muscle

Human atrial muscle fibers were harvested during open-heart surgery from patients who underwent elective coronary bypass surgery and used for contractility studies as previously described [23,24]. In brief, fibers were mounted between a force transducer and a micrometer screw and constantly superfused with oxygenated Krebs–Henseleit (KHS) buffer at 37 °C with a pO_2 of >500 mm Hg. The fibers were electrically stimulated at 1 Hz, 25% above threshold, and were initially stretched to optimal length (L_{max}), which was indicated by maximum isometric force amplitude; length was not adjusted further during the experiment. Fibers were superfused with oxygenated KHS buffer containing different TTX concentrations. These experiments were carried out in the presence of atropine (100 nM) and propranolol (1 μM) to prevent effects of neurotransmitters that might be released from autonomic nerve endings.

3. Results

3.1. Localization of sodium channel α subunits in human right atrium

Human atrial tissue samples from 14 patients were stained with antibodies recognizing individual α subunits of voltage-gated sodium channels to determine their localization. $Na_v1.1$, $Na_v1.3$ and $Na_v1.6$ were observed in puncta on the surface of myocytes (Figs. 1A-E). The number of puncta and the intensity of staining were greatest for $Na_v1.1$ and $Na_v1.6$; puncta were fewer and less intensely stained for $Na_v1.3$. None of these channels was located at the z-lines which were marked by staining for α-actinin (Figs. 1B, C). In contrast, antibodies recognizing $Na_v1.2$ channels stained the cell surface most intensely near the intercalated disks (Fig. 1G); pale striated staining for $Na_v1.2$ was also seen (Figs. 1G, I). Double labeling with connexin 43 to mark intercalated disks confirmed the intercalated disk localization of the high density $Na_v1.2$ staining (Figs. 1G-J). Staining was abolished when primary antibodies were omitted (Fig. 1F).

$Na_v1.4$ and $Na_v1.5$ channels also were observed at the myocyte surface, but in a striated pattern (Figs. 1K, L). The striated $Na_v1.5$ staining was in register with α-actinin marking the z-lines (Figs. 1M-O). This pattern suggests that $Na_v1.5$ channels cluster at a cell surface specialization anchored to the proteins of the z-line. In contrast to $Na_v1.2$, increased staining near intercalated disks was not observed for $Na_v1.4$ and $Na_v1.5$.

Surface staining by $Na_v1.5$ was confirmed with an optical z-series (Figs. 2A–C, green). $Na_v1.5$ staining was observed across the entire width of the myocyte in sections that included the cell surface (Fig. 2A). In progressively deeper sections (Figs. 2B, C), staining was seen at the periphery of the myocyte where the cross section intersected the surface membrane, but was reduced or absent from the cell interior in the center of the cross-section. Connexin 43 is also seen in a band traversing the entire myocyte cross-section in cell surface sections (Fig. 2A, red) but is reduced in intensity and restricted to the edges of the myocyte in the deeper sections (Figs. 2B, C, red).

We further assessed the cell-surface localization of the $Na_v1.5$ sodium channel by co-labeling with an antibody recognizing the SERCA ATPase in the sarcoplasmic reticulum (Figs. 2D–I). Optical sections including the surface or through the center of the cell show $Na_v1.5$ at the surface (Figs. 2D, F) but SERCA ATPase throughout the cross-section of the myocyte in the deeper sections (Figs. 2E, F). The most intense bands of SERCA ATPase staining fall between the bands of $Na_v1.5$ staining that mark the z-lines (Figs. 2G–I), consistent with primary localization of the SERCA ATPase in the sarcoplasmic reticulum between the z-lines. Conversely, the $Ca_V1.2$ channel was found in a striated pattern in the cell interior but in register with $Na_v1.5$ on the myocyte surface (Figs. 2K, L). Staining for $Ca_V1.2$ occurred at each z-line in doublets flanking the staining for $Na_v1.5$ (Figs. 2J, L) and for α-actinin (not shown).

Collectively, these data indicate that $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.5$, and $Na_v1.6$ are all expressed in human atrial myocardium, but are localized in distinct patterns. In Fig. 1 staining for each α subunit isoform is shown at similar brightness to clearly illustrate the pattern of staining for each channel subtype. When imaged quantitatively, staining

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