Setting the Pace with $Na_V1.5$ Channels

Roei Levy, M.Sc. and Melanie Grably, Ph.D.

SCN5A is the gene encoding $Na_V1.5$ voltage-gated Na^+ channels. These membrane proteins are responsible for the initiation and propogation of the excitable cell action potential in the heart. They have been extensively studied over the years due to their critical importance in regulation of cardiac pulsing, and are the prime target for several important anti-arrhythmic drugs. This article briefly reviews the contribution of Alomone Labs products to the understanding of $Na_V1.5$ role and function at the cellular and physiological levels.

Right: Aconitine maintains Na_V channels in the open state.

Introduction

Voltage-gated Na^+ (Na_V) channels are heteromultimeric, transmembrane proteins. They are abundant in the vertebrate heart, skeletal muscle and nerve tissues, where they enable the initiation and propagation of action potentials in excitable cells. They do so by allowing transmission of Na^+ ions in response to membrane voltage alterations, thus executing essential processes such as neuronal firing and muscular contraction, which in turn permit higher events, e.g. locomotion and cognition, to occur[9,18].

A plethora of pathologies (channelopathies), usually inherited, are attributed to defects in one or more mammalian Na_V isoforms; from generalized epilepsy and familial infantile-seizures, through hyperkalemic periodic paralysis and variants of myotonia to congenital long QT syndrome, arrhythmia and even sudden infant death syndrome. Accumulating evidence is also being gathered regarding the crucial role Na^+ channels play in cancer invasion[12], and recently Na_Vs have even been identified as the primary mediators of abnormal gastrointestinal motility[9,18,19].

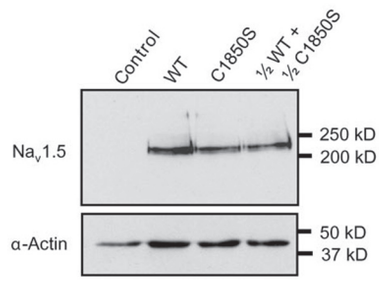
$Na_V1.5$ is expressed almost exclusively in cardiac tissues. It generates the rapid action potential (AP) of myocardia, and is responsible for its conduction and duration and contributes to the plateau of the AP through a continuous inward Na^+ current (late I_{Na}; I_{NaL})[6,33]. The channel alternates between three modes of conformation: *activation*, upon a rapid upward shift in membrane potential from the resting state, rendering the channel in the open conformation, followed by a stable, *inactivation* phase less than a millisecond later, and a *closed* conformation, at a resting membrane potential of about -85 mV. Conductance modifications and channel trafficking ascribed to genetic mutations of $Na_V1.5$ and its regulatory partners are at the base of several inborn cardiac channelopathies[1,3]. The reviewed studies presented below aim to shed light upon some of the more severe mutant phenotypes, malfunctions and mechanisms of $Na_V1.5$.

Heart Malfunctions

Brugada Syndrome/Decreased Na^+ Density Mutants

Brugada syndrome (BrS), a rare clinical cardiac disorder characterized by an abnormal rise of the ST-segment in an ECG scan, usually unmasked by Na^+ channel blockers[20], is an arrhythmical disorder with episodes of ventricular fibrillation, which significantly elevates the risk of sudden cardiac death and sudden infant death syndrome[14,15,20], albeit without structural

Figure 1. C1850S $Na_V1.5$ Mutant Does not Affect Expression Levels of the Channel



Western blot analysis using **Anti-$Na_V1.5$** antibody (#ASC-005) of HEK293 cells expressing Wild Type and mutant $Na_V1.5$ channel shows that the mutation does not affect the expression of $Na_V1.5$.

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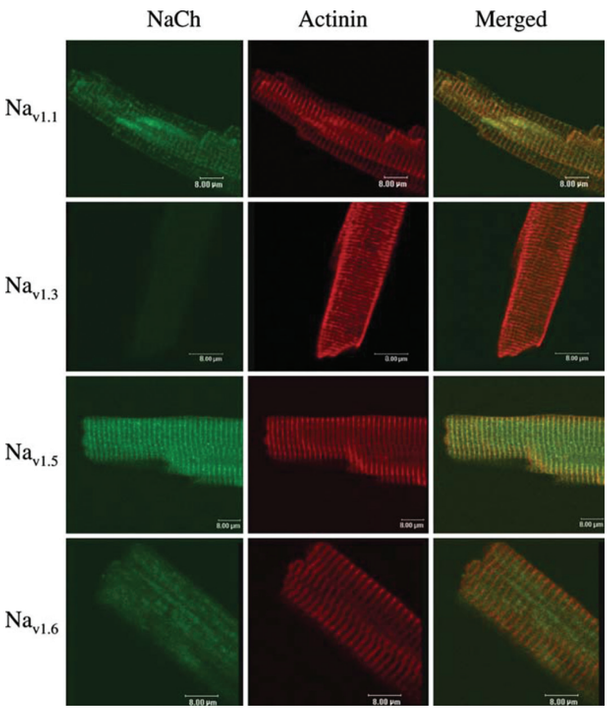
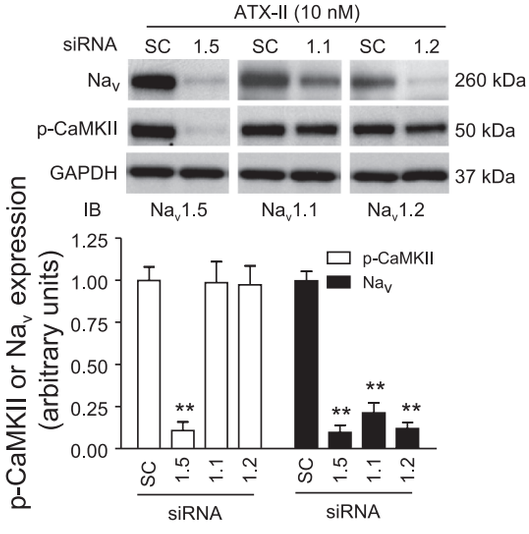
Figure 2. Expression of $Na_V1.1$, $Na_V1.3$, $Na_V1.5$ and $Na_V1.6$ in Rat Cardiac Myocytes



Immunocytochemical staining of rat cardiac ventricular myocytes using **Anti-$Na_V1.1$** (#ASC-001), **Anti-$Na_V1.3$** (#ASC-004), **Anti-$Na_V1.5$** (#ASC-005) and **Anti-$Na_V1.6$** (#ASC-009) antibodies.
Adapted from reference 31 with permission of Oxford Journals.

Figure 3. ATX-II Fails to Activate CaMKII in $Na_V1.5$ siRNA Treated Cells



The decrease in $Na_V1.5$ protein levels by siRNA reduces ATX-II-induced phosphorylation of CaMKII. $Na_V1.5$ levels were monitored by using **Anti-$Na_V1.5$** antibody (#ASC-005). Alomone Labs **ATX-II** (#STA-700) was used to activate $Na_V1.5$ channels.
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heart disease. Although more than 90 mutations leading to BrS have been mapped to $Na_V1.5$, the majority of which reduce I_{Na} either by alternations of its membrane expression density or its biophysical properties, only about 20% of the patients carry a mutation in the gene encoding it. In an effort to map causative factors of this elusive morbidity, Petitprez *et al.*[20] investigated a novel BrS mutation, C1850S, which they identified by a molecular screening of SCN5A in a patient suffering from phenotypical BrS. Following a transfection of HEK293 cells with WT and mutant DNA, results of immunoblots using **Anti-$Na_V1.5$** antibody (#ASC-005) showed similar levels of protein expression between WT and mutant strands (Figure 1), confirming the hypothesis that the mutation does not alter the channel's membrane density, but rather, as was evident by electrophysiological experiments, reduces the peak of I_{Na}, its intermediate-density and the time it takes to inactivate[20].

Using a different approach, Kattygnarath *et al.*[14] studied $Na_V1.5$'s regulatory associates, and focused on MOG1, a small (28 kDa) ubiquitous nuclear protein. MOG1 colocalizes with $Na_V1.5$ at ventricular myocytes and interacts with it through the latter's intracellular loop between domains II and III, and increases the Na^+ current $Na_V1.5$ conveys. E83D, a MOG1 mutant, was transfected into adult rat cardiomyocytes in parallel with its WT counterpart. Immunofluorescence with Anti-$Na_V1.5$ revealed that in E83D-transfected cells, $Na_V1.5$ accumulates around the nucleus, with a significantly lower density in the cell's membrane, even though the MOG1 mutant itself was distributed evenly along these locales. This supports their previous electrophysiological attempts in which the I_{Na} density was reduced in mutant cells[14].

Investigating factors involved in $Na_V1.5$ malfunction, Liu *et al.* hypothesized that NADH is a causative player in the downregulation of $Na_V1.5$, by the observation that a mutation in GPD1-L (involved in NAD-dependent energy metabolism) induces BrS. Treating cells with **Chelerythrine chloride** (#C-400), a known blocker of Protein Kinase C (PKC), they demonstrated that the I_{Na} reduction happens due to an increase in NADH concentration, since chelerythrine inhibited the decrease in Nav1.5 currents via PKC. Using Anti-$Na_V1.5$ in fluorescent imaging, no significant differences were observed in the $Na_V1.5$ membrane density and localization between cells incubated with agents known to increase NADH (thus reduce I_{Na}) and control samples[15].

The D1275N SCN5A mutant is associated with several cardiac arrhythmic disorders. However, heterologous expression of this mutant does not display any functional anomalies, nor does it show altered expression levels. When expressed in a mouse model, the same mutant is expressed to lower levels and displays a decrease in Na^+ currents in cardiomyocytes as shown by both western blot analysis and immunostaining with Anti-$Na_V1.5$. Overall, this study emphasizes the importance of the channel's environment in modulating its activity[28].

Prolonged Late-Na^+ Current Complexities

A related abnormality is the long QT syndrome (LQTS). LQTS, a gain-of-function arrhythmia, is characterized by a lengthened action potential density (APD), a longer QT interval, and susceptibility to polymorphic ventricular tachyarrhythmia (torsade de pointes; TdP)[29]. In this case, $Na_V1.5$ fails to inactivate normally and maintains an elongated action potential plateau due to a prolonged I_{NaL}. Such an increase leads to a disruption of the cellular ionic homeostasis and results in an intracellular Ca^{2+} overload[2,16,21]. Interestingly, a gender-dependent bias is seen in the prevalence of LQTS and BrS: females, who seem to have a lower density of the cardiac K^+ channel α subunit which supplies the current involved in atrial repolarization (I_{Kr}) as well as in its inactivation (I_{To})[25], are more prone to LQTS than males, who instead have a higher risk of BrS. However, the distinct arrhythmia profiles cannot be relied solely on the gender-specific differences in K^+ currents. Speculating that a non-homogenous expression of $Na_V1.5$ is the determining

gender-related risk factor, a study was set out to explore the sex-biased distribution of Na_Vs; immunoblots using **Anti-Pan Na_V** (#ASC-003) revealed an overall even Na_V dispersion between the genders and thus led the authors to base the anomaly on the male hormone, testosterone, which they claimed to be a depressant of the I_{Na} amplitude[4]. However, Lowe *et al.* ruled out any interference of hormones, to show that Na_Vs are expressed evenly among the genders by using Anti-Na_V1.5 in immunoblots. ATX-II alone, a potent Na^+ channel opener, drastically increased the I_{NaL} in females, whereas only moderately did so in males[16]. Their finding enforced a previous one, which also used **ATX-II** (#STA-700) to induce LQTS-like symptoms; this proves a direct relationship between increased I_{NaL} and LQTS[27].

ΔKPQ is a SCN5A mutant which induces a long QT-like phenotype. Different inactivation inhibitors were tested on the mutant and was compared to its wildtype counterpart. Alomone Labs ATX-II was used and surprisingly inactivated the mutant channel by shifting the steady-state inactivation, as opposed to its activating effect on the wild type channel[23].

Although lengthened QT interval often reflects TdP, each cannot reliably indicate the presence of the other. Hondeghem *et al.* used Alomone Labs ATX-II, along with dofetilide (I_{kr} blocker) to show that in isolated Langendorff perfused hearts, TdP occurs with prolonged, normal and even shortened APDs[11].

Connexins and Na_V1.5 are two important factors for impulse propagation in cardiac cells. As these parameters are often affected in cardiac disease, a study was initiated to see how these two factors can together affect conductivity. Heterozygous animals for Na_V1.5 and/or connexin-43 (Cx43) were used; thereby leading to a decrease of up to 50% in protein expression (Anti-Nav1.5 was used in immunohistochemical studies to monitor Na_V1.5 protein levels). The electrophysiological properties of these mutants showed that the deficiency of both conductance and impulse propagation, while caused a reduced Na^+ current, surprisingly benefited the QT interval[24]. In a related study, elevated levels of Cx43 and Na_V1.5 (shown using Anti-Na_V1.5 antibody) in the left ventricle (LV) were spotted in an experiment studying the effects of rabbit's heart biventricular pacing (BIV). Increases in KVLQT1 (a gene coding a cardiac K_V7.1 K^+ channel subtype) protein levels, observed using **Anti-K_V7.1 (KCNQ1)** antibody (#APC-022), explained the shortened QT intervals occasionally seen in artificially paced hearts[22].

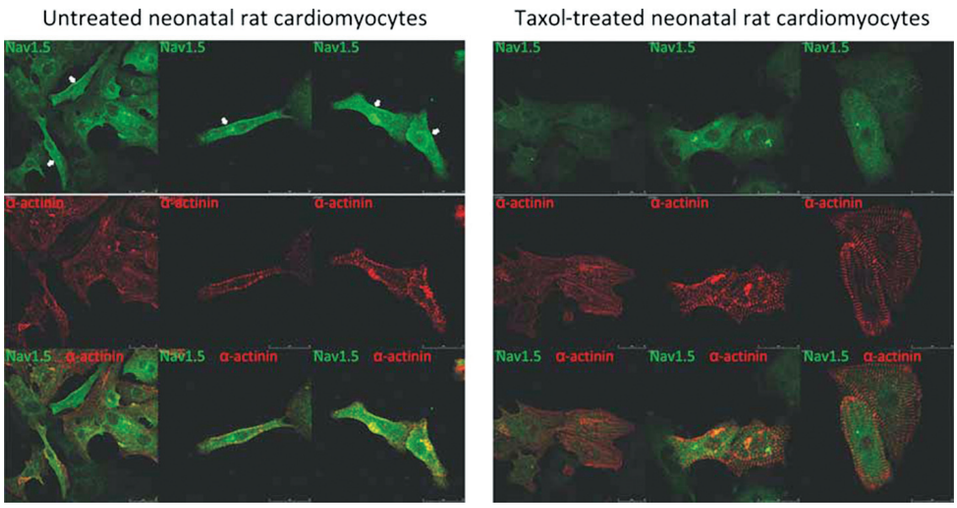
Although Na_V1.5 is the major voltage-gated Na^+ channel isoform expressed in the heart, designated neuronal type Na_V channels, namely Na_V1.1, Na_V1.3 and Na_V1.6 have also been detected in the mammalian heart and contribute 10-20% of the Na^+ current peaks in cardiomyocytes and Purkinje cells[31]. In a study, their contribution to heart failure was investigated. Using a combination of electrophysiological measurements and immunological staining using Alomone Labs **Anti-Na_V1.1** (#ASC-001), **Anti-Na_V1.3** (#ASC-004), Anti-Na_V1.5 and **Anti-Na_V1.6** (#ASC-009) antibodies, the results indicate that most Na_V isoforms are expressed in cardiac ventricular myocytes (Figure 2), and that upon pressure overload induction (i.e. heart failure), there is an increase in TTX-sensitive currents, namely those of Na_V1.1, Na_V1.3 and Na_V1.6 channels. This is reflected by an increase in the expression of Na_V1.1 and Na_V1.6 and a decrease of Na_V1.5 channels[31].

Drugs acting on Na_V1.5

Some antiarrythmic agents, while not altering the QT interval, have nonetheless a critical impact on TdP risk. One such agent, amiodarone, a drug which elevates the risk to TdP in patients with the S1102Y SCN5A polymorphism (which heightenes I_{NaL} peak and duration), was reported to facilitate an already prolonged APD in rabbits all the while reducing its peak (by inhibiting the K^+ current I_{kr} rather than that of Na_V) when acutely administrated at low doses; an alarming finding which called for an immediate reassessment of the drug's safety. The S1102Y SCN5A polymorphism was mimicked by administering Alomone Labs ATX-II[30]. A related study, focusing on mexilethine, a Na_V blocker, showed that in some of the SCN5A gain-of-function mutants, treatment with mexiletine can worsen the symptoms of a LQTS-variant by causing an increased trafficking of Na_Vs to the membrane – as was observed using Anti-Na_V1.5 antibody in immunostaining[21].

Bepridil has multi-channel blocking properties and exhibits anti-electrical remodeling effects in the diseased heart. A study aimed at investigating the long term effects of bepridil in rat cardiomyocytes and heterologous human Na_V1.5 system, found that bepridil inhibited I_{Na} in the short term, and stimulated the channel in the long run. Na_V1.5 channel levels were

Figure 4. Taxol Treatment Reduces Na_V1.5 Expression in NRC Cells



Immunocytochemical staining of neonatal rat cardiomyocytes (NRCs) using **Anti-Na_V1.5** antibody (#ASC-005). Untreated cardiomyocytes (left) show Na_V1.5 staining (green) in the cytoplasm and the membrane surface. Following taxol treatment, less Na_V1.5 staining is observed and the channel is mostly localized to the cytoplasm.
Adapted from reference 7 with permission of Oxford Journals.

monitored using Anti-$Na_V1.5$ and **Anti-$Na_V\beta2$** (#ASC-007) antibodies. The long term enhancing effect was seemingly achieved by inhibiting the proteasome activity in a Ca^{2+}/calmodulin (Ca^{2+}/CaM) dependent signaling pathway; western blot analysis showed an increase in $Na_V1.5$ (but without an upregulation of its beta subunits) upon bepridil treatment, attributed to the proteasome-inhibiting effect of the drug. The authors concluded that while bepridil-induced cardio-aversion does occur in the long term in diseases related to atrial remodeling, it has an unpredicted pharmacological outcome due to its intereference with the Ca^{2+}/CaM pathway[13].

F15845, a newly discovered $Na_V1.5$ channel blocker, was shown to prevent hypoxia-induced contraction of the femoral artery. The Na^+ channel is prominently expressed in the femoral artery, as shown using Anti-$Na_V1.5$ in immunofluorescent assays[5].

Kinases in Cardiac Pathologies

Two recent studies have elucidated the role of calmodulin-dependent protein kinase II (CaMKII) regarding I_{NaL}. In heart failure, CaMKII is up-regulated and maintains a partly positive-feedback relationship with $Na_V1.5$ such that I_{Na} promotes CaMKII auto-phosphorylation via Ca^{2+}, which results in either a prolonged I_{NaL}, or a reduced I_{Na} at low and high heart rates, respectively. I_{NAL} induction via ATX-II treatment led to CaMKII activation which could be reversed by specific siRNA downregulation of $Na_V1.5$ and not by $Na_V1.1$ and $Na_V1.2$ downregulation (Figure 3)[2,32].

Serum-and glucocorticoid-regulated kinase-1 (SGK1), a PI3-kinase member is activated in prolonged APD arrhythmia. Immunoprecipitation experiments validated the binding of SGK1 to $Na_V1.5$ (using in part Anti-$Na_V1.5$ antibody). Whole-cell patch clamp protocols of cardiomycytes expressing constitutively active SGK mutant displayed a 3.6 fold increase in I_{NaL}, compared to wild-type cells[8].

The Effects of Reactive Oxygen Species and NCX

Reactive oxygen species (ROS) leads to an increase in I_{NaL}. The resulting increase in intracellular Na^+ reverses the activity of Ca^{2+}/Na^+ exchanger (NCX) thus leading to Ca^{2+} overload (which results in long-QT and related arrhythmia). In isolated rat heart, administration of Alomone Labs ATX-II had the overall same effect as that of H_2O_2; both caused an increase in ROS, an increase in I_{NaL}, and subsequently a Ca^{2+} overload. However, the mechanisms used are different[26]. A more recent study investigated the effects of ROS on the transcription regulation of $Na_V1.5$. It seems that, upon oxidative stress, Foxo1 transcription factor binds to the promoter region of SCN5A and suppresses its expression. This was apparent in western blot analysis using Anti-$Na_V1.5$; $Na_V1.5$ levels were significantly reduced in the presence of Foxo1, and high when Foxo1 RNA was silenced[17].

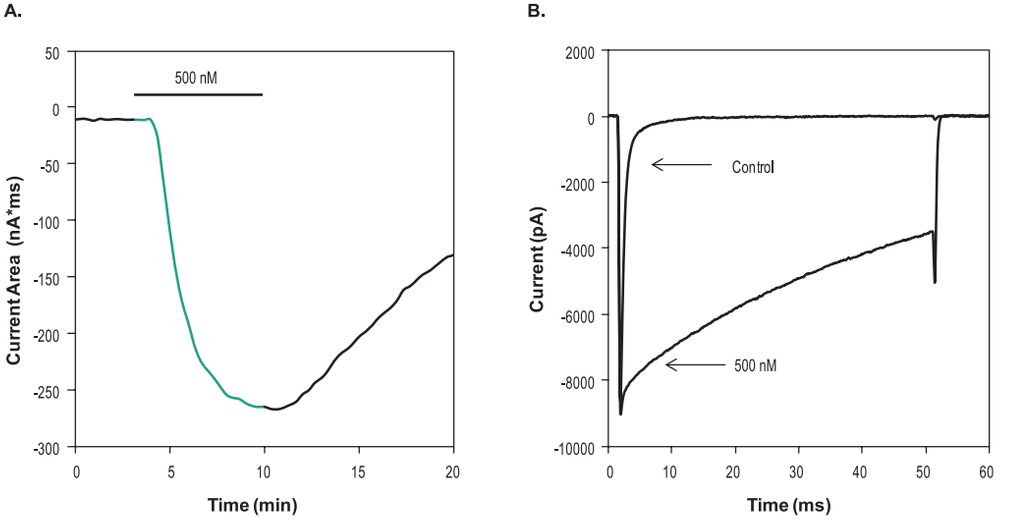
NCX plays an important role in excitation-contraction coupling in the neonatal rabbit heart, where Na^+ influx is large enough to promote Ca^{2+} entry via NCX. This event is possible due to the co-localization between various Na_V channels, including $Na_V1.5$ (detected with **Anti-Human-$Na_V1.5$** antibody (#ASC-013) in neonatal rabbit hearts. Reverse-mode activity of NCX is lost during adulthood because the spatial organization of Na_V channels with respect to NCX changes[10].

$Na_V1.5$ in Cancer

Accumulating evidence suggests that Na_Vs have a pivotal role in the invasiveness of several types of cancer. A study mapped the functionality and expression of $Na_V1.5$ in colon cancer and showed, in immunohistochemical staining using Anti-$Na_V1.5$ antibody, the abundance of the channel in the luminal surface of human malignant colon cells, and its absence in normal analogs[12].

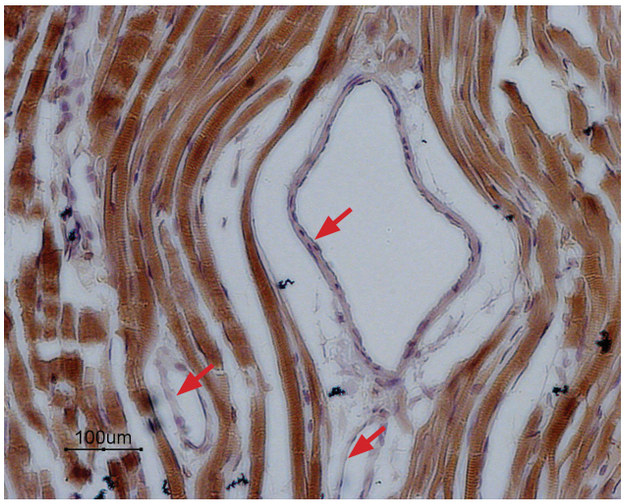
Because of its dependence on the cytoskeletal framework, Na^+ channels can be impaired when treated with anti-cancer drugs like taxol (TXL), which polymerizes tubulin an essential cytoskeletal protein. Indeed, patients treated with the drug often develop cardiac disorders. Casini *et al.* nicely demonstrated electrophysiologically the reduction of I_{Na} peak and density in cardiomyocytes treated with TXL and assured this observation was due to the channel's reduced expression, in immunostaining procedures (Figure 4). They also established the necessity of the β_1 subunit in enhancing I_{Na}[7]

Alomone Labs Jingzhaotoxin-II activates $Na_V1.5$ currents in stably transfected HEK293 cells



A. Time course of **Jingzhaotoxin-II** (#STJ-150) action. Current area was plotted as a function of time. Holding potential was -100 mV and currents were stimulated every 20 seconds by a voltage step of 50 msec from holding potential to -20 mV. 500 nM Jingzhaotoxin-II was perfused in the period marked by the horizontal bar (green), indicating a toxin-dependent decrease in $Na_V1.5$ currents inactivation. B. Superimposed traces of $Na_V1.5$ currents before and during 7 min application of 500 nM Jingzhaotoxin-II.

Expression of $Na_V1.5$ in rat cardiac muscle



Immunohistochemical staining of $Na_V1.5$ in rat *myocardium* paraffin-embedded section using **Anti-Human $Na_V1.5$** antibody (#ASC-013), (1:100). Staining is specific for cardiomyocytes while smooth muscles cells in the artery walls are negative (red arrows). Hematoxilin is used as the counterstain.

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Related Products

Compound	Cat. #
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Jingzhaotoxin-V	STJ-050
Jingzhaotoxin-XII	STJ-100
KC 12291 hydrochloride	K-105
Lidocaine	L-105
Lidocaine hydrochloride	L-145
Lorcainide HCl	L-135
Mexiletine hydrochloride	M-115
ProTx-I	STP-400
ProTx-II	STP-100
Tolperisone hydrochloride	T-115
TTX-Resistant NaV Channel Blocker Explorer Kit	EK-106
$Na_V1.5$ Channel Activators	
Aconitine	A-150
Anthopleurin-C	A-400
ATX-II	STA-700
Jingzhaotoxin-II	STJ-150
Veratridine	V-110
Na_V Channel Activator Explorer Kit	EK-203
Na_V Channel Antibodies	
Anti-$Na_V1.1$	ASC-002
Anti-$Na_V1.1$-ATTO-594	ASC-001-AR
Anti-$Na_V1.3$	ASC-004
Anti-Human $Na_V1.5$	ASC-013
Anti-$Na_V1.5$	ASC-005
Anti-$Na_V1.5$	AGP-008
Anti-$Na_V1.6$	ASC-009
Anti-Pan Na_V	ASC-003
Anti-Pan Na_V	AGP-007
Anti-$Na_V\beta2$	ASC-007
TTX-Resistant Na_V Channel Antibody Explorer Kit	AK-223
$K_V7.1$ Channel Antibody	
Anti-$K_V7.1$ (KCNQ1)	APC-022
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Chelerythrine chloride	C-400

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