Late Na\(^+\) current produced by human cardiac Na\(^+\) channel isoform Na\(_v\)1.5 is modulated by its β\(_1\) subunit

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Abstract  Experimental data accumulated over the past decade show the emerging importance of the late sodium current (\(I_{NaL}\)) for the function of both normal and, especially, failing myocardium, in which \(I_{NaL}\) is reportedly increased. While recent molecular studies identified the cardiac Na\(^+\) channel (NaCh) α subunit isoform (Nav1.5) as a major contributor to \(I_{NaL}\), the molecular mechanisms underlying alterations of \(I_{NaL}\) in heart failure (HF) are still unknown. Here we tested the hypothesis that \(I_{NaL}\) is modulated by the NaCh auxiliary β\(_1\) subunits. tsA201 cells were transfected simultaneously with human Na\(_v\)1.5 (former hH1a) and cardiac β\(_1\) or β\(_2\) subunits, and whole-cell patch-clamp experiments were performed. We found that \(I_{NaL}\) decay kinetics were significantly slower in cells expressing α + β\(_1\) (time constant \(τ = 0.73 ± 0.16\) s, \(n = 14\), mean ± SEM, \(P < 0.05\)) but remained unchanged in cells expressing α + β\(_2\) (\(τ = 0.52 ± 0.09\) s, \(n = 5\)), compared with cells expressing Na\(_v\)1.5 alone (\(τ = 0.54 ± 0.09\) s, \(n = 20\)). Also, β\(_1\), but not β\(_2\), dramatically increased \(I_{NaL}\) relative to the maximum peak current, \(I_{NaT}\) (2.3 ± 0.48%, \(n = 14\) vs. 0.48 ± 0.07%, \(n = 6\), \(P < 0.05\), respectively) and produced a rightward shift of the steady-state availability curve. We conclude that the auxiliary β\(_1\) subunit modulates \(I_{NaL}\) produced by the human cardiac Na\(^+\) channel Na\(_v\)1.5 by slowing its decay and increasing \(I_{NaL}\) amplitude relative to \(I_{NaT}\). Because expression of Na\(_v\)1.5 reportedly decreases but β\(_1\) remains unchanged in chronic HF, the relatively higher expression of β\(_1\) may contribute to the known \(I_{NaL}\) increase in HF via the modulation mechanism found in this study.

Keywords  Whole-cell sodium current · Heterologous expression · Human sodium channel subunits

Introduction

Experimental data accumulated over the past decade show the emerging importance of the late sodium current (\(I_{NaL}\)) for the function of both normal and, especially, failing myocardium, in which \(I_{NaL}\) is reportedly increased [1–3] The importance of the contribution of \(I_{NaL}\) to heart failure (HF) mechanisms has been demonstrated in experiments in which “correction” of \(I_{NaL}\) in failing cardiomyocytes resulted in:

1. rescue of normal repolarization;
2. reduced beat-to-beat action potential duration (APD) variability; and
3. improvement of Ca\(^{2+}\) handling and contractility [1, 3–5].

Accordingly \(I_{NaL}\) has recently emerged as a novel possible target for cardioprotection to treat the failing heart [6, 7].
Voltage-clamp studies have identified several types of single Na\(^+\) channel activity and whole-cell Na\(^+\) currents that could contribute to APD in cardiomyocytes. The variety of Na\(^+\) channel activities identified so far has been classified (for review see Ref. [6]) in terms of the late (or persistent) Na\(^+\) current i.e. \(I_{\text{Na,p}}\) and background Na\(^+\) currents. In contrast with \(I_{\text{Na,p}}\), background Na\(^+\) currents have been poorly characterized and have no clear molecular identity.

Major biophysical and pharmacological characteristics of the whole-cell \(I_{\text{Na}}\) have been studied in great detail in human cardiomyocytes by our research group [3, 8, 9] and can be summarized as follows:

1. potential-independent slow inactivation and re-activation (~0.5 s);
2. steady-state activation and inactivation similar to that for \(I_{\text{Na,1}}\) and
3. low sensitivity to the specific toxins TTX and STX similar to the cardiac Na\(^+\) channel isoform Na\(_{v1.5}\).

A slowly inactivating \(I_{\text{Na}}\) with aforementioned biophysical characteristics has been identified in ventricular cardiomyocytes and cardiac Purkinje cells of dogs [1, 3, 5, 10–12], guinea pigs [13–15], rabbits [16], rats [17] and mice [18]. \(I_{\text{Na}}\) is also produced by the heterologously expressed cardiac Na\(^+\) channel isoform main \(\alpha\)-subunit Na\(_{v1.5}\) [7, 19].

Despite explosive interest in this new component of the Na\(^+\) current (for recent reviews see Refs. [6, 7, 14, 20]) the mechanisms of \(I_{\text{Na}}\) regulation in normal heart and its alterations in HF are not yet understood and are likely to need further collective efforts based on different approaches including detailed biophysical and molecular biology examinations in addition to traditional pharmacological studies. Utilizing antisense inhibition and siRNA technologies our most recent studies explored the molecular identity of \(I_{\text{Na}}\) in ventricular cardiomyocytes [7, 21]. These studies suggested the cardiac Na\(^+\) channel \(\alpha\)-subunit isoform (Na\(_{v1.5}\)) was a major contributor to \(I_{\text{Na}}\).

Although most recent studies have shown that \(I_{\text{Na}}\) is strongly and differently modulated by intracellular Ca\(^{2+}\) in the cardiomyocytes of normal and failing hearts [18, 22], Na\(^+\) channels operate not in isolation but within macromolecular complexes [23, 24], which are critical attributes of Na\(^+\) channel function (in addition to membrane voltage and ion concentrations). The macromolecular complexes include auxiliary \(\beta\)-subunits, phospholipids and elements of the cytoskeleton, each of which can modulate Na\(^+\) channel function including \(I_{\text{Na}}\) (for review see Ref. [7]). The \(\beta\)-subunit gene family has four members—\(\beta_1\) (SCN1B), \(\beta_2\) (SCN2B), \(\beta_3\) (SCN3B), and \(\beta_4\) (SCN4B) (for review see Ref. [24]). Despite high homology between \(\beta_1\) and \(\beta_3\), and \(\beta_2\) and \(\beta_4\) the different functional role of these newly discovered isoforms (\(\beta_3\) and \(\beta_4\)) could not be ruled out. In addition there is a splice variant \(\beta_1^A\) of SCN1B that is expressed in embryonic brain and adult heart in rat [25]. All five \(\beta\)-subunits are expressed in rodent heart and are differently localized to specific sub-cellular domains and cell types. The \(\beta_1\) subunit is non-covalently attached to the \(\alpha\) subunit, and the \(\beta_2\) subunit is covalently linked to the \(\alpha\) subunit by a disulfide bond [26]. Numerous studies indicate a possible role of \(\beta\) auxiliary subunits in modulating Na\(^+\) channel expression and function (for review see Ref. [24]), but the possible implication of \(\beta\)-subunits in \(I_{\text{Na}}\) modulation has not been studied in detail, especially in HF. Our previous studies using the canine chronic HF model showed that in the state of HF the protein level of Na\(_{v1.5}\) is reduced but remains unchanged for \(\beta_1\) and \(\beta_2\) subunits, making these \(\beta\) subunits relatively upregulated [27].

Thus, an intriguing possibility could be that differential expression of \(\alpha\) and \(\beta\)-subunits in normal and failing hearts can contribute, at least in part, to \(I_{\text{Na}}\) alterations observed in HF. Accordingly, in this study, using a heterologous expression system, we specifically tested whether \(I_{\text{Na}}\) is modulated by \(\beta_1\) or \(\beta_2\) co-expression with Na\(_{v1.5}\). Our experiments show that \(\beta_1\) substantially and significantly affects \(I_{\text{Na}},\) whereas the effects of \(\beta_2\) were insignificant. More specifically, the \(\beta_1\) subunit modulates \(I_{\text{Na}}\) by two mechanisms one of which slows \(I_{\text{Na}}\) decay and the other of which increases \(I_{\text{Na}}\) amplitude relative to the peak transient (\(I_{\text{Na,T}}\) ) current.

Methods

Human kidney epithelial cells tsA201 were transiently transfected by Na\(_{v1.5}\) alone and/or simultaneously with human cardiac \(\beta_1\) or \(\beta_2\) subunits (tagged by GFP or fivefold excess of \(\beta\)-encoding cDNA, as previously suggested [28]). We chose tsA201 cells because there was no expression of endogenous \(\alpha\) or \(\beta_1\) and \(\beta_2\) subunits compared with that of some HEK293 cell lines (our unpublished data). Whole-cell recordings were made using the conventional patch-clamp technique. The heterologous expression system provides an unique possibility of measuring the peak transient current (\(I_{\text{Na,T}}\)) and \(I_{\text{Na}}\) at the same physiological [Na\(^+\)]\(_o\) = 140 mM. This is impossible in cardiomyocytes because of voltage-control problems. The \(I_{\text{Na}}\) amplitude was measured as averaged current within 200–220 ms after the onset of depolarization (2 s duration) and was normalized to the peak \(I_{\text{Na,T}}\) (see examples in Fig. 3a, b). The \(I_{\text{Na}}\) decay was evaluated by the single-exponential fit starting 200 ms after the onset of depolarization, as previously suggested [8]. The data points of the peak current in the current–voltage relationships were fitted to the function [29]:

\[
\text{Data Points} \rightarrow \text{Function} [29]
\]
\[ I_{Na}(V_t) = G_{max} \times \frac{(V_r - V_t)/(1 + \exp((V_{1/2G} - V_t)/k_G)))}{(1 + \exp((V_{1/2G} - V_t)/k_G)))} \]

where \( G_{max} \) is a normalized maximum \( Na^+ \) conductance, \( V_r \) is a reversal potential, \( V_t \) is the testing voltage, and \( V_{1/2G} \) and \( k_G \) are the midpoint and slope of the respective Boltzmann functions underlying the NaCh steady-state activation (SSA). The data points were fitted to model equations by use of nonlinear regression (Origin 7.0 software, Microcal Software, MA, USA).

The steady-state availability (or inactivation, SSI) terms \( (V_{1/2A}, \text{the midpoint and } k_A, \text{the slope of the relationship}) \) were measured by a standard double-pulse procedure with the 2-s-duration pre-pulse \( (V_p) \) ranging from \(-130\) to \(-40\) mV. The data points of \( I_{NaT} \) normalized to \( I_{NaT} \) measured at \(-30\) and \(-130\) mV prepulse were fitted to a Boltzmann function \( A(V_p) \):

\[ A(V_p) = 1/(1 + \exp((V_p - V_{1/2A})/k_A)) \]  (2)

Full details can be found in an extended “Materials and methods” section in the online supplement linked with the online version of this article.

Results

The reporter GFP gene expression monitored the transient transfection of the \( \beta \)-subunits (Fig. 1). Evidently both \( \beta_1 \) (Fig. 1a) and \( \beta_2 \) (Fig. 1b) can be detected both perinuclear and, importantly, in membrane compartments of the cells in the optical slices under the confocal microscope. Our confocal imaging thus validated the transfection procedure, ensuring that \( \beta \) subunits are located in the cell membrane together with functional Na channel \( \alpha \) subunits. It is also important to note that we did not make any attempt to quantify the \( \beta \)-subunits expression level based on these results because our objective was to study \( \beta \) subunit effects on \( I_{NaL} \) in all-or-none fashion.

To carefully evaluate \( I_{NaL} \), we averaged 20–50 original current traces, and the “zero” current (obtained after TTX 25 \( \mu \)M application) was subtracted from the current traces (typical examples are shown in Fig. 2). We determined effects of \( \beta \) subunits on \( I_{NaL} \) kinetics and \( I_{NaL} \) relative amplitude compared with that of \( I_{NaT} \) (Fig. 3). We found that co-expression of \( \alpha \) with the \( \beta_1 \) subunit but not the \( \beta_2 \) subunit significantly slows \( I_{NaL} \) decay (Fig. 3a) and substantially increases the relative amplitude of \( I_{NaL} \) (Fig. 3b). Accordingly \( \alpha + \beta_2 \) can be interpreted as a mock control for these studies. The effect of the \( \beta_1 \) subunit on these \( I_{NaL} \) values was independent of the reporter GFP gene. This is evident from comparison of the \( \beta_1 \)-GFP construct with \( \beta_1 \) alone (Fig 3b, c). Furthermore, the \( \beta_1 \) subunit caused a significant rightward shift of the SSI curves for both peak \( I_{NaT} \) and \( I_{NaL} \) (measured at 200 ms; not shown). Representative examples of \( I_{NaT} \) are shown in Fig. 4, statistics for SSI data are given in Table 1. The current–voltage relationship and the steady-state activation values remained unchanged (Fig. 5, Table 1).

Discussion

For the first time we demonstrate that the \( \beta_1 \) subunit can significantly modulate \( I_{NaL} \) produced by the heterologously expressed \( Na_1.5 \). The modulation includes slowed
inactivation, augmented amplitude relative to $I_{NaT}$, and rightward SSI shift.

Although $\beta$ subunits do not form ion-conducting pores, they are important modulators of Na\textsubscript{v} function, expression levels at the plasma membrane (trafficking), and cell adhesion [23, 24]. Recent studies support the emerging significance of the $\beta_1$ auxiliary subunit in modulation of Na\textsubscript{v}1.5 function. It has been shown that the $\beta_1$-subunit:

1. is involved in abnormal NaCh activity associated with the LQT3 mutation [30];
2. aggravates NaCh dysfunction in Brugada syndrome [31];
3. modifies block of NaCh by fatty acids [32] and lidocaine [33]; and
4. modulates trafficking of Na\textsubscript{v}1.5 [34].

As to modulation of the late Na\textsuperscript{+} channel activity by the $\beta$ subunits, there are only a few controversial reports. Both $\beta_1$ and $\beta_3$ subunits exhibit dual and opposite effects on the

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**Fig. 2** Experimental approach used to elucidate $I_{NaL}$ of heterologously expressed Na\textsubscript{v}1.5. **a, b** Typical examples of the $I_{Na}$ currents recorded in tsA201 cells transiently transfected by $\alpha + \beta_1$-GFP. Shown are averaged traces from 50 sweeps before and after TTX (25 $\mu$M) application to assess “zero” current. **c** Difference $I_{NaL}$ current obtained by subtraction of “zero” current. Note different currents and time scales in **a**, **b**, and **c** to demonstrate peak ($I_{NaT}$) and $I_{NaL}$. $V_h = -120$ mV, $V_m = -30$ mV, 23°C

**Fig. 3** Effects of the $\beta$ subunits on the $I_{NaL}$ produced by the heterologously expressed human cardiac NaCh isoform $\alpha$ subunit (Na\textsubscript{v}1.5). **a** Representative examples of superimposed current traces recorded in tsA201 cells transfected with cDNA encoding human cardiac NaCh $\alpha$-subunit alone or together with $\beta_1$ ($\alpha + \beta_1$) or $\beta_2$ ($\alpha + \beta_2$). Shown are averaged (10–20) currents with a single-exponential fit to $I_{NaL}$ decay (solid lines) starting 200 ms after the onset of depolarization. The time constant ($\tau$) values are given in the panel. The current amplitudes are relative to the peak $I_{NaT}$. The voltage-clamp procedure given in the inset. **b, c** Statistical analysis of the $I_{NaL}/I_{NaT}$ ratio (**b**) and the decay time course (**c**) changes in response to coexpression of $\alpha$ with $\beta$ subunits. The statistically significant difference ($P$) in panels **b** and **c** was evaluated by ANOVA followed by the Bonferroni’s post hoc test. Bars in panels **b** and **c** represent means ± SE, $n$ number of cells. There was no significant difference between $\alpha + \beta_1$-GFP compared with $\alpha + \beta_1$ (1:5 cDNA ratio) or for $\alpha$ alone compared with $\alpha + \beta_2$-GFP for both **b** and **c** panels.
decay time course of the Na\(^+\) current produced by heterologously expressed rat brain αIIA (Na\(_v\)1.2) [35]. The current decay was assessed by use of two exponential models and the fast time constant, \(\tau_1\), was accelerated whereas the slow time constant, \(\tau_2\), was increased more than twofold (10.9 vs. 25 ms) by \(\beta_1\) and \(\beta_3\), respectively. In line with this finding it has been shown that expression of the \(\beta_1\) subunit increased slowly, inactivating current (called persistent current by the authors) produced by the new epilepsy-related Na\(_v\)1.1 mutant D1866Y [36]. These data are in line with our findings reported here.

Transient expression of Na\(_v\)1.5 into the HEK293 cell line stably expressing \(\beta_1\) subunit reduced a non-inactivating current component measured 750 ms after depolarization onset [37]. A similar non-inactivating Na\(^+\) current has been also reported in rat cardiomyocytes; it was highly TTX-sensitive and could be augmented by hypoxia or cyanides [38, 39]. In both cases, when the non-inactivated current was measured, the authors used intracellular solutions deprived of ATP and containing artificial (non-physiological) anion fluoride. It has long been known that fluoride can retard Na\(^+\) channel inactivation significantly.

**Table 1** Steady-state activation (SSA) and inactivation (SSI) data for \(I_{\text{Na}}\) in the heterologously expressed cardiac Na\(_v\)1.5 (\(\alpha\)) without or with its auxiliary \(\beta\) subunits

| Conditions         | SSI data | SSA data |
|-------------------|----------|----------|
|                   | \(V_{1/2}\) (mV) | \(K_A\) (mV) | \(n\) | \(V_{1/2}\) (mV) | \(K_G\) (mV) | \(n\) |
| \(\alpha\) alone  | –88.2 ± 0.9 | –6.2 ± 0.2 | 11 | –35.4 ± 1.2 | 5.5 ± 0.2 | 11 |
| \(\alpha + \beta_1\) + GFP | –85.1 ± 1.1* | –5.7 ± 0.4 | 13 | –36.1 ± 2.1 | 6.4 ± 0.4 | 11 |
| \(\alpha + \beta_1\) (1:5) | –81.8 ± 0.9* | –5.8 ± 0.4 | 12 | –34.7 ± 1.2 | 5.9 ± 0.2 | 7 |
| \(\alpha + \beta_2\) + GFP | –88.3 ± 1.8 | –6.1 ± 0.4 | 9 | –36.8 ± 1.2 | 5.9 ± 0.3 | 5 |

SSI and SSA data were obtained from \(I_{\text{Na}}\) data fit to Eqs. 1 and 2 (“Methods”), respectively. Data represent means ± SE, \(n\) stands for the cell number

* \(P < 0.05\) versus \(\alpha\) alone. Statistically significant differences between results within the experimental groups were evaluated by ANOVA followed by Bonferroni’s post hoc test and were considered significant at \(P < 0.05\)
as was shown in internally perfused axons [40], and in cardiac cells [41]. Metabolic regulation of Na\(^+\) channel inactivation and its rundown was demonstrated in neonatal cultured rat cardiomyocytes [42]. Therefore, this non-inactivating \(I_{Na}\) component is likely to be related to these non-physiological experimental conditions. In contrast, our current recordings were performed in close-to-physiological conditions (physiological [Na\(^+\)] and with no fluoride) and they did not reveal any non-inactivating current either in heterologously expressed Na\(_v\)1.5 clone (see Fig. 2, and Ref. [19]) or in cardiomyocytes from human hearts [3, 8].

In addition to the non-inactivating current discussed above, a background Na\(^+\) current that could be recorded at very negative membrane potentials (−120 mV) has been reported in rabbit cardiac Purkinje cells and ventricular cardiomyocytes [43]. In contrast, recent studies [11, 12] show that canine cardiac Purkinje cells exhibit slowly inactivating \(I_{NaL}\), similar to that described in ventricular cardiomyocytes of humans and dogs [3, 8]. Unlike background Na\(^+\) currents, this \(I_{NaL}\) in Purkinje cells possesses steady state inactivation and is not activated at resting membrane potentials. Furthermore, non-inactivating Na\(^+\) current was not present in human cardiomyocytes [3, 8]. Our single-channel data, in fact, excluded the presence of the non-inactivating component under close-to-physiological conditions for both Na\(_v\)1.5 clone and for human cardiomyocytes [2, 19]. Thus, the non-inactivating or background Na\(^+\) currents are more likely to be species-dependent and/or were recorded in the presence of artificial anion fluoride and absence of ATP in the intracellular milieu. The molecular and genetic origins of background currents in cardiac cells remain unknown, but Denis Noble in his recent review [6] suggested that they could result from a leak form of Na\(^+\)–K\(^+\) ATPase [44] or from NCX [45]. Accordingly, it is important to emphasize that we report here for the first time the modulatory effect of the \(\beta_1\) subunit on cardiac-type late Na\(^+\) current \(I_{NaL}\) (also known as persistent Na\(^+\) current \(I_{pNa}\)) rather than on background non-inactivated currents of yet unknown nature reported in some previous studies.

The potency of the \(\beta_1\) subunit to modulate \(I_{NaL}\) shown herein has been confirmed in the native cell environment by our preliminary study in normal dog cardiomyocytes in which antisense inhibition of SCN1B significantly accelerated \(I_{NaL}\) decay ([46], see Fig. 8A in Ref. [7]). It has been also shown that at the protein level Na\(_v\)1.5 is down-regulated whereas \(\beta_1\) remained unchanged in HF, pointing toward relative higher membrane content of \(\beta_1\) [27], but the SSI shift was not found in HF [2, 3, 29]. The SSI shift is dependent on variety of factors, including intracellular [Ca\(^{2+}\)], cytoskeleton, and membrane lipid content, that may affect SSI in different ways negating the \(\beta_1\)-related effect ([22], see review [7]). These data together with the findings of our study suggest a potential mechanism for the contribution of \(\beta_1\) to HF-related \(I_{NaL}\) alterations [1, 3]. Furthermore, in addition to the \(I_{NaL}\) decay slowing, \(\beta_1\) can also change \(I_{NaL}\) via its well-known effect of SSI shift. This \(\beta_1\)-induced SSI shift under physiological conditions (i.e. at a resting potential of \(\sim -80\) mV) may have profound effect on \(I_{NaL}\) enhancement during action potentials, as
more Na\(^{+}\) channels operating in late modes become available.

The next important question is how, specifically, the β\(_1\) subunit interacts with Na\(^{+}\) channel to produce the observed \(I_{\text{NaL}}\) changes. One possibility, however, could be related to the C-terminus (CT). The role of the CT in regulating Na\(_{\text{v}}\)1.5 and human cardiomyocytes show that late NaCh I and thus provides a possible molecular mechanism for the \(I_{\text{NaL}}\) modulation found here.

Single-channel studies in heterologously expressed Na\(_{\text{v}}\)1.5 and human cardiomyocytes show that late NaCh activity is arranged in two major gating modes—late scattered mode (LSM) and “burst” mode (BM) [19]. Numerical evaluation based on the Markovian chain model revealed that BM + LSM is responsible for the intermediate phase (40–300 ms) whereas LSM is responsible for the ultra-late (>300 ms) phase on \(I_{\text{NaL}}\) inactivation [2]. In this study we analyzed the amplitude and decay of \(I_{\text{NaL}}\) after 200 ms, thus presumably the function of LSM mode. Therefore, the β\(_1\) subunit may increase the probability of occurrence of these modes (i.e. make modal switch more probable), thus increasing relative \(I_{\text{NaL}}/I_{\text{NaT}}\).

We conclude that the auxiliary subunit β\(_1\) modulates \(I_{\text{NaL}}\), produced by the human cardiac Na\(^{+}\) channel Na\(_{\text{v}}\)1.5 by slowing its decay and increasing \(I_{\text{NaL}}\) amplitude relative to \(I_{\text{NaT}}\). Because expression of Na\(_{\text{v}}\)1.5 reportedly decreases but β\(_1\) remains unchanged in chronic HF, the relatively higher expression of β\(_1\) may contribute to known \(I_{\text{NaL}}\) increase in HF via the modulation mechanism found in this study.

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References

1. Undrovinas AI, Maltsev VA, Sabbah HN (1999) Repolarization abnormalities in cardiomyocytes of dogs with chronic heart failure: role of sustained inward current. Cell Mol Life Sci 55:494–505. doi:10.1007/s000180050306
2. Maltsev VA, Undrovinas AI (2006) A multi-modal composition of the late Na\(^{+}\) current in human ventricular cardiomyocytes. Cardiovasc Res 69:116–127. doi:10.1016/j.cardiores.2005.08.015
3. Maltsev VA, Silverman N, Sabbah HN, Undrovinas AI (2007) Chronic heart failure slows late sodium current in human and canine ventricular myocytes: implications for repolarization variability. Eur J Heart Fail 9:219–227. doi:10.1016/j.ejheart.2006.08.007
4. Maltsev VA, Sabbah HN, Tanimura M, Lesch M, Goldstein S, Undrovinas AI (1998) Relationship between action potential, contraction-relaxation pattern, and intracellular Ca\(^{2+}\) transient in cardiomyocytes of dogs with chronic heart failure. Cell Mol Life Sci 54:597–605. doi:10.1007/s000180050187
5. Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN (2006) Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. J Cardiovasc Electrophysiol 17:S169–S177. doi:10.1111/j.1540-8167.2006.00401.x
6. Noble D, Noble PJ (2006) Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. Heart 92:i1–i5. doi:10.1136/hrt.2005.078782
7. Maltsev VA, Undrovinas A (2008) Late sodium current in failing heart: friend or foe? Prog Biophys Mol Biol 96:421–451. doi:10.1016/j.pbiomolbio.2007.07.010
8. Maltsev VA, Sabbah HN, Higgins RSD, Silverman N, Lesch M, Undrovinas AI (1998) Novel, ultraslow inactivating sodium current in human ventricular cardiomyocytes. J Mol Cell Cardiol 33:923–932. doi:10.1006/jmcc.1998.0584
9. Maltsev VA, Sabbah HN, Undrovinas AI (2001) Late sodium current is a novel target for amiodarone: studies in failing human myocardium. J Mol Cell Cardiol 33:923–932. doi:10.1006/jmcc.2001.1355
10. Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C (2001) Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. Am J Physiol 281:H689–H697
11. Vassalle M, Bocchi L, Du F (2007) A slowly inactivating sodium current (INa2) in the plateau range in canine cardiac Purkinje single cells. Exp Physiol 92:161–173. doi:10.1113/expphysiol.2006.035279 (Epub 19 Oct 2006)
12. Bocchi L, Vassalle M (2008) Characterization of the slowly inactivating sodium current INa in canine cardiac single Purkinje cells. Exp Physiol 93:347–361. doi:10.1113/expphysiol.2007.040881 (Epub 9 Nov 2007)
13. Sakmann BF, Spindler AJ, Bryant SM, Linz KW, Noble D (2000) Distribution of a persistent sodium current across the ventricular wall in guinea pigs. Circ Res 87:910–914
14. Belardinelli L, Shroyck JC, Fraser H (2006) Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. Heart 92:iv6–iv14. doi:10.1136/hrt.2005.078790
15. La C, You Y, Zhabyeyev P, Pelzer DJ, McDonald TF (2006) Ultraviolet photoalteration of late Na+ current in guinea-pig ventricular myocytes. J Membr Biol 210:43–50. doi:10.1007/s00232-005-0844-6
16. Wu L, Shroyck JC, Song Y, Belardinelli L (2006) An increase in late sodium current potentiates the proarrhythmic activities of low-risk QT-prolonging drugs in female rabbit hearts. J Pharmacol Exp Ther 316:718–726. doi:10.1124/jpet.105.094862 (Epub 18 Oct 2005)
17. Chattou S, Coulombe A, Diacono J, Le Grand B, John G, Feuvray B et al (2002) Slowly inactivating component of sodium current in ventricular myocytes is decreased by diabetes and partially inhibited by known Na+-H+ exchange blockers. J Mol Cell Cardiol 32:1181–1192. doi:10.1016/j.yjmcc.2000.11.151
18. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P et al (2006) Ca/calmodulin-dependent protein kinase II regulates cardiac Na channels. J Clin Invest 116:3127–3138
19. Maltsev VA, Kyle JW, Silverman NA, Sabbah HN (2002) Gating of the late Na+ channel in normal and failing human myocardium. J Mol Cell Cardiol 34:1477–1489. doi:10.1016/j.yjmcc.2002.2100
20. Josephson ME, Rosen MR, Tomaselli GF (2008) The year in arrhythmias—2007: part I. Heart Rhythm 5:742–748. doi:10.1016/j.hrthm.2008.03.012 (Epub 14 Mar 2008)
21. Maltsev VA, Kyle JW, Mishra S, Undrovinas A (2008) Molecular identity of the late sodium current in adult dog cardiomyocytes identified by Nav1.5-antisense inhibition. Am J Physiol Heart Circ Physiol 295:H667–H676. doi:10.1152/ajpheart.00111.2008
22. Maltsev VA, Reznikov V, Undrovinas NA, Sabbah HN, Undrovinas AI (2008) Modulation of the late sodium current by Ca2+-calmodulin, and CaMKI I in normal and failing dog cardiomyocytes: similarities and differences. Am J Physiol Heart Circ Physiol 294:H1597–H1608. doi:10.1152/ajpheart.00484.2007
23. Nerbonne JM, Kass RS (2005) Molecular physiology of cardiac Na channels: similarities and differences. Am J Physiol Heart Circ Physiol 288:H1609–H1624. doi:10.1152/ajpheart.00002.2005
24. Meadows LS, Isom LL (2005) Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. Cardiovasc Res 67:448–458. doi:10.1016/j.cardiores.2005.04.003
25. Kazen-Gillespie KA, Ragsdale DS, D’Andrea MR, Mattei LN, Rogers KE, Isom LL (2000) Cloning, localization and functional expression of sodium channel β1A subunits. J Biol Chem 275:1079–1088. doi:10.1074/jbc.275.2.1079
26. Messner DJ, Catterall WA (1986) The sodium channel from rat brain. Role of the β1 and β2 subunits in saxitoxin binding. J Biol Chem 261:211–215
27. Zicha S, Maltsev VA, Nattel S, Sabbah HN, Undrovinas AI (2004) Post-transcriptional alterations in the expression of cardiac Na+ channel subunits in chronic heart failure. J Mol Cell Cardiol 37:91–100. doi:10.1016/j.yjmcc.2004.04.003
28. Qu Y, Rogers JC, Chen SF, McCormick KA, Scheuer T, Catterall WA (1999) Functional roles of the extracellular segments of the sodium channel alpha subunit in voltage-dependent gating and modulation by beta1 subunits. J Biol Chem 274:32647–32654. doi:10.1074/jbc.274.46.32647
29. Maltsev VA, Sabbah HN, Undrovinas AI (2002) Down-regulation of sodium channel in chronic heart failure: effects of long-term therapy with carvedilol. Cell Mol Life Sci 59:1561–1568. doi:10.1007/s00018-002-8529-0
30. An RH, Wang XL, Kerem B, Benhorin J, Medina A, Goldmuntz E et al (1998) Novel QT-3 mutation affects Na+ channel activity through interactions between alpha- and beta1-subunits. Circ Res 83:141–146
31. Makita N, Shirai N, Wadsg DW, Sasaki K, George ALJ, Kanno M et al (2000) Cardiac Na+ channel dysfunction in Brugada syndrome is aggravated by β1-subunit. Circulation 101:54–60
32. Xiao YF, Wright SN, Wang GK, Morgan JP, Leaf A (2000) Coexpression with β1-subunit modifies the kinetics and fatty acid block of hH1a Na+ channels. Am J Physiol 279:H335–H46
33. Makielski JC, Limberis J, Fan Z, Kyle JW (1999) Intrinsic lidocaine affinity for Na channels expressed in Xenopus oocytes depends on α (hH1 vs. rSkM1) and β1 subunits. Cardiovasc Res 42:503–509. doi:10.1006/card.2000.0288.2004
34. Zhou J, Yui J, Hu NN, George ALJ, Murray KT (2000) Activation of protein kinase A regulates cardiac Na channels in Xenopus oocytes. Circ Res 87:33–38
35. Morgan K, Stevens EB, Shah B, Cox PJ, Dixon AK, Lee K et al (2000) beta 3: An additional auxiliary subunit of the voltage-sensitive sodium channel that modulates channel gating with distinct kinetics. Proc Natl Acad Sci USA 97:2308–2313. doi:10.1073/pnas.030262197
36. Spaumanns J, Kearney JA, de Haan G, McEwen DP, Escayg A, Aradi I et al (2004) A novel epilepsy mutation in the calcium channel SCN1A identifies a cytoplasmic domain for beta subunit interaction. J Neurosci 24:10022–10034. doi:10.1523/JNEUROSCI.2034-04.2004
37. Valdivia C, Nagatomo T, Makielski J (2002) Late Na currents affected by alpha subunit Isoform and beta1 subunit co-expression in HEK293 cells. J Mol Cell Cardiol 34:1029. doi:10.1006/jmcc.2002.2040
38. Saint DA, Ju YK, Gage PW (1992) A persistent sodium current in rat ventricular myocytes. J Physiol 453:219–231
39. Ju YK, Saint DA, Gage PW (1996) Hypoxia increases persistent sodium current in rat ventricular myocytes. J Physiol 497:337–347
40. Moe et al (1978) Inactivation of the sodium permeability in squid giant nerve fibres. Prog Biophys Mol Biol 33:207–230. doi:10.1016/0033-8994(78)90029-4
41. Kohlhardt M (1991) Gating properties of cardiac Na+ channels in cell-free conditions. J Membr Biol 122:11–21. doi:10.1007/BF01872735
42. Kohlhardt M, Fichtner H, Frobe U (1989) Metabolites of the glycolytic pathway modulate the activity of single cardiac Na+ channels. FASEB J 3:1963–1967
43. Zilberter YI, Starmer CF, Starobin J, Grant AO (1994) Late Na+ channels in cardiac cells: the physiological role of background Na+ channels. Biophys J 67:153–160. doi:10.1016/S0006-3495(94)80464-3
44. Ariga P, Gadsby DC (2004) Large diameter of palytoxin-induced Na/K pump channels and modulation of palytoxin interaction by Na/K pump ligands. J Gen Physiol 123:357–376. doi:10.1523/jgp.200308964 (Epub 15 Mar 2004)
45. Hilgemann DW (2004) New insights into the molecular and cellular workings of the cardiac Na+/Ca2+ exchanger. Am J Physiol Cell Physiol 287:C1167–C1172. doi:10.1152/ajpcell.00288.2004
46. Undrovinas AI, Maltsev VA (2002) Molecular basis for late Na+ current. Knockdown of Na+ channel subunits in adult cardiomyocytes by antisense oligonucleotides. Biophys J 82:89a
47. Motoike HK, Liu H, Glaaser IW, Yang AS, Tateyama M, Kass RS (2004) The Na⁺ channel inactivation gate is a molecular complex: a novel role of the COOH-terminal domain. J Gen Physiol 123:155–165. doi:10.1085/jgp.200308929
48. Abriel H, Kass RS (2005) Regulation of the voltage-gated cardiac sodium channel Nav1.5 by interacting proteins. Trends Cardiovasc Med 15:35–40. doi:10.1016/j.tcm.2005.01.001
49. Chang SY, Satin J, Fozzard HA (1996) Modal behavior of the \( \mu \)1 Na⁺ channel and effects of coexpression of the beta 1-subunit. Biophys J 70:2581–2592. doi:10.1016/S0006-3495(96)79829-6
50. Malhotra JD, Kazen-Gillespie K, Hortsch M, Isom LL (2000) Sodium channel beta subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact. J Biol Chem 275:11383–11388. doi:10.1074/jbc.275.15.11383
51. Undrovinas AI, Shander GS, Makielski JC (1995) Cytoskeleton modulates gating of voltage-dependent sodium channel in heart. Am J Physiol 269:H203–H214
52. Chauhan VS, Tuvia S, Buhusi M, Bennett V, Grant AO (2000) Abnormal cardiac Na⁺ channel properties and QT heart rate adaptation in neonatal ankyrin(B) knockout mice. Circ Res 86:441–447
53. Zimmer T, Benndorf K (2007) The intracellular domain of the beta 2 subunit modulates the gating of cardiac Na v 1.5 channels. Biophys J 92:3885–3892. doi:10.1529/biophysj.106.098889 (Epub 2007 Mar 16)