Investigations of the Na\textsubscript{\(\alpha\beta1b\)} sodium channel subunit in human ventricle; functional characterization of the H162P Brugada syndrome mutant

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Yuan L, Koivumäki JT, Liang B, Lorentzen LG, Tang C, Andersen MN, Svendsen JH, Tfelt-Hansen J, Maleckar M, Schmitt N, Olesen MS, Jespersen T. Investigations of the Na\textsubscript{\(\alpha\beta1b\)} sodium channel subunit in human ventricle; functional characterization of the H162P Brugada syndrome mutant. Am J Physiol Heart Circ Physiol 306: H1204–H1212, 2014. First published February 21, 2014; doi:10.1152/ajpheart.00405.2013.—Brugada syndrome (BrS) is a rare inherited disease that can give rise to ventricular arrhythmia and ultimately sudden cardiac death. Numerous loss-of-function mutations in the cardiac sodium channel Na\textsubscript{1.5} have been associated with BrS. However, few mutations in the auxiliary Na\textsubscript{\(\alpha\beta1\)-4} subunits have been linked to this disease. Here we investigated differences in expression and function between Na\textsubscript{\(\alpha\beta1\)} and Na\textsubscript{\(\alpha\beta1b\)} and whether the H162P/Na\textsubscript{\(\alpha\beta1b\)} mutation found in a BrS patient is likely to be the underlying cause of disease. The impact of Na\textsubscript{\(\alpha\beta1b\)} subunits was investigated by patch-clamp electrophysiology, and the obtained in vitro values were used for subsequent in silico modeling. We found that Na\textsubscript{\(\alpha\beta1b\)} transcripts were expressed at higher levels than Na\textsubscript{\(\alpha\beta1\)} transcripts in the human heart. Na\textsubscript{\(\alpha\beta1\)} and Na\textsubscript{\(\alpha\beta1b\)} coexpressed with Na\textsubscript{1.5} induced a negative shift on steady state of activation and inactivation compared with Na\textsubscript{1.5} alone. Furthermore, Na\textsubscript{\(\alpha\beta1b\)} was found to increase the current level when coexpressed with Na\textsubscript{1.5}, Na\textsubscript{\(\alpha\beta1b/\)H162P} mutated subunit peak current density was reduced by 48% (−645 ± 151 vs. −334 ± 71 pA/pF), V\textsubscript{1/2} steady-state inactivation shifted by −6.7 mV (−70.3 ± 1.5 vs. −77.0 ± 2.8 mV), and time-dependent recovery from inactivation slowed by >50% compared with coexpression with Na\textsubscript{\(\alpha\beta1\)} wild type. Computer simulations revealed that these electrophysiological changes resulted in a reduction in both action potential amplitude and maximum upstroke velocity. The experimental data thereby indicate that Na\textsubscript{\(\alpha\beta1b/\)H162P} results in reduced sodium channel activity functionally affecting the ventricular action potential. This result is an important replication to support the notion that BrS can be linked to the function of Na\textsubscript{\(\alpha\beta1\)} and is associated with loss-of-function of the cardiac sodium channel.

Brugada syndrome; electrophysiology; sodium current; ventricular arrhythmia; computer simulation

THE Na\textsubscript{1.5} ION CHANNEL PROTEIN, encoded by SCN5A, forms the \(\alpha\)-subunit of the cardiac voltage-gated sodium current (\(I\textsubscript{Na}\)). Mutations in SCN5A have been shown to play a central role in several cardiac rhythm disorders, including Brugada syndrome (BrS), long QT Syndrome (LQTS), conduction disease, and lone atrial fibrillation (AF) (6, 21, 28). BrS is characterized by ST-segment elevation in V1–V3 on the electrocardiogram (ECG) and by an increased risk of ventricular arrhythmia (2, 3).

Surface expression and gating of Na\textsubscript{1.5} are modified by Na\textsubscript{\(\alpha\beta\)} subunits, which are encoded by four different genes, SCN1-4B (17). These Na\textsubscript{\(\alpha\beta1\)-4} auxiliary subunits are all composed of large extracellular immunoglobulin-like domains, a single transmembrane segment, and an intracellular COOH-terminal domain (17). Na\textsubscript{\(\alpha\beta1\)}, encoded by SCN1B, has been reported to increase sodium current levels (25) and to change inactivation kinetics (8). The SCN1B gene has a prominent alternative splice variant, SCN1Bb, which encodes the Na\textsubscript{\(\alpha\beta1b\)} subunit (24) described to be secreted (22) in the absence of Na\textsubscript{1.5} but retained at the membrane when the \(\alpha\)-subunit is coexpressed.

In heterologous mammalian expression systems, Na\textsubscript{\(\alpha\beta1\)} has been reported to enforce a number of different changes in Na\textsubscript{1.5} channel gating. Some reports have revealed a negative shift in steady-state activation and inactivation (14, 30, 31), while another report did not observe such a shift (12). Further, the peak \(I\textsubscript{Na}\) amplitude has either been found to be increased (12, 30, 31) or to be the same (14), and discrepancy concerning the time-dependent recovery from inactivation has also been published (12, 31). Functional differences between the SCN1B and SCN1Bb splice variants have not been directly investigated.

Mutations in the genes encoding the Na\textsubscript{\(\alpha\beta\)} subunits have, in several clinical studies, been associated with different cardiac arrhythmias. SCN1B and SCN3B mutations have been linked to BrS (11, 31), one mutation in SCN4B has been associated with LQTS (18), and mutations in SCN1-3B have been associated with AF (20, 30). Furthermore, mutations in SCN3B and SCN4B were found in a cohort of sudden infant death syndrome (SIDS) cases (27).

To date, 12 genes have been suggested to cause BrS (5). Most of these mutations affect the sodium current, foremost by compromising Na\textsubscript{1.5} function, but loss-of-function mutations in the L-type calcium channel and gain-of-function of the transient outward potassium current \(I\textsubscript{to}\) have also been reported.

Our group has previously published two Na\textsubscript{\(\alpha\beta1b\)} mutations, H162P and R214Q, identified in patients with a Brugada type 1 ECG pattern (19). At the same time, Hu et al. (12) identified R214Q in BrS and SIDS patients. They found that R214Q
induced a reduction in the peak $I_{Na}$ and an increase in $I_{Na}$. Here, we compare RNA expression levels in human hearts and electrophysiological characteristics of Na$_{<\beta1}$ and Na$_{<\beta1}$. Furthermore, we incorporate patch-clamp data obtained for the H162P/Na$_{<\beta1}$ mutation into a ventricular action potential computer model to address the functional consequences of this mutation on human ventricular electrophysiology.

**METHODS**

**Tissue and cDNA preparation.** The steady-state mRNA expression of SCN1-4B was investigated in human myocardial tissue samples. From six patients (mean age: 64 yr) undergoing valvular surgery, biopsies from atria and ventricle were obtained. RNA extraction and cDNA synthesis were performed as previously described (26). The study fulfilled principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee of Copenhagen and Frederiksberg (KF 0131322). All included patients gave written informed consent.

**Quantitative PCR.** Quantitative real-time PCR (qPCR) was performed as previously described (4). The predesigned gene expression assay from Applied Biosystems Hs00962353_g1 was used for quantification of SCN1B (complementary to exon-5). The primers and probes targeting SCN1B (3′-end of exon-3), SCN2B, SCN3B, and SCN4B were designed and synthesized by Applied Biosystems, following submission of intron spanning sequences using Primer Express 3.0 software. Cyclophilin D (Assay Rn01458749_g1) was used for normalization.

**Molecular biology.** The plasmid hNa$_{<\beta1}$,5 in pcDNA3 has been previously described (21). The plasmid pIREs-hβ1-CD8 carrying human Na$_{<\beta1}$,1 isoform 1 (Na$_{<\beta1}$, SCN1B1, NM_001037) was generously donated by H. Abriel (U Lausanne, Switzerland). The coding sequence was PCR amplified and cloned into the mammalian expression vector pcDNA3.1. cDNA encoding human Na$_{<\beta1}$,1 isoform 2 (Na$_{<\beta1}$b, SCN1Bb, NM_199037) was obtained by reverse transcription of human right atrium cDNA and subsequent amplification of the coding sequence. RNA purification and cDNA synthesis of a specimen from a 46-yr-old male who had undergone valvular surgery were performed as described previously (5). cDNAs for Na$_{<\beta1}$ subunits cloned into pcDNA3.1 were engineered with a Kozak consensus site to optimize initiation of translation (15, 16).

The point mutation H162P in hNa$_{<\beta1}$b was introduced using mutated oligonucleotide extension (PfuTurbo Polymerase; Stratagene, La Jolla, CA) from the plasmid template harboring the cDNA of interest, digested with DpnI (Fermentas, St. Leon-Roth, Germany), and transformed into Escherichia coli XL1 Blue cells. All plasmids were verified by complete DNA sequencing of the cDNA insert (Macrogen, Seoul, Korea).

**In vitro electrophysiology.** CHO-K1 cells were transiently transfected with 1 μg hNa$_{<\beta1}$,1 alone or cotransfected with 1 μg hNa$_{<\beta1}$,1, hNa$_{<\beta1}$,b, or different combinations of β1-transcripts (wild-type or H162P) with 0.5 μg DNA of each plasmid. eGFP (0.2 μg) was used as a reporter gene. Transfection was performed using Lipofectamine 2000 (Invitrogen, Naerum, Denmark) according to the manufacturer’s instructions. Patch-clamp experiments were performed at room temperature (20–22°C) 2–3 days after transfection. Patch-clamp recordings were conducted using an internal solution containing the following (in mmol/l): 60 CsCl, 70 Cs-aspartate, 11 EGTA, 1 MgCl$_2$, 1 CaCl$_2$, 10 HEPES, and 5 Na$_2$-ATP pH 7.2 with CsOH; and external solution: 130 NaCl, 2 CaCl$_2$, 1.2 MgCl$_2$, 5 CsCl, 10 HEPES, and 5 glucose pH 7.4 with CsOH. Measurements were made with Pulse software (HEKA Elektronik, Lambrecht, Germany) and using an EPC-9 amplifier (HEKA Elektronik) sampling at 20 kHz and filtering at 5 kHz. Borosilicate glass pipettes were pulled on a DPZ-Universal puller (Zeitz Instrumente, Munich, Germany). The pipettes had a resistance of 1.5–2.5 MΩ when filled with intracellular solution. The series resistances recorded in the whole cell configuration were 2–5 MΩ and were compensated (80%). The seal resistance in all experiments was >1.0 GΩ. No leak subtraction was applied. All experiments were performed on a minimum of three batches of transfected cells. Data analyses were performed as previously described (23).

**Results.** Data are presented as means ± SE. Student’s unpaired t-test and two-way ANOVA with the Bonferroni correction were used to compare means. A value of $P < 0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity.

**In silico modeling.** Both $I_{Na}$ and action potential experiments were performed with the well-established human ventricular cell model developed by Ten Tusscher and Panfilov (29). Simulation protocols were similar to in vitro measurements. In the $I$–$V$ curve experiment, the virtual cell was clamped to a test potential, starting from a prepotential of $–100$ mV, with a pulse frequency of 0.2 Hz. In action potential experiments, the virtual cell was paced using a standard current stimulus with a frequency of 1 Hz. A quasi-steady state was reached by eliciting 100 action potentials before measurements were recorded. In the pacing experiment with stepwise increasing frequency, the frequency was increased in 0.25-Hz steps from 1 to 4 Hz, corresponding to basic cycle lengths (BCL) = [1,000, 800, 667, 571, 500, 444, 400, 364, 333, 308, 286, 267, and 250] ms. Pacing was maintained for 30 s for each BCL before the action potential traces were recorded and the frequency was stepped up.

Mutated, both homozygous and heterozygous, version of the virtual cell, were obtained by 1) shifting the $V_{1/2}$ of steady-state inactivation to left, 2) increasing the time constants for release from inactivation for the fast and slow processes, and 3) decreasing the maximum conductance of $I_{Na}$ to match the in vitro findings.

**Results.**

**mRNA expression of the Na$_{\beta}$ subunits in human heart.** We performed qPCR experiments to assess the relative abundance of Na$_{\beta}$ subunits in human heart. The analyses showed that all mRNAs encoding the different subunits are expressed in both atria and ventricle (Fig. 1). However, the two splice variants of SCN1B are expressed more abundantly than SCN2B, SCN3B, and SCN4B. Interestingly, the SCN1Bb splice variant is found at a higher expression level than SCN1B in both atria ($P < 0.001$) and ventricle ($P < 0.05$), indicating a probable functional role for the resulting Na$_{<\beta1}$b protein in human heart.

![Fig. 1. Expression profile of SCN1B–SCN4B in nondiseased human heart tissues using quantitative real-time PCR (qPCR). Graph represents the relative expression levels. Atrium (black bars) and ventricle (gray bars). Data are expressed as means ± SE; n = 6.](http://ajpheart.physiology.org/)

- **Fig. 1.** Expression profile of SCN1B–SCN4B in nondiseased human heart tissues using quantitative real-time PCR (qPCR). Graph represents the relative expression levels. Atrium (black bars) and ventricle (gray bars). Data are expressed as means ± SE; n = 6.
Figure A: Current-voltage relationships for different sodium channel configurations.

Figure B: Normalized currents as a function of membrane potential.

Figure C: Normalized currents over time with voltage steps.

Figure D: Normalized currents over voltage for specific time points.

Figure E: Normalized currents over time with voltage steps and time intervals.

Figure F: Decay time constants as a function of membrane potential.

Figure G: Rise time constants as a function of membrane potential.
Electrophysiological effects of Na\(_{\beta 1}\) and Na\(_{\alpha\beta 1}\) on I\(_{Na}\).

To address the functional role of the two Na\(_{\beta 1}\) subunits, we first investigated potential differences between \(\beta 1\) and the alternatively spliced \(\beta 1b\) subunit on Na\(_{1.5}\) gating. CHO-K1 cells were transiently transfected and whole cell currents were recorded. Coexpression of Na\(_{1.5}\) with \(\beta 1\) significantly increased the peak current density compared with both, Na\(_{1.5}\) alone and Na\(_{\alpha\beta 1}\) + Na\(_{1.5}\) (Fig. 2 and Table 1). In addition, coexpression of Na\(_{1.5}\) with \(\beta 1, \beta 1b\), or their combination shifted the voltage dependence of both activation (\(P < 0.05\)) and inactivation (\(P < 0.01\)) to more hyperpolarized potentials compared with Na\(_{1.5}\) alone (Fig. 2 and Table 1).

**Electrophysiological characterization of H162P/Na\(_{\alpha\beta 1}\).**

The H162P/Na\(_{\alpha\beta 1}\) mutant was found in a Caucasian male who, at age of 47, had a spontaneous type 1 ECG pattern (19). The H162P mutation is located in extracellular domain in the part of the protein originating from the alternatively spliced exon 3A (Fig. 3). To investigate the functional impact of H162P, we generated an expression plasmid carrying the mutant and measured currents by whole cell patch clamping (Fig. 4). The H162P/\(\beta 1b\) mutant enforced a negative shift in steady-state inactivation of \(-6.7\) mV compared with wild-type Na\(_{\alpha\beta 1}\) when coexpressed with Na\(_{1.5}\) (Table 2). Peak sodium current amplitude was decreased by 48%. Furthermore, the time-dependent recovery from inactivation of both the slow and the fast components was drastically slowed with 75% \((\tau_{\text{slow}})\) and 46% \((\tau_{\text{fast}})\), respectively. Onset of (or fast decay) inactivation was not altered and neither was steady-state activation nor time to peak changed with this mutant. To mimic the heterologous situation in the patient, H162P was also evaluated by coexpressing it with Na\(_{\beta 1}\) and Na\(_{\alpha\beta 1}\). In both heterologous combinations peak current amplitude was reduced as for homogenous H162P. Steady-state inactivation and time to half recovery from inactivation values were intermediate of homogenous wild-type and mutant Na\(_{\alpha\beta 1}\), although only significant different from wild type for H162P + Na\(_{\alpha\beta 1}\) at time to half recovery from inactivation. As Na\(_{\beta 1}\) subunits have been suggested to modify the transient outward current I\(_{to}\) (8) we tested whether H162P/\(\beta 1b\) induced a change in the current kinetics of hKv4.3 in Xenopus laevis oocytes. Performing a two-way ANOVA on the IV relationship demonstrated that coexpression of Na\(_{\alpha\beta 1}\)-wild type or Na\(_{\alpha\beta 1}\)-H162P significantly reduced the peak current measured at \(\pm 30\) and \(\pm 40\) mV compared with expression of K\(_{4.3}\) alone. However, no significant difference was found between wild-type and H162P Na\(_{\alpha\beta 1}\) (10.7 \(\pm\) 3.32 \(\mu\)A (K\(_{4.3}\)) vs. 9.0 \(\pm\) 2.2 \(\mu\)A (K\(_{4.3}\) + Nav\(_{\alpha\beta 1}\)) vs. 8.5 \(\pm\) 2.6 \(\mu\)A (K\(_{4.3}\) + Nav\(_{\alpha\beta 1}\)H162P); \(n = 15, 15, 17\); means \(+ SD\) measured at \(\pm 40\) mV (data not shown). A reduced sodium peak current combined with a negative shift in steady-state inactivation indicate a loss-of-function phenotype. An increased time-dependent release from inactivation could be speculated to have an effect during high pacing rates where the diastolic interval is short. The results thereby indicate that it is possible that the H162P mutation could significantly alter I\(_{Na}\) under specific conditions.

In silico analyses. The in vivo functional consequences of the biophysical parameters altered by the H162P/\(\beta 1b\) mutant were addressed employing the Tusscher and Panfilov (29) human ventricular cell model in simulation experiments. By including measurements obtained for wild-type and mutant Na\(_{\alpha\beta 1}\) in patch-clamp experiments into the mathematical model, we were capable of analyzing potential proarrhythmic effects of H162P/\(\beta 1b\). In addition, we also investigated the effect of homozgyous Na\(_{\alpha\beta 1}\) expression in the ventricular cell model. Figure 5, A and B, confirms that we can recapitulate the biophysical properties in silico. When incorporating the changed sodium current into the model, simulations revealed a reduced peak current as well as a slowed upstroke velocity of the action potential compared with control simulations. This is most pronounced for the homozgyous H162P/\(\beta 1b\), while in the heterozygous model, which mimics the patient phenotype, an intermediate effect is found (Fig. 5, C and D). Interestingly, when the Na\(_{\beta 1}\) parameters in the model are used, upstroke velocity and peak voltage similar to the heterologous H162P + Na\(_{\alpha\beta 1}\) expression are found. When the action potential morphology as a function of

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**Table 1. Biophysical parameters of Na\(_{\alpha\beta 1}\) isoforms on I\(_{Na}\)**

<table>
<thead>
<tr>
<th></th>
<th>Peak Current at (-10) mV</th>
<th>Steady-State Activation</th>
<th>Steady-State Inactivation</th>
<th>Time to Half Recovery from Inactivation at (-85) mV, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(pA/pF)</td>
<td>(V_{1/2}), mV</td>
<td>(k)</td>
<td>(V_{1/2}), mV (k)</td>
</tr>
<tr>
<td>Na(_{1.5})</td>
<td>(-364 \pm 75)</td>
<td>(-16.2 \pm 1.0)</td>
<td>6.4 (\pm) 0.3</td>
<td>17</td>
</tr>
<tr>
<td>Na(_{1.5} + \beta 1)</td>
<td>721 (\pm) 61</td>
<td>-22.2 (\pm) 1.8</td>
<td>7.3 (\pm) 0.6</td>
<td>9</td>
</tr>
<tr>
<td>Na(_{1.5} + \beta 1/2)</td>
<td>-394 (\pm) 76</td>
<td>-21.9 (\pm) 0.9</td>
<td>7.1 (\pm) 0.4</td>
<td>9</td>
</tr>
<tr>
<td>Na(_{1.5} + \beta 1b)</td>
<td>-645 (\pm) 151 (\pm)</td>
<td>-21.3 (\pm) 1.6</td>
<td>6.6 (\pm) 0.3</td>
<td>20</td>
</tr>
</tbody>
</table>

Data are means \(\pm\) SE. Parameters were obtained from fitting individual experiments illustrated in Fig. 2. \(V_{1/2}\), midpoint potential; \(k\), slope k value; \(r\), time constant; \(n\), number of cells. \(* P < 0.05; \dagger P < 0.01\) vs Na\(_{1.5}\); \(\ddagger P < 0.05\) vs. Na\(_{1.5} + \beta 1\).
pacing frequency is examined, the model indicates that \( I_{Na} \) is reduced at shorter BCL for the heterozygous state compared with the wild-type model, due to the fact that the time-dependent recovery from inactivation is slower for H162P/β1b. The impact of this increased inactivation on the action potential is a reduced upstroke velocity (dV/dt_{max}) by 41% at a BCL of 333 ms (325 vs. 193 mV/ms) and 66% at a BCL of 250 ms (292 vs. 99 mV/ms), a reduced action potential amplitude by 10 mV at a BCL of 333 ms (113 vs. 103 mV) and by 11 mV at a BCL of 250 ms (107 vs. 96 mV), and an almost lack of phase 1 repolarization (Fig. 5, E, F, I, and J). Na,β1 showed a decreased upstroke velocity (333 ms: 208 mV/ms; 250 ms: 153 mV/ms) and action potential amplitude (333 ms: 105 mV; 250 ms: 97 mV; Fig. 5, G and H). In summary, computer simulations confirm the experimental findings showing that the phenotypic changes enforced by H162P/β1b significantly alter the properties of the ventricular action potential by functionally reducing the sodium current.

**DISCUSSION**

Functional characterization of mutations found in cardiac pathologies such as LQTS and BrS have been instrumental in understanding a number of proteins involved in conducting electrical current in the heart. Na,β subunits have long been known to interact with Na,1.5; however, how these subunits regulate the α-subunit in the different compartments of the heart remains elusive. SCN1B is spliced into two major variants, giving rise to the Na,β1 and Na,β1b subunits. Here we analyzed the relative expression levels and electrophysiological differences of these two variants. These investigations were followed by a patch-clamp characterization of the H162P/Na,β1b mutation found in a BrS patient, and the obtained results were used to simulate the functional consequences of this mutation on the ventricular action potential.

In qPCR analyses, we found that the steady-state mRNA levels of SCN1B and SCN1Bb transcripts were more abundant in atria, and for SCN1Bb also in ventricle, compared with SCN2-4B transcripts. Furthermore, SCN1Bb was expressed at a two- to threefold higher amount in both atria and ventricle than SCN1B. In line with this, Kazan-Gillespie et al. (13) have previously shown that the rat variant of Na,1b, named Na,1A, was expressed at high levels in the heart and by immunohistochemical analyses documented membrane expression in atrial cells. However, in contrast to our data, Watanabe and colleagues (31) detected a relative low expression level of SCN1Bb compared with SCN1B in human ventricle. As the methods used are comparable, this discrepancy is difficult to explain but may be due to the chosen RNA target sequences. Due to the lack of reliable antibodies, the level of expression at the protein level in human tissue remains elusive. However, the high abundance of SCN1B促进了 us to investigate whether the two β-subunits have different impacts on the Na,1.5 current.

Patch-clamp analyses in CHO-K1 cells showed that both Na,β1 isoforms modulate Na,1.5 gating by shifting the voltage dependence of activation and inactivation to more negative potentials, as reported in previous studies (14, 31). Furthermore, an increased peak current density was observed with Na,β1b. The changes of biophysical properties of \( I_{Na} \) upon coexpression of Na,β1b are in line with previous studies showing that this subunit is retained at the cell surface by Nav1.5 subunits (22). The peak \( I_{Na} \) density was not affected by coexpression of Na,β1 subunits in our study. This is in conflict

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**Fig. 3.** Coding exons and topology of hNa,β1 and hNa,β1b. A: the 2 different isoforms of SCN1B: SCN1B (NM_001037.3), encoding Na,β1 (or Na,β1 iso a), and SCN1Bb (NM_199037.2), encoding Na,β1b (or Na,β1 iso b). Extension of exon 3 generates an alternate transcript encoding Na,β1B. The green region indicates the unique sequence of exon 3A. B: topology showing the 2 different Na,β1 isoforms. Yellow dots indicate our novel mutation (H162P) and the numbers of cells measured are presented in Table 2. Error bars represent the means ± SE.
Characterization of H162P/Na,β1b

A

Na\(_{1.5+{\beta_1b}}\)  

Na\(_{1.5+{\frac{1}{2}}H162P+{\frac{1}{2}{\beta_1b}}}\)  

Na\(_{1.5+{\frac{1}{2}}H162P+{\frac{3}{2}{\beta_1}}}\)  

Na\(_{1.5+H162P}\)

B

\(\begin{array}{c}
\text{Vm (mV)} \\
\text{Normalized Currents (pA/pF)}
\end{array}\)

C

\(\begin{array}{c}
\text{Normalized Currents}
\end{array}\)

D

\(\begin{array}{c}
\text{Normalized Currents}
\end{array}\)

E

\(\begin{array}{c}
\text{Normalized Currents}
\end{array}\)

F

\(\begin{array}{c}
\tau_{\text{fast}} (\text{ms})
\end{array}\)

G

\(\begin{array}{c}
\tau_{\text{slow}} (\text{ms})
\end{array}\)
with the results of Watanabe and colleagues (30, 31) but consistent with the study performed by Ko et al. (14). The H162P/Nav1.5β1b mutation was found in a patient with at Brugada type I ECG pattern (19). As BrS has been associated with a reduced conduction velocity, which is increasingly pro-

### Table 2. Biophysical parameters of Na,β1b/H162P associated with Brugada syndrome

<table>
<thead>
<tr>
<th>Na,1.5 + β1b</th>
<th>Peak Current at −10 mV</th>
<th>Steady-State Activation</th>
<th>Steady-State Inactivation</th>
<th>Time to Half Recovery from Inactivation at −85 mV, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/Pn/PF</td>
<td>V1/2, mV</td>
<td>k</td>
<td>V1/2, mV</td>
<td>To0.5, HS</td>
</tr>
<tr>
<td>−645 ± 95†‡</td>
<td>21.3 ± 1.6</td>
<td>6.6 ± 0.3</td>
<td>70.3 ± 1.5</td>
<td>9.6 ± 11†‡</td>
</tr>
<tr>
<td>−349 ± 59*</td>
<td>20.8 ± 1.7</td>
<td>6.6 ± 0.2</td>
<td>73.7 ± 1.6</td>
<td>154 ± 28*</td>
</tr>
<tr>
<td>−354 ± 67*</td>
<td>20.8 ± 1.3</td>
<td>6.9 ± 0.3</td>
<td>72.6 ± 1.4</td>
<td>16 139 ± 31</td>
</tr>
<tr>
<td>−334 ± 71*</td>
<td>20.6 ± 2.0</td>
<td>6.9 ± 0.5</td>
<td>70.0 ± 2.8*</td>
<td>164 ± 29*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = number of cells. Parameters were obtained from fitting individual experiments illustrated in Fig. 4. *P < 0.05 vs. Nav1.5 + β1b; †P < 0.05 vs. Nav1.5 + ½β1b/H162P + ½β1b; ‡P < 0.001 vs. Nav1.5 + ½β1b/H162P + ½β1b.

fact, sympathetic activation by isoproterenol is used as treatment. However, as our results indicate that H162P/Nav1.5β1b increasingly compromises Ina at increasing frequencies, it may be that isoproterenol would not be the correct treatment for patients carrying this mutation.

The arrhythmias observed in BrS patients have also been suggested to be related to arrhythmia occurring due to early repolarization in the right ventricle rather than conduction abnormalities (9). It is suggested that a rather large Ina current in the epicardial layer and an almost absent Ina in the endocardial layer gives rise to a transmural gradient of repolarization. When the sodium current is reduced, as has been established for numerous BrS SCN5A mutations, there is a risk of a very early repolarization in the epicardial myocytes, and, as the action potential duration is unaltered on the endocardial side, phase 2 reentrant arrhythmias may arise. Our computer simulations show that action potential amplitude is reduced for the heterozygous mutation, whereby the balance between the inward sodium current and the transient outward potassium current is altered. Hence, the H162P/Nav1.5β1b could also play a prominent role in a phase 2 reentrant arrhythmia due to early repolarization of the action potential.

**Limitations.** We use heterologous expression in noncardiac derived culture cells and focused on the electrophysiological properties of the Na,1.5α-subunit and the Na,β1/Na,β1b β-subunits, i.e., we did not address nonconductive function of Na,β1b. These cells will not have the same intracellular regulatory properties as cardiac myocytes and a number of parameters such as phosphorylation, and surface targeting/stabilization may therefore be different. Furthermore, in computer simulations, we have programmed the model according to the patch-clamp data we obtained for Na,β1b and Na,β1b/H162P (heterozygous in model), respectively, and thereby have not included the impact of Na,β1 on the Na,1.5 current.

**Conclusion.** In the present study, we find that SCN1Bβ is expressed at a higher level than SCN1B in the human heart. This observation, together with an increased Na,1.5 current when coexpressed with Na,β1b, compared with Na,β1, suggests that the Na,β1b alternatively spliced variant may play an important role in the heart. By characterization of the Na,β1b/H162P mutation found in a BrS patient and following incorporating the in vitro data in a ventricular cell model, we find that this mutation profoundly reduces the action potential amplitude and conduction velocity. These changes are considered to be proarrhythmic, and the Na,β1b/H162P mutation is thereby found to be a mutation increasing the risk of ventricular arrhythmia.
Fig. 5. Effects of Na,β1, Na,β1b WT and H162P mutant on I_{Na} and action potential (AP) characteristics in silico. A: steady-state activation and inactivation as a function of voltage. B: I–V relationship. C: action potentials at basic cycle lengths (BCL) = 1,000 ms for all 3 isoforms. D: zoomed presentation of the upstroke of APs for homozygous Na,β1b (healthy, black trace), homozygous Na,β1 (beta1, pink), heterozygous Na,β1b + H162P (het, blue), and homozygous H162P (homo, red). E–J: peak I_{Na} and AP amplitude gradually decrease with shorter BCLs; an effect that is much more pronounced for Na,β1 (G and H) and in the heterozygous mutant (I and J) compared with the WT Na,β1b isoform (E and F).
REFERENCES