A molecular basis for gating mode transitions in human skeletal muscle Na⁺ channels

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Recombinant sodium channel α subunits expressed in Xenopus oocytes display an anomalously slow rate of inactivation that arises from channels that predominantly exist in a slow gating mode [1,2]. Co-expression of Na⁺ channel β subunit with the human skeletal muscle Na⁺ channel α subunit increases the Na⁺ current and induces normal gating behavior in Xenopus oceans. The effects of the β subunit can be explained by an allosterically induced conformational switch of the α subunit protein that occurs upon binding the β subunit. This binding alters the free energy barriers separating distinct conformational states of the channel. The results illustrate a fundamental modulation of ion channel gating at the molecular level, and specifically demonstrate the importance of the β subunit for gating mode changes of Na⁺ channels.

1. INTRODUCTION

Co-expression of brain or muscle recombinant Na⁺ channel α subunit with low molecular weight mRNA in Xenopus oocytes induces a fast (normal) gating mode and increases the macroscopic Na⁺ current without changing the single channel conductance [1,2]. It has been speculated that a modulatory factor encoded in the low molecular weight mRNA may be an enzyme or an accessory peptide such as a β subunit [3]. A recent report indicated that a recombinant β subunit from rat brain increased Na⁺ current and induced fast inactivation of rat brain II Na⁺ channel α subunits expressed in oocytes [4]. The observed changes in rat brain sodium current upon co-expression with a β subunit may have arisen from an increase in the number of expressed channels or possibly by a change in channel gating, however, the precise mechanism has not been determined. It is not known whether β subunits interact with other Na⁺ channel isoforms in general or specifically with the skeletal muscle Na⁺ channel. In order to explore the properties and gating mechanisms of human Na⁺ channels that may be relevant to human disease states, we have utilized the recently cloned human skeletal muscle Na⁺ channel [5] (hSkM1) α subunit and a recombinant β1 cDNA.

2. MATERIALS AND METHODS

2.1. PCR cloning of β₁ cDNA

A rat heart β₁ subunit cDNA was isolated using reverse transcrip-

2.2. Two-electrode voltage clamp

Defolliculated oocytes were injected with 40 nl (4-20 ng) of 5' capped cRNA and were电压 clamped 16-48 h after injection using standard two microelectrode voltage clamp techniques as previously described [6]. The ND-96 bath solution contained (mM): NaCl 96, KCl 1.8, CaCl₂, 1 MgCl₂, 5 HEPES (pH 7.50). Currents were filtered at 5 kHz (-3 dB) by a four-pole Bessel filter and sampled at 50 kHz. Electrodes were filled with 3 M KCl. Electrode resistances were 2-4 MΩ for voltage recording electrodes and 0.15-0.3 MΩ for current passing electrodes. Membrane capacitance was measured by integrating the current induced by small voltage jumps from -100 to -110 mV. Linear leak was subtracted by using a linear least squares regression to leak currents generated between 100 and 60 mV. Membrane capacitance transients were subtracted using a subthreshold scaled signal averaged tracing or a tracing in which channels had been inactivated.

2.3. Patch clamp recordings

Outside-out patches were obtained by standard methods [7]. Membrane currents were filtered at 5 kHz (3 dB) by a four-pole Bessel filter before sampling at 20 kHz. Capacitative and linear leak currents were subtracted by averaging the traces without activity. The patches were superfused with ND-96 solution. The patch electrodes contained (mM): NaCl 10, CsF 110, CsCl 20, MgCl₂ 2.0, EGTA 2 and HEPES 10; pH 7.35.
$V_{1/2}$ represents the half maximal voltage: $y(V) = (1 + e^{V - V_{1/2}})^{-1}$. The time courses of the falling phase of the macroscopic Na$^+$ current 'apparent inactivation', as well as onset and recovery from inactivation were fitted with exponential functions:

$y(t) = A_0 + \Sigma(B_j \exp(-t/\tau_j))$.

Simulations were carried out in FORTRAN on a microcomputer. In this model, a global change in rate constants occurred upon binding of the $\beta_1$ subunit such that the forward rate constants were increased and the backward rate constant from the open state was decreased by 5 fold. Each state is separated by an energy barrier the height of which depends exponentially upon thermodynamic factors such as temperature, electrodynamic and electrostatic interactions within the protein as well as the transmembrane electrical field:

$k_{ij} = \nu \cdot \exp(-\Delta G/kT)$

where $\Delta G_{ij}$ is the energy barrier height separating states i and j. $\Delta G$ contains all factors that contribute to the barrier height including both voltage-dependent and non-voltage-dependent terms. $\Delta G$ can be partitioned into a voltage-independent term, $\Delta G_{ini}$ that reflects the barrier height when the field strength is zero, and additional terms that account for the electric field effects. The global change in barrier heights upon binding of the $\beta_1$ subunit accounts for both the change in kinetics and the increase in current amplitude. The value of $\nu$ is approximately $6 \times 10^{12}$ s$^{-1}$ and it represents the attempt rate for crossing barriers: it is an upper limit for transition frequencies. At room temperature, $RT$ is 0.587 kcal/mol, thus the activation energy barrier height (well to peak) for a rate constant of 1,000 s$^{-1}$ is 13 kcal/mol. Data are presented as means and standard errors.

3. RESULTS AND DISCUSSION

The first important observation is illustrated in Fig. 1 which demonstrates that the $\alpha$ subunit alone spends a fraction of time in the normal fast gating mode. This was apparent from both the onset of inactivation (Fig. 1A) and its recovery (Fig. 1B). Fig. 1B shows that channels produced by the $\alpha$ subunit alone recover from inactivation very slowly ($38 \pm 1\%; \tau_{slow} = 1.2 \pm 0.32$ s, $n = 4$) although a fraction of the channels recover rapidly. However when the $\alpha$ subunit is co-expressed with the $\beta_1$ subunit, not only did the current inactivate faster (Fig. 1C), the channels also recover from inactivation rapidly (88% $\pm$ 1; $\tau = 6.5 \pm 0.9$ ms, $n = 4$). Injection of oocytes with $\beta_1$ alone had no effect which demonstrates that $\beta_1$ alone did not induce expression of an endogenous channel. Fig. 1D shows that $\beta_1$ increases the level of Na$^+$ current compared to the $\alpha$ subunit alone, but only small effects on the apparent voltage dependence of steady state inactivation were observed ($\alpha$ $V_{1/2}$: $-51 \pm 0.7$ mV, $n = 13$; $\alpha + \beta_1$, $V_{1/2}$: $-55 \pm 0.65$ mV, $n = 14$) [4].

To further assess the effects of the $\beta_1$ subunit on the magnitude of the expressed Na$^+$ current, oocytes ex-
expressing α alone or α + β1 were voltage clamped altern-atively within a 6 h period. The normalized current
density (at -20 mV) was significantly (P < 0.01) in-
dcreased during co-expression of the α and β1 subunits:
α subunit 21.1 ± 1.3 μA/μF, n = 14, α + β1 35.4 ± 2.3
μA/μF, n = 13. In additional experiments using differ-
ent batches (2) of oocytes and different membrane po-
tentials, the normalized membrane conductance was in-
creased from 0.58 ± 0.12 mS/μF to 1.3 ± 0.26 mS/μF
(n = 12, P < 0.05). We could not account for these in-
creases in macroscopic Na+ current by an increase in the
single channel conductance. Excised patch clamp exper-
iments were performed on oocytes expressing the α + β1,
subunits in order to determine the single-channel con-
ductance. Fig. 2A shows an ensemble averaged current
recorded from an outside-out membrane patch. These
channels exhibited fast gating behavior and the single
channel conductance was 21 pS (Fig. 2B) which is iden-
tical to the single-channel conductance of the α subunit
alone [2,8].

Our results demonstrate that human skeletal muscle
sodium channels do not have an absolute requirement
for the β1 subunit in order to exhibit normal gating
behavior (Fig. 1A). Our data show that co-expression of
the β1 and the α subunit produce the same effects as
the unknown factor in the low molecular weight mRNA
[2]. When the β1 subunit is co-expressed with the α
subunit, the equilibrium between gating modes appears
shifted such that the faster gating mode dominates.
These two types of macroscopic inactivation arise from
gating mode shifts and not from multiple populations
of ion channels being expressed since they are seen in
patches with a single channel [2].

We considered whether the observed changes in gat-
ing kinetics could also account for the increase in peak
Na+ current. Many voltage-gated ion channels from
excitable membranes display heterogeneous patterns of
behavior referred to as gating modes [9,10]. Gating
mode changes play an important role to modify cellular
electrical activity in both normal and diseased states of
excitable cells [10–13,17,18]. Brain, cardiac and skeletal
muscle sodium channels display at least two distinct
gating modes [1,10,12,13,16,18] but the fast gating
mode is dominant in situ. Simulations using Eyring the-
ory energy barrier models for the gating transition rate
constants indicated that the effects of β1 on both kinetics
and current amplitude could be explained entirely by a
change in the probability of channel opening upon bind-
ing of the β1 subunit. In the Eyring reaction rate theory,
the gating transition rate constants describe the energy
barrier heights for a transition from one kinetic state to
another. Lowering these barrier heights results in a
faster transition. Since gating of the channel involves a
rearrangement of atoms in the protein, the structure(s)
responsible must achieve sufficient energy from thermal
motion or other sources to traverse the energy barrier
that separates different states. We estimated the height
of these barriers from reaction rate theory by:
\[ \Delta G = -RT \cdot \ln(k_u/k_i) \]

where \( u \) is a rate constant, \( k \) is Boltzmann’s constant, \( T \) is temperature, \( \kappa \) is a transmission coefficient (assumed to be 1) and \( h \) is Planck’s constant. We assume that binding of the β1
subunit induces a global effect on the structure of the
α subunit. This is supported by experiments where neu-
rotoxin binding and action at three distinct sites on the
channel are modified by β1 association [3]. During chan-
nel activation, if we lower the barrier heights for all
forward transition rates by 1.6 kT and increase the tran-
sition barrier height for the rate constant leaving the
open state by 1.6 kT (~ 0.93 kcal/mol) when the β1
subunit binds, we can account for changes in gating and
current magnitude (Fig. 3). These profound changes in
channel behavior upon binding the β1 subunit suggest that relatively small energetic changes are required to extensively modulate gating. The energetic requirement for the changes are less than many reactions that occur readily at room temperature: e.g., carbon-carbon bond rotation (3 kcal/mol), hydrogen bonds (5 kcal/mol) or protonation reactions (2–13 kcal/mol). This result also explains the ability of the α subunit alone to exhibit the fast gating mode albeit less frequently than when the β1 subunit is present.

Changes in Na⁺ channel gating may underlay the aberrant sodium channel behavior seen in human disease states [11] and Na⁺ channel mutations are known to occur in patients with these disorders [14,19]. Our results suggest that an additional focus on subunit interactions must also be considered while exploring the mechanisms of ion channel based diseases.

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