The Extracellular Domain of the β1 Subunit Is Both Necessary and Sufficient for β1-like Modulation of Sodium Channel Gating

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The type IIA voltage-gated sodium Na⁺ channel from rat brain is composed of a large, pore-forming α subunit and the auxiliary subunits β1 and β2. When expressed in Xenopus oocytes, the β1 subunit modulates the gating properties of the type IIA α subunit, resulting in acceleration of both inactivation and recovery from inactivation and in a negative shift in the voltage dependence of fast inactivation. The β1 subunit is composed of an extracellular domain with a single immunoglobulin-like fold, a single transmembrane segment, and a small intracellular domain. A series of chimeras with exchanges of domains between the Na⁺ channel β1 and β2 subunits and between β1 and the structurally related protein myelin P0 were constructed and analyzed by two-microelectrode voltage clamp in Xenopus oocytes. Only chimeras containing the β1 extracellular domain were capable of β1-like modulation of Na⁺ channel gating. Neither the transmembrane segment nor the intracellular domain was required for modulation, although mutation of Glu158 within the transmembrane domain altered the voltage dependence of steady-state inactivation. A truncated β1 subunit was engineered in which the β1 extracellular domain was fused to a recognition sequence for attachment of a glycosylphosphatidylinositol membrane anchor. The β1ec-glycosylphosphatidylinositol protein fully reproduced modulation of Na⁺ channel inactivation and recovery from inactivation by wild-type β1. Our findings demonstrate that extracellular domain of the β1 subunit is both necessary and sufficient for the modulation of Na⁺ channel gating.

EXPERIMENTAL PROCEDURES

Plasmid Construction—cDNAs encoding the rat brain β1 subunit (5), the rat brain β2 subunit (6), and rat myelin P0 (15) were subcloned into plasmid pSP64T for analysis of Na⁺ channels by expression in Xenopus oocytes. Plasmid pSP64T-β1 was modified such that extraneous restriction sites were removed from the polylinker, a silent ClaI site was introduced into the β1 cDNA at a position 14 nucleotides 5' from the proposed junction of the β1 extracellular (β1ec) and transmembrane (β1tm) domains (pSP64T-β1ClaI), and a silent NotI site was introduced at a position 12 nucleotides 3' of the junction between the cDNA encoding the β1tm and intracellular (β1ic) domains, to form plasmid pSP64T-β1ClaINotI.

Construction of β1-β2 and β1-P0 Chimeras—In general, chimeras in which entire domains were exchanged either between the β1 and β2 subunits or between β1 and P0 were constructed by polymerase chain reaction (PCR), amplification of cDNA encoding the replacement do-

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main, followed by subcloning into the appropriate pSP64T-based receiving plasmid. The 5’ forward and 3’ reverse PCR primers were designed with “tails” that contained small segments of nucleotides derived from the receiving plasmid that flank the region being replaced, resulting in in-frame fusions between replacement cDNAs and the cDNA of the recipient vector. Restriction endonucleases fragment the engineered silent sites, which were then incorporated into the PCR primers for use in the subsequent subcloning step.

Chimera β1|β2 was constructed by replacing the cDNA encoding β1m in plasmid pSP64T-β1m, with cDNA encoding the β2m domain. The 5’ forward and 3’ reverse primer tails contained flanking β1m sequences, with introns retained in the new construct. The 3’ reverse primer fragment was then subcloned into HpaI/KpnI-digested pSP64T, to form plasmid pSP64T-β1|β2m.

To construct chimera β1|β2m, β2m-w was amplified by PCR. The 5’ primer tail contained a silent ClaI site at a position analogous to that found in pSP64T-β1|ClaI, fused in frame to nucleotides encoding the β2m domain. The 3’ reverse primer was as described for chimera β1|β2m. The β2m PCR product was then subcloned into ClaI/KpnI-digested pSP64T-β1m to form plasmid pSP64T-β1|β2m.

Chimera β1P0|β1 was constructed by replacing the cDNA encoding β1m with that of P0m. The P0m PCR product contained 5’ and 3’-flanking sequences derived from the β1m domain with the silent ClaI site and β1m with a silent NolI site, respectively. The PCR product was digested with ClaI and NolI and then ligated to ClaI/NolI-digested pSP64T-β1m to form plasmid pSP64T-β1P0m.

To construct chimera P0β1|β1, P0m cDNA was PCR amplified using a 5’ PCR primer containing a 5’ NolI site, which mimicked an intrinsic NolI site located at the start codon of β1m. Nucleotides encoding the β1m domain, including a NolI site intrinsic to β1m, were added in frame to the 3’ reverse primer used to make the P0m PCR product. The P0m PCR product was then subcloned into NolI/NdeI-digested pSP64T-β1m to form plasmid pSP64T-P0β1m.

Chimera P0β1|0P0 was made by replacing the cDNA encoding β1m in pSP64T-P0β1m with cDNA encoding the P0m. The 5’ end of the P0m PCR product contained cDNA encoding a portion of the β1m domain, including an intrinsic NolI site, fused in frame to the P0m cDNA. A KpnI site was incorporated at the 3’ end of the P0m PCR product. The P0m PCR product was then subcloned into NdeI/KpnI-digested pSP64T-P0β1m.

To facilitate the construction of chimera P0β1|0P0, myelin P0 cDNA was subcloned into plasmid pSP64T in a manner such that all extraneous AavI sites were deleted. In this subcloning, a single AavI located in the P0m domain was retained, and a ClaI site 5’ to the P0m cDNA was carried over from the pSKK polyclinker. The β1m domain was then amplified with a ClaI site added 5’ to the cDNA, and the PCR product was fused in frame to a portion of the P0m cDNA with the intrinsic AavI site. The β1m PCR product was then subcloned into ClaI/NdeI-digested pSP64T-P0β1m.

To facilitate the construction of chimera β1P0β1, P0m cDNA was PCR amplified using a 5’ PCR primer containing a 5’ NolI site, which mimicked an intrinsic NolI site located at the start codon of β1m. Nucleotides encoding the β1m domain, including a NolI site intrinsic to β1m, were added in frame to the 3’ reverse primer used to make the P0m PCR product. The P0m PCR product was then subcloned into NolI/NdeI-digested pSP64T-β1m to form plasmid pSP64T-P0β1m.

Chimera β1P0|β1 was constructed by replacing the cDNA encoding β1m with that of P0m. The P0m PCR product contained 5’ and 3’-flanking sequences derived from the β1m domain with the silent ClaI site and β1m with a silent NolI site, respectively. The PCR product was digested with ClaI and NolI and then ligated to ClaI/NolI-digested pSP64T-β1m to form plasmid pSP64T-β1P0m.

To construct chimera P0β1|β1, P0m cDNA was PCR amplified using a 5’ PCR primer containing a 5’ NolI site, which mimicked an intrinsic NolI site located at the start codon of β1m. Nucleotides encoding the β1m domain, including a NolI site intrinsic to β1m, were added in frame to the 3’ reverse primer used to make the P0m PCR product. The P0m PCR product was then subcloned into NolI/NdeI-digested pSP64T-β1m to form plasmid pSP64T-P0β1m.
channels with rapid recovery from inactivation observed at the 1:30 ratio may be due to formation of homo-oligomers of P0 associating with the channel (22). Neither of these effects resembles the effect of the β1 subunit on Na⁺ channel function.

Because of the structural similarity and functional difference between β1 and myelin P0, we constructed chimeras that exchanged domains between these two proteins and analyzed their functional effects on α subunits. The functional characteristics of chimera P0β1P0 (Fig. 3A) closely resembled those of P0. The kinetics of inactivation and the voltage dependence of inactivation were similar to α subunits alone (Fig. 3B, traces a and c; Fig. 3C). The recovery from inactivation of α11α + P0β1P0 channels was accelerated slightly compared with that of α11α alone because of an increase in the fraction of current recovering with the fast time constant (Fig. 3D and Table I).

Co-expression of the inverse chimera, β1P0β1 (Fig. 3A), yielded fast inactivating Na⁺ channels that were similar to those observed in oocytes injected with α11α + wild-type β1 transcripts (Fig. 3B, compare traces b and d). The voltage dependence of steady-state fast inactivation was shifted to more negative potentials, like that of channels containing the wild-type β1 subunit (Fig. 3C). Recovery from inactivation was fast, with no apparent slow component (Fig. 3D and Table I).

The most obvious difference between channels containing the wild-type β1 and those containing the β1P0β1 chimera was the rate at which the channels were expressed. Channels composed of α11α and β1P0β1 subunits expressed more slowly than their wild-type counterparts, requiring 3–4 days post-injection to reach peak currents comparable with those of α11α + wild-type β1 channels (data not shown). The results of these studies demonstrate that although cell surface expression is delayed, the β1P0β1 chimera associates with the pore-forming α11α subunits.
**Na⁺ Channel Modulation by the β1 Extracellular Domain**

Parameters for voltage dependence of fast inactivation and recovery from inactivation

Oocytes were injected with an α₁IA:auxiliary subunit ratio of 1:10 (w/w), unless otherwise indicated. Electrophysiological protocols were as described under “Experimental Procedures.” Values for means and standard errors are given.

| RNA Transcripts Injected | Voltage dependence of fast inactivation | Recovery from inactivation |
|--------------------------|----------------------------------------|----------------------------|
|                          | n            | V₁/₂          | F₁/₂     | t₁/₂     | F_slow | t_slow |
| α₁IA alone              | 8            | -38.5 ± 0.2  | 0.55 ± 0.02 | 3.17 ± 0.17 | 0.43 ± 0.01 | 217 ± 12 |
| α₁IA + β1               | 6            | -45.6 ± 0.2  | 1.00 ± 0.05 | 2.65 ± 0.15 | NA      | NA      |
| α₁IA + P0               | 5            | -39.9 ± 0.3  | 5 (1:10) | 0.55 ± 0.02 | 4.86 ± 0.21 | 0.41 ± 0.01 | 244 ± 17 |
| α₁IA + P0β1P0           | 4            | -40.0 ± 0.1  | 3 (1:30) | 0.78 ± 0.00 | 4.06 ± 0.03 | 0.20 ± 0.00 | 203 ± 16 |
| α₁IA + β1P0β1           | 5            | -48.7 ± 0.7  | 4       | 0.78 ± 0.01 | 2.83 ± 0.10 | 0.28 ± 0.00 | 220 ± 40 |
| α₁IA + P0β1P0β1         | 5            | -35.1 ± 0.2  | 4       | 1.00 ± 0.04 | 3.73 ± 0.19 | NA      | NA      |
| α₁IA + β1P0β1P0         | 3            | -49.1 ± 0.3  | 4       | 1.00 ± 0.09 | 2.69 ± 0.28 | NA      | NA      |
| α₁IA + β1E158K          | 3            | -49.9 ± 0.3  | 3       | 1.00 ± 0.04 | 3.73 ± 0.17 | NA      | NA      |
| α₁IA + β1E158Q          | 3            | -49.9 ± 0.3  | 3       | 1.00 ± 0.03 | 3.70 ± 0.15 | NA      | NA      |
| α₁IA + β1E158G1         | 7 (1:20)     | -48.0 ± 0.2  | 7 (1:20) | 1.00 ± 0.06 | 2.56 ± 0.18 | NA      | NA      |

*NA, not applicable.

Fig. 3. Electrophysiological properties of Na⁺ channels containing chimeras of β1 and myelin P0 with exchanged transmembrane domains. A, schematic representations of the domain structures of the mature Na⁺ channel β1 subunit, myelin P0, and chimeras P0β1P0 and β1P0β1 are given. The loop in the extracellular domains represents the Ig-like motif. β1-derived portions of the chimeras are gray, whereas the P0-derived portions are stippled. The amino acid sequences of the chimera junctions are provided in single-letter codes. P0-derived segments are underlined. B, Xenopus oocytes were injected with in vitro transcribed RNA encoding the Na⁺ channel α₁IA subunit alone or in combination with RNA encoding the β1 subunit, myelin P0 or the β1-P0 tm chimeras depicted in panel A. Na⁺ currents were recorded as described in the legend to Fig. 1B. Traces a (α₁IA alone) and b (α₁IA + β1) are as described in the legend to Fig. 1B. Normalized representative traces are given for α₁IA + chimera P0β1P0 (trace c), and α₁IA + chimera β1P0β1 (trace d). C, voltage dependence of steady-state fast inactivation for channels containing β1-P0 chimeras, relative to that for control channels. Steady-state fast inactivation of Na⁺ channels expressed in Xenopus oocytes was measured as described under “Experimental Procedures.” Averaged normalized inactivation curves α₁IA alone (filled circles; n = 8), α₁IA + β1 (filled square; n = 6), α₁IA + P0β1P0 (open triangle; n = 4), and α₁IA + β1P0β1 (open inverted triangle; n = 5) are shown. Fits and error bars are as described in the legend to Fig. 1B. D, normalized, averaged recovery from inactivation profiles for channels containing β1-P0 transmembrane chimeras and control channels. Recovery from inactivation was examined as described under “Experimental Procedures.” Symbols are as defined for C. Solid lines represent curve fits of the averaged recovery values. Recovery curves for α₁IA + β1 and α₁IA + β1P0β1 channels were well fit with one exponential, whereas all remaining curves were fit with the sum of two exponentials. The derived recovery fractions, time constants, and number of cells examined for each condition are given in Table I.

Unit and modulates channel function normally, supporting the conclusion that the transmembrane segment of the β1 subunit is not required for modulation of channel gating.

Effects of β1K158 in the Transmembrane Segment on the Voltage Dependence of Steady-State Inactivation—Chimera β1P0β1, when co-expressed with the α₁IA subunit, caused a negative shift in the voltage dependence of fast inactivation of approximately 10.2 mV, significantly greater than the 7.1 mV shift observed for α₁IA + wild-type β1 (Table I). Comparison of the proposed transmembrane segments of the Na⁺ channel β1 subunit and myelin P0 shows that the β1 transmembrane domain contains a negatively charged Glu at position 158 of β1, whereas the corresponding residue in the P0 membrane-spanning segment is hydrophobic (Fig. 4A, underlined letter). We
examined the influence of β1E158 on the voltage dependence of steady-state fast inactivation by neutralizing the negative charge by conversion to Gln (β1E158Q) and by replacing the negative Glu with a positively charged Lys (β1E158K). As shown in Fig. 4B, the replacement of β1E158 with a Gln or Lys had no detectable effect on the kinetics of inactivation. Recovery of channels from inactivation was only slightly slowed as a result of the β1E158Q and β1E158K mutations (Fig. 4C and Table I). However, both of the mutant β1 subunits conferred an enhanced negative shift in the voltage dependence of steady-state inactivation, compared with the shift conferred by the wild-type β1 subunit (Fig. 4D and Table I). Evidently, neutralization or replacement of the negatively charged β1E158 mimics the effects of the replacement of the β1 transmembrane domain with that of myelin P0.

Secondary Structure of Myelin Protein P0 and the β1 Subunit—To further examine the role of the β1 extracellular domain, chimera β1P0P0 was constructed in which only the extracellular domain was derived from β1 (Fig. 5A). Currents expressed from cells co-injected with transcripts encoding the α1A subunit and β1P0P0 were smaller on average than those obtained from cells injected with only the α1A transcript, reaching 0.75–1.0 μA 5 days post-injection compared with 1.5–8.0 μA for α1A. Although the peak currents were small, channels from α1A + β1P0P0-expressing oocytes were fast inactivating and resembled channels containing the wild-type β1 subunit in all parameters tested (Fig. 5, B–D, and Table I). As observed for the β1P0β1 chimera and β1E158K mutations, the voltage dependence of steady-state inactivation was shifted to more negative potentials than for wild-type β1 (Fig. 5C and Table I). Recovery from inactivation was fast for these channels, indicating that the β1P0P0 chimera associated well with the α1A subunit and fully modulated its gating (Fig. 5D). In contrast, chimera P0β1β1 (Fig. 5A), in which the extracellular domain of β1 was replaced with P0, did not significantly influence the kinetics of Na⁺ channel gating (Fig. 5B). In addition, the voltage dependence of inactivation for α1A+β0P0β1 channels was shifted to more positive potentials than that of α1A alone, opposite to the negative shift conferred by the wild-type β1 subunit (Fig. 5C and Table I). Recovery from inactivation for channels expressed in α1A+β0P0β1-injected oocytes resembled that observed for α1A alone (Fig. 5D and Table I). However, recovery from inactivation was significantly accelerated when the P0β1β1 chimera RNA was injected at 40-fold weight excess relative to α1A transcript (Fig. 5D and Table I). Aside from this small effect on recovery at high expression levels, the data from β1-P0 chimeras support the conclusion that the functional effects of β1 are conferred entirely by its extracellular domain.

The expression of the predicted transmembrane segments from the Na⁺ channel β1 subunit and myelin protein P0. The amino acid sequences are given in single-letter codes and are shown with the putative membrane stop transfer positions aligned. The Glu at position 158 of the β1 subunit is underlined. B. Xenopus oocytes were injected with in vitro transcribed RNA encoding the Na⁺ channel α1A subunit alone or in combination with RNA encoding the wild-type β1 subunit, β1E158Q or β1E158K. Na⁺ currents were measured as described in the legend to Fig. 1B. Traces a (α1A, alone) and b (α1A + β1) are as described in the legend to Fig. 1B. Normalized representative traces are given for a α1A + β1E158Q (trace c) and a α1A + β1E158Q (trace d). C. Normalized, averaged recovery from inactivation profiles for channels containing mutant β1 subunits and control channels. Recovery from inactivation was measured as described under "Experimental Procedures." Filled circle, α1A; open circle, α1A + β1; open triangle, α1A + β1E158Q; open inverted triangle, α1A + β1E158K. Solid lines represent curve fits of the averaged recovery values. The recovery curves for a α1A + β1, α1A + β1E158Q and a α1A + β1E158K channels were well fit with one exponential, whereas the α1A curve was fit with the sum of two exponentials. The derived recovery fractions, time constants, and number of cells examined for each condition are given in Table I. D, the voltage dependence of steady-state fast inactivation is plotted for channels containing mutant β1 subunits, relative to that of control channels. Symbols are as defined for C. The voltage dependence of steady-state fast inactivation of Na⁺ channels was measured as described under "Experimental Procedures." The inactivation curves shown are normalized averages. Fits and error bars are as described in the legend to Fig. 2B.
sequence from HPAP (17, 18) (Fig. 6A). GPI-anchored fusion proteins have been successfully used to study a variety of cell surface proteins (18, 23, 24). In GPI-anchored proteins, the recognition site for GPI attachment is large, usually over 30 amino acids. The GPI moity is attached to an Asp residue within the recognition sequence, and the following 30 C-terminal amino acids. The GPI moiety is attached to an Asp residue for GPI attachment is large, usually over 30 amino acids. GPI-anchored fusion proteins have been successfully used to study a variety of cell surface proteins (18, 23, 24). In GPI-anchored proteins, the recognition site for GPI attachment is large, usually over 30 amino acids. The GPI moiety is attached to an Asp residue within the recognition sequence, and the following 30 C-terminal amino acids. GPI-anchored fusion proteins have been successfully used to study a variety of cell surface proteins (18, 23, 24).

DISCUSSION

Previous work has highlighted the importance of the β1 extracellular domain in modulation of Na⁺ channel function (8, 11, 12) and led to the working hypothesis that the Ig fold motif in the β1 extracellular domain serves as a scaffold to present molecular determinants of β1 for interaction with the α subunit. This interaction causes an increase in the fraction of channels that gate in a fast mode (8). Negatively charged residues predicted to lie in the A/A’ β strand on one edge of the Ig fold were shown to be important for modulation of gating mode (8). The extracellular loop IVS2-S6 in the α subunit has also been identified as a point of attachment of the β1 subunit (12, 27). In contrast with these experiments, studies in which the transmembrane domain of the β1 subunit was expressed as

FIG. 5. Electrophysiological properties of Na⁺ channels containing chimeras of β1 and myelin P0 with exchanged extracellular domains. A, schematic representations of the domain structures of the Na⁺ channel β1 subunit, myelin P0, and chimeras P0β1β1 and β1P0P0. Components of the schematics are as described in the legend to Fig. 3A. Letters in parentheses following the construct name refer to electrophysiological traces shown in B. Note that extracellular domain of β1 (141 amino acids) is larger than that of P0 (123 amino acids). B, Xenopus oocytes were injected with in vitro transcribed RNA encoding the Na⁺ channel α1A subunit alone or in combination with RNA encoding the wild-type β1 subunit, chimera P0β1β1, or chimera β1P0P0. Na⁺ currents were measured as described in the legend to Fig. 1B. Traces a (α1A alone) and b (α1A + β1) are as described in the legend to Fig. 1B. Normalized representative traces are given for α1A + P0β1β1 (trace c) and α1A + β1P0P0 (trace d). C, normalized, averaged steady-state fast inactivation measured as described under “Experimental Procedures.” Filled circle, α1A alone (n = 8); filled square, α1A + β1 (n = 6); open triangle, α1A + P0β1β1 (n = 5); open inverted triangle, α1A + β1P0P0 (n = 5). Fits and error bars are as described in the legend to Fig. 2B. D, normalized, averaged recovery from inactivation time courses are given for channels containing β1-P0 extracellular chimeras and control channels. Recovery from inactivation was examined as described under “Experimental Procedures.” Symbols are defined as in panel C. Solid lines represent curve fits of the averaged recovery values. Recovery curves for α1A + β1 and α1A + β1P0P0 channels were well fit with one exponential, whereas all remaining curves were fit with the sum of two exponentials. The derived recovery fractions, time constants, and number of cells examined for each condition are given in Table I.
a separate protein suggested that the transmembrane domain could modulate sodium channel gating by itself (13). Moreover, human heart β subunits containing a mutation in the intracellular C-terminal tail are linked to long QT syndrome and are functionally abnormal only when co-expressed with the β1 subunit (14). In the experiments described here, we have further examined the possibility that the modulation of Na⁺ channel gating conferred by the β1 subunit is indeed due to extracellular interactions using chimeric and lipid-anchored β1 subunits.

The Transmembrane Domain of the β1 Subunit Is Not Sufficient for Na⁺ Channel Modulation—If the transmembrane domain were necessary for β1 function, one would expect to observe loss of function of the β1P0/β1 chimera and for β1ec-GPI. Alternatively, if the transmembrane domain were sufficient for β1 function, one would expect to observe β1-like function for the P0/β1P0 chimera. Our results show the opposite. The β1P0/β1 chimera and β1ec-GPI conferred wild-type levels of β1 modulation of Na⁺ channel gating, whereas the P0/β1P0 chimera lacked β1 function. The expression of channels containing the β1P0/β1 chimera was delayed, probably because of problems in folding problems caused by the longer, P0-derived transmembrane segment. We did observe a small but significant acceleration of recovery from inactivation in oocytes expressing the α3EC subunit in combination with high levels of either the P0β1P0 or P0β1β1 chimeras. In general, the Na⁺ currents mediated by these chimeras in oocytes having fast recovery kinetics were very small (<1 μA; data not shown).

Because both of these chimeras contain the P0 extracellular domain, it is likely that residues within the P0 Ig fold motif can accelerate the recovery process but with much lower efficacy than the wild-type β1 subunit. This idea is plausible, because there are several short regions of sequence identity within the extracellular domains of β1 and P0 (8). The results of our experiments with chimeras demonstrate that the transmembrane domain of the β1 subunit cannot support modulation of sodium channel gating and therefore that its role in interactions with and modulation of the α subunit is secondary to that of the extracellular domain. This conclusion is further supported by our results showing that β1ec-GPI is fully active in channel modulation.

Removal of a Negatively Charged Residue in the β1 Transmembrane Domain Causes a Larger Hyperpolarizing Shift in the Voltage Dependence of Steady-state Inactivation—We observed that the replacement of the β1 transmembrane segment with that of myelin protein P0, as well as the complete removal of the transmembrane domain, resulted in a more pronounced negative shift in the voltage dependence of steady-state fast inactivation than that measured for channels containing the wild-type β1 subunit. These effects could be mimicked by neutralizing or charge replacement mutations at position β1E158 located within the transmembrane segment. Therefore, although it is unusual for a charged residue to be found in a transmembrane segment, β1E158 does appear to play a significant role in determining the voltage dependence of inactivation. This suggests that β1E158 is situated where it can alter the
electric field sensed by the gating charges that control inactivation or can interact with them directly or indirectly through the protein structure.

The Extracellular Domain of the Na+ Channel β1 Subunit Is Sufficient for Modulation of Gating Mode—We found that chimera β1P0, in which the only extracellular domain is derived from β1, modulates channel function in a manner indistinguishable from that of wild-type β1. This agrees with previous experiments with skeletal muscle Na+ channels and a β1-β2 chimera that contained the extracellular domain plus six adjacent transmembrane amino acid residues derived from β1 (12). Our data narrow the region required for β1 function in β1-β2 chimeras to residues in the extracellular domain. In addition, the β1 extracellular domain is functional when spliced to the transmembrane and intracellular domains of myelin protein P0. Chimera β1P0P0 yielded channels with fast inactivation, negatively shifted voltage dependence of steady-state inactivation, and rapid recovery from inactivation. On the other hand, channels containing chimera P0β1P0 were slow to inactivate, showed no negative shift in the voltage dependence of inactivation, and had slow recovery from inactivation. Taken together, the results of the β1-β2 and β1-P0 chimeras strongly implicate the β1 extracellular domain as the region involved in the modulation of Na+ channel gating.

In further support of the conclusion that only the extracellular domain of the β1 subunit is necessary, we found that the GPI-anchored β1 extracellular domain is able to mimic the effects of the intact β1 subunit for all parameters tested. This is a clear demonstration that the action of the β1 subunit on channel modulation is primarily or exclusively via extracellular contacts. Our data from experiments assessing recovery from fast inactivation show that the β1ec-GPI protein fully shifts the δIIA subunit to the fast gating mode. Moreover, the association of β1ec with the α subunit is stable for several days, consistent with stable, high affinity interaction like the native β1 subunit.

In addition to causing changes in the gating kinetics of the Na+ channel, the β1 subunit has also been shown to enhance Na+ channel expression in both Xenopus oocytes and mammalian cells (6, 28). It would be interesting to determine which domain of the β1 subunit is responsible for this increase in channel cell surface expression. Chimeras between the β1 subunit and myelin P0 proved difficult to analyze in this respect. In general, the chimeras between β1 and P0 expressed poorly, yielding Na+ currents that were smaller on average than those observed for channels composed of only the δIIA subunit. This dilemma was circumvented by the successful expression of Na+ channels containing the GPI-anchored β1 extracellular domain. These channels expressed well, with current amplitudes comparable with those observed for α with wild-type β1 subunits. The data suggest that the β1 extracellular domain is sufficient for increasing channel cell surface expression as well as modulation of gating.

Mechanism of Modulation of Gating by β1 Subunits—It is surprising that the extracellular domain of β1, independent of the transmembrane and intracellular segments, can alter Na+ channel gating. Activation of Na+ channels involves outward movement of the S4 transmembrane segments, which serve as voltage sensors (29, 30). Fast inactivation is largely an intracellular event involving closure of an inactivation gate formed by the intracellular loop between domains III and IV (31–34). Because the β1 subunit is unlikely to interact directly with either of these gating structures, it seems reasonable to propose that the β1 extracellular domain influences or evokes a conformational change in the α subunit and thereby affects channel gating indirectly through the protein structure. Polypeptide toxins from scorpions and sea anemones are also able to modulate Na+ channel gating by interaction with a site on the extracellular face of the channel, and, in that case, direct interaction of the bound toxins with the S3-S4 loops at the extracellular end of the S4 gating segments is proposed to mediate the actions of the toxins via a voltage-sensor trapping model (35, 36). Likewise, small mutations in the S3-S4 loops of potassium channels have profound effects on voltage-dependent gating (37). The β1 subunit may also influence the movement of one or more S4 gating segments, perhaps by interacting with the S3-S4 loop of one or more homologous domains and, in that way, alter the kinetics and voltage dependence of channel gating.

Significance of the Extracellular Domain of the β1 Subunit in Vivo—It was recently discovered that a mutation of a Cys residue predicted to be disulfide-linked in the Ig fold in the extracellular domain of β1 is responsible for an inherited form of febrile seizure (38). This finding demonstrates the physiological importance of the Na+ channel β1 subunit and again emphasizes the role of the Ig fold in the β1 extracellular domain in regulation of Na+ channel function. Further molecular and structural analysis may reveal both the mechanism of action of the β1 subunits and the molecular basis for this inherited seizure syndrome.

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