Sodium Channel β1 Subunits Promote Neurite Outgrowth in Cerebellar Granule Neurons*

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Many immunoglobulin superfamily members are integral in development through regulation of processes such as growth cone guidance, cell migration, and neurite outgrowth. We demonstrate that homophilic interactions between voltage-gated sodium channel β1 subunits promote neurite extension in cerebellar granule neurons. Neurons isolated from wild-type or β1(−/−) mice were plated on top of parental, mock-, or β1-transfected fibroblasts. Wild-type neurons consistently showed increased neurite length when grown on β1-transfected monolayers, whereas β1(−/−) neurons showed no increase compared with control conditions. β1-Mediated neurite extension was mimicked using a soluble β1 extracellular domain and was blocked by antibodies directed against the β1 extracellular domain. Immunohistochemical analysis suggests that the β1 and β4 subunits, but not β2 and β3, are expressed in cerebellar Bergmann glia as well as granule neurons. These results suggest a novel role for β1 during neuronal development and are the first demonstration of a functional role for sodium channel β subunit-mediated cell adhesive interactions.

Intercellular communications mediate critical developmental events in neurons. Interactions between integrins, cadherins, and immunoglobulin superfamily cell adhesion molecules (IGSF CAMs) on opposing cells result in events such as growth cone guidance and neurite extension. For example, NCAM- and L1-CAM-mediated cell adhesive interactions result in signal transduction pathways involving kinase activation, modulation of local, submembrane calcium concentrations, gene transcription, and ultimately, neurite extension (1–4). Some IGSF CAMs such as myelin-associated glycoprotein and contactin balance the growth promoting activity of other molecules through inhibition of neuritogenesis (5, 6). Thus, it is the concerted effort of growth-promoting and growth-inhibitory molecules on neuronal and non-neuronal cells that act to influence the developing nervous system.

Postnatal cerebellar development involves migration of cerebellar granule neurons from the external germinial layer to the rapidly developing granule cell layer. During migration, a granule neuron develops several neurites, two of which ultimately become parallel fibers of the cerebellar molecular layer (7). Whereas most cell migration and neuritogenesis in the cerebellum is complete within the second postnatal week, migration of granule neurons, growth of the granule cell layer, and extension of parallel and vertical fibers continues through postnatal day 21 (P21) (7, 8).

Voltage-gated sodium channels are composed of a central, pore forming α-subunit and one or two β subunits (9). Whereas α alone is sufficient to form the ion-conducting pore, current density, channel kinetics, gating mode, and channel cell surface density are influenced by β subunit expression (9, 10). There are five known β subunits: β1, β1A, β2, β3, and β4. β1, β1A, and β3 are non-covalently linked to the pore-forming α subunit, while β2 and β4 are disulfide linked to α. Based on structural and amino acid homologies, β subunits are IGSF CAMs (11). β1 and β2 exhibit homophilic and heterophilic adhesion and interact with extracellular matrix molecules (12–16). β1- and β2-mediated homophilic cell adhesion results in recruitment of ankyrin to points of cell-cell contact, which in β1 can be inhibited by phosphorylation of a single, intracellular tyrosine residue (12, 17). β1 and β2 mRNAs are expressed as early as P1 in brain. Although β1 and β2 mRNA expression is initially low in cerebellum, their expression is more robust from P14 through adulthood (18). β3 mRNA expression is detected in brain as early as embryonic day 10. β3 then decreases in brain after P3 in certain areas (including the cerebellum) but remains high in the hippocampus and striatum (18). In adult rats, β4 mRNA is expressed in the cerebral cortex, cerebellar purkinje cells, hippocampus, caudate putamen, and globus pallidus (19). However, the expression profile of β4 at earlier time points is not known.

Here we examine the role of β-subunits in postnatal cerebellar granule cell neurite extension. We examine the effects of β1, β2, and β4 presented on the surface of fibroblast monolayers, or in soluble form, on neurite growth promotion in cerebellar granule cells isolated from β1(+/+) or β1(−/−) mice. We show that β1 promotes, whereas β2 reduces the level of basal neurite extension. The β4 subunit has no measurable effect on neurite outgrowth. Previous studies used heterologous expression systems, such as Drosophila S2 cells, to show β subunit-mediated cell adhesive interactions (12). The present study is the first functional demonstration of β-subunit-mediated cell adhesion in neurons.

The observation that an IGSF CAM can promote neurite outgrowth is far from novel. What is novel, is that proteins that participate in voltage-dependent ion channel gating can also function as CAMs that participate in extracellular and intracellular signal transduction leading to neurite extension. We...
propose that β subunits, as CAMs, participate in inter- and intracellular communication and thus may play critical roles in neuronal development.

**EXPERIMENTAL PROCEDURES**

**Cerebellar Dissociation—β1(+/+) and β1(−/−) mice were generated and maintained as previously described, in accordance with the guidelines of the University of Michigan Committee on the Use and Care of Animals (20). Animals used in this study were bred from β1(+/−) mice that had been repeatedly backcrossed to C57Bl/6 mice for at least 10 generations, creating congenic strains. The β1(+/+) and β1(−/−) mice used in each individual experiment were age-matched littermates. Following cervical dislocation, cerebella from β1(+/+) or β1(−/−) mice at the ages indicated in the figure legends were quickly excised and placed in ice-cold Hibernate A/B-27 with 1-glutamine. Tissue was cut into small pieces and cerebellar granule cells were dissociated according to the method described by Brewer with slight modifications (21). Briefly, cerebellar tissue was subjected to trypsin treatment (1 mg/ml) in Hibernate A (Brain) for 30 min at 37 °C with shaking at 250 rpm. Following trypsinization, tissue was allowed to settle for 2–5 min and Hibernate A/trypsin was aspirated. Tissue was resuspended in 2 ml of Hibernate A/B-27 and triturated using a fire-polished glass pipette or P 1000. The suspensions were isolated by centrifugation for 15 min at 800 × g using a 4-ml Opti-Prep (Axis-Shield) density gradient (35, 25, 20, and 15%) made with Hibernate A/B-27. Fraction 3, which is highly enriched in neurons, was collected and resuspended in 12 ml of Neurobasal A/B-27 with 0.5 mm l-glutamine, 10 μm gentamycin, and 5 nm/ml FGF-β, then plated at 2 × 10^5 cells/well. Cell viability was measured using the trypan blue exclusion assay.

**Immunocytochemical Analysis of CHL Cells—Confluent monolayers of 1610 Chinese hamster lung (CHL) (13) or CHL-β1 (13) cells were fixed in 4% paraformaldehyde for 40 min at room temperature. Following fixation, cells were blocked with a solution containing 5% nonfat dry milk and 1% bovine serum albumin. Cells were then incubated for 1 h with a primary monoclonal antibody to GAP 43 (Chemicon, 1:500) followed by goat anti-rabbit Alexa Fluor 594 (Molecular Probes) at 1:500 dilution was used as the secondary antibody. Monolayers were visualized using a Zeiss Axioshot fluorescence microscope at the Microscopy and Image Analysis Core facility at the University of Michigan. Digital images were processed using Adobe Photoshop.

**Creation of CHLβ4 Stable Cell Line—**Rat brain total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcriptase-PCR was performed using the Titan One RT-PCR kit (Roche Diagnostics) with primers corresponding to the 5' and 3' ends of the rat β4 cDNA sequences (GenBank™ accession numbers BK001030 and XM_236199; 5'-CTGGTCCACAGTGCCTATCATGACGAC and 3'-CAACATCCTCGACGACGACGACGAC).AGO-1 mRNA was evaluated using primers: 5'-CAACGTAGGCGCTTGCGAGAACAGGG and 3'-ATCGAATTCACATTACAGAAGATGTGCGC. The resulting reverse transcriptase-PCR product was used as the template in a second PCR using nested β4 primers: 5'-CAACGTAGGCGCTTGCGAGAACAGGG and 3'-ATCGAATTCACATTACAGAAGATGTGCGC. The resulting PCR product was gel-purified, subcloned into pcDNA3.1/hygromycin (Invitrogen), and sequenced to confirm identity with β4. CHL cells were transfected with pcDNA3.1/hygromycin (Invitrogen) and stable cell colonies were selected in the presence of 500 μg/ml hygromycin (Invitrogen). Northern blot analysis of the resulting CHL-β4 cell lines was performed using full-length β4 antisense cDNA as the probe label with digoxigenin. 10 μg of total RNA was extracted from each cell line, and loaded onto an RNA gel. Following transfer, hybridization and detection was as previously described (20). These results were confirmed by Western blot analysis with an anti-β4 antibody (1:200, gift from Dr. W. A. Catterall) followed by an anti-rabbit horseradish peroxidase-conjugated secondary antibody.

**Neurite Outgrowth Assay—**Parental (CHL), pcDNA3 mock-transfected (CHL-mock), or stably transfected β1 (CHL-β1), β2 (CHL-β2), β1β2 (CHL-β1β2), Na1.2β1 (CHL-β1), Na1.2β2 (CHL-β1β2), Na1.2.1β2 (CHL-β1β2β1) subunit expressing 1610 CHL cells were plated at 4 × 10^5 cells/well in 8-well chamber slides and grown for 24 h (13, 14). Upon the establishment of confluent monolayers, freshly dissociated cerebellar granule cells were plated (2 × 10^6 cells/well) on top of cell monolayers and allowed to grow in Neurobasal A/B-27 with l-glutamine, 10 μg/ml gentamycin, and 5 nm/kg FGF-β for 19–22 h. Cells were fixed with 4% paraformaldehyde and visualized using a primary monoclonal antibody to GAP 43 (Chemicon, 1:500) followed by Alexa Fluor 488-conjugated anti-mouse antibody (Molecular probes). Successful cloning was confirmed by restriction digest with EcoRI and BamHI as well as dideoxy sequencing. The newly constructed clones were screened using PCR with pcDNA3.1. Western blot analysis was carried out on 5 μl of tissue culture medium exposed for 22 h to a single confluent well of either uninduced CHLSolβ1 cells or 1 mg/ml tetracycline-induced CHL or CHLSolβ1 cells (200 μl total volume). Blots were probed with mouse anti-c-myc antibody (Santa Cruz, 1:500) for 1 h at room temperature. Secondary horseradish peroxidase-conjugated, anti-mouse antibody (Cell Signaling, 1:2,000) was incubated with blots for 1 h prior to washing and cholinesterase detection using West Dura reagent (Pierce).

**Affinity Purification of the CHLSolβ1 Subunit—**Confluent T-75 flasks of CHLSolβ1 cells were induced to express the soluble β1 subunit using 1 mg/ml tetracycline overnight. Collected media was centrifuged at 500 × g to remove cells and debris. Media was then collected and incubated for 1–2 h with nickel metal hydride-agarose (Qiagen). Non-specific interactions with the nickel-agarose were disrupted using a washing buffer containing 500 mM NaCl, 20 mM NaHPO4, and 20 mM imidazole (pH 8.0) prior to elution. The CHLSolβ1 protein was eluted from nickel-agarose using 200 mM imidazole in the wash buffer.

**Immunohistochemistry—**P12 or P18 C57Bl/6 mice were anesthetized by administering an intraperitoneal injection of 0.2 ml of sodium pentobarbital (50 mg/ml, Abbott Laboratory) and perfused intracardi-
neurite distribution for a given neurite length. Neurite length distributions for neurons grown on CHL (solid line) or CHL-mock (dotted line) monolayers overlap, indicating a similar percentage of neurites at any given neurite length under these conditions. The rightward shift of the neurite length distribution for granule neurons grown on CHL-β1 monolayers (dashed line) indicates an increase in the percentage of neurites at any given length. For B and C, 13 independent experiments (50 neurons measured per experiment) were performed for each condition. Error bars represent the S.E. Values are statistically significant for p < 0.05 using a one-way analysis of variance with a Dunnett's post test.
next used an antiserum specific to the Ig loop region of β1 (anti-β1ex) to examine the specificity of the observed increases in neurite outgrowth. We reasoned that if the observed effects were truly β1 specific, involving extracellular, cell adhesive events, then neurite outgrowth on CHL-β1 monolayers should be blocked by the addition of antibodies that recognize the β1 Ig loop domain. When 5 μg/ml β1ex antibody, directed against the A-A’ face of the β1 Ig loop domain (12) (illustrated in Fig. 5A), was added to co-cultures, we observed a blockade of β1-stimulated neurite extension from granule neurons grown on CHL-β1 monolayers compared with neurons grown on CHL-β1 monolayers but in the absence of antibody (Fig. 3). Comparison of the β1 amino acid sequence with the crystal structure of myelin P0 predicts that the anti-β1ex antibody epitope lies in a region that is critical for trans-homophilic cell adhesion (23, 24) (Fig. 5A). We observed no changes in neurite length when β1ex antibodies were added to granule neurons grown on CHL monolayers. The addition of non-immune IgG had no effect on granule neurons grown on either CHL or CHL-β1 monolayers. These results, taken together with our previous results, suggest that β1 may play a role in neurite extension from acutely dissociated P14–P21 cerebellar granule cells.

**Neurite Outgrowth Is Impaired in β1(−/−) Neurons—** SCN1B encodes the sodium channel β1 subunit and is located on chromosomes 7A3 in mice and 19q13.1 in humans (25, 26). β1(−/−) mice exhibit a dramatic behavioral phenotype including severe seizures, ataxia, and lethality by approximately P21 (20). The β1 extracellular domain (anti-β1ex) reduced neurite length of granule neurons grown on CHL-β1 monolayers but not granule neurons grown on CHL monolayers, as indicated. Addition of rabbit nonimmune IgG (Sigma) had no effect on neurite length. Six independent experiments (50 neurons measured per experiment) were performed for each condition. Error bars represent the S.E. Values are statistically significant for p < 0.05 using a one-way analysis of variance with a Dunnett’s post test.

**Fig. 4.** Granule neurons isolated from β1(−/−) mice do not show enhanced neurite outgrowth in response to β1. A, granule neurons from β1(−/−) mice grown on CHL or CHL-β1 monolayers show similar mean neurite lengths of 51.2 and 49.3 μm, respectively. β1(+/-) granule neurons grown on CHL-β1 monolayers have a mean neurite length of 77.8 μm. B, neurite distribution for given neurite length. Neurite length distributions overlap for β1(−/−) granule neurons grown on CHL (solid line) or CHL-β1 (dashed line) monolayers. In contrast, the neurite length distribution for β1(+/-) granule neurons grown on CHL-β1 monolayers (dot-dash line) shows an increase in the percentage of neurites at any given length. Seven independent experiments (50 neurons measured per experiment) were performed for each condition. Error bars represent the S.E. Values are statistically significant for p < 0.05 using a one-way analysis of variance with a Dunnett’s post test.
struct truncated at the extracellular juxtamembrane region (CHLSolβ1, Fig. 5A). This truncation allows for secretion of the β1 extracellular domain (c-myc-β1) into the tissue culture media. Treatment of cells with 1 μg/ml tetracycline for 22 h followed by Western blotting with anti-c-myc antibody shows that this cell line expresses high levels of c-myc-β1 subunits (Fig. 5B). Uninduced cells express a low level of c-myc-β1 protein. However, as shown below, this level of expression was not sufficient to promote neurite extension above control levels. Utilizing CHLSolβ1 cells as a monolayer, we examined the effect of the recombinant β1 extracellular domain on neurite outgrowth. Fig. 5C illustrates that c-myc-β1 increased granule cell neurite length to a similar level as full-length β1 subunits. We obtained similar results when granule cells were grown on untransfected CHL monolayers in the presence of 100 nM nickel-nitrotriacetic acid-purified CHLSolβ1 subunit added to the cell culture medium (Fig. 5D). Together, these results indicate that the β1 extracellular domain, including the Ig loop and juxtamembrane region, is sufficient to induce cerebellar granule cell neurite outgrowth in this system.

β2 Reduces Basal Neurite Extension, whereas β4 Has No Effect on Neurite Extension in Granule Neurons—To date, there are five known β-subunits β1, β1A, β2, β3, and β4 (9, 19, 27). β1 and β2 share similar functions as CAMs because both mediate homophilic adhesion, recruit ankyrin, and interact with tenasin-C and -R. Because β1 promotes neuritogenesis in our assay system, we were interested to investigate whether β2 or β4 might also function in a similar capacity. Utilizing monolayers of CHL cells stably transfected with β2 or β4 subunits (CHL-β2, CHL-β4), we examined whether the expression of β2 or β4 affected neurite length. CHL cells transfected with β4 subunit cDNA showed robust mRNA and protein expression, as assessed by Northern and Western blot, respectively (Fig. 6A). As shown in Fig. 6B, expression of β4 by CHL cells had no observable effects on granule cell neurite extension.

In contrast to cerebellar granule neurons grown on CHL-β1 or CHL-β4 monolayers, those grown on CHL monolayers expressing β2 consistently had shorter neurite lengths than neurons grown on untransfected CHL monolayers (Fig. 7). This apparent inhibitory effect of the β2 subunit was not nearly as
robust as that observed for β1-enhanced outgrowth. Relative to neurons grown on CHL monolayers, those grown on CHL-β2 monolayers showed an average 1.2-fold decrease in neurite length from control with a mean neurite length of 28.3 ± 2.5 versus 34.5 ± 3.2 μm for granule cells grown on CHL monolayers. These results suggest that the β1 and β2 subunits may be able to act antagonistically to regulate neuronal development.

**Effect of Sodium Channel α Subunit Coexpression**—We demonstrated previously that β1 and β2 subunits function as CAMS in vitro, both in the presence and absence of the pore-forming α subunit, Na,1,2 (12). The results presented above show that β subunits expressed on a cell monolayer in the absence of α subunits regulate neurite extension from cerebellar granule neurons. We next examined whether αβ subunit coexpression by the monolayer would affect the observed β subunit-mediated effects on cerebellar neurite outgrowth, for example, by masking a critical site on β. Using acutely dissociated granule neurons from P14–P21 mice, we observed significant increases in neurite length from granule neurons grown on CHL-αβ1 cells compared with untransfected monolayers. This effect was comparable with that observed for β1 alone (Table I). Consistent with our previous results in S2 cells, the present results show that β1-β1 homophilic interactions are not modulated by α subunit association. In contrast, we found no significant differences in neurite length for CHL-αβ2, CHL-αβ1β2, or CHL-β1β2 cells compared with CHL monolayers. Interestingly, these results suggest that β1-β2 interactions may prevent β1-β1 homophilic adhesion and subsequent signal transduction. Alternatively, the effect of β2 subunits to inhibit neurite extension may be dominant over β1-mediated promotion of neurite outgrowth.

**Developmental Regulation**—Our results thus far demonstrate that sodium channel β1 and β2 subunits modulate neurite extension in P14–P21 cerebellar granule neurons via trans-homophilic cell adhesive interactions. We next investigated whether neurons isolated from older animals exhibited similar responses. Table I shows the results of β subunit-mediated neurite extension from cerebellar granule neurons isolated from P22–P26 mice. In these cells, β1 subunits presented by the monolayer in the absence of α or β2 subunits (CHL-β1) produced a ~1.5-fold increase in neurite length, similar to that observed for P14–P21 neurons. However, in contrast to experiments performed with neurons isolated from P14–P21 mice, CHL-αβ1 monolayers did not promote neurite extension, suggesting that the response of the neurons to sodium channel subunits presented by the substrate changes with development.

**Immunolocalization of β Subunits in Cerebellum**—During late migration from the external germinal layer to the developing granule cell layer, granule neurons appose Bergmann glia and travel en masse to their final destination. *In situ* hybridization studies have suggested that β1 mRNA is present in the molecular layer of the cerebellum from P14 through adulthood in rats (18). If sodium channel β1 subunits were expressed on Bergmann glial plasma membranes or on the plasma membranes of adjacent granule or Purkinje neurons, then they might serve as in vivo equivalents of the β1-transfected fibroblast monolayers used in our co-culture system. To test this, we performed immunohistochemistry on P12 or P18 mouse cerebellum to determine whether Bergmann glia express sodium channel β subunits. RC-2 is the marker of choice for Bergmann glia in the early phases of development prior to birth, however, immunoreactivity for this antigen is absent following the second postnatal week (27). From P14 to adult-
3. Sodium Channel β1 Subunit-mediated Neurite Outgrowth

Table I
Average neurite length of P14–P21 and P22–P26 mouse cerebellar granule neurons grown on CHL cell monolayers expressing the indicated sodium channel α (Na1.2) and β subunits.

| Monolayer | CHL | CHL | β1 | β1 | αβ1 | αβ1 | αβ2 | αβ2 | β1β2 | β1β2 | αβ1β2 | αβ1β2 |
|-----------|-----|-----|----|----|-----|-----|-----|-----|------|------|-------|-------|
| Age       | P14–P21 | P22–P26 | P14–P21 | P22–P26 | P14–P21 | P22–P26 | P14–P21 | P22–P26 | P14–P21 | P22–P26 | P14–P21 | P22–P26 |
| -Fold difference (versus CHL) | 1.0 | 1.0 | 1.4 | 1.5 | 1.6 | 1.0 | 1.0 | 0.9 | 1.0 | 0.8 | 1.0 | 1.2 |
| S.E.      | 0.0 | 0.0 | 0.06 | 0.08 | 0.09 | 0.1 | 0.06 | 0.08 | 0.04 | 0.07 | 0.1 | 0.21 |

Discussion

Sodium channel β1 and β2 subunits regulate neurite extension from cerebellar granule neurons. We observed a level of β1-enhanced neurite outgrowth that was consistently on the order of 1.4–1.7-fold when compared with control monolayers, levels similar to those published for a variety of IGSF molecules (3, 30). This β1-mediated effect was specific and could be blocked by addition of an antibody directed against the A-A' face of the β1 Ig domain, suggesting that trans-homophilic adhesion involving this region may be responsible for our observed results. The soluble β1 extracellular domain was as effective in promotion of neurite extension as full-length β1 subunits. Neurons isolated from β1(−/−) mice did not respond to β1-expressing monolayers. Thus, we propose that, similar to L1-CAM and NCAM, β1-β1 trans-homophilic cell adhesive interactions stimulate neurite extension via activation of second messenger cascades in the neuron (4).

In contrast to β1, β2, when presented by the substrate, reduced the extent of basal neurite outgrowth from wild-type cerebellar granule neurons. This is an important finding because neuronal development involves a balance between growth-promoting and growth-inhibiting events. The observation that β2 reduces neurite extension may be related to its homology to the third Ig loop and juxtamembrane region of F3/contactin, an IGSF CAM capable of strong neurite growth inhibition in monolayer assays (6, 31). Whereas mRNA for β2 is found within the molecular layer of the cerebellum in adult rats, in situ hybridization studies as well as the present results indicate that P14–P21 GFAP positive cells, such as Bergmann glia, are devoid of detectable β2 expression (18, 32). We suggest that β1 and β2 may act in an antagonistic manner if presented as substrates to the same neuronal cell.

Co-expression of Na1.2 with either or both β subunits revealed that there are differences in the response of cerebellar granule neurons to β1, depending on their level of maturation. Granule neurons isolated from P14–P21 mice responded similarly to β1-expressing and to αβ1-expressing monolayers, whereas neurons isolated from P22–P26 mice displayed enhanced neurite extension in response to cells expressing β1 alone but not to those expressing the αβ1 complex. Perhaps different domains of β1 are required for neurite extension as

Fig. 8. Immunohistochemical analysis of sodium channel β subunit expression in mouse cerebellum. Mouse brains were prepared for immunohistochemistry as described under “Experimental Procedures.” Sections were costained for sodium channel β1, or β4 subunits as indicated in the figures (green) and for GFAP (red). Yellow areas as well as white arrows indicate colocalization of β-subunits and GFAP. Scale bar = 50 μm.
neuronal development progresses, such that α-β association at later time points results in masking of a critical site for β1-β1 interaction. Alternatively, perhaps neuronal signal transduction resulting from β1-β1 trans adhesion changes with development, such that the signal induced by β1 in complex with α is different from that induced by β1 alone at later stages of maturation. Interestingly, coexpression of β2 with β1, regardless of a subunit expression on the monolayer (CHL-αβ1/β2 or CHL-β1/β2), prevented β1-stimulated outgrowth at both developmental stages. We propose that cis-heterophilic β1/β2 association in the plasma membrane of the substrate cell may result in masking of a β1 domain that is critical for β1-β1 trans-homophilic adhesion. Alternatively, β2-mediated signal transduction, resulting in the reduction of neurite extension, may be dominant over the β1-mediated response. Thus, β1 expressed alone or in complex with α by substrate cells early in development (P14–P21) appears to promote neurite extension. Subsequent β2 expression and/or α-β1 association by substrate cells at later developmental stages may serve as a molecular switch to attenuate β1-mediated outgrowth.

We observed that neurons isolated from β1(-/-) mice do not extend neurites in response to β1 presented by the monolayer. These results demonstrate that the mechanism responsible for β1-mediated neurite extension requires β1 expression on the neuron as well as on the substrate cell. Studies using NCAM(-/-) or L1-CAM(-/-) mice indicate that neurons lacking these CAMs exhibit higher levels of pathfinding errors and greater degrees of desilicafication compared with wild-type (33–35). β1(-/-) mice exhibit a dramatic behavioral phenotype including seizures, ataxia, and lethality by approximately P21 (20). The molecular phenotype of β1(-/-) mice includes decreases in action potential conduction velocity, disruption of nodal architecture, decreases in the number of mature nodes of Ranvier in the optic nerve, and altered patterns of sodium channel expression in the hippocampus. Although the observed seizure activity may be caused by altered sodium channel isoform expression in the hippocampus, our present results suggest that errors in neurite extension and pathfinding may also contribute. A human mutation in SCN1B that causes generalized epilepsy with febrile seizures plus type 1 results in the reduction of neurite extension and, as CAMs, participate in inter- and intracellular communication. Together, these functions make β subunits critical players in neuronal development.

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12. Malhotra, J. D., and Z dataList(12, 15). Signaling mechanisms responsible for β1-mediated homophilic cell adhesive interactions (37). Neuronal pathfinding errors may also underlie the generalized epilepsy with febrile seizures plus 1 epileptic phenotype. Finally, pathfinding errors in the cerebellum of β1(-/-) mice may contribute to the observed ataxic phenotype.

Our observations that sodium channel β subunits promote neurite outgrowth suggest the existence of β subunit signaling complexes in neurons. In addition to the pore-forming α subunits, sodium channel β1 subunits have been shown to associate with tenascin-R, contactin/F3, neurofascin-186, ankynir, and the receptor protein-tyrosine phosphatase β (12–14, 16, 38). β2 associates with tenasin-C, tenascin-R, and ankynir, but not with contactin/F3 (12, 15). Signaling mechanisms responsible for β1-mediated promotion of neurite extension may involve a variety of pathways previously proposed for other CAMs such as mitogen-activated protein or Rho kinases. FGF-R (receptor-dependent and -independent models have been proposed to explain CAM-mediated neurite outgrowth (1, 30). In the FGF-R-dependent model, FGF-R and IGSF CAMs interact in a cis-heterophilic manner following initial IGSF CAM trans-homophilic adhesion. Resulting outgrowth occurs via local increases in N- and L-type Ca2+ channel activity (40). These binding events are thought to involve interacting CAM homology domains on the FGF-R and IGSF CAMs (39). The extracellular domains of β1 and β2 lack CAM homology domains, as described for L1-CAM, NCAM, and N-cadherin, and thus may act through FGF-R-independent signaling events, for example, lipid raft-associated cascades (30). Alternatively, sodium channel β subunits may act indirectly through CAM homology domain-containing molecules. We have shown that β1 and N-cadherin interact in heart (41). Posttranslational modifications such as polyisylation have been shown to be important determinates in NCAM-mediated neurite outgrowth and neuronal fasciculation (4). Sodium channel β subunits are heavily glycosylated (42). Thus, the extent and type of glycosylation in β1 and β2 during different stages of development or in different cell types may be important in their ability to modulate neurite outgrowth. In summary, we propose that sodium channel β subunits modulate electrical signal transduction and, as CAMs, participate in inter- and intracellular communication. Together, these functions make β subunits critical players in neuronal development.
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