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Expression and regulation of voltage-gated sodium channel β1 subunit protein in human gliosis-associated pathologies

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Abstract Auxiliary β1 subunits of voltage-gated sodium channels (NaChs) critically regulate channel activity and may also act as cell adhesion molecules (CAMs). In a recent study we have shown that the expression of β1 NaCh protein is increased in reactive astrocytes in a rat epilepsy model of mesial temporal lobe epilepsy. The present study was undertaken to examine whether changes of NaCh β1 subunit protein expression are also associated with structural changes occurring in human reactive astrocytes under different pathological conditions in vivo, as well as in response to changing environmental conditions in vitro. Strong β1 astroglial immunoreactivity was present in human brain tissue from patients with astrogliosis. The overexpression of β1 protein in reactive glia was observed in both epilepsy-associated brain pathologies (temporal lobe epilepsy, cortical dysplasia), as well as non-epileptic (cerebral infarction, multiple sclerosis, amyotrophic lateral sclerosis, meningo-encephalitis) disorders. The up-regulation of β1 subunit protein in astrocytes can be reproduced in vitro. β1 protein is highly expressed in human astrocytes cultured in the presence of trophic factors, under conditions in which they show morphology similar to the morphology of cells undergoing reactive gliosis. The growth factor-induced overexpression of β1 protein was abrogated by PD98059, which inhibits the mitogen-activated protein kinase pathway. These findings demonstrate that the expression of NaCh β1 subunit protein in astrocytes is plastic, and indicate a novel mechanism for modulation of glial function in gliosis-associated pathologies.

Keywords Pathology · Astrogliosis · Epilepsy · Cell culture · Growth factors

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Introduction

Voltage-gated sodium channels (NaChs) are composed of a central pore-forming α subunit and auxiliary β subunits. Although the α subunit is primarily responsible for the ion permeability and voltage sensing, the β subunits critically modulate channel function, as well as channel membrane expression levels [11, 12, 22, 24, 29, 31, 39]. In addition, it has been shown recently, that β-subunits may function as cell adhesion molecules (CAMs), interacting with extracellular matrix proteins [21, 23, 53]. While the role of neuronal NaChs has been extensively studied, the role of NaCh in glial cells is still unclear [52]. Multiple types of NaCh α and β subunit mRNAs are expressed in both neurons and glial cells [7, 12, 32, 34, 52]. In most of these cell types, the expression of NaChs is dynamic, with levels and composition of various NaCh subtypes changing during development, in response to injury and upon exposure to neurotrophins [7]. Several studies using the patch-clamp technique have shown the presence of sodium current in astrocytes in culture, as well as in acute tissue slices from different brain regions [4, 5, 8, 47]. In particular, attention has been focused on the NaCh properties of reactive astrocytes [6, 9, 10]. However, little information is available about the molecular characteristics of the channels in reactive astrocytes. Regulation of NaCh β mRNA levels has been observed in rat astrocytes stimulated in vitro [35]. In a recent study we observed a per-

sistent increase of NaCh β1 subunits specifically in reactive astrocytes in a rat model for temporal lobe epilepsy [18]. In the present study we examined the expression of NaCh β1 subunit protein in human pathological conditions associated with gliosis. Knowledge of the expression patterns of these β1 subunits in various pathological conditions could give us insight in their possible role in either modulation of excitability and/or structural reorganization. Adult human-derived astrocytes in culture were also used to study the regulation of β1 protein expression in response to specific changes in the astroglial cell's microenvironment.

Materials and methods

Human tissue

Tissue samples and pathology reports were retrieved from the files of the Department of Neuropathology of the Academical Medical Center (University of Amsterdam). Autopsy and surgical specimens of human brain tissue from patients with non-neoplastic brain pathologies associated with reactive gliosis [five cases with temporal lobe epilepsy with hippocampal sclerosis (TLE), five cases of focal cortical dysplasia, three cases with focal cerebral infarction, three cases with meningo-encephalitis, three cases with multiple sclerosis and five cases with amyotrophic lateral sclerosis] were included in the study for immunocytochemical analysis of the NaCh β1 protein. Control brain tissue (including normal cortex and white matter from the temporal, frontal, parietal regions and spinal cord) was obtained from six age-matched patients who died from a non-neurological disease. All autopsies were performed within 16 h after death.

Tissue preparation

All specimens used in the study for immunocytochemistry were fixed in 10% buffered formalin and embedded in paraffin. Paraffin-embedded tissue was sectioned at 5 μm on a sliding microtome and mounted on organosilane (3-aminopropylethoxysilane; Sigma) -coated slides. Representative sections of all specimens were processed for hematoxylin-eosin staining, as well as for immunocytochemical reactions, as described below.

Immunocytochemistry

Immunocytochemistry was performed with a polyclonal antibody (anti-$\beta1_{ex}$; 1:100 dilution) generated against the extracellular domain of NaCh β1 subunit (KRRSETTAETFTEWTFR). This antibody, synthesized by the Protein and Carbohydrate Structure Core facility at the University of Michigan, has been described and characterized previously [18, 28, 53]. A polyclonal antiserum (anti-$\beta1_{IN}$; 1:50) against an intracellular domain of β1 subunit (LAITSESKENCTGVQVAE; [28]) was also used. Antibodies against glial fibrillary acidic protein (GFAP; polyclonal rabbit; Dako, Denmark; 1:2,000), vimentin (mouse clone V9; Dako; 1:400) and HLA-DR (mouse clone Tal1B5, Sigma, USA; 1:100) were used in the routine immunocytochemical analysis of reactive gliosis. Paraffin-embedded sections were deparaffinated in xylene and after rinses in ethanol (100% and 95%) were incubated with 1% H_2O_2 diluted in methanol for 20 min. Slides were then washed with phosphate-buffered saline (PBS; 10 mM, pH 7.4). For the detection of β1 protein, the slides were placed into sodium citrate buffer (0.01 M, pH 6.0) and heated in a microwave oven at 650 W for 10 min. The slides were allowed to cool for 20 min in the same solution at room temperature and then washed in PBS. At this point, they were incubated in PBS containing 10% normal goat serum for 15 min prior to the incubation with the primary antibody (30 min room temperature and overnight at 4°C). Single labeling was carried out using avidin-biotin peroxidase method (Vector Elite, Calif.) and 3,3'-diaminobenzidine (DAB) as a chromogen. Sections of human tissue were counterstained with hematoxylin. A similar staining pattern was obtained in control brain tissue using two different polyclonal anti-β1 antibodies (anti-$\beta1_{ex}$ and anti-$\beta1_{IN}$). Control human sections incubated without the primary antibody or replacing it with pre-immune sera were essentially blank. Sections that were incubated in the presence of the primary antibody (anti-$\beta1_{ex}$) preadsorbed with 100-fold excess of the respective peptide, showed only a very light background staining (Fig. 1C, insert)

Evaluation of immunostaining

Using a light microscope all sections were examined by two observers independently. The intensity, the cellular localization, and the percentage of immunoreactive astroglial cells were examined in human sections of control brain/spinal cord and brain/spinal cord with reactive gliosis. We rated the degree of staining and the proportion of immunopositive astrocytes on a semiquantitative point scale as indicated in Table 1. β1-immunoreactive staining was semiquantitatively evaluated using a four-point scale (–, no; ±, weak; +, moderate; ++, strong staining). The percentage of immunoreactive astroglial cells (number of labeled astrocytes per total number of astrocytes) was also stratified into four groups: (1) <1%; (2) 1–10%; (3) 11–50; (4) >50%. Ten representative fields of two labeled sections of each specimen were examined at a magnification of ×250, using an ocular grid as previously described [2, 3, 49]. Astrocytes were differentiated from neuronal and microglial cells on the basis of morphology by staining of a serial section with antibodies against GFAP, vimentin and HLA-DR. Normal astrocytes were differentiated from reactive astrocytes on the basis of morphology and the absence of vimentin immunoreactivity. As previously reported [1, 25] vimentin allowed the detection of reactive hypertrophic astrocytes.

Astrocytes-enriched human cell cultures

Human brain tissue for cell cultures was obtained at autopsy (with short postmortem interval; <6 h) from three adult patients (case 1, 84 years; case 2, 31 years; case 3, 67 years) without evidence of neurological disorders. Resected tissue samples from temporal cortex were collected in Dulbecco's modified Eagle's medium (DMEM)/HAMF10 (1:1) medium (Gibco, Grand Island, N.J.). Cell isolation

Fig. 1 NaCh β1 subunit IR in reactive human astrocytes. **A–C** Expression of β1 immunoreactivity in control normal brain (**A** white matter; **B** hilar region and **C** CA1 region of the hippocampus). Only weak or undetectable β1 IR is present in astroglial cells. In control hippocampus, NaCh β1 IR is detected in neuronal cells (**B**, **C**). *Insert* in **C**: Immunostaining of control hippocampus (CA1) with the anti-β1 antibody preadsorbed with an excess of the corresponding peptide. **D**, **E** Prominent β1 expression is observed in reactive astrocytes (*arrows*) of mesial temporal lobe epilepsy (*MTLE*; **D** hilus, **E** CA1 region). *Insert* in **D**: Merged image showing NaCh β1 expression (*red*) in reactive vimentin-positive astrocytes (*green*). **F** NaCh β1 expression in focal cortical dysplasia (*FCD*). Large dysplastic neurons (*arrow*), balloon cells (*asterisk*) and astrocytes (*arrowhead*) are intensely stained. **G** β1 expression in reactive astrocytes (*arrows*) is seen in chronic active plaques of a patient with multiple sclerosis (*MS*). **H** Low expression of β1 protein is detectable in the white matter of control spinal cord. **I** Increased β1 expression is observed in reactive spinal cord astrocytes (*arrows*) of a patient with amyotrophic lateral sclerosis (*ALS*) (*NaCh* sodium channel, *IR* immunoreactivity). Counterstaining with hematoxylin. *Bars* **A** (also for **B–E** 85 μm; **F** 60 μm; **H** (also for **G** and **I**) 90 μm

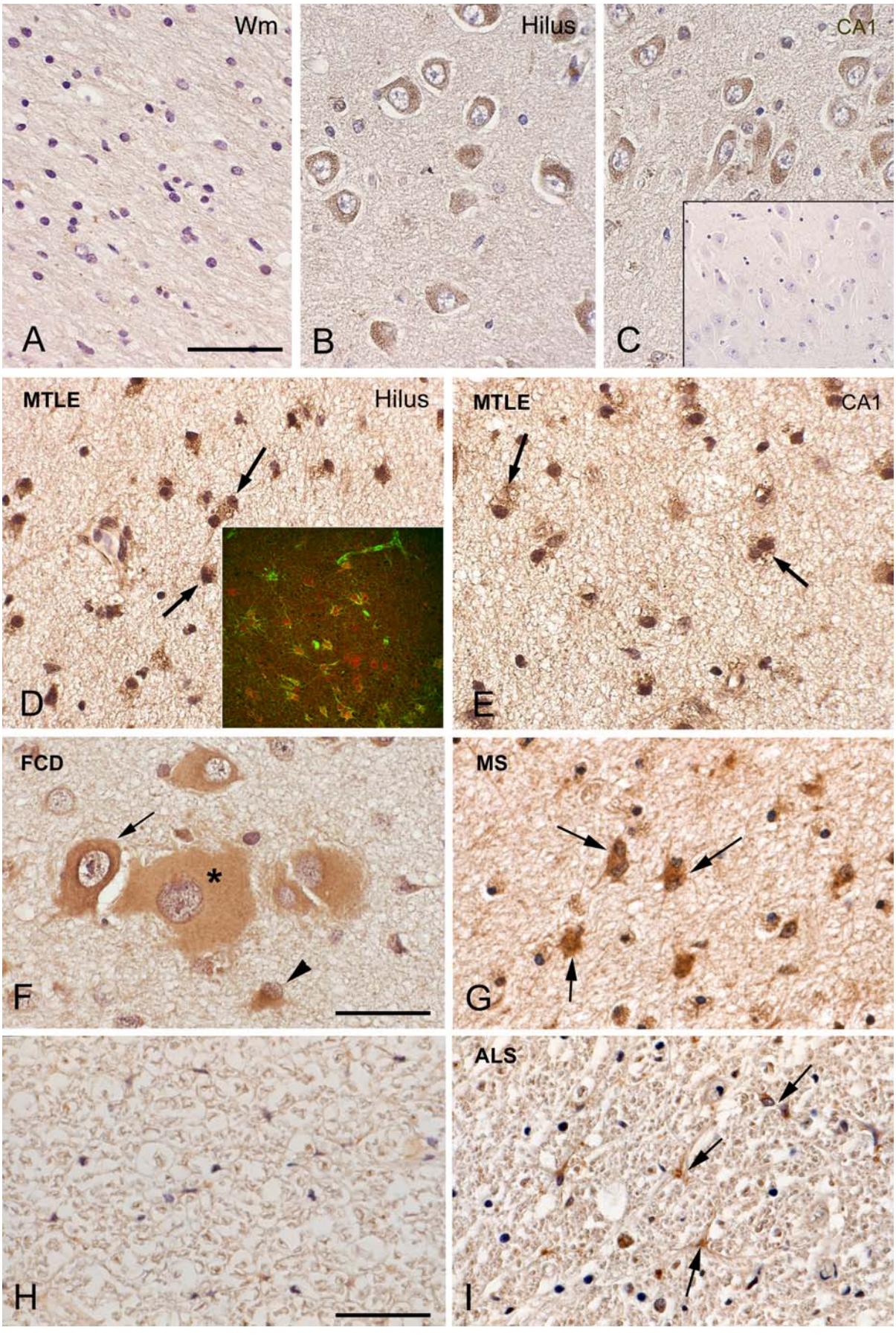
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Table 1 Distribution of NaCh β1-subunit protein immunoreactivity in astrocytes in human brain pathologies associated with gliosis. β1immunoreactivity is given as: –, none detectable; ±, weak; +, moderate; ++, strong. Percentage of β1-immunopositive cells with astroglial morphology is given as: (1) <1%; (2) 1–10%; (3) 11–50; (4) >50% (*NaCh* sodium channel, *TLE* temporal lobe epilepsy, *FCD* focal cortical dysplasia, *FCI* focal cerebral infarction, *ME* meningo-encephalitis, *MS* multiple sclerosis, *ALS* amyotrophic lateral sclerosis)

Control		TLE	FCD	FCI	ME	MS	ALS
Cortex	*Spinal cord*						
-/± (1)	±/+ (2)	+/++ (3)	++ (4)	++ (3)	++ (3)	++(4)	+/++(3)

was performed as previously described [14]. Briefly, after removal of meninges and blood vessels, tissue was dissociated by incubation at 37°C for 20 min in Hanks' balanced salt solution containing 2.5 mg/ml trypsin (Sigma, St. Louis, Mo.) and 0.1 mg/ml bovine pancreatic DNase I (Boehringer Mannheim, Germany). Tissue was triturated and washed with DMEM/HAMF10 medium, supplemented with 50 U/ml penicillin and 50 µg/ml streptomycin and 10% fetal calf serum (FCS). Cell suspension (containing approximately 0.5 g wet weight tissue/10 ml culture medium) was plated into 80-cm^2 flasks (Falcon, Lincoln Park, N.J.) and maintained in a 5% $C0_2$ incubator at 37°C for 2 h, to allow monocytes/macrophages to adhere to the bottom of the flask. The supernatant was then plated into poly-L-lysine (PLL; 15 µg/ml, Sigma) -precoated 80-cm^2 flasks. After 48 h, the culture medium was replaced by fresh medium and subsequently cultures were fed once a week. Cultures usually reached confluence after 2–3 weeks. Secondary astrocyte cultures were established by trypsinizing confluent cultures and sub-plating onto PLL-precoated 25-cm^2 flasks (2×10^4 cell/ml; for Western blot analysis or for the generation of serial passages) and simultaneously onto PLL-precoated 12-mm coverslips (Sigma) in 24-well plates (Falcon; 2×10^4 cell/well; for immunocytochemistry). More than 98% of the cells in primary culture, as well as in the successive 12 passages were strongly immunoreactive for the astrocytic marker GFAP (present results; [14]). In the present study astrocytes were used for Western blot and immunocytochemical analyses at passage 3. After 24 h in culture with DMEM/HAMF10 medium, containing 10% FCS, astrocytes were rinsed and either switched to serum-free medium or fed again with DMEM/HAMF10 medium plus 10% FCS. For serum-free medium, we used a modification of the minimally supplemented serum-free medium or of the astrocytes-defined medium, both described in [30]. Minimally supplemented serum-free medium (SF) comprised DMEM/HAMF10 supplemented with transferrin (100 µg/ml), D-biotin (1 µg/ml), sodium selenite (5.2 ng/ml), heparan sulfate (0.5 µg/ml) and insulin (10 µg/ml). The astrocytes-defined medium (ADM) used consisted of SF (described above) supplemented with basic fibroblast growth factor (bFGF; 10 ng/ml) and/or epidermal growth factor (EGF; 10 ng/ml). The efficacy of the two growth factors was analyzed by culturing astrocytes with various concentrations of either EGF or bFGF (1, 2.5, 5, 10, 20, 30 ng/ml). Astrocytes prepared from case 2 and 3 were used to investigate the involvement of mitogen-activated protein kinase (MAPK) pathway in the regulation of β1 subunit expression using the MAPK pathway inhibitor PD98059 (30 µM; Calbiochem, La Jolla, Calif.). Astrocytes were maintained under the different growth conditions for 3 days before immunocytochemical or immunoblot analysis.

Immunocytochemistry

Coverslips with adherent cells were rinsed in PBS (pH 7.4) and fixed for 30 min in 4% paraformaldehyde in PBS. After rinsing, cultures were treated with 1% H_2O_2 for 10 min and incubated in PBS containing 10% normal goat serum for 15 min prior to the incubation with the primary antibodies (anti-$\beta1_{ex}$; 1:100; or anti-EGFR, 1:200; with anti-GFAP, monoclonal mouse, Chemicon, 1:500; or anti-vimentin, 1:50).

For the double labeling, cultures and human specimens (after the incubation with the primary antibodies), were incubated for 2 h with a 1:200 dilution of Alexa 488 goat anti-mouse IgG antisera (Molecular probes, Eugene, Ore.) and CY3 goat anti-rabbit IgG antisera (Zymed, Calif.). Cultures and sections were analyzed on laser scanning confocal microscope equipped with argon-ion laser.

Western blot analysis

Western blot analysis was performed on samples of cultured astrocytes. Cultured astrocytes were harvested at 4°C, homogenized in lysis buffer containing 10 mM TRIS (pH 8.0), 150 mM NaCl, 10% glycerol, 1% NP-40, 5 mM ethylenediamine tetraacetic acid (EDTA) and protease inhibitor cocktail (Boehringer), sonicated, and centrifuged in a microfuge at maximal speed for 5 min; pellets were resuspended in lysis buffer. Protein content was determined using the bicinchoninic acid method [46]. Samples were diluted to a concentration of 2 mg protein/ml in SDS/bromophenol blue loading buffer, and boiled for 5 min. For electrophoresis, equal amounts of proteins were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred to nitrocellulose paper for 1 h, using a semi-dry electroblotting system (Bio-Rad, Transblot SD), and incubated in 50 mM TRIS-HCl, 0.1% Tween-20, 154 mM NaCl, pH 7.5 (TTBS), containing 5% non-fat dry milk and 1% bovine serum albumin (BSA) for 1 h. Samples were then incubated overnight in TTBS, 3% BSA, 0.1% sodium azide, containing the primary antibody [anti-$\beta1_{ex}$, polyclonal rabbit (1:1,500); anti-β-actin, monoclonal mouse, Sigma, 1:1,000). After several washes in TTBS, the membranes were incubated in TTBS, 5% non fat dry milk, 1% BSA, containing the goat anti-rabbit or anti-mouse coupled to horseradish peroxidase (1:1,500; Dako) for 2 h. Immunoreactive bands were visualized using an enhanced chemiluminescence kit (ECL, Amersham). The levels of each protein were evaluated by measuring optical densities (OD) of the protein bands using Scion Image for Windows (beta 4.02) image-analysis software. Expression of β-actin (as reference protein) in the same protein extracts did not change. The specificity of the anti-$\beta1_{ex}$ in human brain tissue was tested by preincubating the antibody with 100-fold excess of the corresponding antigenic peptide, and by Western blots of the total homogenates of human control cortex (Fig 3A).

Results

NaCh β1 subunit immunoreactivity in human brain tissue

To examine whether NaCh β1 subunit protein up-regulation is a general feature of reactive astrocytes, we analyzed human brain tissue from patients with different brain pathologies associated with gliosis. Using morphological analysis and glial markers such as GFAP or vimentin we confirmed that all cases included in the study showed prominent reactive astrogliosis. In the lesioned areas of all the different diseases examined, the intensity of β1 subunit immunoreactivity appeared increased in cells with typical astroglia morphology. Activated mi-

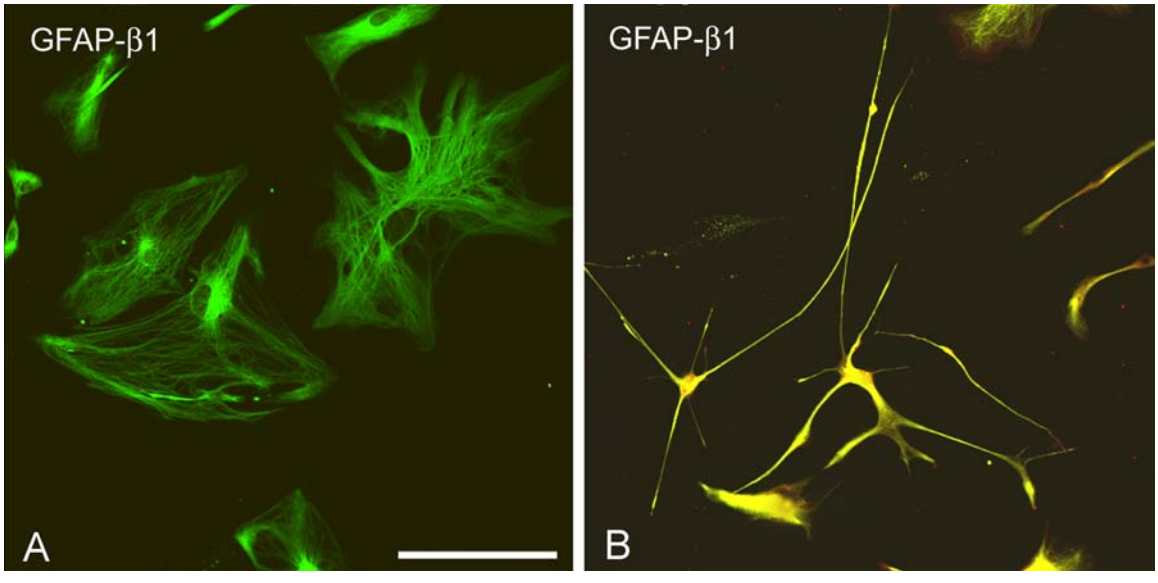
croglial cells did not express detectable levels of β1 protein. The overall results, showing the relative proportion of astrocytes positive for the β1 protein and the intensity of staining indicates an overall increase of β1 protein in reactive tissue, compared to control brain (Table 1). In control adult brain (in the absence of vimentin-positive glial cells), there was only weak expression of β1 in astrocytes in both white and gray matter (Table 1; Fig. 1A–C). In control human hippocampus β1 immunoreactivity was predominantly observed in neuronal cells (Fig. 1B, C). Weak expression of β1 protein was detectable in the white matter of the spinal cord (Table 1; Fig. 1H). Prominent β1 expression was observed in epilepsy-associated pathologies. In TLE patients, increased β1 immunoreactivity was observed in reactive (vimentin-positive) astrocytes in the hilar and in the CA1 region of the hippocampus (Fig. 1D, E). In the cortical dysplasia cases, strong β1 staining was detected in both the glial and the neuronal components of the lesion, including reactive astrocytes, balloon cells and dysplastic neurons (Fig. 1F). Changes in β1 expression were also detected in non-epileptic disorders associated with gliosis. Reactive astrocytes in active plaques of patients with multiple sclerosis (MS; Fig. 1G), as well as in the spinal cord of patients with amyotrophic lateral sclerosis (ALS; Fig. 1I) displayed moderate to strong β1 immunoreactivity.

Effect of growth factors on NaCh β1 subunit protein expression in cultured human astrocytes

Astrocyte-enriched human cell cultures growing in a serum-free chemically defined medium were used to study the regulation of β1 protein expression in response to changes in the glial cell's microenvironment. Human astrocytes growing in SF demonstrated a flat polygonal morphology (Fig. 2A). In contrast, (as previously reported for rat astrocytes [30]) addition to serum-free medium of specific growth factors (such as bFGF and EGF) produced a highly branched, stellate morphology, similar to the morphology of reactive astrocytes in vivo (Fig. 2B). In addition to these morphological changes, the presence of growth factors increased the expression of β1 immunoreactivity (Fig. 2B). Highly branched reactive astrocytes in vitro showed also strong EGF receptor immunoreactivity (data not shown).

Western blot analysis confirmed that the expression level of β1 protein in astrocytes is modulated by the composition of the extracellular environment (Fig. 3B). There was little β1 protein detectable in astrocytes cultured in the presence of 10% FCS or in SF. In contrast, the antibody recognized a strong band at approximately 36 kDa in an equivalent amount of protein from astrocytes exposed to either EGF or bFGF (Fig. 3B). EGF appeared more efficacious in the up-regulation of β1 protein than bFGF in astrocytes cultured with various concentrations of either EGF or FGF. EGF produced its maximal effect at a concentration of 5–10 ng/ml, while bFGF was most effective at 20–30 ng/ml. The OD measurements of the β1 expression levels in astrocytes (from two different patients) cultured under different growth conditions are shown in Fig. 3D. PD98059, a selective inhibitor of MAPK kinase (MEK) blocked the up-regulation of β1 protein observed in astrocytes cultured in the presence of growth factors (Fig. 3C, E).

Fig. 2 Expression of NaCh β1 subunit protein in astrocytes. Confocal laser scanning microscopic merged images of GFAP (*green*) and NaCh β1 subunit-positive (*red*) human astrocytes in culture. **A** GFAP-positive astroglial cells growing in SF with a flat polygonal morphology and low expression of β1 subunit. **B** Addition of specific growth factors (bFGF and EGF) to SF produced a highly branched, stellate morphology and increased the expression of β1 IR (*yellow*) (*SF* minimally supplemented serum-free medium). *Bar* **A** (also for **B**) 200 μm



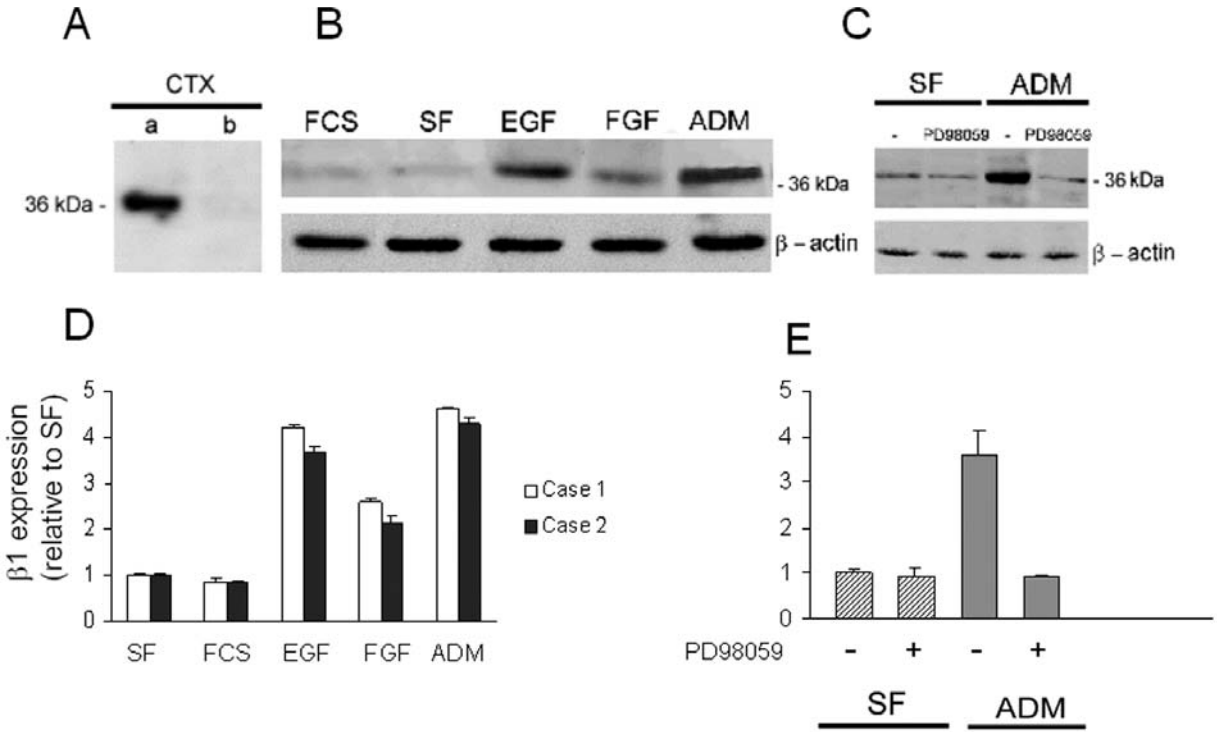


Fig. 3 The expression level of β1 protein in astrocytes depends upon the composition of the extracellular environment. **A.** The antibody (anti-$\beta1_{ex}$) recognized a strong band at approximately 36 kDa in normal human brain cortex (*CTX*; *a*). The immunoreactive band for β1 was completely abolished by preadsorption of the antibody with the corresponding peptide (*b*). **B** Representative immunoblot shows faint β1 bands in astrocytes cultured in the presence of 10% FCS or in SF. Exposure to either 10 ng/ml EGF or 10 ng/ml bFGF (concentrations used in the ADM) can induce β1 protein expression. **C** Effect of the MEK inhibitor, PD98059 on β1 protein expression in astrocytes growing in ADM. All of the lanes in **C** are from the same gel (case 3). PD98059 blocked the up-regulation of β1 protein observed in ADM, but did not affect the β1expression in SF. **D** Optical density measurements of the β1 expression levels in astrocytes (from two different patients, cases 1 and 2) cultured under different growth conditions. Data are expressed relative to the levels observed in SF cultures and are mean ± SEM from separate experiments performed in triplicate. **E** Optical density measurements of the β1 expression levels in astrocytes (from two different patients, cases 2 and 3) cultured in the presence or in the absence of PD98059 (30 μM). Data are expressed relative to the levels observed in SF cultures and are the mean ± SEM from separate experiments performed in triplicate. Proteins (40 μg/lane) were subjected to Western blot analysis using a specific anti-β1 antibody. Expression of β-actin (as reference protein) is shown in the same protein extracts. By comparison with SF, cultures growing in medium containing growth factors (EGF, FGF, ADM), in the absence of the MEK inhibitor PD98059, exhibited significant increase in β1 protein ($P<0.05$ compared with SF; ANOVA with a Fisher Protected Least Significant Difference post hoc analysis) (*EGF* epidermal growth factor, *bFGF* basic fibroblast growth factor, *ADM* astrocyte-defined medium)

Discussion

The present study shows that conditions associated with astrogliosis are related with an increased expression of the β1 protein in reactive astrocytes of human brain. The regulation of the expression appears to be plastic and under influence of extracellular environmental signals, such as growth factors. The significance of these findings is discussed below.

NaCh β1 protein is up-regulated in human brain tissue from patients with various brain pathologies associated with astrogliosis

We have found increased expression of β1 protein in human gliotic tissue associated with a number of pathological conditions, including TLE, focal cortical dysplasia, cerebral infarction, meningo-encephalitis, multiple sclerosis and ALS. The strong β1 immunoreactivity was exclusively observed in astrocytes from gliotic tissue. In astrocytes from adult normal brain there was only weak expression of β1 protein, although β1 immunoreactivity was more readily detected in spinal cord astrocytes. Spinal cord astrocytes are known to express higher densities of NaChs in vitro than astrocytes cultured from other brain regions [47]. Particularly interesting is the over-expression of β1 proteins in dysplastic neurons and balloon cells of focal cortical dysplasia, in view of its complex functions including regulation neuronal hyperexcitability. In a previous study we found an increased β1 expression in epileptic rat hippocampus [18]. Considering the modulating role of this subunit in NaCh inactivation and activation kinetics, a changed expression might contribute to changes in excitability of reactive astrocytes. An increased glial excitability with "spiking" activity has been found in human astrocytic tumor cells [9], in cells from

human oligodendroglial tumors [38], and in neoplastic glial cells of human glioblastomas [26], suggesting that these glial cells themselves may be an etiological factor for epileptic seizures. Interestingly, preliminary studies in our laboratory indicate strong expression of β1 subunit also in astrocytic tumors. However, since we also observed increased expression in pathological conditions that are not generally associated with increased excitability (ALS, MS), it is unlikely that an increased β-subunit expression simply leads to increased excitability. Since β-subunits may also function as CAMs, its expression in reactive astrocytes probably includes a more prominent role in structural reorganization [21, 23, 53]. Clearly, future studies must evaluate the specific sodium current (in)activation properties of normal astrocytes and of reactive cells measured in slices obtained after surgical removal of epileptic brain tissue. Changes in the functional properties of ion channels in acute hippocampal slices obtained from patients with temporal lobe epilepsy have been recently reported [20].

β1 protein expression responds to specific changes in the astrocytes's microenvironment in vitro

In vitro studies were performed to identify the molecules that mediate the up-regulation of β1 protein in reactive astrocytes. The effects of growth factors previously implicated in activation of glial cells on the expression of β1 protein were examined in astrocytes-enriched human cell cultures growing in a serum-free chemically defined medium. Under these culture conditions we had a precise control over the concentration of individual factors. Since our culture does not contain neurons, it cannot reproduce the complex glial-neuronal interactions that underlie gliosis in vivo. However, glial scars in vivo are also devoid of neurons, providing a relatively pure glial environment.

The present in vitro results demonstrate that the expression of β1 subunit can be modified according to the growth factor composition in the extracellular environment. We have shown that EGF and bFGF will increase β1 protein expression levels. Although EGF and bFGF are astrocyte mitogens [48], the observed increase in β1 protein is not simply related to an increase in cell number because astrocytes growing in the minimally supplemented SF did not show detectable β1 immunoreactivity. Moreover, the mitogenic effect was also accounted for by the Western blot analysis by running equal amount of protein per lane.

FGF and EGF-like growth factors, as well as their receptors have been shown to be elevated in a variety of pathological states associated with gliosis [13, 17, 37, 40]. Addition of these growth factors to human astrocytes in culture produces morphology that is reminiscent of reactive astrocytes evoked by injury in vivo. It is important to note here that differential NaCh β1 subunit mRNA expression has been reported in stellate and flat astrocytes cultured from rat cortex, showing β1 mRNA expression only in stellate astrocytes [33]. Moreover, treatments (such as exposure to cAMP analogs) associated with changes in cell morphology and induction of markers of living reactive astrocytes [41, 51], are able to increase the NaCh β subunit mRNA levels [35].

The observation that factors that induce astrocyte differentiation are also able to stimulate β1 subunit expression, suggests that common mechanisms may mediate this phenomenon. Considerable evidence is available about the signal transduction cascade initiated via EGF or bFGF receptors. Several pathways can be activated, including the MAPK pathway [19, 54]. This pathway has been shown to control astrocytic differentiation and to mediate several astrocytic functions [15, 27, 42, 43, 55]. We examined the involvement of MAPK pathway in the regulation of β1 subunit expression using PD98059, which is a selective inhibitor of MAPK kinase (MEK), the enzyme that activates MAPK [16]. The observation that PD98059 blocked the up-regulation of β1 protein in astrocytes cultured in the presence of growth factors suggests the involvement of this signaling pathway. In future studies, it will be interesting to further examine additional signaling pathways (e.g., the phosphatidylinositol-1-kinase pathway) activated by exposure to growth factors or cAMP analogs that could also contribute to the regulation of astroglial β1 subunit expression.

Whether the up-regulation of β1 subunits could play a critical role in the signal cascade for astroglial activation and related immune response under pathological conditions is at present unknown. The role of β1 subunits in the regulation of astrocytes function deserves further investigation in view of their dual functions as modulators of NaCh activity and as cell surface glycoproteins, which may act as CAMs independently of α subunits [21].

Modulation of the expression levels of astroglial β1 subunits by growth factors may be also relevant during brain development. The expression of the different NaCh β subunits is regulated independently of α throughout development [23, 44, 45]. The expression of EGF, bFGF and their receptors in astrocytes in vivo [36, 48] supports the possibility that astroglial β1 subunit expression is developmentally controlled and depends on the microenvironment of the cell, as seen in vitro.

Conclusions

The present study demonstrates that NaCh β1 subunit protein is expressed in human astrocytes in vivo. Its expression is plastic and associated with changes in astroglial cell morphology occurring in various pathological conditions associated with gliosis. In vitro experiments indicate that growth factors, cytokines and other factors released by neurons and glia in response to injury may regulate the levels of specific glial β1 protein, modifying the astrocytic response over a prolonged time. This regulation may represent a novel mechanism for modulation of astroglial function and for changes in glial-neuronal communication in gliosis-associated pathologies.

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