

β 1B subunit of voltage-dependent Ca^{2+} channels is predominant isoform expressed in human neuroblastoma cell line IMR32

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Received 3 October 1997; revised version received 13 November 1997

Abstract Human neuroblastoma cells (IMR32) respond to treatment with either dibutylryl-cAMP or nerve factor by acquiring a neuronal phenotype which is accompanied by a marked increase in the density of neuronal (N-type) VDCC currents. Using IMR32 cells as a model for neuronal differentiation, we were interested in examining possible changes in the level of expression of the α 1B subunit of N-type calcium channels as well as beta subunit isoforms. Upon differentiation with dibutylryl-cAMP and 5-bromo-2-deoxyuridine for 16 days, we observed a dramatic increase in α 1B protein which initiated between day 8 and 10. Day 10 evidenced maximal expression of α 1B protein, which was followed by an interval of relatively constant expression of α 1B (day 12 to day 16). Monitoring beta subunit expression using a pan specific anti-beta antibody (Ab CW20), we observed an increase in expression of a single 82 kDa beta subunit. The predominant 82 kDa beta subunit expressed throughout the course of differentiation was identified as the β 1b isoform using a panel of beta subunit specific antibodies. Of significance, neither the β 2 nor β 3 isoforms were detected in full differentiated IMR32 cells. Contrary to a previous report on the absence of neurotypic expression of VDCC beta subunits in a second model for in vitro differentiation, NGF-treated rat pheochromocytoma cells (PC12 cells) [1], we report the regulated expression of the β 1b protein in differentiated IMR32 cells suggesting a cell specific function for this beta subunit which parallels the acquisition of the neuronal phenotype. The restrictive expression of the β 1b in IMR32 cells may reflect a cell-type specific function that extends beyond its role as an auxiliary subunit of VDCC complexes.

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Key words: Calcium channel; Beta subunit; Differentiation; Neuronal phenotype; Neuroblastoma

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Abbreviations: VDCC, voltage-dependent calcium channel; α 1 subunit, 190–240 kDa protein subunit of VDCC; α 1B subunit, 230 kDa protein subunit of N-type VDCC; β 1b, β 2, β 3 and β 4 subunits: 50–85 kDa subunits of VDCC; [¹²⁵I]CTX, [¹²⁵I]Tyr-22-omega-conotoxin GVIA; Na,K-ATPase, sodium and potassium ion-stimulated adenosine triphosphatase; mAb 9A7, monoclonal antibody to Na,K-ATPase alpha subunit; α 1, α 2, α 3, 110–105 kDa alpha subunit isoforms of the Na,K-ATPase; BdUR, 5-bromo-2-deoxyuridine; dibutylryl-cAMP, dibutylryl-adenosine 3',5'-cyclic monophosphate; ECL, enhanced chemiluminescence; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; KPi, monobasic potassium phosphate; PAGE, polyacrylamide gel electrophoresis; NGF, nerve growth factor; PMSF, phenylmethanesulfonyl fluoride; protease inhibitor cocktail, 1 mM DTT, benzamidine at 189 μ g/ml, leupeptin at 2 μ g/ml, aprotinin at 2 μ g/ml, pepstatin at 2 μ g/ml, 0.2 mM PMSF, and calpain inhibitors I and II at 1 μ g/ml; TBS, Tris-buffered saline

1. Introduction

The process of neurodifferentiation involves changes in cell morphology, the extension of neurites, and the establishment of synaptic connections requiring the acquisition of proper cellular 'machinery', including secretory apparatus, receptors and ion channels. While the immature central and peripheral nervous systems provide authentic starting points from which to investigate this process, the complexity of molecular events and the array of different signaling processes make it extremely difficult to study specific factors that influence differentiation. Therefore, several cell lines have been developed to model neurodifferentiation and include rat pheochromocytoma cells (PC12) and human neuroblastoma cells (IMR32) [2]. Both of these cell lines in the presence of nerve growth factor (NGF) and other agents are induced to obtain a sympathetic neuronal phenotype [3]. It is known that the differentiation of excitable cells is accompanied by changes in electrical properties, including the increased expression of potassium [4], sodium and calcium channels [5,6]. PC12 cells exhibit both dihydropyridine (DHP)-sensitive L-type, ω -conotoxin-sensitive N-type channels [7] and have been recently shown to also express an ω -agatoxin-IVA-sensitive (P/Q) type component [1]. The preferential localization of DHP-sensitive L-type channels to the cell soma, with N-type channels present in growth cones from these cells hinted at a possible role of the N-type channel in neurite development [8]. An early role of calcium current in the regulation of soma and neurite outgrowth, rather than solely as a trigger for neurotransmitter release has been suggested by earlier experiments [9].

A similar pattern of expression and localization of the N-type VDCC has been observed in rat brain wherein N-type channels were detected along nerve processes and nerve terminals by immunohistochemical localization [10]. Immunohistochemical and biochemical characterization of the N-type calcium channel complex from brain shows it to consist of at least three subunits (α 1, β , α 2/ δ) [11–14]. To date at least four different beta subunit genes (β 1, β 2, β 3, β 4) have been cloned [15–19]. The apparent molecular weights of the translated beta subunit cDNAs fall into 'high' molecular weight beta subunits (β 1 and β 2, with anticipated apparent molecular weights of approximately 66 kDa and 69 kDa, respectively) and 'low' molecular weight beta subunits (β 3 and β 4, with apparent molecular weights of approximately 55 kDa and 58 kDa, respectively) [15–19]. Reports that investigated the subunit composition of N-type [12] and L-type VDCC [20] in brain homogenates have concluded that the beta subunits do not preferentially assemble with specific subunits. Moreover, de-

spite the intense interest in determining the structural features which contribute to the formation of the specific $\alpha 1$, $\alpha 2/\delta$ and β subunit complexes, the underlying trafficking and sorting mechanisms remain elusive [12,21]. In a more simplified system such as rat PC12 cells, the N-type calcium channel is predominantly associated with the $\beta 2$ and $\beta 3$ subunits [1]. However, it was difficult to determine if this association simply reflects the relative abundance of these beta isoforms in PC12 cells rather than a specific $\alpha 1\beta$ -beta subunit enrichment. Interestingly, despite increases in the level of $\alpha 1\beta$ subunit (ω -conotoxin binding subunit of the N-type VDCC) expressed upon NGF-induced differentiation, there was no change in the relative contribution of either $\beta 2$ or $\beta 3$ subunit to the immunoprecipitated N-type VDCC complex [1].

There is very little known concerning N-type calcium channel beta subunit composition or the role it might play in IMR32 cell differentiation. In differentiating IMR32 cells, the transient appearance of a low voltage-activated calcium channel is followed by a marked increase in the appearance of N-type calcium channels as measured electrophysiologically and monitored by [125 I]CTX binding [22]. The purpose of this study is to examine the expression of the $\alpha 1\beta$ subunit as well as the resident beta subunits which are present in IMR32 cells with an eye towards determining the subunit structure of the endogenous N-type VDCC complex. Here we report that cAMP-induced IMR32 cells show a specific increase in $\beta 1b$ subunit expression which parallels the increase in N-type calcium channel expression during neurodifferentiation.

2. Materials and methods

Aprotinin, pepstatin, calpain inhibitors I and II, and choline chloride were obtained from Calbiochem. Enhanced chemiluminescence kit (ECL) was purchased from Amersham, unlabeled omega GVIA conotoxin from Peninsula Laboratories, [125 I]Tyr-22- ω -conotoxin GVIA from New England Nuclear. Nitrocellulose membranes (0.45 μ m) were obtained from S and S. All secondary antibodies were from Boehringer Mannheim. Bovine serum albumin was from United States Biochemicals, Inc. HEPES was from Research Organics. BCA protein assay reagents and Sulfolink columns were obtained from Pierce, dialysis tubing (12–14 kDa cut-off) was obtained from Fisher. All other reagents were obtained from Sigma Chemicals.

2.1. Production of anti-peptide antibodies to VDCC $\alpha 1\beta$ and β subunits

Anti- $\alpha 1\beta$ subunit antibodies (Ab CW14) were raised to amino acid residues 1051–1069 in the rat $\alpha 1\beta$ [23] which were present in all N-type $\alpha 1$ subunit cDNAs cloned to date [24–26]. Anti-beta subunit 'generic' antibodies (Ab CW20) were raised to a highly conserved sequence (ESYTSRPSDSDVSLEEDRE) present in all beta subunits cloned to date. The anti- $\beta 4$ antibody was raised to a 16 amino acid peptide (ENYHNERKSRNRLS) specific to the $\beta 4$ subunit [27]. These peptide antigens were synthesized to include a unique cysteine residue to be used both in the unambiguous attachment of peptide to carrier protein (maleimide-derivatized KLH) and to the affinity column (Sulfolink). The peptides were purified, and coupled to maleimide-activated KLH and used in the production of polyclonal sera in rabbits bled twice per month (15–20 ml/bleed) and tested after four weeks.

2.2. Preparation of peptide columns immobilized via CYS residue and affinity purification of anti- $\alpha 1\beta$ (CW14), anti- $\beta 4$ and anti- β generic (Ab CW20) antibodies

The free, reduced peptides (1.5 to 3 mg in 2 ml volume) were coupled to a Sulfolink column (Pierce) according to the manufacturer's instructions. The coupling efficiency was 94–99% as determined by dot blotting and Ellman's assay. Antibodies were affinity purified by diluting crude antisera with 3% BSA in 1 \times TBS and incubating with peptide column for 90 min at room temperature. The column was

then washed sequentially with TBS, 30 mM glycine pH 5, and 80 mM glycine pH 4. The affinity purified antibodies were eluted from the column with 200 mM glycine pH 2.5 and immediately neutralized by the addition of 1 M Tris pH 12.0. The antibody fractions were pooled and dialyzed overnight against TBS plus 1 mM EGTA. The antibodies were dialyzed against an additional volume of TBS, aliquoted and stored at -80°C . Affinity purified Ab CW20 reacted with all recombinant beta subunit isoforms expressed in HEK293 cells (data not shown) and affinity purified Ab CW14 reacted with the 230 kDa $\alpha 1\beta$ subunit present in human, rat, rabbit and mouse samples (data not shown). The anti- $\beta 1b$, anti- $\beta 2$, and anti- $\beta 3$ isoform specific antibodies used in this study have been characterized elsewhere [20,28].

2.3. Differentiation of human neuroblastoma cells (IMR32 cells)

IMR32 human neuroblastoma cell line was obtained from the ATCC and grown in Eagle's minimal essential medium supplemented with 100 units of penicillin per ml and 100 μ g of streptomycin and 10% fetal bovine serum. Cells were plated at a density of $10^4/\text{cm}^2$ in plastic dishes or flasks and cultured at 37°C in a 5% CO_2 humidified atmosphere. Differentiation of IMR32 cells was carried out by treating IMR32 cells with a mixture of 1 mM dibutyryl-cAMP and 2.5 μ M 5-BdUR for a period of 16 days as described [29]. Cells were harvested into PBS plus copious protease inhibitors prepared as follows: PMSF (1/1000 dilution from 200 mM stock in ethanol), calpain inhibitors I and II (1/1000 dilution from 4 mg/ml stock), benzamide (1/500 dilution of 200 mM stock), aprotinin (1/500 dilution from 1 mg/ml stock), leupeptin (1/500 dilution from 1 mg/ml stock), pepstatin (1/500 dilution from 1 mg/ml stock). The IMR32 cell differentiation and parallel Western blot analyses were carried out in three independent experiments on cells of different passage number with similar results.

2.4. Preparation of tissue homogenates and membranes

Adult rats were killed by euthanasia and the forebrains and other organs removed and immediately placed in 50 mM HEPES pH 7.4, 1 mM EGTA plus protease inhibitors as described [11]. For subsequent use in Western blot analysis, all samples were stored in -20°C at concentrations of 2 mg/ml in sample buffer (5 \times sample buffer: 325 mM Tris pH 7.0, glycerol (25% v/v), mercaptoethanol (25% v/v), SDS (10%) in 100 μ l aliquots. The samples were frozen once, used and then discarded.

2.5. Methods for [125 I]CTX binding

[125 I]CTX ([125 I-Tyr-22]- ω -conotoxin) GVIA (specific activity 2000 Ci (81.4 TBq)/mmol) binding was assayed by published procedures [30] which use filtration over PEI-soaked glass fiber filters (Whatman GF/B) to separate bound from unbound ligand in the presence of bovine serum albumin (BSA). Unlabeled CTX added was from 0.1 pM to 50 nM in the presence of constant [125 I]CTX (4.2–5 pM). The values reported in the text represent the average of more than five independent determinations done in duplicate \pm S.E.M.

2.6. Western blot analysis and general methods

Gels were transferred to nitrocellulose at 0.45 A for 17 to 22 h, the filters blocked in 5% powdered milk in 1 \times TBS+0.01% sodium azide+0.05% Tween. The primary antibody was diluted in 3% BSA and 1 \times TBS and incubated with the filter overnight at 4°C . Various preparation of affinity purified anti- $\alpha 1\beta$ and anti- β antibodies were

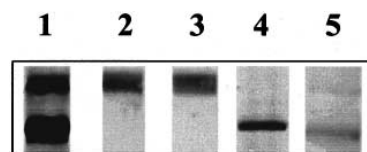


Fig. 1. Identification of rat brain β subunits by generic anti-beta antibody and beta isoform specific antibodies. Rat forebrain (75 μ g/lane) was resolved on a 4–17% gradient gel, transferred to nitrocellulose and probed with Ab CW20 (lane 1) and isoform specific antibodies to the $\beta 1b$ (lane 2), $\beta 2$ (lane 3), $\beta 3$ (lane 4) and $\beta 4$ (lane 5). The position of the subunits was detected with goat anti-rabbit secondary antibodies conjugated to HRP and visualized by ECL as described in Section 2.

used throughout the course of these experiments with identical results. The filters were washed 3 times with $1 \times$ TBS with constant shaking at room temperature. The secondary antibody was diluted in 3% BSA in $1 \times$ TBS and the filter was incubated for 45 min at room temperature with constant shaking. The filters were washed as before. The antigen was visualized using the ECL detection method as described by the suppliers. Membrane protein and soluble protein were measured by standard procedures [31]. Bovine serum albumin was used as a standard in all cases. Gel electrophoresis was carried out according to standard procedures [32].

3. Results

3.1. Expression of beta subunits in adult rat brain as detected by beta generic and beta isoform specific antibodies

Prior to carrying out these studies to determine the pattern of expression of beta subunits in differentiating IMR32 cells as detected by our beta generic antibody, Ab CW20, we needed to compare the pattern of reactivity obtained with Ab CW20 to a panel of antibodies specific for the individual beta isoforms [20,28]. As shown in Fig. 1, the Ab CW20 yields a signal which can be discriminated into 'high' and 'low' molecular weight beta subunits. Using the antibodies specific for the $\beta 1b$, $\beta 2$, $\beta 3$ and $\beta 4$ isoforms, it is clear that the $\beta 1b$ and $\beta 2$ isoforms (lanes 2 and 3) comprise the upper band of Ab CW20 reactivity while the $\beta 3$ and $\beta 4$ (lanes 4 and 5) comprise the lower band of antibody reactivity. It is of interest that the $\beta 3$ and $\beta 4$ isoforms tended to migrate as narrow bands while the mobility of the $\beta 1b$ and $\beta 2$ isoforms was broad and diffuse.

3.2. Increased expression of N-type $\alpha 1B$ and β subunits in differentiating IMR32 cells

Ab CW14, an anti-peptide antibody which reacts with a 230 kDa protein present only in neuronal samples, and does not cross-react with the L-type VDCC present in skeletal muscle, was used to determine the expression level of $\alpha 1B$ protein in human IMR32 cells. Western blot analysis of differentiating IMR32 reveals a dramatic upregulation of $\alpha 1B$ between day 8 and day 10 of treatment with cAMP analogs (Fig. 2A). This dramatic upregulation of N-type $\alpha 1B$ subunit protein parallels the acquisition of [125 I]CTX binding sites during differentia-

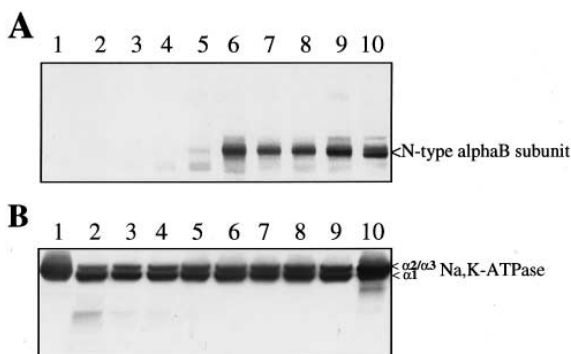


Fig. 2. Increased expression of N-type $\alpha 1B$ subunit following differentiation of IMR32 cells. IMR32 cells were differentiated as described in Section 2. Cells were harvested as described and resolved on a 4–17% gradient gel, transferred to nitrocellulose and probed with Ab CW14 (1/100 dilution, Panel A) or mAb 9A7 (1/10 000 dilution, Panel B). The samples are as follows: lane 1: day 0; lane 2: day 2; lane 3: day 4; lane 4: day 6; lane 5: day 8; lane 6: day 10; lane 7: day 12; lane 8: day 14; lane 9: day 16; lane 10: adult rat forebrain. The protein concentration was 150 μ g/lane.

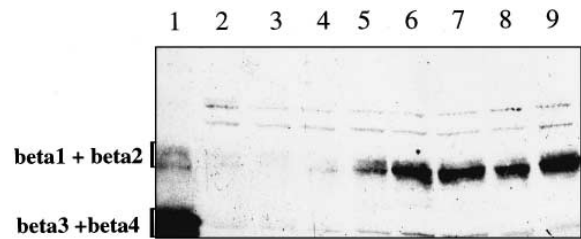


Fig. 3. A single beta subunit is predominantly expressed in differentiating IMR32 cells. IMR32 cells were differentiated as described in Section 2; Cells were harvested as described and resolved on a 4–17% gradient gel, transferred to nitrocellulose and probed with Ab CW20 (1/200 dilution). The samples are as follows: lane 1: adult rat forebrain; lane 2: day 0; lane 3: day 2; lane 4: day 4; lane 5: day 6; lane 6: day 10; lane 7: day 12; lane 8: day 14; lane 9: day 16. The protein concentration was 150 μ g/lane. The position of the authentic beta isoforms present in the rat brain samples are indicated.

tion as determined by Scatchard analysis (undifferentiated IMR32 cells $K_d = 10.5$ pM with $B_{max} = 3.5 \pm 1.0$ fmol/mg and day 14 differentiated IMR32 cells $K_d = 17.5$ pM with $B_{max} = 17.5 \pm 2$ fmol/mg). The increase in $\alpha 1B$ expression is even more striking when compared to either the constitutive pattern of expression of Na,K-ATPase $\alpha 1$ isoform (the lower band in the immunoreactive doublet shown in Fig. 2B) or the differentiation-dependent increase in expression of the $\alpha 3$ isoform (the upper band in the immunoreactive doublet shown in Fig. 2B) [33].

Our attention then focused on the pattern of beta isoforms expressed in differentiating IMR32 cells (Fig. 3). Ab CW20 reacts with all recombinant beta subunit isoforms expressed in HEK293 cells (data not shown) and evidences a pattern of reactivity in rat forebrain which is a composite of the reactivity obtained with beta isoform specific antibodies (Fig. 1). Using Ab CW20, we detected a single immunoreactive protein of approximately 82 kDa in differentiated IMR32 cells (Fig. 3). This apparent size is consistent with it being either the $\beta 1b$ or $\beta 2$ isoform (Fig. 1). In addition, there was a faint signal which corresponded to a beta subunit of 55 kDa which could be indicative of one of the smaller beta subunits such as the $\beta 1c$, $\beta 3$ or $\beta 4$ isoforms or perhaps a proteolytic remnant of the $\beta 1b$. There was a very good temporal correlation between the intensity of the 82 kDa beta subunit and the appearance of the $\alpha 1B$ subunit.

3.3. The $\beta 1b$ subunit increases upon differentiation of IMR32 cells

To identify the 82 kDa beta subunit which was observed to greatly increase upon differentiation of IMR32 cells and to analyze the pattern of expression of beta subunits in developing rat brain, we used antibodies which specifically recognize the $\beta 1b$, $\beta 2$ and $\beta 3$ isoforms. As shown in Fig. 4, the $\beta 1b$ isoform was detected as the major beta isoform and was shown to increase in representative samples of differentiating IMR32 cells. In addition to the 82 kDa $\beta 1b$ detected, there was an unidentified protein with a higher apparent molecular weight which was also detected by the anti- $\beta 1b$ antibody in all IMR32 samples and the rat forebrain sample. The immunostaining of $\beta 1b$ and this unidentified protein was fully reversed by excess peptide. This unidentified protein is currently under investigation. There was a very faint signal obtained using anti- $\beta 3$ antibodies in the differentiated IMR32 cell sample

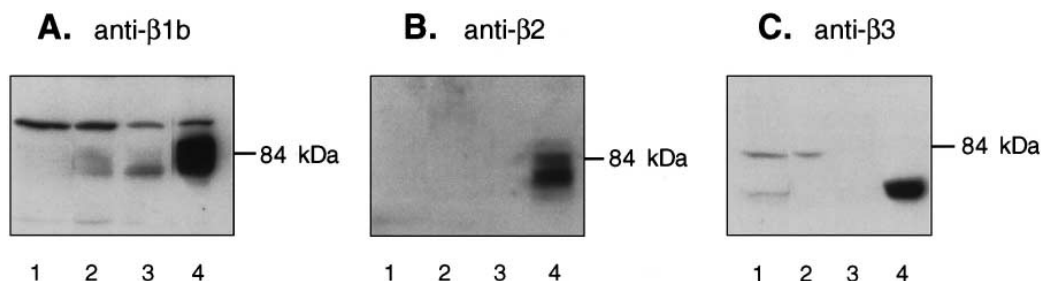


Fig. 4. Detection of beta isoforms in differentiating IMR32 cells. IMR32 cells were differentiated as described in Section 2, resolved by PAGE on 10% acrylamide gels and transferred to nitrocellulose. Adult rat forebrain was included as a positive control. Representative panels were probed with the following beta isoform specific antibodies: Panel A: anti- β 1b (1 μ g IgG/ml), Panel B: anti- β 2 (1 μ g IgG/ml); and Panel C: anti- β 3 (1 μ g IgG/ml). The representative samples are as follows: lane 1: differentiated IMR32 cells (day 4); lane 2: differentiated IMR32 cells (day 10); lane 3: differentiated IMR32 cells (day 14); lane 4: adult rat forebrain. The protein concentration was 150 μ g/lane.

(Fig. 4, lane 3). Interestingly, the anti- β 3 antibody identified a second protein with an apparent molecular weight of 82 kDa in day 4 and day 10 differentiated IMR32 cells. While the β 2 isoform was not detected in IMR32 cells, adult rat brain was positive for β 2 expression. It can thus be concluded from these experiments that the β 1b is the predominant protein detected by Ab CW20 in the differentiated IMR32 cell samples.

Of additional note is the difference in the mobility of the β 1b expressed in IMR32 cells compared to the β 1b endogenous to rat brain (Fig. 4, panel A). The beta subunit present in IMR32 cells as detected by both the anti- β 1b antibody (Fig. 4) and the beta generic antibody Ab CW20 (Fig. 3) is a discrete band as resolved by SDS-PAGE. In contrast, the immunoreactive β 1b protein in adult rat brain migrates broadly (Figs. 3 and 4).

4. Discussion

The human neuroblastoma cell line IMR32 has recently become an accepted cellular model for the acquisition of the neuronal phenotype in culture [2]. IMR32 cells exhibit a variety of calcium channel activities (L-, N- and T-type) [22] suggesting that this cell line harbors a multitude of calcium channel α 1 subunit isoforms, which when assembled with beta subunits, constitute the active channel complexes. The beta subunit which comprises these assembled calcium channel complexes remains undetermined.

The acquisition of active N-type VDCC as previously described [22,34,35] coincide well with our results on the temporal expression of the α 1B subunit during IMR32 cell differentiation. It is interesting to point out that the level of α 1B reached a maximum level of expression at a time (day 10) which coincides with maximal expression of N-type current [22] and then, following a slight decrease observed on day 12, the level of α 1B was maintained at a constant level (day 12–day 16). This is an interesting observation as previous studies have suggested that changes in the level of N-type VDCC throughout differentiation reflected changes in protein turnover rates [34]. Similar effects upon [125 I]CTX-labeled N-type VDCC stability were seen in SY5Y cells differentiated in the presence of retinoic acid [34].

The central issue we sought to address in this study was to identify the beta subunit that is expressed in parallel with α 1B. Prior studies on the purified N-type channel complex from adult rabbit brain first suggested an exclusive association of the β 3 isoform with the α 1B [12]. However, it was subsequently shown that the N-type calcium channel can associate

with different beta subunits (β 1b, β 3, β 4) [21]. In differentiating PC12 cells, both the β 2 and β 3 isoforms were detected in association with the α 1B subunit, but their level of expression did not change upon treatment with NGF [1]. Therefore, to streamline our efforts to probe for beta subunit content in IMR32 cells, it was critical to these studies to employ an anti-beta antibody (Ab CW20) which could monitor all beta subunits simultaneously.

The data presented in this study demonstrate the singular expression of the β 1b protein in differentiating IMR32 cells. Significantly, neither the β 2 nor the β 3 were detected in differentiated IMR32 cells. The coordinate expression of the α 1B subunit with the β 1b strongly suggests that the resident N-type VDCC is comprised of the α 1B and β 1b. Our observation of dramatic increases in α 1B and β 1b protein expressed during IMR32 cells differentiation supports previous studies which sought an explanation for the overall increase in calcium channel expression measured by [125 I]CTX radioligand binding [34]. In contrast to the dramatic increase in expression of the α 1B and the β 1b subunits demonstrated in this study, previous reports have identified the α 2 subunits in undifferentiated IMR32 cells [36]. Interestingly, in vitro expression studies have demonstrated α 2- δ coexpression modulates the binding affinity of CTX when coexpressed with both α 1B and β 1b message in human embryonic kidney cells (HEK293 cells) [37]. Therefore, it is of interest to further investigate the time course of expression of the IMR32 cell α 2 subunit in response to differentiation.

The comparable level of total α 1B and the β 1b subunits throughout differentiation may therefore reflect their coordinate expression as well as their association. A mechanism for the regulation of cell surface expression of N-type channels in IMR32 cells might also be controlled through the level of beta subunit expression as in the case of alpha/beta assembly found for Na,K-ATPase [38]. The alteration in surface expression of [125 I]CTX receptors which occurred upon introducing anti-sense β 1b oligonucleotides into IMR32 cells [39] implicates the β 1b in regulating the density of surface N-type VDCC and is consistent with the specific increase in β 1b expression associated with N-type channel expression in our experiments. The impact of this single population of β 1b subunits upon the subunit composition of the other VDCC requires further investigation.

Until recently, the heterogeneity in beta subunit isoforms has prompted studies focused primarily upon determining their role in calcium channel regulation and modulation. However, another role for beta subunits has come to light

from studies in which the expression of beta has been eliminated by either anti-sense competition [40] or genomic 'knock-outs' [41]. Inhibition of beta subunit synthesis by knocking out endogenous beta subunits expression in *X. laevis* oocytes prevented both channel formation and $\alpha 1E$ expression [40]. It was speculated that at low levels of beta subunit expression provided by the oocyte allowed for a beta subunit 'chaperone' effect for the transport of the $\alpha 1$ to the cell surface while higher levels of beta subunit expression were required for a mature $\alpha 1E$ -beta subunit complex. In case of the genomic 'knock-out' of the beta subunit in skeletal muscle, the absence of the beta subunit lead to the absence of $\alpha 1_s$ expression, disorganization of the T-tubule membrane and decreased L-type current [41]. In contrast, in dysgenic mice which do not express $\alpha 1_s$, the beta subunit is transported to the membrane [41]. Similarly, there is a requirement for $\alpha 1_s$ and beta subunit interaction for the localization of the DHP receptor to the plasma membrane of transfected non-muscle cells [42]. Thus, the differentiation-dependent expression of the $\beta 1b$ subunit in IMR32 cells may reflect an increased demand for specific proteins which function in the trafficking of VDCC subunits through the cell. In conclusion, it is interesting to speculate that the unitary expression of the $\beta 1b$ subunit in IMR32 cells may reflect a role for this protein which extends beyond its role as a component of a mature VDCC channel.

Acknowledgements: The authors gratefully acknowledge Dr. Gary Landreth, Dept. of Neuroscience, CWRU for the use of his tissue culture facility, Ms. Catherine M. Begg for expert technical assistance, and funding from the LHIMRF (M.W.M.), NIH (M.W.M.), American Heart Association (M.W.M.), and the Deutsche Forschungsgemeinschaft Ha 1773/3-1 (H.H.). M.W.M. is an Established Investigator of the AHA.

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