Mol. Cells *27*, 113-118
DOI/ 10.1007/s10059-009-0012-4

Communication

Molecules and Cells

Valproic Acid Induces Transcriptional Activation of Human GD3 Synthase (hST8Sia I) in SK-N-BE(2)-C Human Neuroblastoma Cells

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In this study, we have shown the transcriptional regulation of the human GD3 synthase (hST8Sia I) induced by valproic acid (VPA) in human neuroblastoma SK-N-BE(2)-C cells. To elucidate the mechanism underlying the regulation of hST8Sia I gene expression in VPA-stimulated SK-N-BE(2)-C cells, we characterized the promoter region of the hST8Sia I gene. Functional analysis of the 5′-flanking region of the hST8Sia I gene by the transient expression method showed that the -1146 to -646 region, which contains putative binding sites for transcription factors c-Ets-1, CREB, AP-1 and NF-κB, functions as the VPA-inducible promoter of hST8Sia I in SK-N-BE(2)-C cells. Site-directed mutagenesis and electrophoretic mobility shift assay indicated that the NF-κB binding site at -731 to -722 was crucial for the VPA-induced expression of hST8Sia I in SK-N-BE(2)-C cells. In addition, the transcriptional activity of hST8Sia I induced by VPA in SK-N-BE(2)-C cells was strongly inhibited by SP600125, which is a c-Jun *N*-terminal kinase (JNK) inhibitor, and GÖ6976, which is a protein kinase C (PKC) inhibitor, as determined by RT-PCR (reverse transcription-polymerase chain reaction) and luciferase assays. These results suggest that VPA markedly modulated transcriptional regulation of hST8Sia I gene expression through PKC/JNK signal pathways in SK-N-BE(2)-C cells.

INTRODUCTION

Valproic acid (VPA), a simple branched-chain fatty acid, is widely used in the treatment of epilepsy and bipolar disorders due to its anticonvulsant activity (Rogawski and Loscher, 2004). Like other short chain fatty acids such as phenylbutyrate and phenylacetate, VPA possesses anti-cancer properties which are exerted through several mechanisms including inhibition of cell growth as well as the induction of cellular differentiation and apoptosis (Blaheta et al., 2005; Johannessen, 2000). Recent studies have suggested that these effects are at least partly due to VPA-mediation through the inhibition of histone deacetylases (HDACs), which causes hyperacetylation and subsequent de-repression and transcriptional activation of genes that are silenced in cancer (Gottlicher et al., 2001; Gurvich et al., 2004; Phiel et al., 2001).

Gangliosides are sialic acid-containing glycosphingolipids found in the outer leaflets of vertebrate plasma membranes; they are particularly abundant in cells of the central nervous system (Svennerholm, 1980). They play important roles in multiple biological processes, such as cell-cell interaction, adhesion, cell differentiation, growth control, and receptor function (Hakomori and Igarashi, 1993; Varki, 1993). In addition, they have been studied as brain tissue markers in various mammals (Hakomori and Igarashi, 1993; Svennerholm, 1980), as well as tumor markers in neuroectoderm-derived cells (Hakomori, 1981) and neuroblastoma cells (Cheung et al., 1985).

Recent reports demonstrated that VPA could modulate the mRNA expression of two polysialyltransferases (ST8Sia II and IV) responsible for polysialylation of neural cell adhesion molecule (NCAM) in human neuroblastoma cells (Beecken et al., 2005). NCAM is downregulated during the migratory phase of brain tumor invasion, whereas ganglioside expression is upregulated (Gratsa et al., 1997), but it is not known whether VPA-mediated changes in sialyltransferase gene expression are responsible for this ganglioside upregulation.

To answer this question, we designed the present study, in which we investigated whether VPA modulated human GD3 synthase (hST8Sia I) mRNA expression, and whether such an effect correlated with ganglioside GD3 biosynthesis in human neuroblastoma cells. We demonstrated that VPA did indeed induce hST8Sia I mRNA expression in the human neuroblastoma cell line SK-N-BE(2)-C. Furthermore, we functionally characterized the mechanism by which the promoter region of hST8Sia I upregulated transcription of a luciferase reporter in response to VPA.

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Received June 13, 2008; revised October 7, 2008; accepted October 13, 2008; published online October 13, 2008

Keywords: human GD3 synthase, SK-N-BE(2)-C, transcription factor, Valproic acid

MATERIALS AND METHODS

Cell cultures

The human neuroblastoma cell line SK-N-BE(2)-C, obtained from American Type Culture Collection (USA) was maintained at 37°C in a 5% CO_2 incubator and cultured in Dulbecco's modified Eagle's medium (DMEM; WelGENE Co., Korea) containing 1 mM sodium pyruvate and 1x MEM nonessential amino acids, supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 10% (v/v) fetal bovine serum (Gibco BRL, Life Technologies, USA). To induce expression of the hST8Sia I gene with VPA, SK-N-BE(2)-C cells were cultured for various time periods in the presence of 5 mM VPA.

Evaluation of ganglioside GD3 surface expression by VPA

To analyze ganglioside GD3 levels on the cell membrane, SK-N-BE(2)-C cells were seeded on round cover slips (pretreated with 2% 3-aminopropyl-triethoxysilan) in a 8-well multi-plate. Upon reaching confluence, cells were given 5 mM VPA for 6 to 12 h. After various incubation times, cell cultures were washed twice with distilled PBS, fixed with 4% room-temperature PFA (paraformaldehyde), washed again with PBS, and blocked (5% BSA in PBS). Cells were then incubated overnight at 4°C with a GD3 monoclonal antibody (mouse IgM, Kappa-chain, clone: GMR19; Seigakagu; Japan) diluted 1:100 with 5% BSA in PBS. After another wash, cells were labeled with 1:100 FITC-conjugated goat anti-mouse IgM (Sigma; USA) as a secondary antibody. The samples were mounted with glycerol and analyzed by confocal laser-scanning microscope (LSM 510; Zeiss; Germany).

Reverse transcription-polymerase chain reaction (RT-PCR)

Using Trizol reagent (Invitrogen; USA), total RNA was isolated from SK-N-BE(2)-C cells treated with a vehicle control or VPA, and 1 μg of the RNA was subjected to reverse transcription with oligo (dT), utilizing a Takara RNA PCR kit (Takara Bio; Japan) according to the manufacturer's protocol. The resulting cDNA was amplified by PCR with specific hST8Sia I primers (460 bp), 5′-TGTGGT-CCAGAAAGACATTTGTGGACA-3′ (sense) and 5′-TGGAGT-GAGGTATCTTCACATGGGTCC-3′ (anti-sense); β-actin (247 bp), 5′-CAAGAGATGGCCACGGCTGCT-3′ (sense) and 5′-TCCTTCTGCATCCTGTCGGCA-3′ (anti-sense) was also amplified as a loading control. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. The intensity of the bands obtained from the RT-PCR product was estimated with a Scion Image Instrument (Scion Corp.; USA). The values were calculated as a percent of the control and are expressed as means ± SD.

Quantitative real-time PCR

Total cellular RNAs and single-stranded cDNAs were prepared, as described above, from SK-N-BE(2)-C cells treated with a vehicle control or VPA. Real-time PCR was performed on a Rotor Gene 3000 (Corbett Research) with the QuantiTect® SYBR®Green PCR Master Mix (Qiagen). Samples were standardized to the β-actin mRNA level. The transcripts of the hST8Sia I gene were detected by the quantitative real-time PCR approach using the primers 5′-TTCAACCTCTCTCTT-CCCACA-3′ (sense) and 5′-TCTTCTTCAGAATCCCACCATT-3′ (antisense) from hST8Sia I sequences (GenBank accession no. D26360.1). The transcript copy number of the hST8Sia I gene was normalized to the β-actin transcript copy number for each sample. As a standard control, the β-actin gene was amplified by the primers 5′-ACCCACTCCTCCACCTTTGAC-3′ (sense) and 5′-CCTGTTGCTGTAGCCAAATTCG-3′ (antisense) from human β-actin (GenBank accession no. NM_001101.3). Real-time PCR amplification of the hST8Sia I and β-actin genes was carried out for 50 cycles of 94°C for 10 s, 58°C for 15 s, and 72°C for 15 s.

Cell proliferation assay

Cell proliferation was investigated using a commercially available proliferation kit II (XTT; Boehringer Mannheim GmBH, Mannheim. Germany). Briefly, the cells were suspended in culture medium, plated in 96-well culture plates at a density of 3×10^4 cells per well, and incubated for 24 h in a 37°C humidified incubator under an atmosphere of 5% CO_2. The medium was replaced with 100 μl of serum-free DMEM culture medium containing various concentrations (0-10 mM) of VPA. After incubation for 12 h, each well received 50 μl of the XTT reaction solution, which was sodium 3′-[1-(phenyl-aminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate and *N*-methyl dibenzopyrazine methyl sulfate; mixed in a 50:1 ratio (5 ml of XTT-labeling reagent and 100 μl of electron coupling reagent). After 4 h of incubation in 37°C and 5% CO_2 incubator, absorbance was measured on an ELISA plate reader (Bio-Rad; USA) at the test wavelength of 490 nm. All determinations were confirmed using at least three identical replicates. The data shown are the means ± SD of three independent experiments.

Transfection and luciferase assay

The luciferase reporter plasmids used herein, namely pGL3-2646/-646 and its derivatives (pGL3-1146/-646 to pGL3-2246/-646) with base substitutions in the CREB, AP-1, c-Ets-1, NF-κB binding sites, have been described elsewhere (Kang et al., 2006). To analyze hST8Sia I promoter activity in response to VPA treatment, SK-N-BE(2)-C cells (3.0×10^5 cells/well) were seeded in 24-well tissue culture plates and allowed to grow to 70% confluence, at which point they were transiently co-transfected with 0.5 μg of the indicated reporter plasmid and 50 ng of the control *Renilla* luciferase vector pRL-TK (Promega; Madison, WI, USA), using 1 μl Lipofectamine 2000 (Invitrogen). After a 12-h recovery in normal medium without VPA, the medium was changed to medium containing 5 mM VPA and incubated for an additional 12 h, after which cells were collected and treated with passive lysis buffer (Promega). Firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions, and a GloMax™ 20/20 luminometer (Promega). Firefly luciferase activity of the reporter plasmid was normalized to *Renilla* luciferase activity and expressed as a fold induction over the empty pGL3-Basic vector, used as a negative control. Independent triplicate experiments were performed for each plasmid.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed using a gel shift assay system kit (Promega) according to the manufacturer's instructions. Nuclear extracts of VPA-induced SK-N-BE(2)-C cells were prepared as described previously (Kang et al., 2006), and the protein concentrations of the extracts were determined using a Bio-Rad protein assay kit. EMSA probes were constructed as follows: Double-stranded oligonucleotides synthesized using for a set of oligonucleotides (Kang et al., 2006) encompassing binding site for the transcription factors NF-κB were end-labeled with [γ-^{32}P] dATP using T4 polynucleotide kinase and used as probes for EMSA.

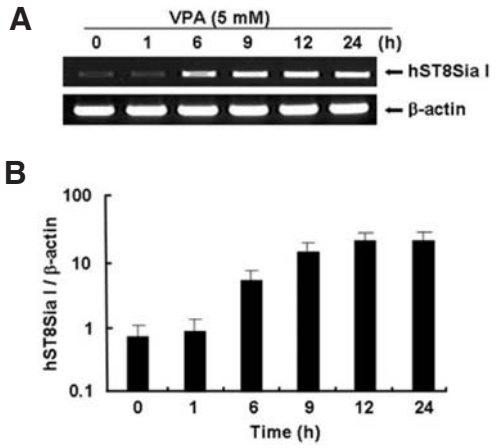

Fig. 1. Effects of VPA on levels of hST8Sia I mRNA expression in SK-N-BE(2)-C cells. (A) Total RNA from SK-N-BE(2)-C cells was isolated after 0, 1, 6, 9, 12 or 24 h of VPA treatment, and hST8Sia I mRNA was detected by RT-PCR. As an internal control, parallel reactions were performed to measure the levels of the housekeeping gene β-actin. (B) hST8Sia I mRNA expression was analyzed by quantitative real-time PCR. The transcript copy number of hST8SIa I was normalized to the β-actin transcript copy number for each sample. Experiments were repeated five times to ascertain reproducibility of results. The *error bar* indicates standard error.

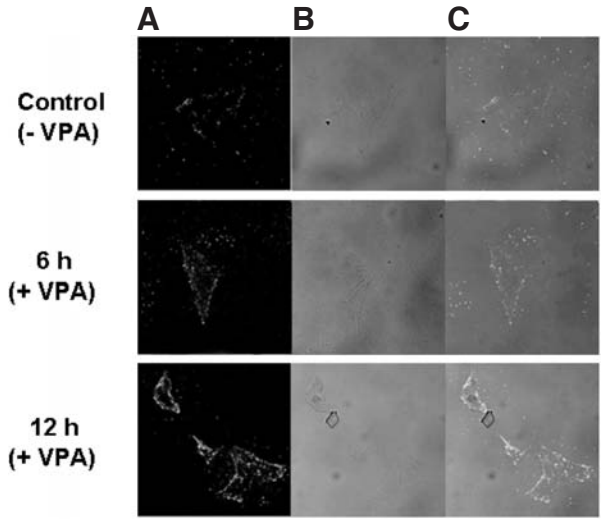

Fig. 2. Confocal analysis of VPA-mediated ganglioside GD3 levels. SK-N-BE(2)-C cells were grown in standard medium or in medium containing 5 mM VPA for 6 h or 12 h. A monoclonal antibody (GMR19) was used to analyze the resulting GD3 ganglioside levels. VPA treatment of the cells gradually induced a strong GD3 upregulation between 6 and 12 h, but the control without VPA induction for 12 h exhibited no response. (A), immunofluorescence image; (B), phase-contrast image; (C), merge

RESULTS

VPA induced hST8Sia I gene expression, and subsequent GD3 expression, in SK-N-BE(2)-C cells

To determine whether stimulation with VPA upregulated the hST8Sia I gene in SK-N-BE(2)-C cells, we treated the cells with varying doses of VPA for varying periods of time, and we examined the expression level of hST8Sia I mRNA using RT-PCR (reverse transcription-polymerase chain reaction) and quantitative real-time PCR. As shown in Fig. 1, hST8Sia I mRNA levels were higher in SK-N-BE(2)-C cells that received VPA. During stimulation with 5 mM VPA, hST8Sia I mRNA increased up to 24 h in a time-dependent manner. These results clearly showed that the expression of hST8Sia I was stimulated by VPA. Then, the SK-N-BE(2)-C cells were treated with various concentrations of VPA and the resulting cell viability was measured using the XTT assay. The resulting survival curve showed that VPA did not exert any cytotoxic effects on the proliferation of the cells. The addition of 5 mM VPA did not alter the viability of the cells, while treatment with 10 mM VPA reduced 12% of the cell viability only when compared to the control (data not shown).

To investigate whether VPA-mediated hST8Sia I induction increased cellular levels of the ganglioside GD3, we used immunofluorescent confocal microscopy to visualize GD3 expression in VPA-stimulated SK-N-BE(2)-C cells. We observed a higher GD3 expression in VPA-treated cells than in control cells not subjected to VPA treatment (Fig. 2).

Analysis of transcriptional activity of hST8Sia I promoter by VPA in SK-N-BE(2)-C cells

To determine whether the 5′-flanking sequence of the hST8Sia I gene contained a VPA-responsive promoter, we prepared luciferase constructs carrying serial 5′deletions of the hST8Sia I promoter, transfected them into SK-N-BE(2)-C cells, and then treated the transfected cells with VPA. We monitored the subsequent expression of the luciferase reporter gene using the dual-luciferase reporter assay system, after which we measured luciferase activity with a luminometer. As shown in Fig. 3A, cells harboring the pGL3-1146/-646 construct showed a remarkable increase in luciferase activity after VPA treatment, about nine-fold higher than untreated transfected cells. In contrast, VPA stimulation did not alter the luciferase activity in cells expressing the pGL3-basic (negative control) or other 5′-deleted hST8Sia I promoter constructs. These results clearly suggest that the region containing nucleotides -1146 to -646 played an important role in the expression of hST8Sia I and its functions as the VPA-inducible promoter in SK-N-BE(2)-C cells.

Identification of VPA-responsive element in nucleotide -1146 to -646 region of hST8Sia I promoter

Previous studies conducted in our lab demonstrated that the region from -1146 to -646 contained putative binding sites such as c-Ets-1, AP-1, CREB and NF-κB binding sites (Kang et al., 2006; 2007). To determine whether these binding sites contributed to VPA-induced expression of hST8Sia I in SK-N-BE(2)-C cells, four mutants (pGL3-1146/-646mtCREB, mtAP-1, mtNF-κB and mtc-Ets-1) were used, which contained the exact same construct as wild type pGL3-1146/-646 except that combined nucleotides within these binding sites had been changed (Kang et al., 2006). A series of substituted mutations of luciferase constructs (Fig. 3B) were transfected into SK-N-BE(2)-C cells and luciferase assays were carried out. The activity of each construct was compared to that of pGL3-basic and wild type (pGL3-1146/-646) as negative and positive controls, respectively. In VPA-treated cells, pGL3-1146/-646mtNF-κB of four constructed mutations markedly reduced transcriptional activity to more than 3-fold of pGL3-1146/-646wt, whereas the activities of the pGL3-1146/-646mtCREB, mtAP-1 and mtc-Ets-1 constructs were not decreased (Fig. 3B). These results indicate that this NF-κB site is crucial for the VPA-induced expression of

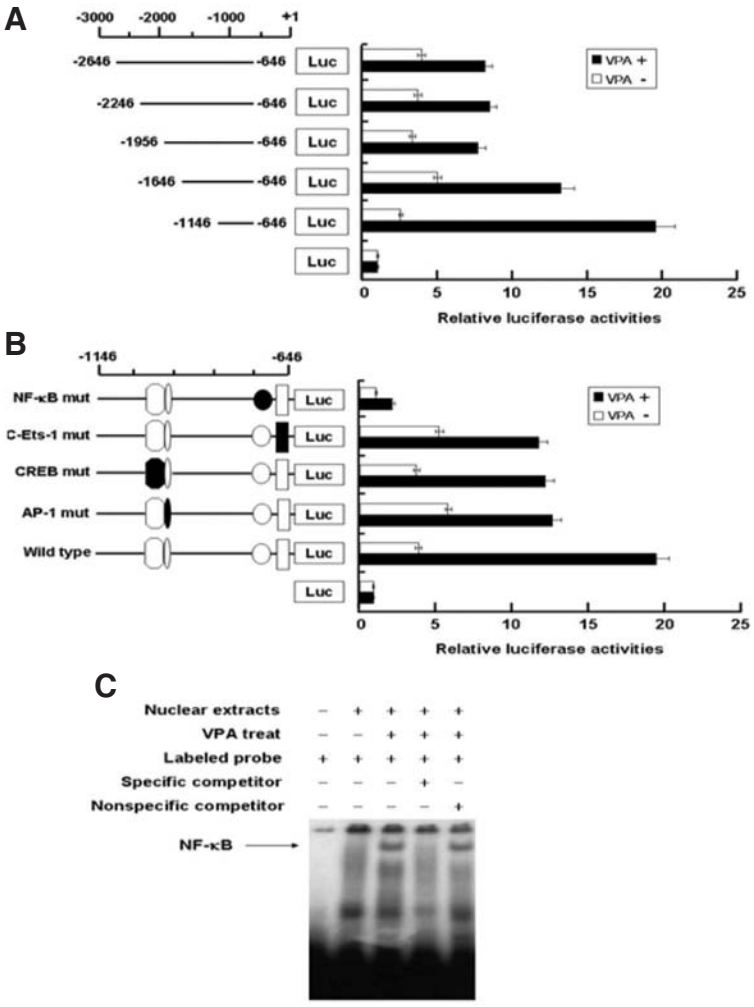

Fig. 3. Analysis of hST8Sia I promoter activity in SK-N-BE(2)-C cells and EMSA with nuclear extracts and the NF-κB sequence of the hST8Sia I promoter. The schematic diagrams represent DNA constructs (A) containing various lengths of the wild type hST8Sia I promoter, or constructs (B) with mutants c-Ets-1, AP-1, CREB and NF-κB sequences in the 5′-flanking region, upstream of a luciferase reporter gene; the transcription start site is designated +1. The pGL3-basic construct, which did not contain a promoter or an enhancer, was used as a negative control. Each construct was transfected into SK-N-BE(2)-C cells, with pRL-TK co-transfected as an internal control. The transfected cells were incubated in the presence (solid bar) or absence (open bar) of 5 mM VPA for 12 h. Relative firefly luciferase activity was measured using the Dual-Luciferase Reporter Assay System, and all firefly activity was normalized to the *Renilla* luciferase activity derived from pRL-TK. The values represent the means ± SD of three independent experiments with triplicate measurements. (C) Nuclear extracts isolated from SK-N-BE(2)-C cells after treatment with 0 mM or 5 mM VPA for 12 h were incubated with ^{32}P-labeled wild type probe, unlabeled wild type probe, or unlabeled mutant NF-κB probe. For competitive inhibition experiments, a 50-fold molar excess of unlabeled wild type or unlabeled mutant NF-κB oligonucleotide was used. The DNA-protein complexes were analyzed on a 4% non-denaturing polyacrylamide gel.

hST8Sia I, and that NF-κB binding to this site is involved in VPA-stimulated SK-N-BE(2)-C cells.

To verify whether NF-κB played a role in VPA-induced expression of hST8Sia I in SK-N-BE(2)-C cells, we performed EMSA on nuclear extracts from VPA-treated cells, using a double-stranded ^{32}P-labeled oligo fragment (20 bp) containing the NF-κB consensus sequence as the probe. In VPA-treated nuclear extracts, we detected a DNA-protein complex that was not detected in the untreated nuclear extracts (Fig. 3C). This complex completely disappeared after specific competitive inhibition with a 50-fold molar excess of unlabeled oligo fragment, but not after a 50-fold molar excess of an unlabeled mutant NF-κB oligonucleotide (a nonspecific competitor). These results indicated that VPA induced an interaction between the NF-κB protein and this region of DNA to induce hST8Sia I expression.

Transcriptional activation of hST8Sia I via JNKs pathway in SK-N-BE(2)-C cells induced by VPA

We also investigated whether VPA-induced transcriptional activity of a pGL3-1146/-646-containing NF-κB was stimulated via PKC and JNK signal pathways. RT-PCR showed that expression of hST8Sia I mRNA was increased in VPA-induced SK-N-BE(2)-C cells, compared to untreated SK-N-BE(2)-C cells (Fig. 4A). Both protein kinase C (PKC) (GÖ6976) and JNK (SP600125) inhibitors resulted in a decrease of hST8Sia I expression in the VPA-stimulated SK-N-BE(2)-C cells. However, enhanced expression of hST8Sia I in VPA-stimulated SK-N-BE(2)-C cells was not inhibited by phosphatidylinositol-3 kinase (PI-3K), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated protein kinase (ERK) inhibitors (wortmannin, SB203580 and U0126), respectively, compared to SK-N-BE(2)-C cells induced by VPA in the absence of chemical inhibitors, as evidenced by RT-PCR. As shown in Fig. 4B, the activity of pGL3-1146/-646 was increased in VPA-stimulated SK-N-BE(2)-C cells, compared to untreated SK-N-BE(2)-C cells. The promoter activity of pGL3-1146/-646 in SK-N-BE(2)-C cells stimulated by VPA was not significantly inhibited by PI-3K, ERK and p38 MAPK inhibitors, respectively, compared to SK-N-BE(2)-C cells induced by VPA in absence of chemical inhibitors, as evidenced by luciferase promoter assay. However, both PKC and JNK inhibitors resulted in a marked decrease of pGL3-1146/-646 activity in SK-N-BE(2)-C cells induced by VPA. These results indicated that promoter activity and mRNA transcription of the hST8Sia I gene were regulated by PKC and JNK signaling pathways.

DISCUSSION

In this study, we demonstrated for the first time that VPA upregulated the expression of hST8Sia I mRNA in human

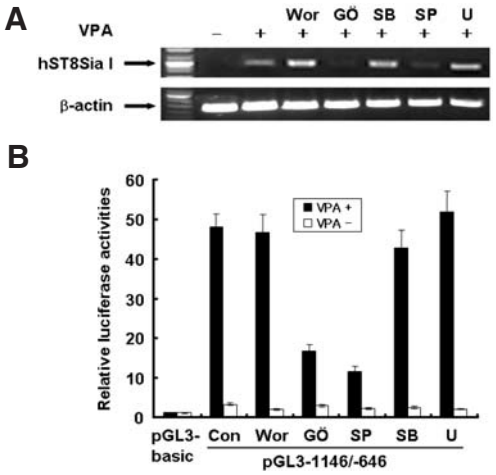

Fig. 4. Transcriptional activation of hST8Sia I through PKC/JNK-signal pathways in VPA-induced SK-N-BE(2)-C cells. (A) SK-N-BE(2)-C cells were treated with GÖ6976 (5 μM), U0125 (5 μM), wortmannin (200 nM), SP600125 (10 μM), and SB203580 (20 μM) inhibitors in the absence or presence of VPA (5 mM) for 12 h in serum free DMEM medium, respectively. Total RNA from these cells was isolated and hST8Sia I mRNA was detected by RT-PCR analysis. Beta-actin was included as an internal control. (B) The pGL3-1146/-646 was cotransfected into SK-N-BE(2)-C cells with pRL-TK as the internal control. The transfected cells were incubated in the presence and absence of 5 mM VPA with each inhibitor for 12 h. All firefly activity was normalized to the *Renilla* luciferase activity derived from pRL-TK. The values represent the mean ± SD for three independent experiments with triplicate measurements.

neuroblastoma cells. Moreover, this increase was time-dependent. The induced hST8Sia I mRNA signal was detected after 6 h of VPA treatment, and then this signal increased up to the 24-h time point. As a downstream consequence, VPA treatment markedly increased levels of the ganglioside GD3 in SK-N-BE(2)-C cells, as shown by immunofluorescent confocal microscopy, and our results suggested that the VPA-induced increase in hST8Sia I expression exhibited a close temporal relation to the GD3 induction. We also revealed that the promoter region of the hST8Sia I gene contained VPA-responsive element(s), which supported the idea that hST8Sia I gene induction would eventually direct GD3 formation in response to VPA.

Previous studies have shown that VPA induces apoptosis and differentiation of neuroblastoma cells both *in vivo* and *in vitro* (Cinatl et al., 1996; 1997; Stockhausen et al., 2005). VPA also reportedly inhibited the growth and differentiation of SK-N-BE(2)-C cells, and these effects were associated with altered expression of differentiation genes and decreased adhesion of cells to the endothelium (Cinatl et al., 2002). In line with these reports, we observed that VPA induced morphological differentiation of SK-N-BE(2)-C cells, but that it had no significant effects on cell viability even after a 24-h treatment of the cells with 10 mM VPA.

We also unraveled a part of the transcriptional regulation mechanism that underlies hST8Sia I gene induction in response to VPA signaling. In order to investigate VPA-responsive elements involved in the enhanced expression of the hST8Sia I gene in SK-N-BE(2)-C cells, we first sought to identify the region within the hST8Sia I promoter that was critical for VPA-induced gene expression. We isolated the region between -1146 and -646 as the core promoter; this region was required for transcriptional activation of hST8Sia I in VPA-induced SK-N-BE(2)-C cells. Previous reports from out lab revealed several transcription factor binding sites such as c-Ets-1, AP-1, CREB and NF-κB binding sites in this region (Kang et al., 2006; 2007). We have also demonstrated that only the NF-κB binding site at position at -731 to -722 in this region contributes to hST8Sia I promoter activity in Fas-induced Jurkat T cells (Kang et al., 2006) and human melanoma SK-MEL-2 cells (Kang et al., 2007). In agreement with these findings, our present site-directed mutagenesis and EMSA analyses indicated that binding to this NF-κB element mediated VPA-dependent upregulation of hST8Sia I gene expression.

NF-κB is a crucial transcription factor that controls the expression of various genes involved in immune and inflammatory responses, cell cycle progression, apoptosis, and oncogenesis (Chen et al., 2001; Ghosh and Karin, 2002). Although VPA enhances AP-1 mediated gene expression by activating the MAP kinase-signaling pathway in human neuroblastoma SH-SY5Y cells (Chen et al., 1999; Yuan et al., 2001), NF-κB-mediated gene expression by VPA stimulation in human neuroblastoma cells have not been reported. Therefore, it is important to elucidate which signaling pathways are upstream of this NF-κB-mediated enhanced expression of the hST8Sia I gene. In the present study, our data showed that PKC and JNK were induced by VPA in SK-N-BE(2)-C cells. The transcriptional activation of hST8Sia I was associated with PKC/JNK pathways induced by VPA in SK-N-BE(2)-C cells, which has not been reported yet. PKC and JNKs inhibitors blocked the expression of hST8Sia I, but PI-3K, PKC, p38 MAPK, and ERK inhibitors did not decrease the expression levels of hST8Sia I in SK-N-BE(2)-C cells stimulated by VPA. These results suggest that transcriptional activity of hST8Sia I may be related to PKC/JNK-dependent pathways.

Although the precise mechanisms involved in the constitutive activation of NF-κB leading to a transcriptional upregulation of the hST8Sia I gene are unknown, we have demonstrated here for the first time that the PKC/JNK-dependent NF-κB activation regulates the expression of hST8Sia I in VPA-stimulated SK-N-BE(2)-C cells.

ACKNOWLEDGMENT

This study was supported by research funds from Dong-A University.

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