JOURNAL OF BIOSCIENCE AND BIOENGINEERING
Vol. 105, No. 2, 164–167. 2008
DOI: 10.1263/jbb.105.164



# Augmentation of Polysialic Acid by Valproic Acid in Early Postnatal Mouse Hippocampus and Primary Cultured Hippocampal Neurons

Takamitsu Natori,[1] Fumiaki Kodaira,[1] Takae Hirasawa,[1]
Yong Ying Gao,[1] and Kaoru Nagai[1]*

*Department of Epigenetic Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan*[1]

Received 11 October 2007/Accepted 22 November 2007

**We examined the effects of valproic acid (VPA) on hippocampal neurons. Prenatal VPA exposure significantly increased polysialic acid (PSA) expression in the early postnatal mouse hippocampus. Moreover, VPA treatment significantly enhanced PSA expression in primary cultured hippocampal neurons and stimulated neurite growth. Our results suggest that VPA exposure *in ovo* affects hippocampal development.**

[**Key words:** polysialic acid, valproate, neurite growth, hippocampus]

Polysialic acid (PSA) is a polysaccharide of α-2,8-linked sialic acid residues primarily expressed in the nervous system. PSA is abundantly expressed during development and its expression level gradually decreases during the course of maturation (1). PSA, which is usually attached to the neural cell adhesion molecule (NCAM), has been reported to contribute to neuronal precursor cell migration (2), neural differentiation (3), axon guidance (2), and synaptic plasticity (4) in the developing nervous system. It has been reported that PSA-NCAM promotes the activity-dependent remodeling of the synapse associated with *N*-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) in the CA1 region of hippocampal cultures (5). In other studies, it has been reported that serious defects, including postnatal growth retardation and premature death, occur in PSA-negative/NCAM-positive mice. These defects were recovered by the additional deletion of NCAM (6). Therefore, PSA plays a crucial role in the regulation of neural network formation and development.

Valproic acid (VPA) is an antiepileptic drug with a broad spectrum of anticonvulsant effects in cases of generalized epilepsy (7). In recent studies, it has been reported that prenatal VPA exposure leads to postnatal autistic features in mice (8), and in some studies, it has been reported that VPA induces abnormality in the hippocampus (9, 10) and cerebellum (11). These observations suggest that VPA exposure during critical periods might disrupt critical aspects of neuronal development, including neuronal migration and synaptogenesis in specific brain areas such as the hippocampus. However, there has been little research on the relationship between VPA-induced hippocampal abnormality and PSA expression level. Since PSA contributes to neuronal migration and synaptic connectivity, the abnormal expression of PSA might affect anatomical and physiological features. Moreover, it has been reported that PSA expression is modulated by VPA in human tumor cells (12). We therefore sought to determine whether VPA affects PSA expression in the brain during neuronal development.

We investigated the expression level of PSA in the brain of prenatally VPA-treated mice during the postnatal period. All the animal experiments were approved by the University of Yamanashi Animal Care and Use Committee. C57BL/6 mice were subjected to a single intraperitoneal injection of 600 mg/kg sodium valproate on the 12th day after conception, and control C57BL/6 mice were injected with phosphate-buffered saline (PBS) at the same time. The brains were obtained from the offspring on postnatal days 0, 5, 10, 20, and 30, and were dissected into the cerebrum, cerebellum, hippocampus, and striatum. The PSA expression in each brain region was quantified by dot blotting. Each tissue sample was placed in PBS with 1% SDS and 1% protease inhibitor cocktail (Nacalai Tesque, Kyoto) and sonicated until the pellet was invisible. The protein in the samples was quantified with a protein quantification kit (Dojindo, Kumamoto). The samples (2.5 μg of total protein per spot) were spotted onto a Hybond $N^+$, positively charged nylon transfer membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK) set on a dot blotting apparatus (Bio-Rad, Hercules, CA, USA). The spots were washed with PBS, and the membrane was blocked with 1% BSA in PBS at room temperature (RT) for 30 min. The membranes were incubated with primary mouse anti-PSA (1:1000 dilution) or mouse anti-β-actin (1:1000 dilution) antibodies in PBS for 2 h at RT. They were then incubated with biotin-conjugated goat anti-mouse IgG+IgM for 30 min at RT. The membrane was subsequently treated using a streptavidin biotin complex peroxidase kit according to the manufacture's instructions (Nacalai Tesque).

* Corresponding author. e-mail: kaoru@yamanashi.ac.jp
phone: +81-(0)55-273-9557 fax: +81-(0)55-273-9561

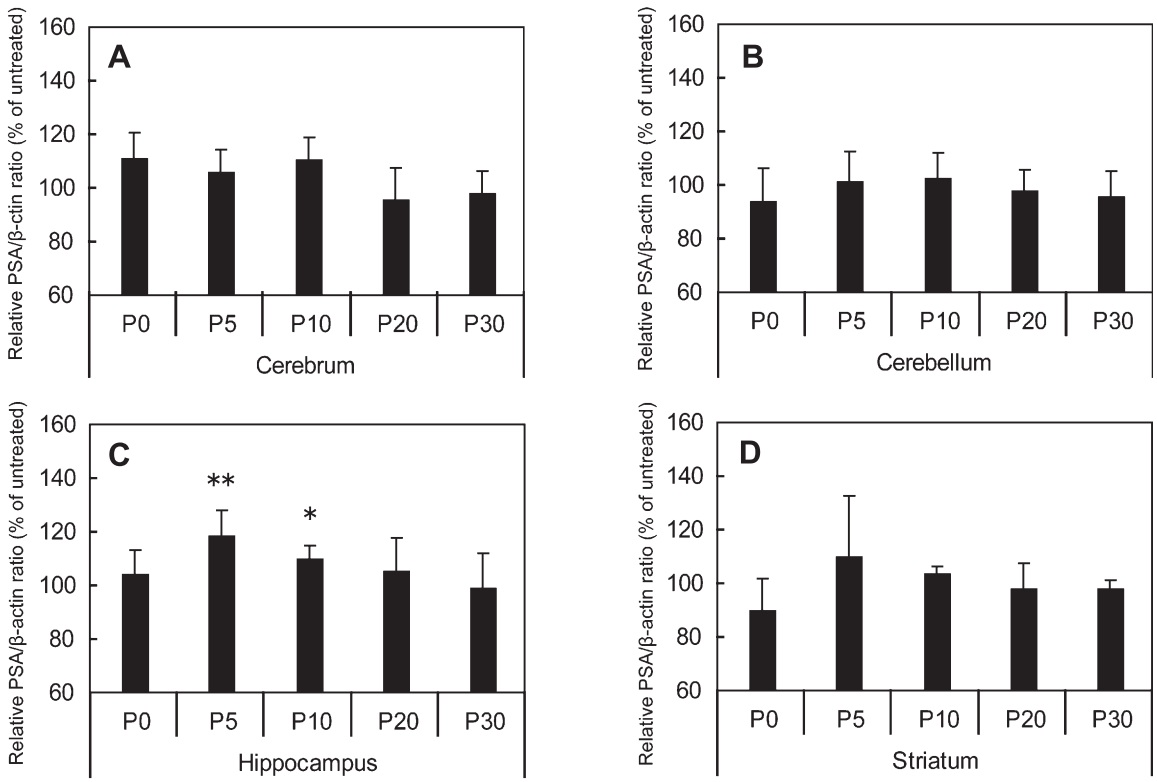


FIG. 1. Effects of prenatal VPA exposure on postnatal PSA expression in brain. The expression of PSA was quantified by dot blotting in the cerebrum (A), cerebellum (B), hippocampus (C) and striatum (D). The results were normalized to those for β-actin expression. The values are represented as the mean±SD (n=4) of the relative percentage of PSA expression compared with that in the untreated mice. ** $p<0.01$, * $p<0.05$, unpaired t-test. P0, P5, P10, P20, and P30; postnatal days 0, 5, 10, 20, and 30, respectively.

The specific immunoreactivity was developed with an ECL kit (GE Healthcare) and quantified using the computerized imaging program Quantity One (Bio-Rad). The relative expression level ratios for PSA/β-actin on postnatal days 0, 5, 10, 20, and 30 of the tissue samples (n=4), compared with the control values (n=4) were as follows: 110.8±9.7%, 105.8±8.7%, 110.3±8.6%, 95.3±12.0%, and 97.5±9.0% in the cerebrum (Fig. 1A); 93.5±12.8%, 101.3±11.2%, 102.3±10.0%, 97.8±8.1%, and 95.5±9.7% in the cerebellum (Fig. 1B); 103.8±9.6%, 118.5±9.7%, 109.5±5.2%, 105.0±12.9%, and 98.8±13.3% in the hippocampus (Fig. 1C); and 89.5±12.4%, 109.5±23.1%, 103.3±3.3%, 97.8±9.4% and 98.0±3.4% in the striatum (Fig. 1D), respectively. The values were represented as mean±SD of relative expression levels compared with the values of the untreated mice. We observed no significant effect of VPA on the ratio of PSA/β-actin expression levels in the cerebrum, cerebellum and striatum. However, the ratio was significantly increased on postnatal days 5 and 10 in the hippocampus ($p<0.01$, $p<0.05$, respectively, unpaired t-test) (Fig. 1C). Our data suggests that PSA expression in the hippocampus is regulated by a specific mechanism that is affected by VPA. In the adult brain, PSA is present only in restricted areas, such as the hippocampus and olfactory bulb. This suggests that the specific regulating mechanism of PSA expression is present in the hippocampus. It is considered that PSA plays crucial roles during the prenatal and early postnatal stages, when the neuronal circuits are being actively constructed. In this study, the alteration of PSA expression occurred in an early postnatal period (Fig. 1). Our observation in the hippocampus raises a query about whether a higher PSA expression disrupts neuronal maturation. The overexpression of PSA synthase in chick retinas results in a severe disruption of retinal morphogenesis (13). This indicates that an abnormally high PSA expression level also dysregulates neuronal circuit formation. Our findings suggest that altered PSA expression in the hippocampus correlates with abnormal neuronal circuit formation, which might be a cause of mental disorders.

Next, we examined the PSA expression levels in primary hippocampal neuronal cells and glial cells treated with VPA. Hippocampal tissues were dissected from mice brains on embryonic day 18. Primary cultured hippocampal neuronal cells and glial cells were plated on a poly-L-lysine (Sigma, St. Louis, MO, USA) -coated tissue culture dish (diameter; 100 mm) with a cell density of $2\times10^6$ cells/dish. The neuronal cells were grown in neurobasal medium (Invitrogen, Carlsbad, CA, USA) supplemented with 1% B27 (Invitrogen), 2 mM L-glutamine (Invitrogen), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma) for 24 h and subsequently treated with VPA (0.5 or 1 mM) (n=5) or left untreated (control) (n=5) for 6 d. The glial cells were grown in Dulbecco's modified Eagles medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma) for 14 d. After passage to kill neurons, the cells were cultured for