THE EFFECT OF VALPROIC ACID ON CHANGES IN METHYLATION PATTERN OF HISTONE H3 INDUCED BY PRENATAL MAM ADMINISTRATION IN mPFC

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INTRODUCTION

Several data indicate that epigenetic regulation of gene expression might be involved in schizophrenia etiology. Our previous study showed the changes in the methylation pattern of histone H3 in the medial prefrontal cortex (mPFC) in neurodevelopmental model of schizophrenia based on the prenatal administration of methylazoxymethanol (MAM) at embryonic day 17 (E17). Thus, the aim of the present study was to determine whether the repeated valproic acid (VA) administration at pre-puberty can affect the observed changes of histone H3 methylation at lysine 9 (H3K9) at 30 day old (P30) and at lysine 4 (H3K4) at 60- or 70 day old rats (P60 or P70) receiving prenatally MAM. VA acting as an inhibitor of histone deacetylases can influence the epigenetic mechanism and might affect the methylation process of histone H3

METHODS

Animals and treatment

Pregnant dams (Wistar Harlan rat) were obtained from animal provider (Charles River, Germany) at embryonic day 15 (E15) and were housed individually in polycarbonate cages. They randomly assigned to the experimental groups and at E17, pregnant females were injected with 22 mg/kg/ml methylazoxymethanol acetate (MAM, Midwest Research Institute, Kansas City, USA) or its saline (0.9 % NaCl, 1 ml/kg) intraperitoneally (i.p.). The offspring were weaned 21 days after birth, except the animals killed at postnatal day 15 (P15), and only males were used in our experiments. Rats were housed by groups of five with ad libitum access to food and water with an artificial 12/12-h light/dark cycle (lights on at 7 a.m.). The different experimental groups always consisted of animals derived randomly from different litters to avoid litter effects. Experiments were conducted on rats at postnatal days 15, 30, 45, 60 and 70 (P15, P30, P45, P60 and P70, respectively). Valproic acid sodium salt (Sigma, Poland) was given at the dose 250 mg/kg, sc, twice a day,in early adolescence (from 23rd to 29th day of rat life) and in late adolescence (form 53rd to 59th of life) and the western blot analysis were performed at P30, P60 and P70.

Western Blotting

The brains were removed after decapitation, cooled on ice, and sliced into 1 mm coronal sections using a rodent brain matrix (Ted Pella, INC). The nuclear extract of the isolated mPFC was obtained using ProteoExtract Subcellular ProteomeExtraction kit as was recommended by the manufacture (Calbiochem, Darmstadt, Germany). The protein concentration in the extracts were determined using QuantiPro BCA Assay kit (Sigma, Poland). Samples of equal protein contents were adjusted to an equal volume with 50 mM Tris (pH 6.8), containing 2% SDS, 8% glycerol, and 2% 2-mercaptoethanol with bromophenol blue as a marker and boiled for 5 min. Protein extracts (6µg protein/lane) were separated by 12.5% SDS-PAGE and transferred to nitrocellulose membranes using an electrophoretic transfer system (BioRad). Then, the membranes were cut into two parts, the lower part was taken to determine the histone H3 and its modification forms proteins, and the other proteins with higher molecular weight than 17 kDA molecular weight were determined on the second part of membranes. The blots were then incubated overnight at 4C with the following primary antibodies: rabbit di-methyl histone H3 Lys 9 (1:1000), rabbit tri-methyl histone H3 Lys 4 (1:1000), rabbit JARID1C (1:1000), rabbit ASH2L (1:2000), rabbit LSD1 (1:1000), rabbit G9a/EHMT (1:200) obtained from Cell Signalling. Immune complexes were detected using appropriate peroxidase-conjugated secondary antibodies: anti-rabbit IgG (1:1000, Roche). The reaction was visualized by ECL (Enhanced Chemiluminescence) (Lumi-LightPlus Western Blotting Kit, Roche). Chemiluminescence was recorded and evaluated with a luminescent image analyzer (Fugifilm LAS-1000). The relative levels of immunoreactivity were quantified using Image ProPlus (Media Cybernetics) software. Molecular weights of immunoreactive bands were calculated on the basis of the migration of molecular weight markers (Bio-Rad Laboratories) using Image Gauge (Fujifilm) software.

Statistics

The results are presented as group mean±SEM. Statistical evaluation was performed by two-way variance (ANOVA) followed by Newman-Keuls post hoc test using the Statistica program.

CONCLUSIONS

VA administration in early adolescence did not completely prevent the increase at H3K9me2 induced by MAM at P30. However it blocked the decrease in H3K4me3 induced by MAM in post puberty (P60, P70). The above effect could be regulated by the level of histone methyltransferase ASH2L.
In contrast, VA administration in late adolescence did not prevent the decrease in H3K4me3 at P60 but blocked the observed decrease of the level at P70. The effect induced by VA at P70 might dependend in histone demethylase JARID1C level. The obtained results indicate that VA administration in adolescence might prevent changes in gene expression in post-puberty controlled by methylation at H3K4.

SUPPORT

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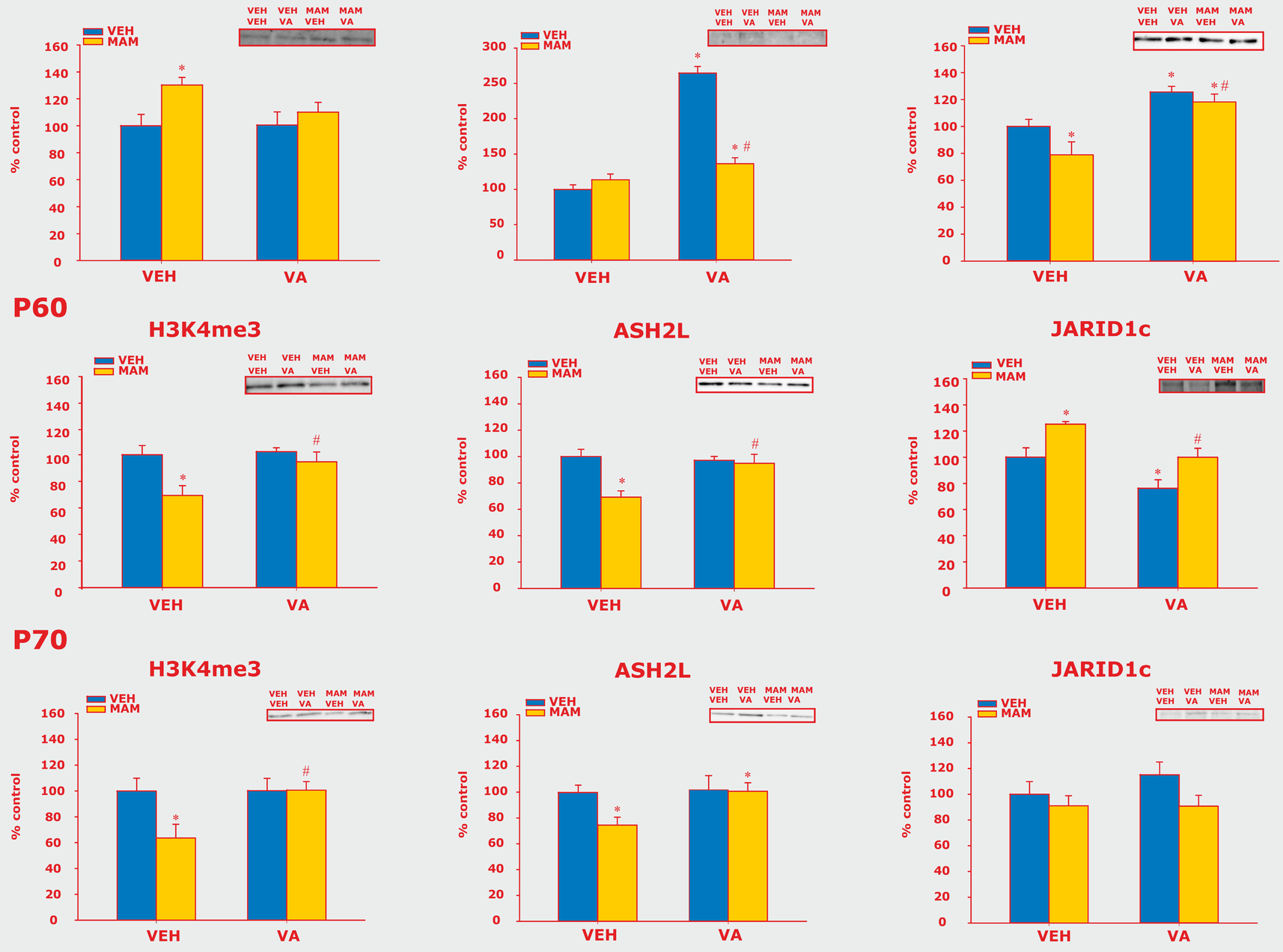
Methylation pattern of histone H3 in postnatal life

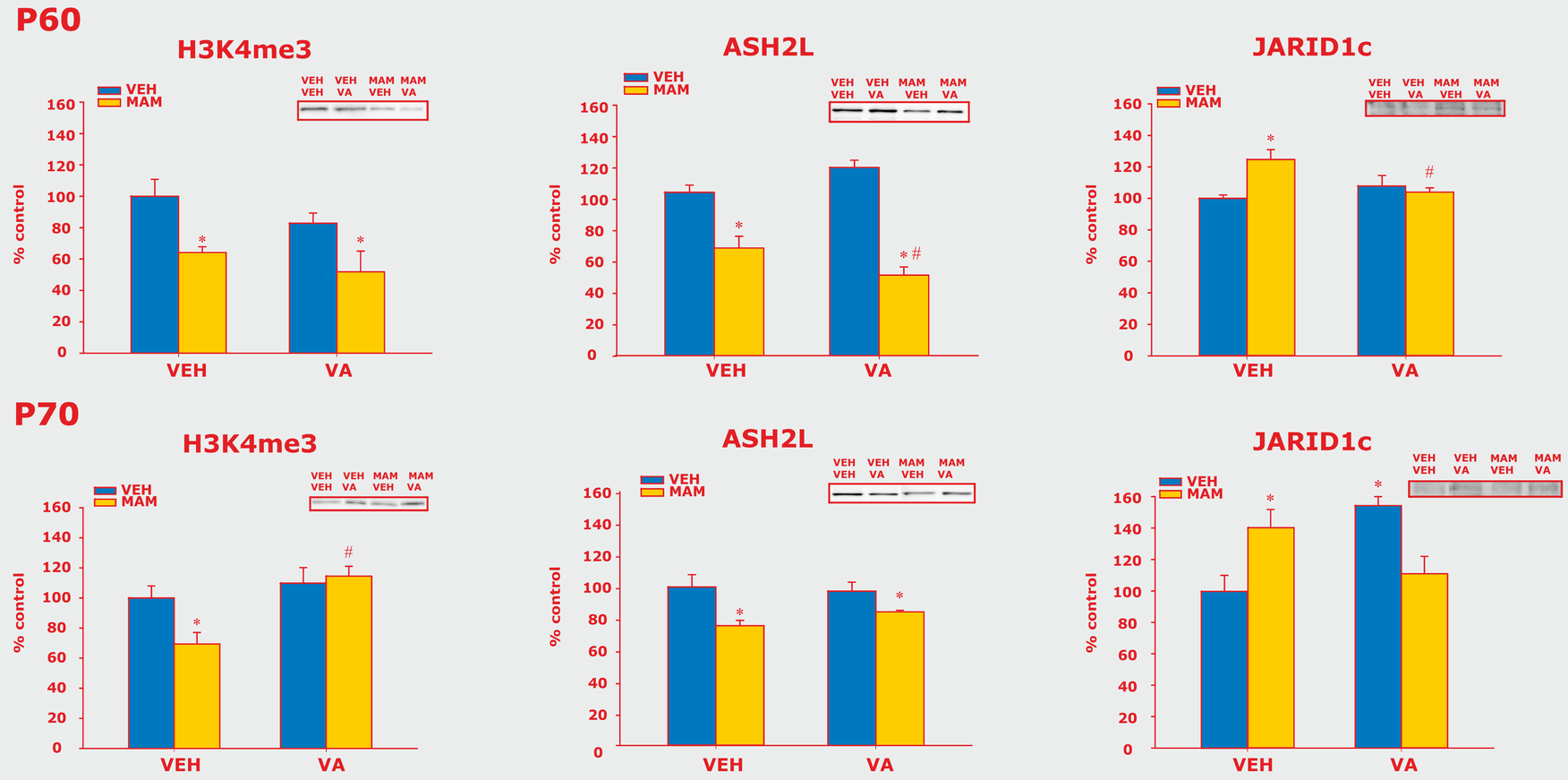
The effect of prenatal MAM administration on levels of H3K4me3 or H3K9me2 during rat development. Asterisks indicate statistical significance when compared to the appropriate control group, p<0.05, n=5.

Valproic acid administration in early adolescence



The effect of VA administration on the changes in level of H3K4me3, H3K9me2, LSD1, G9a, ASH2L, JARID1c induced by prenatal MAM administration. VA was given in early adolescence (23rd- 29th day of life) and western blot were processes at P30, P60, P70. (*) statistical significance versus VEH+VEH, (#) statistical significance versus MAM+VEH, p < 0.05, n=5.

Valproic acid administration in late adolescence



The effect of VA administration on the changes in level of H3K4me3, ASH2L, JARID1c induced by prenatal MAM administration. VA was given in late adolescence (53rd- 59th day of life) and western blot were processes at P60, P70. (*) statistical significance versus VEH+VEH, (#) statistical significance versus MAM+VEH, group, p < 0.05, n=5.