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DNA hypomethylating agents 5-aza-2′-deoxycytidine and valproate increase neuronal 5-lipoxygenase mRNA

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Abstract

DNA methylation regulates gene expression. Neuronal 5-lipoxygenase expression increases during aging and during the proliferation of immature rat cerebellar granule neurons. In these cultures, we investigated the effects of hypomethylating agents 5-aza-2′-deoxycytidine (AdC) and valproate on 5-lipoxygenase mRNA. Both drugs increased 5-lipoxygenase mRNA in proliferating cells; only valproate was effective in differentiated neurons. We propose that neuronal 5-lipoxygenase expression can be affected by aging-altered DNA methylation and by hypomethylating drugs, such as the anticonvulsant valproate.

Keywords: DNA methylation; Cerebellar granule neuron; Histone deacetylase

Recent studies point to an important role for DNA methylation in the epigentic regulation of gene expression in the central nervous system (CNS) (Tucker, 2001). DNA can be covalently modified by methylation at the 5 carbon position of cytosine residues at CpG dinucleotides. DNA methylation of promoter-containing CpG islands is associated with transcriptional inactivation, i.e., gene silencing. Thus, typically, the same gene is not methylated in the tissue where it is expressed; it is methylated in a tissue or cells where it is not expressed. During development, aberrant DNA methylation may have deleterious consequences for CNS structure and function (Tucker, 2001). In an already developed CNS, DNA methylation may lead to modifications in gene expression and brain functioning. Alterations in DNA methylation can be induced pharmacologically, which is generally confined to cancer treatments (Szyf, 2001).

Uhl et al. (2002) found that DNA methylation of the 5-lipoxygenase promoter regulates the expression of this gene. 5-Lipoxygenase is an enzyme involved in the synthesis of biologically active metabolites of arachidonic acid. In the brain, 5-lipoxygenase expression is up-regulated during aging and has been associated with neurodegeneration (Manev et al., 2000). In primary cultures of rat cerebellar granule neurons, 5-lipoxygenase is required for the proliferation of immature cells and its expression decreases as neurons differentiate (Uz et al., 2001). In human myeloid cell lines, 5-lipoxygenase promoter is methylated in 5-lipoxygenase-negative cell lines and unmethylated in 5-lipoxygenase-positive cells. Treatment of 5-lipoxygenase-negative cells with the demethylating agent 5-aza-2′-deoxycytidine (AdC) triggered the expression of 5-lipoxygenase primary transcripts and mature mRNA (Uhl et al., 2002). Since our previous work has established a role for 5-lipoxygenase in primary cultures of rat cerebellar granule neurons (Uz et al., 2001), we used this model to investigate the action of hypomethylating agents on neuronal 5-lipoxygenase expression.

AdC becomes effective when incorporated into DNA and is used to induce hypomethylation in proliferating cells. In our model, only immature cells proliferate (Uz et al., 2001). A cell-cycle-independent hypomethylation can be triggered by valproic acid (valproate) (Alonso-Aperte et al., 1999; Phiel et al., 2001), a drug used clinically as an anticonvulsant and mood stabilizer. The mechanism of valproate's action involves a direct inhibition of histone deacetylase (Phiel et al., 2001). This inhibition causes a loss of DNA methylation (Selker, 1998). Thus, we used both AdC and valproate plus immature and differentiated neuronal cultures.

Primary cultures of rat cerebellar granule neurons were prepared as described previously (Uz et al., 2001). The cells

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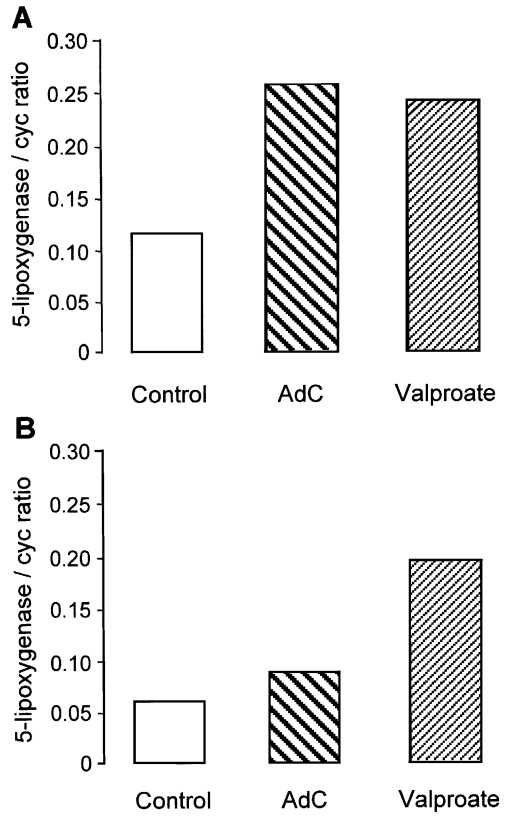

Fig. 1. Stimulatory effects of AdC and valproate on 5-lipoxygenase mRNA content in primary cultures of rat cerebellar granule neurons. Treatment was initiated (A) 1 h after seeding (immature, proliferating cultures), or (B) 8 days after plating (mature, differentiated cultures). mRNA was assayed 6 days after the beginning of treatment, i.e., at 6 days in vitro (DIV) (A) or at 14 DIV (B). Both 5-lipoxygenase and cyclophilin (cyc) mRNA contents were measured with the internal standards (Uz et al., 2001); their values were measured as attomol/μg total RNA and were used to calculate 5-lipoxygenase/cyc ratios; the bars represent 5-lipoxygenase mRNA corrected by the content of cyclophilin mRNA. Note that both AdC and valproate increased 5-lipoxygenase/cyclophilin ratios in proliferating cultures (A) but only valproate was effective in differentiated cultures (B).

were maintained in a serum-free medium (10×10^6 cells/10 ml/10-cm culture dish). Either 1 h (immature cells) or 8 days (differentiated neurons) after plating, cultures were treated with AdC (100 nM; Sigma) or valproate (1 mM; 2-propylpentanoic acid; Sigma). The treatment was repeated (including medium replacement) every 24 h for 3 days. After the last treatment, cells remained in the same medium for three additional days. These cells were harvested in guanidine isothiocyanate for total RNA extraction and quantitative competitive reverse transcription/polymerase chain reaction assay of 5-lipoxygenase and cyclophilin mRNA levels (for details, see Uz et al., 2001).

In proliferating cultures, both AdC and valproate increased 5-lipoxygenase mRNA (attomol/μg total RNA; measured with internal standards) (control = 80, AdC = 158, valproate = 171). In differentiated cultures, only valproate was effective (control = 50 ± 8, AdC = 58 ± 10, valproate = 160 ± 10*; $n = 3$; * $p < 0.05$). Fig. 1 shows the cyclophilin-corrected 5-lipoxygenase values.

Our finding that AdC is effective in proliferating but not in differentiated cultures is consistent with the requirement for AdC to be incorporated into DNA to cause hypomethylation, whereas valproate-stimulated 5-lipoxygenase expression in differentiated cultures reflects valproate's cell-cycle-independent neuronal action. The only published evidence of AdC-stimulated 5-lipoxygenase expression refers to a human cell line (Uhl et al., 2002). Although the rat 5-lipoxygenase promoter has not been cloned, our data suggest that similar to the human 5-lipoxygenase promoter, it is susceptible to regulation via DNA methylation.

We demonstrated that neuronal 5-lipoxygenase expression can be increased by hypomethylating agents AdC and valproate, suggesting a role for DNA methylation in regulating CNS 5-lipoxygenase. It is possible that aging-altered DNA methylation causes aging-associated 5-lipoxygenase up-regulation in the brain (Manev et al., 2000). Alternatively, CNS-acting compounds, i.e., valproate, could influence brain functioning by altering the expression of genes such as 5-lipoxygenase.

Acknowledgements

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