Neuroscience 265 (2014) 147–157

HISTONE DEACETYLASE INHIBITORS VALPROIC ACID AND SODIUM BUTYRATE ENHANCE PROSTAGLANDINS RELEASE IN LIPOPOLYSACCHARIDE-ACTIVATED PRIMARY MICROGLIA

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Abstract—Modifications of histone deacetylases (HDACs) may be involved in microglia-driven neuroinflammatory responses. Recent studies suggest that several inflammatory molecules can regulate the extent of neurodegeneration and regeneration in the central nervous system (CNS). In the present study, we investigated the effects of HDAC inhibitors (HDACi) valproic acid (VPA) and sodium butyrate (NaBut) on the release of prostaglandins (PGs) in lipopolysaccharide (LPS)-activated microglia. We found that VPA and NaBut significantly enhanced LPS-induced release of PGE_2, PGD_2 and 8-iso-$PGF_{2\alpha}$. In addition, both compounds increased cyclooxygenase-2 and microsomal prostaglandin E synthase immunoreactivity and gene expression in LPS-stimulated microglia. Interestingly, treatment of activated microglia with HDACi also enhanced the gene expression and the release of different pro-inflammatory cytokines. Microglia activation with LPS leads to IκB-α degradation, as well as p38, ERK1/2 and JNK MAPKs phosphorylation and thus activation, which is not affected by treatment with VPA and NaBut. Furthermore, VPA and NaBut treatment induced histone acetylation at H3-K18 in microglia. We suggest that VPA and NaBut-driven increase in PGs release in LPS-activated microglia might be regulated at the transcriptional level and involves histone hyperacetylation. Our data demonstrate that VPA and NaBut are able to modulate microglia responses to inflammatory insults and thus possibly can regulate the CNS degenerative and regenerative processes.

Key words: cyclooxygenase-2, microsomal prostaglandin E synthase-1, microglia, prostaglandin, histone deacetylase inhibitors.

INTRODUCTION

Transcription is a crucial process in living cells and is regulated by fine-tuned mechanisms of histone proteins modifications. Among these mechanisms, histone acetylation has an important role in transcriptional balance of several genes. This regulation of gene expression is managed by two classes of enzymes known as histone acetyl transferases (HATs) and histone deacetylases (HDACs). These enzymes have opposing activity and thereby maintain a balance between gene incitement and repression. In general, HATs are responsible for activation of gene transcription by relaxation of the chromatin structure, while HDACs repress gene transcription by compressing chromatin structure (Mizzen and Allis, 1998). Functions of HATs and HDACs can also be reversed in different cells, which represent the more complex and dynamic nature of these enzymes (Reid et al., 2005).

Recently, HDACs have been suggested as potential therapeutic targets for the treatment of various diseases such as cancer, diabetes, autoimmune and neurodegenerative disorders (Morrison et al., 2007; Lawless et al., 2009). Many HDAC inhibitors (HDACi) are developed and approved clinically for the treatment of various disorders. For example, HDACi valproic acid (VPA) and sodium butyrate (NaBut) are used for the treatment of epilepsy and sickle cell anemia, respectively (Dover et al., 1994; Trinka, 2007). The therapeutic effects of HDACi have been attributed to the pre and post-transcriptional regulation of several proteins (Chuang et al., 2009; Fessler et al., 2013). However, the exact mechanisms of action of these compounds are still not completely understood.

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Abbreviations: 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; BBB, blood–brain barrier; COX, cyclooxygenase; ERK1/2, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; HAT, histone acetyl transferase; HDAC, histone deacetylase; HDACi, HDAC inhibitors; IκB, inhibitor of κB; IL, interleukin; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, Mitogen-activated protein kinase; mPGES-1, microsomal prostaglandin E synthase-1; MTT, 3-(4,5-dimetylthiazol-2-yl)-2,5 diphenyltetrazolium bromide; NaBut, sodium butyrate; NF-κB, nuclear factor κB; PG, prostaglandin; qPCR, quantitative real-time PCR; SAHA, suberoylanilide hydroxamic acid; SDS, sodium dodecyl sulfate; TSA, trichostatin A; TNF, tumor necrosis factor; VPA, valproic acid.

http://dx.doi.org/10.1016/j.neuroscience.2014.01.037

The role of inflammation has been greatly acknowledged in the pathogenesis of several neurological disorders. It is known that the CNS resident microglia actively participate in the regulation of different inflammatory events (Minghetti and Levi, 1998). Microglia can be activated by the ligation of different molecules on their specialized cell surface receptors and, in response, release several cytokines, chemokines, and prostaglandins (PGs). However, if these molecules have only protective or degenerative functions in the CNS is still questionable (Wee Yong, 2010).

Experimental evidence has indicated that HDACi can modulate the inflammatory responses in different cells. For instance, HDACi have been shown to inhibit the release of interleukin (IL)-1β-induced PGE_2 and to lower the levels of cyclooxygenase (COX)-2 enzyme in human chondrocytes and to reduce the levels of these molecules in phorbol-12-myristate-13-acetate-stimulated cancer cell lines (Yamaguchi et al., 2005; Chabane et al., 2008). Moreover, different HDACi can augment or inhibit the release of pro-inflammatory mediators like nitric oxide and cytokines in the CNS-derived glial cells (Suuronen et al., 2003; Faraco et al., 2009). These findings draw the attention toward an indispensable part of HDACi action in regulating cellular inflammatory responses.

Although some studies have suggested different effects of HDACi, there is still a debate whether these compounds have a potentiating or inhibitory role in the regulation of activated microglia/macrophage-produced pro-inflammatory mediators (Usami et al., 2008; Dietz and Casaccia, 2010). To our interest, data presenting the effects of HDACi in lipopolysaccharide (LPS)-induced PGs release in microglia are still lacking. In this study, we investigated the effects of two HDACi, VPA and NaBut, on LPS-induced COX-2 and microsomal prostaglandin E synthase (mPGES)-1 expression, as well as on the release of PGs in primary microglia. Moreover, we studied the effects of HDACi on the expression and the release of different cytokines. Interestingly, we revealed that VPA and NaBut enhanced PGs and cytokines release in LPS-activated microglia.

EXPERIMENTAL PROCEDURES

All experiments and animal procedures were performed according to the guidelines of ethics committee of University of Freiburg Medical School. All efforts were made to minimise the number of animals used and the stress given.

Primary microglia cultures

Primary microglia cultures were prepared from brains of neonatal Wistar rats as previously described (Akundi et al., 2005) or from C57BL/6 mice. Briefly, brains were carefully removed and cerebral cortices were collected GmbH, Cölbe, Germany) and, freed from meninges. Further, forebrains were minced in Hank's balanced salt solution (PAA Laboratories GmbH, Cölbe, Germany) and dissociated by passing through a nylon cell strainer with 70-μm pores (BD Biosciences, Heidelberg, Germany). Cells were collected by centrifugation, resuspended in Dulbecco's-modified Eagle's medium containing 10% fetal bovine serum (FBS) (PAA Laboratories GmbH, Cölbe, Germany) and 1% penicillin/streptomycin (10,000 U/ml and 10 mg/ml, respectively) (Sigma Aldrich, Deissenhofen, Germany) and cultured at the density of 5×10^5 cells/ml on 10-cm cell culture dishes (Falcon, Heidelberg, Germany) in 5% CO_2 at 37 °C. After 12–14 days, floating microglia were removed from mixed (astrocyte–microglia) primary cultures and re-seeded into cell culture plates at the density of 2×10^5 cells/ml. After 1 h non-adherent cells were removed by changing the medium. The next day fresh medium was added and cells were used for different experiments.

Microglia cultures treatment

To test the effects of VPA and NaBut (Sigma Aldrich, Deissenhofen, Germany) on LPS-induced COX-2/mPGES-1 synthesis and production of PGs and cytokines, microglia were pre-incubated with different concentrations of VPA (0.1–1 mM) and NaBut (0.01–1 mM) for 30 min. Thereafter, LPS (10 ng/ml; from *Salmonella typhimurium*, Sigma Aldrich) was added for an additional 24 h for protein, PGs and cytokines synthesis. The incubation time of 24 h was selected since at this time point COX-2 and mPGES-1 are induced in LPS-activated rat microglia (de Oliveira et al., 2008). For quantitative PCR of COX-2 and mPGES-1, LPS was added for 4 h. For analysis of cell-signaling molecules by Western blot, LPS was added for 30 min. In each experiment, VPA and NaBut alone were also tested for any effect on microglia as control.

MTT (3-(4,5-Dimetylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay for cell viability

MTT cell viability assay was performed as described previously (Olajide et al., 2013). Briefly, cells were cultured in 96 well plates at the density of 25×10^3 cells/well in DMEM medium containing 10% FBS and 1% P/S. Cells were treated with different concentrations of VPA (0.5 and 1 mM) and NaBut (0.1 and 1 mM) for 24 h. After incubation, medium was removed and 100 μl MTT solution (5 mg/ml in medium) was added to each well and cells were incubated for 2 h at 37 °C. The principle of the assay is that in living cells yellow tetrazolium salt MTT is converted into blue colored formazan crystals by the enzyme mitochondrial dehydrogenase which is taken as a measure of cell viability (Sylvester, 2011). Afterward MTT solution was removed from the cells and formazan product was dissolved by adding 100 μl DMSO:ethanol (1:1) solution. The optical density was measured at 570 nm using a 96 well plate reader (Dynex Magellan Biosciences, Houston, Texas, USA). Values obtained were normalized and presented as the percentage of untreated control.

Western blot

After the respective experimental setup, microglia were washed with phosphate-buffered saline (PBS) and lysed