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Valproic acid in normal therapeutic concentration has no neuroprotective or differentiation influencing effects on long term expanded murine neural stem cells

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Summary The antiepileptic drug valproic acid (VPA) has shown neuroprotective effects in different cell types including mesencephalic neural primary cultures. Furthermore, an influence on neural differentiation and neurite outgrowth has been described. Nevertheless, results in this regard are contradictory and data on long term expanded neural stem cells are missing. This is why we investigated possible neuroprotective effects of VPA on fetal mesencephalic neural stem cells (fmNSCs) in vitro, using the neurotoxic agent 1-methyl-4-phenyl-pyridin (MPP+). We also examined potential VPA effects on cell expansion and differentiation and the underlying signaling pathways.

In our study, we could exclude any relevant toxic effects of 100 μg/ml and 200 μg/ml VPA on fmNSCs during expansion and differentiation for up to 96 h. MPP+ treatment in concentrations of 30 and 60 μM MPP+ significantly decreased the survival rate of fmNSCs during expansion and differentiation. In all used concentrations, VPA did neither reverse these MPP+ effects when applied simultaneously with MPP+ nor after pre-treatment with VPA for 24 h. In contrast, MPP+ effects were emphasized by VPA pretreatment for 24 h when applied during cell expansion. Concerning the self-renewing capacity of fmNSCs, measured by BrdU and Ki67 staining, we did not find any significant influence of VPA. Additionally there was no significant influence of therapeutic VPA dosages on astroglial (GFAP), oligodendroglial (GalC) and neuronal (MAP2) differentiation, measured by immunostaining after 10 days of differentiation.

Summing up, we did not find any neuroprotective effects of VPA on fmNSCs in vitro, neither during expansion nor during cell differentiation. Also the self-renewing and differentiation

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potential of the used fmNSCs was not altered. These findings have implications for the large community of patients having to take VPA on a chronic base, especially in the light of knowledge that a regular cell replacement out of hippocampal adult stem cells is mandatory for the maintenance of normal cognition through adulthood.

Introduction

Valproic acid (VPA) is a branched short chain fatty acid that enhances the activity of the neurotransmitter gamma amino butyrate (GABA) in the human brain, alters N-methyl-D-aspartate-mediated excitation and blocks Na^+ channels, Ca^{2+} channels and voltage gated K^+ channels (Chateauvieux et al., 2010). Furthermore, it acts as an inhibitor of the transcriptional repressor histone deacetylase (HDAC) (Phiel et al., 2001; Hsieh et al., 2004). VPA is clinically used as an antiepileptic and mood stabilizing drug in the treatment of different epilepsy syndromes and convulsive seizures, bipolar disorders, migraine headache and depression (Tunnicliff, 1999; Johannessen, 2000). Furthermore several clinical studies are ongoing, investigating the HDAC inhibitory properties of VPA and its potential actions on cancers and in HIV therapy (Chateauvieux et al., 2010). Though the therapeutic properties and side effects of VPA are known since the 1970s (Peterson et al., 2005), the question whether a long term treatment may be harmful for the patient was never satisfyingly answered. Some state, that VPA in contrary may even have neuroprotective effects. On cellular level neuroprotective and anti-inflammatory effects have as well been reported as neuronal differentiation and neurite outgrowth promoting effects. Many different cell types were tested, among them also mesencephalic primary cultures (Di Daniel et al., 2005; Jin et al., 2005; Peng et al., 2005; Chen et al., 2006a,b; Yamauchi et al., 2007, 2008; Yamauchi et al., 2009; Yuan et al., 2009). Also the question, in which dosage VPA may be neurotoxic or neuroprotective is controversially discussed. Some groups reported differentiation inhibiting and cell death promoting effects at lower concentrations of 0.5—10 mM (Jin et al., 2005; Shen et al., 2005), but neither a toxic nor neuroprotective effect at higher concentrations of 15 and 20 mM (Jin et al., 2005; Shen et al., 2005). In contrast to these findings, other publications reported increased cell death in dosages >3 mM, but no influence on cell death at concentrations of 0.3 and 1 mM (Hsieh et al., 2004) or no influence on cell survival at low concentrations of 0.05 and 0.2 mM, but neuroprotective effects at a concentration of 0.6 mM (Peng et al., 2005). Because of the many different cell types used and the wide range of different VPA concentrations, these varying results are difficult to interpret.

The described neuroprotective effects of VPA are thought to be mediated by inhibition of the release of pro-inflammatory factors (Peng et al., 2005), by inhibitory effects on the GSK-3/PI3-kinase/Akt/NFkB pathway (Mora et al., 2002; Jin et al., 2005) as well as by the release of neurotrophic factors as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) from astrocytes (Chen et al., 2006a,b). Contrary to these findings, one group described pro-apoptotic effects of VPA by inhibition of Akt (Chen et al., 2006a,b). So even here conflicting results are reported. In our own group we previously did not find any cell death inducing effects by pharmacological inhibition of the PI3-kinase/Akt/NFkB pathway at different positions (Sabolek et al., 2006, 2009) in long term expanded mesencephalic neural progenitor cells.

In own previous studies, we investigated the stage-dependent vulnerability of fmNSCs toward the neurotoxins 1-methyl-4-phenylpyridin (MPP+), 6-hydroxydopamine (6-OHDA) and the free radical generator H_2O_2 (Sabolek et al., 2008). We found the most consistent results for MPP+, showing concentration dependent increased cell death at concentrations >10 μM (Sabolek et al., 2008). MPP+ is the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces parkinsonian symptoms in different species because of its selective dopaminergic toxicity in the substantia nigra. This effect in dopaminergic neurons is in mediated by intracellular MPP+ accumulation via transmembrane transportation through the dopamine transporter molecule (DAT) and consecutive inhibition of the mitochondrial complex I (NADH-Q reductase). This leads to the buildup of free radicals and toxic molecules and ends up in cell destruction. Still, there are also known DAT-independent mechanisms, leading to a unspecific, non-dopaminergic toxicity via generation of free radicals and binding at sites other than complex I (Storch et al., 1999; Sabolek et al., 2008). This second described effect, the non-dopaminergic toxicity at concentrations >10 μM, is the effect we were making use of in the here presented study using long term expanded mesencephalic neural progenitor cells not expressing the DAT (Sabolek et al., 2008). As this toxic mechanism gives us a well established, predictable and linear toxicity in our used cell system, we found MPP+ to be a good toxic system to investigate possible neuroprotective effects of VPA on fmNSCs.

In the present study we investigated whether VPA in standard therapeutic levels has cell death inducing effects for itself and whether VPA has the potential to inhibit MPP+ induced cell death in long term expanded fmNSCs. We also examined the influence of VPA on cell expansion and differentiation and the underlying signal cascades.

Experimental procedures

Isolation and propagation of fetal mesencephalic neural stem cells from rat (fmNSCs)

Midbrain precursors from E14.5 rat embryonic brain were prepared as described previously (Storch et al., 2003). In brief, pregnant females (Wistar rats; Charles-River, Braunschweig, Germany) were sacrificed and the dissected