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Chronic ethanol exposure changes dopamine D2 receptor splicing during retinoic acid-induced differentiation of human SH-SY5Y cells

Catrin Wernicke[1], Julian Hellmann[2], Ulrich Finckh[3], Hans Rommelspacher[1,2]

[1]Charité, Universitätsmedizin Berlin, Campus Charité Mitte, Klinik für Psychiatrie und Psychotherapie, Dorotheenstr. 94, 10117 Berlin, Germany

[2]Charité, Universitätsmedizin Berlin, Campus Benjamin Franklin, Klinik für Psychiatrie und Psychotherapie, Sektion Klinische Neurobiologie, Eschenallee 3, 14050 Berlin, Germany

[3]Institut für Humangenetik, Universitätsklinikum Eppendorf, Hamburg, Germany

Correspondence: Catrin Wernicke, e-mail: catrin.wernicke@charite.de

Abstract:
There is evidence for ethanol-induced impairment of the dopaminergic system in the brain during development. The dopamine D2 receptor (DRD2) and the dopamine transporter (DAT) are decisively involved in dopaminergic signaling. Two splice variants of DRD2 are known, with the short one (DRD2s) representing the autoreceptor and the long one (DRD2l) the postsynaptic receptor. We searched for a model to investigate the impact of chronic ethanol exposure and withdrawal on the expression of these proteins during neuronal differentiation. RA-induced differentiation of human neuroblastoma SH-SY5Y cells seems to represent such a model. Our real-time RT-PCR, Western blot, and immunocytochemistry analyses of undifferentiated and RA-differentiated cells have demonstrated the enhanced expression of both splice variants of DRD2, with the short one being stronger enhanced than the long one under RA-treatment, and the DRD2 distribution on cell bodies and neurites under both conditions. In contrast, DAT was down-regulated by RA. The DAT is functional both in undifferentiated and RA-differentiated cells as demonstrated by [^{3}H]dopamine uptake. Chronic ethanol exposure during differentiation for up to 4 weeks resulted in a delayed up-regulation of DRD2s. Ethanol withdrawal caused an increased expression of DRD2l and a normalization of DRD2s. Thus the DRD2s/DRD2l ratio was still disturbed. The dopamine level was increased by RA-differentiation compared to controls and was diminished under RA/ethanol treatment and ethanol withdrawal compared to RA-only treated cells. In conclusion, chronic ethanol exposure impairs differentiation-dependent adaptation of dopaminergic proteins, specifically of DRD2s. RA-differentiating SH-SY5Y cells are suited to study the impact of chronic ethanol exposure and withdrawal on expression of dopaminergic proteins during neuronal differentiation.

Key words:
chronic ethanol, alcohol withdrawal, dopamine D2 receptor, splice variants, development, differentiation, DAT, human neuroblastoma cells

Abbreviations: BCA – bicinchoninic acid, DAPI – 4',6-diamidino-2-phenylindole, DAT – dopamine transporter, DBH – dopamine-β-hydroxylase, DMSO – dimethylsulfoxide DRD2 – dopamine D2 receptor, FBS – fetal bovine serum, FITC – fluoresceine isothiocyanate, HEK cells – human embryonic kidney cells, hPBGD – human porphobilinogen deaminase housekeeping gene, l – long, nCAM – neural-cell-adhesion-molecule, NET – norepinephrine transporter, PMSF – phenylmethylsulfonylfluorid, RA – retinoic acid, RT – reverse transcriptase, s – short, TEA – triethanolamine, TRITC – tetramethylrhodamine isothiocyanate

Introduction

The mesolimbic dopaminergic system exerts key functions in rewarding properties of ethanol and other drugs [16, 25, 62, 64]. It was demonstrated that the DRD2 is important for the reinforcing and motivating effects of ethanol as DRD2 knock-out mice demonstrated reduced ethanol drinking [37] and ethanol-conditioned place preference [10]. In humans, the levels of DRD2 in the nucleus accumbens and the amygdala were lower in alcoholics compared to healthy subjects [20, 41, 58]. Furthermore, healthy relatives of subjects with alcohol dependence demonstrated higher levels of DRD2 in the respective brain regions compared to healthy persons without family history of alcohol dependence [61]. Whether the reduced DRD2 level in alcoholics is a cause or consequence of alcohol consumption was not clear for a long time. It was repeatedly demonstrated that DRD2 expression influences ethanol consumption: Animal studies revealed that over-expression of DRD2 in the nucleus accumbens reduces ethanol self-administration [53–55], and that reduced DRD2 expression enhances ethanol consumption [4, 35]. Moreover, association studies revealed putative low expressing alleles to be associated with alcohol dependence [26]. There is also evidence that ethanol consumption influences DRD2 expression: One-week ethanol treatment of male Wistar rats reduced D2-like receptor density by 43% in the striatum [60]. Furthermore, Sari et al. [43] demonstrated increased DRD2 binding density in the anterior regions of the nucleus accumbens shell and core but not in the striatum in alcohol-preferring rats which had access to ethanol for 14 days compared to those which had access to water only. Also Kim et al. [24] reported on an increase of DRD2 mRNA in the caudate putamen and nucleus accumbens of rats after chronic intake of ethanol. In rhesus monkeys moderate-level alcohol exposure in early gestation and during whole gestation reduced the striatal DRD2 binding/dopamine synthesis ratio in adulthood, whereas middle-to-late gestation ethanol exposure increased this ratio [45]. In mice, prenatal ethanol exposure did not alter DRD2 binding but increased responses to dopaminergic drugs [7]. Thus, the consequence of ethanol consumption on DRD2 density seems to depend on amount, duration, and time point of ethanol consumption. There is a strong evidence that ethanol will have the greatest influence in periods of brain development, like the fetal and adolescence periods.

There is another aspect, which has to be considered; two isoforms of DRD2 exist derived from alternative splicing of exon 6, which results in a 29 amino acids deletion in the third intracellular loop of the short isoform (DRD2s) compared to the long one (DRD2l) [15]. The long isoform is preferentially involved in postsynaptic response, whereas the short isoform predominantly exerts presynaptic autoinhibition of dopamine synthesis and release [23, 33, 59]. Furthermore, it was demonstrated that the splice variants couple to different G-proteins [34]. A reduced DAT activity results in a higher concentration of dopamine in the synaptic cleft and *vice versa*. Thus, the balance between the actions of both splice variants of DRD2 and DAT is critically involved in dopaminergic neurotransmission and long-term adaptation of dopaminergic pathways.

We were searching for a suited cell model for studying the impact of long-term exposition of ethanol on the regulation of both splice variants of DRD2. As primary mesencephalic embryonic cells derived from animals have a limited survival period [17], a permanent cell line would have the advantage of an unlimited survival. The human neuroblastoma SH-SY5Y cell line seemed to represent a suited model for several reasons [5]. It consists of a mixture of neuron-like and epithelium-like cell populations. Retinoic acid (RA) treatment induces differentiation into the neuron-like phenotype with long neurite-like processes and a network of cell-cell connections whereas epithelium-like cells are suppressed [14, 21, 22]. It was demonstrated, that SH-SY5Y cells express tyrosine hydroxylase [6], and DRD2 [14]. Furthermore, the expression of DAT [38] but also of norepinephrine transporter (NET) [56, 66] and dopamine-hydroxylase (DBH) [6] was reported. In view of the expression of proteins characteristic for dopaminergic neurones, we investigated whether SH-SY5Y cells express both isoforms of DRD2 and whether the expression of DRD2 and DAT are influenced by RA-induced differentiation and chronic ethanol. Furthermore, the levels of dopamine were determined.

Materials and Methods

Chemicals

Cell culture: Minimal essential medium with Earl's salt (MEM-Earle) supplemented with 2.2 g/l $NaHCO_3$, fetal