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Biological Mechanisms in Alcohol Dependence—New Perspectives

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Abstract — Neurobiological research in alcohol dependence has led to a new understanding of this addictive disease. While some important mechanisms like alterations in the mesolimbic reward system or changes in the hypothalamus—pituitary—adrenocortical axis have been well studied, other possible neurobiological mechanisms are still unrevealed. This applies for the role of specific neuroendocrine pathways like the appetite-regulating system and the modification of gene expression, particularly the influence of genetic variants of transcription factors or epigenetic mechanism like DNA methylation or histone acetylation. This review describes the current knowledge regarding these factors, focusing particularly on the role of appetite- and volume-regulating hormones, the role of genetic variants of specific transcription factors and the function of epigenetic alterations in the genomic sequence of candidate genes for alcohol dependence. A further understanding of the influence of transcription factors and epigenetic regulation may help to elucidate the pathophysiological mechanisms in the neurobiology of alcohol dependence.

INTRODUCTION

Research on neurobiological mechanisms in the pathogenesis of alcohol dependence in recent years has led to a fundamental change in the understanding of this disease as a neurobiological disorder. Therefore, studies have shown that particularly alcohol seeking behaviour is closely associated with changes in dopaminergic transmission in the mesocorticolimbic brain circuits (Soderpalm et al., 2009; Tupala and Tiitinen, 2004). But also other central nervous systems like the opioid system (Mendez and Morales-Mulia, 2008; Rodriguez-Arias et al., 2010), glutamatergic- and GABA-ergic neurotransmission (Bleich and Hillemacher, 2009; Colombo et al., 2004; Heinz et al., 2009), the serotonergic system (Johnson, 2004) and changes in neuroendocrinological function have been described (Clarke et al., 2008; Hillemacher et al., 2007a; Kiefer and Wiedemann, 2004). Beside changes in neurotransmission and endocrinology, studies also focused on genetic and epigenetic alterations in alcohol dependence, describing a series of candidate genes for alcohol dependence (Bleich and Hillemacher, 2009; Ducci and Goldman, 2008; Gelernter and Kranzler, 2009). This review will focus on recent research regarding changes of appetite-regulating hormones in alcohol dependence and the importance of alterations in gene transcription due to epigenetic mechanisms and altered function on transcription factors.

THE ROLE OF APPETITE REGULATION IN ALCOHOL DEPENDENCE AND CRAVING

Recent investigations have focused on the role of the appetite-regulating system in alcohol dependence (Kiefer and Wiedemann, 2004; Pelchat, 2002). Regarding neuroendocrinological alterations of appetite-regulating neuropeptides, leptin and ghrelin have received most attention (Addolorato et al., 2006; Kiefer et al., 2001a, b; Kraus et al., 2005). However, other appetite-regulating pathways have also been investigated in the context of alcohol dependence, which are not in the focus of this review. These include, e.g. gut-liver-brain pathways including alterations in the secretion of insulin and other appetitive hormones like, for example thyroid hormones. For further discussion of these pathways see e.g. Ronis et al., 2007, and Leggio, 2009.

Leptin

Leptin is an adipocytokine secreted by white adipose cells. While leptin has numerous ways of action (and probably still not all unrevealed up to now), one main mechanism seems to be the action in the arcuate nucleus of the mediobasal hypothalamus. There, leptin inhibits the action of neurons expressing the appetite-stimulating neuropeptide agouti-related peptide and neuropeptide Y, and activates the appetite-reducing transmitters POMC (proopiomelanocortin) and cocaine- and amphetamine-regulated transcript (for review on the action of leptin, see Schwartz and Porte, 2005). First studies by Kiefer et al. (2001a, c) described an increase of leptin plasma levels in alcohol-dependent patients being associated with elevated alcohol craving measured by a visual analogue scale. Other studies showed that leptin plasma concentrations were elevated during chronic alcohol consumption (Nicolas et al., 2001) with a normalization during abstinence (Wurst et al., 2003). Using a large study sample of 189 alcohol-dependent patients at the beginning of alcohol detoxification, we were able to confirm this previous data for both genders, finding a highly significant association between leptin serum levels and alcohol craving measured by the Obsessive Compulsive Dinking Scale (Hillemacher et al., 2007a). While pathophysiology behind the association between leptin and craving remain unrevealed and speculative, one important hypothesis focuses on the influence of leptin on activating secretion of POMC, which through post-translational modification leads to different active derivates, including adrenocorticotropic hormone (ACTH) and β-endorphin (Muschler et al., 2010). Both ACTH, influencing the hypothalamic–pituitary–adrenocortical (HPA) axis and β-endorphins, affecting the mesolimbic reward system, have been closely linked with craving and anxiety in alcohol dependence (Junghanss et al., 2003; Kiefer et al., 2002; Kiefer and Wiedemann, 2004).
Adiponectin and resistin

Also other adipocytokines like adiponectin and resistin have been subject to recent research. Recent pre-clinical and clinical studies showed an increase of both serum levels under alcohol intake (Pravdova et al., 2007; Siersma et al., 2004). In a first study, we analysed a sample of 88 patients at admission for alcohol detoxification and after 1 week of withdrawal treatment in comparison to 89 healthy controls (Hillemacher et al., 2009b). Findings of this study showed that the extent of alcohol craving, obtained using the Obsessive Compulsive Drinking Scale (OCDS), was associated negatively with adiponectin serum levels in male alcoholic patients on a significant level. Adiponectin and resistin levels were both significantly elevated in patients with alcohol dependence at both dates (admission and after 1 week of treatment) compared with the healthy control group. In the course of withdrawal and early abstinence, adiponectin decreased significantly during the 7-day period while resistin serum levels showed a slight, not significant increase. These findings provide first evidence that also other adipocytokines may be involved in the pathophysiology of alcohol dependence and alcohol seeking behaviour. However, the pathophysiology behind these findings remains unclear. It fits well that adiponectin, known as an opponent of leptin in physiology behind these findings remains unclear. It fits well that adiponectin, known to act as an opponent of leptin in appetite regulation, is inversely associated with craving. Several studies showed effects of adiponectin on the CNS (Kos et al., 2007) by an involvement in the central nervous appetite regulation via enhancement of the AMPK (AMP-activated protein kinase) activity in the arcuate hypothalamus (Kadowaki et al., 2008; Qi et al., 2004). It can be hypothesized that these effects on the hypothalamic level may influence mesolimbic neurotransmission and therefore alcohol craving, while profound studies are necessary to back up this hypothesis.

Ghrelin

Furthermore, the gastrointestinal and also appetite-regulating peptide ghrelin has received attention regarding a possible role in the neurobiology of alcohol dependence. This appetite-regulating neuropeptide is expressed in specialized cells in the pancreas and stomach and exerts different mechanisms of action. The main mechanisms of action seem to be the stimulation of the expression of growth hormone and the activation of the cholinergic-dopaminergic reward link in the brain reward system (Inui et al., 2004; Jerlhag, 2008; Jerlhag et al., 2006; Mondal et al., 2005). Preclinical studies have also focused on a possible role of ghrelin antagonists in the treatment of alcohol-dependent behaviour with promising results for further pharmacological approaches (Jerlhag et al., 2009), which has also been discussed in more detail in a recent review by Leggio (2010). Although the role of ghrelin in the central nervous reward system is well studied in animals, results regarding humans are contradictory (Addolorato et al., 2006; Kim et al., 2005; Kraus et al., 2005). While Kraus et al. described elevated ghrelin serum levels in alcohol-dependent patients at the beginning of alcohol withdrawal, Addolorato et al. found lowered ghrelin levels in actively drinking alcoholics. Similar results were described by Badaoui et al. (2008), who found decreased plasma ghrelin levels as well as lowered ghrelin levels in fundic biopsies in alcohol-dependent patients. Additionally, findings from the Addolorato study showed a positive association between ghrelin serum levels and alcohol craving. In an own investigation regarding different types of alcohol dependence by using Lesch’s typology of alcohol dependence (Lesch and Walter, 1996), we found a trend for an association between ghrelin and craving scores particularly in patients of Lesch’s type 1 (Hillemacher et al., 2007c). Furthermore, a previous investigation described elevated ghrelin serum levels in abstinent patients (Kim et al., 2005), which may point to long-lasting disturbance of ghrelin metabolism in alcohol dependence. Taken together, human studies on alterations of ghrelin metabolism in alcohol dependence are contradictory and difficult to interpret, as most of these studies investigated different patients groups (e.g. psychiatric patients vs. internal patients) and settings and at variable time-points of withdrawal/abstinence.

INFLUENCE OF TRANSCRIPTION FACTORS ON ENDOCRINOLOGICAL MECHANISMS IN ALCOHOL DEPENDENCE

Many factors influencing protein transcription have been identified in the past. One of these is the influence of transcription factors, adhering to the DNA and promoting or inhibiting mRNA transcription. However, little is known regarding the role of transcription factors in the regulation and expression of transmitters relevant for addictive behaviour. One recent investigation from our group focused on the role of the CAG trinucleotide repeat of the androgen receptor (AR). The AR, located on chromosome Xq11-12, belongs to a family of ligand-activated nuclear transcription factors. Two isoforms of the AR have been identified (AR-A, AR-B), both encoded by the same gene (Wilson and McPhaul, 1994; Zhou et al., 1994). Testosterone or dihydrotestosterone binds to the testosterone-binding domain of the AR, building a complex (Mooradian et al., 1987). This complex binds to the DNA, acting as a transcription factor for various transmitters, including leptin and POMC (Fig. 1).

However, the function of the AR is regulated partially by a trinucleotide CAG repeat in the encoding sequence of the
receptor gene (Chamberlain et al., 1994). A higher number of CAG repeats inhibit the possibility of the AR–testosterone complex to bind to the DNA and so inhibits the activating role on mRNA transcription. Clinically, the expansion (>40) of the CAG repeat has been associated with neurological diseases (i.e. Kennedy’s disease) (La Spada et al., 1991) and impaired sperm production (Tut et al., 1997). A relatively small number of CAG repeats in the AR gene is associated with a higher risk of depression (Seidman et al., 2001), adrenopausal symptoms (Härkönen et al., 2003), prostate cancer, benign prostate hyperplasia, young-onset rheumatoid arthritis and with a lower risk of infertility (Westberg et al., 2001). So, it can be hypothesized that genetic differences regarding the number of CAG repeats in the encoding sequence of the AR (which as described above is important for the transcription of relevant transmitters for addictive behaviour like POMC and leptin) may influence (a) the risk of alcohol dependence in general or (b) may be associated with alcohol craving. In a first study, we investigated 112 male alcohol-dependent patients and 50 healthy controls regarding the number of CAG repeats (Lenz et al., 2009). We found no significant difference in terms of CAG repeat length comparing patients with controls, which leads to the assumption that the CAG repeat length is not generally associated with the risk of alcohol dependence. However, we found a significant negative association between the number of repeats and the extent of alcohol craving, measured with the OCDS. A lower number of CAG repeats, leading to a higher binding capacity of the transcription factor, are associated with elevated craving scores in our population. This result leads to the question in which way the investigated polymorphism of the AR influences alcohol craving and whether there is a flexible link between the static genetic model of the CAG repeats and alcohol-seeking behaviour.

In further analysis, however, we were able to show that this association is at least partially mediated by the appetite-regulating peptide leptin (Lenz et al., 2010). We found both, a significant \( P < 0.001 \) association between a lower number of CAG repeats and leptin serum levels and an association (also \( P < 0.001 \)) between leptin serum levels and OCDS score. Using a path analysis the study showed that 40\% of the association between CAG repeats and craving are mediated via leptin, probably by influencing leptin mRNA transcription (Fig. 2).

There are only few studies on the role of transcription factors in alcohol dependence. A recent study of Kiefer et al. (2010) investigated the role of a single nucleotide polymorphism, rs13273672, an intronic SNP in the gene for GATA-binding protein 4 (GATA4). GATA4 has been described to modulate gene transcription of atrial natriuretic peptide. Interestingly, in this study the investigated SNP in the gene of GATA4 was associated with relapse in alcohol-dependent patients. Therefore, it can be supposed that genetic variations in GATA4 may influence the risk of relapse in alcohol dependence via modulation of atrial natriuretic peptide (ANP) plasma levels, similar to the described polymorphism of the AR and its influence on craving via leptin plasma levels.

Evidently, these first studies on the role of transcription factors in alcohol dependence should not be overinterpreted. However, they provide an interesting new approach to further understand alterations in gene regulation and transcription in alcohol dependence. Furthermore, genetic variants in the encoding sequence of transcription factors like the AR, which are able to influence transcription of different relevant neuropeptides, may have a more important impact for addictive behaviour than genetic variants of solitary candidate genes.

**EPIGENETIC GENE REGULATION IN ALCOHOL DEPENDENCE**

*Mechanisms of epigenetic modification*

Besides the influence of transcription factors on gene description, epigenetic mechanisms also have to be taken into account. It is known that the long-term regulation of gene expression is influenced by epigenetic mechanisms, such as DNA methylation, histone modifications and chromatin restructuring, providing a molecular memory of gene and environmental interactions (Rodenhiser and Mann, 2006). Disruption of the heritable methylation patterns in DNA can lead to alterations in chromatin structure and alterations in gene expression (Smith and Crocitto, 1999). Disturbances of the epigenetic control—and particularly of DNA methylation—have been discussed to play a role in the pathophysiology of several psychiatric disorders such as eating disorders (Frielings et al., 2007), depression (Hillemacher et al., 2007b), schizophrenia (Abdolmaleky et al., 2005, 2006; Bleich et al., 2007), drug addiction (Renthal and Nestler, 2008) and alcohol dependence (Bleich et al., 2006; Bönsch et al., 2005).

DNA methylation influences gene transcription by binding a methyl group to a CpG island in the genomic sequence. CpG sequences are spread throughout the genome and are mostly methylated, whereby CpG islands in the promoter regions of genes are usually less methylated. In the majority of cases, a higher methylation of the genomic sequence leads to an inactivation of the referring gene, while less methylation leads to activation (Doerfler, 1983; Egger et al., 2004; Holliday, 1987). Methyl groups bound to the genomic sequence reduce the DNA-binding capacity for transcription factors and so lower the transcription ability of the referring gene. However, in some cases methyl groups do not only reduce the DNA-binding capacity but also are able to enhance transcription factors’ attachment to promoter regions. For example, methyl-binding proteins (Mbs1-4 and MeCP2) bind specially methylated CpG islands exclusively (Lopez-Serra et al., 2006). MeCP2 activates histone methyl-transferases and histone acetylases that is followed by a reduction of gene expression. This means that MeCP2 can be regarded as a mediator between those two epigenetic phenomena—methylation and acetylation (Fukas et al., 2003; Nan et al., 1998; Rodenhiser and Mann, 2006).

![Fig. 2. Function of CAG repeats in the encoding sequence of the AR on leptin transcription and craving.](image-url)
Research on epigenetic alterations in alcohol dependence

Regarding alcohol dependence, several studies showed changes of promotor-specific DNA methylation of several candidate genes. One of these candidate genes investigated lately is the alpha synuclein gene. Studies have shown that the expression of alpha synuclein is enhanced in different brain areas of rats whose alcohol preference is inbred (Liang et al., 2003). Alpha synuclein is known to be involved in dopaminergic neurotransmission (Perez et al., 2002), which has been suggested to be a main mechanism mediating withdrawal and craving associated with alcohol dependence. An increased expression of alpha synuclein mRNA has been described in alcohol-dependent patients, with a correlation to obsessive alcoholic craving (Bönsch et al., 2004). Further studies showed an elevated DNA methylation in the promoter region of the alpha synuclein gene, investigating peripheral mononuclear cells of patients with alcohol dependence compared with healthy controls. It can be hypothesized that this described DNA hypermethylation in the promoter regions of the alpha synuclein gene leads to a down-regulation of alpha synuclein expression, followed by a disturbed dopaminergic neurotransmission (Bönsch et al., 2005).

Another topic in the field of epigenetic alterations in alcohol dependence has been changes in the methylation of the promoter region of HERP (homocysteine-induced endoplasmatic reticulum protein). HERP is an endoplasmatic reticulum resident membrane protein, which regulates Ca\(^{2+}\) homeostasis and thus protects endothelial and neuronal cell integrity against oxidative stress. A recent study described an elevated promoter DNA methylation within the HERP gene in peripheral blood cells of patients with alcohol dependence. Also, HERP mRNA expression was lowered in this study sample, compared with healthy controls (Bleich et al., 2006). Furthermore, HERP mRNA expression was negatively correlated with its promoter methylation (Bleich et al., 2006). This association was reproduced in cell culture experiments incubating neuronal cells with homocysteine (Lenz et al., 2006). In these cell experiments, it was shown that amino acid response element and cyclic-AMP response element-binding protein act as important transcription factors for the expression of HERP. Taken together, these findings may lead to the hypothesis that suppressed expression of HERP under conditions of chronic alcohol consumption may be partially responsible for an elevated rate of seizures, vascular incidents, and other neurological damages.

Also, neuroendocrinological changes due to alcohol dependence might be regulated at least partially by epigenetic mechanisms. However, little is known about epigenetic regulation of endocrinological changes in alcohol dependence. A recent study showed alterations of the promoter-related DNA methylation of ANP and vasopressin precursor genes and the related mRNA expression of these genes in patients at the beginning of alcohol detoxification (Hillemacher et al., 2008). Findings of this investigation showed significantly decreased promoter-related DNA methylation of ANP and significantly elevated promoter-related DNA methylation of vasopressin in peripheral blood cells of the patients group, compared with healthy controls. Furthermore, DNA methylation of ANP was significantly correlated with the extent of craving measured with the OCDS.

A recent investigation focused on alterations of DNA methylation in the promoter region of the DAT (dopamine transporter) gene (Hillemacher et al., 2009a). DAT is responsible for the reuptake of dopamine from the synaptic gap and has therefore a crucial importance for dopaminergic neurotransmission. Findings of this study show a significant hypermethylation in the sequence of the DAT promoter in alcohol-dependent patients compared with the healthy control group, and a negative association between DAT methylation and alcohol craving measured with the OCDS.

Other studies focused on alterations of DNA methylation of the genomic sequence of the N-methyl-d-aspartate 2b receptor subtype (NR2B). An animal study showed recently that chronic but not acute alcohol consumption in mice leads to a hypomethylation of specific areas in the genomic sequence of the NR2B, leading to a receptor up-regulation (Marutha Ravindran and Ticku, 2005). A human study, investigating DNA from peripheral blood cells, showed a significant association between high life time drinking and high daily alcohol intake with lower DNA methylation of NR2B in alcohol-dependent patients undergoing alcohol withdrawal (Biermann et al., 2009). These studies showed that the up-regulation of NR2B in alcohol dependence may be at least partially explained by modification of genomic DNA methylation.

Another recent investigation regarding epigenetic alterations in alcohol dependence focused on epigenetic alterations in the genetic sequence of the polypeptide pro-opiomelanocortin (POMC). POMC is modified post-translationally into several active hormones but particularly into ACTH, which plays an important role in the regulation of the HPA axis. Dysfunction of the HPA axis due to alcohol consumption (Richardson et al., 2008) and in alcohol-dependent patients has been shown in various investigations (Junghanns et al., 2003; Rasmussen et al., 1998). Muschler et al. recently showed that the DNA methylation status in the gene sequence of POMC at single CpG sites differ between patients with alcohol dependence and healthy controls and identified a specific cluster of CpG islands showing a significant association with alcohol craving (Muschler et al., 2010). These results may be considered a hint towards the hypothesis that epigenetic alterations—and particularly changes in DNA methylation—may contribute to the described dysregulation of the HPA axis in alcoholism.

CONCLUSION AND PERSPECTIVES

While the knowledge about the role of epigenetic alterations (due to changes in DNA methylation or histone modifications) and the importance of altered function of transcription factors in alcohol dependence is still limited and must be regarded with caution, the described recent investigations open a new and intriguing field for research. Most investigations performed up to now have been association studies so that the pathophysiology behind still remains hypothetical. However, changes in gene transcription—by epigenetic modifications or altered function of transcription factors—may be important to understand interactions between genetic and environmental factors for the genesis and maintenance of alcohol-seeking behaviour.
While epigenetic alterations and particularly changes in DNA methylation are discussed in a wide range of psychiatric disorders (Abdolmaleky et al., 2005; Frieling et al., 2010; Hillemacher et al., 2007b), they may be of special interest in alcohol dependence for two specific reasons: compared with other psychiatric disorders like schizophrenia or depression, in alcohol dependence a particular agent—ethanol—can be associated with changes in DNA methylation, e.g. by alterations in the homocysteine pathway (Bleich and Hillemacher, 2009; Bleich et al., 2004). Furthermore, these epigenetic alterations may not only be of importance in alcohol dependence but also may contribute to the genesis of alcohol associated malignant diseases like live cancer or colorectal cancer (Baylin, 2005; Giovannucci et al., 1995; Herceg and Hainaut, 2007; McCabe and Caudill, 2005; Seitz and Stickel, 2007; Zhang et al., 2007).

While the described studies are not more than a first step towards a further understanding of the role of epigenetic alterations and the importance of transcription factors in alcohol dependence they provide an important field for further investigations. This may lead to a further understanding of gene–environment interactions in the neurobiology of alcoholism and possibly to more effective therapeutical options in the future.

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