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EPIGENETIC MECHANISMS OF ALCOHOLISM AND STRESS-RELATED DISORDERS

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Abstract

Stress-related disorders, such as anxiety, early life stress and posttraumatic stress disorder appear to be important factors in promoting alcoholism, as alcohol consumption can temporarily attenuate the negative affective symptoms of these disorders. Several molecules involved in signaling pathways may contribute to the neuroadaptation induced during alcohol dependence and stress disorders, and among these, brain-derived neurotrophic factor (BDNF), corticotropin releasing factor (CRF), neuropeptide Y (NPY) and opioid peptides (i.e. nociceptin and dynorphin) are involved in the interaction of stress and alcohol. In fact, alterations in the expression and function of these molecules have been associated with the pathophysiology of stress-related disorders and alcoholism. In recent years, various studies have focused on the epigenetic mechanisms that regulate chromatin architecture thereby modifying gene expression. Interestingly, epigenetic modifications in specific brain regions have been shown to be associated with the neurobiology of psychiatric disorders, including alcoholism and stress. In particular, the enzymes responsible for chromatin remodeling (i.e. histone deacetylases and methyltransferases, DNA methyltransferases) have been identified as common molecular mechanisms for the interaction of stress and alcohol and have become promising therapeutic targets to treat or prevent alcoholism and associated emotional disorders.

Keywords

Alcohol; Anxiety; Stress; Epigenetic; HDAC; histone methylation; DNA methylation

1. Introduction

Alcoholism is a chronic, relapsing brain disorder that is characterized by compulsion to seek alcohol, loss of control in limiting alcohol intake, and experiencing a negative emotional

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Conflict of interest:

SCP reports that a US patent application entitled "Histone acetyltransferase activators and histone deacetylase inhibitors in the treatment of alcoholism" (serial number 60/848237 filed on September 29th, 2006) is currently pending. Other authors reported no biomedical financial interests or potential conflicts of interest.

state during withdrawal (Koob, 2014; Koob, & Volkow, 2010). Genetic and environmental factors interact and appear to be equally important in the development of alcohol addiction (Schuebel et al., 2016; Starkman, Sakharkar, & Pandey, 2012). Stress and other psychiatric disorders, such as anxiety and mood disorders, can increase susceptibility to the development of alcohol use disorder (AUD; Figure 1); in fact, the presence of stress-related disorders leads subjects to consume alcohol in an attempt to attenuate the negative affective symptoms seen during addiction (Figure 1; Becker, 2012; Bolton, Robinson, & Sareen, 2009; Kushner, Abrams, & Borchardt, 2000).

Several adverse life events have the ability to either initiate or exacerbate AUD. For example, it has been demonstrated that exposure to early life stress (ELS) and other common forms of stress, such as acute or chronic stress and posttraumatic stress disorder (PTSD), are predictive factors of AUD (Enoch, 2011; Müller et al., 2015; Sinha, 2007). ELS can promote alterations in the hypothalamic-pituitary-adrenal (HPA) axis, lead to changes in brain morphology and in gene expression of the mesolimbic reward pathway, all of which are implicated in the development of alcohol addiction (Enoch, 2011). Furthermore, negative affective states, such as anxiety and depression, are often comorbid with AUD (Koob, 2014; Shorter, Hsieh, & Kosten, 2015). In this regard, it has been reported that in the United States almost 1 in 2 patients suffering with PTSD also have a diagnosis of AUD (Pietrzak et al., 2011).

Alcohol exposure can affect both the brain reward and stress systems (Koob, & LeMoal, 2001; Koob, & Volkow, 2010). The mesocorticolimbic dopamine system has been implicated in the acute reinforcing effect of several drugs of abuse, including alcohol. Furthermore, neuroadaptations produced in the HPA axis and extrahypothalamic brain stress systems due to chronic ethanol exposure are involved in the withdrawal/negative affect state of alcoholism (Koob, 2013, 2014). In particular, several studies report that neurobiological changes in the extended amygdala are critical for the interaction between alcohol addiction and stress-related disorders such as anxiety (Koob, 2008; Koob, & Volkow, 2010; Pandey, 2003; 2004). In fact, both stress and addiction can induce similar epigenetic modifications underlying changes in neurochemical pathways and synaptic plasticity in various structures of the extended amygdala suggesting a common link between alcoholism and stress-related disorders (Cadet, 2016; Moonat, & Pandey, 2012; Schuebel et al., 2016; Spanagel, Noori, & Heilig, 2014; Stamatakis et al., 2014).

Modifications in the epigenome regulate gene expression affecting neuronal differentiation, maturation, and plasticity (Karpova, Sales, & Joca, 2016; Murrell, Rakyan, & Beck, 2005). The term “epigenetic” refers to chemical modifications of the DNA and histone proteins around which the DNA is wrapped (Holliday, 2006). Together, the DNA-protein complex is known as chromatin. Therefore, chemical alterations contribute in regulating the chromatin state, making it more or less accessible to transcription factors (Kouzarides, 2007). Histone chemical modifications occur mainly at lysine (K) amino acid residue located in the tails of histones 3 and 4 (H3 and H4); however, other histones, such as H2A and H2B, can be modified as well. Covalent alterations include acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, citrullination and ADP-ribosylation, and modulate the chromatin structure differently (Kouzarides, 2007; Krishnan et al., 2014). Among these,

histone acetylation and methylation are the most studied chemical modifications. For example, histone lysine acetylation is associated with the transcriptionally active state of the chromatin and is regulated by two classes of enzymes; histone acetyltransferases (HATs) and histone deacetylases (HDACs), which add and remove acetyl groups respectively (Kouzarides, 2007; Strahl, & Allis, 2000). Based on the lysine residue targeted, the addition of methyl groups seems to have different meanings with respect to transcription; for instance, the mono- and tri-methylation of lysine 4 on histone 3 (H3K4) is related to gene activation, whereas di- and tri-methylation of H3K9 and H3K27 are considered repressive markers. Interestingly, the mono-methylation of H3K9 and H3K27 modulates gene transcription in an opposite way compared to di- and tri-methylation of these same residues, suggesting that the valence of modifications is also important in regulating the chromatin structure (Kouzarides, 2007; Strahl, & Allis, 2000). Epigenetic alterations have been observed during alcohol dependence (Krishnan et al., 2014; Starkman et al., 2012), in several psychiatric disorders (Guidotti et al., 2014; Jawahar et al., 2015; Renthall et al., 2007), and in animal models of alcoholism exhibiting emotional disorders, such as anxiety and stress (Cadet, 2016; Moonat, & Pandey, 2012).

Besides histone modifications, DNA methylation is a common epigenetic alteration able to affect the chromatin structure (Holliday, 2006). DNA methylation is represented by the addition of a methyl group to the DNA sequence, in particular to the cytosine nucleotides, and is regulated by DNA methyltransferases (DNMTs). When DNA is methylated, chromatin condenses and the transcription complex will not be able to bind DNA, thus silencing the gene expression (Klose, & Bird, 2006). Moreover, methyl-CpG binding domain proteins (MBDs) can recognize portions of the DNA sequence enriched with methylated cytosine and guanidine nucleotides (CpG). In turn, these proteins recruit enzymes responsible for further epigenetic alterations that contribute to the condensed state of the chromatin (Boyes, & Bird, 1991; Nan et al., 1998). Alcohol, as well as stress, can lead to epigenetic modifications that may be associated with synaptic remodeling and behavioral phenotypes such as anxiety and depression (Moonat, & Pandey, 2012). This review aims to summarize recent findings on the epigenetic mechanisms regulating gene expression alterations during alcoholism and stress. First, we will provide an overview of the molecules regulating synaptic plasticity that are known to be related to both alcohol dependence and stress disorders and then discuss their regulation by epigenetic mechanisms, particularly histone acetylation and methylation and DNA methylation, underlying these disorders (Figures 2 & 3).

2. Common molecules of alcohol addiction and stress

Several genes are involved in the neuroadaptational mechanisms induced by both alcohol dependence and stress disorders (Spanagel et al., 2014). As mentioned above, addictive processes involve both the brain reward and stress systems (Koob, 2013, 2014). Despite it is known that alcohol dependence and stress-related disorders share similar neuronal pathways, a full characterization of the mechanisms regulating the activity of genes (i.e. epigenetic mechanisms) is still emerging, adding layers of molecular complexity to the regulation of their function.

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Around twenty years ago, Koob and colleagues proposed an elegant “allostatic theory” for alcohol addiction, in which prolonged and excessive alcohol exposure is able to produce adaptive changes in brain function resulting in a deviation of the regulatory system from its homeostatic level (Koob, 2003; Koob & Le Moal, 1997, 2001; Roberts et al., 2000). Positive and negative reinforcement seem to play an important role in this allostatic process, as well as the recruitment of the brain stress system (Koob, 2003). Several neurotransmitters are involved in mediating stress processes, including corticotropin releasing factor (CRF), dynorphin (DYN) and its receptor, the κ opioid (KOP) receptor, substance P, norepinephrine, vasopressin and hypocretin. On the other hand, anti-stress systems like neuropeptide Y (NPY) and nociception/orphanin FQ (N/OFQ) physiologically counterbalance the effect of the pro-stress molecules, and prolonged alcohol exposure can disrupt the balance between these regulatory mechanisms (Koob, 2015).

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NPY is a neuropeptide that is highly expressed in the amygdaloid regions and implicated in alcohol drinking and anxiety-like behaviors. Several pieces of evidence indicate that higher levels of NPY are able to attenuate, whereas lower levels promote, alcohol consumption (Gilpin et al., 2003; Gilpin, Stewart, & Badia-Elder, 2008; Thorsell, Slawecki, & Ehlers, 2005; Zhang et al., 2010). Moreover, it is also well known that NPY antagonizes the behavioral consequences of stress and has a great anxiolytic-like effect (Heilig, 2004). Interestingly, it has been hypothesized that NPY and CRF exert opposite effects in the negative emotional state induced by drug addiction (Koob, 2003; Gilpin, 2012; Heilig, & Koob, 2007).

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Another gene highly implicated in the neurobiology of alcoholism and stress is brain-derived neurotrophic factor (BDNF). In the adult brain, this neurotrophic factor regulates synaptic transmission and plasticity (Gonzalez et al., 2016; Poo, 2001; Tao et al., 1998). BDNF induces an increase in the cytosolic content of calcium (Berninger et al., 1993; Stoop, & Poo, 1996) that can affect the vesicle exocytosis of several neurotransmitters in the synaptic space (Pozzo-Miller et al., 1999). BDNF can in turn modulate cAMP-responsive element binding (CREB) protein activity; in fact, by interacting with the tropomyosin receptor kinase B (TrkB) receptors, BDNF activates the mitogen-activated protein kinase (MAPK) cascade (Shaywitz, & Greenberg, 1999; Waltereit, & Weller, 2003). This activation results in the recruitment and phosphorylation of several transcription factors, including CREB, which in turn enhances the expression of other genes such as activity related cytoskeleton-associated (Arc) protein and regulate synaptic plasticity (Pandey et al., 2008b; Ying et al., 2002). In addition, functional changes induced by BDNF can result into morphological and structural changes (i.e. spine density), since its role in neuron differentiation and synaptic remodeling has been well established (McAllister, Katz, & Lo, 1997; Tanaka et al., 2008; Zagrebelsky, & Korte, 2014).

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BDNF and Arc are both CREB target genes (Pandey, 2004; Tao et al., 1998) and together this signaling cascade plays an important role in mediating alcohol drinking and anxiety-like behavior (Pandey, 2003, 2004; Pandey, Roy, & Zhang, 2003). Dysregulation of BDNF expression most likely due to epigenetic changes is observed during alcohol dependence (Pandey et al., 2008a, 2015; You et al., 2014). Additionally, alcohol-preferring (P) rats, show high innate alcohol preference and anxiety-like behavior compared to nonpreferring (NP)

rats (Gilpin et al., 2003; Pandey et al., 2005). CREB phosphorylation, BDNF and Arc expression are lower in the central and medial nucleus of the amygdala (CeA and MeA) of P as compared to NP rats (Pandey et al., 2005; Prakash, Zhang, & Pandey, 2008; Moonat et al., 2011). Interestingly, alcohol exposure can increase BDNF expression due to increased histone acetylation of BDNF and Arc genes in amygdaloid structures, and produces anxiolysis in P but not in NP rats (Moonat et al., 2011, 2013). In addition, decreasing the expression of BDNF in the CeA and MeA but not in the basolateral amygdala (BLA) produced anxiety-like behaviors and promoted alcohol intake in Sprague-Dawley (SD) rats (Pandey et al., 2006). Furthermore, acute alcohol exposure can increase the phosphorylation of CREB, CREB binding protein (CBP) levels and expression of BDNF and Arc in the CeA and MeA of SD rats (Pandey, 2004; Pandey et al., 2008a,b). On the other hand, withdrawal after chronic ethanol exposure decreased CREB phosphorylation, CBP levels and expression of BDNF and Arc in the CeA and MeA of SD rats and infusion of BDNF into CeA attenuated development of anxiety-like behaviors (Pandey et al., 2008a,b). Together these results suggest that deficits in CREB signaling can lead to chromatin remodeling via CBP and reductions in BDNF and Arc expression in the amygdaloid circuitry thereby promoting excessive alcohol drinking and heightened anxiety-like behavior (Figure 2).

Alterations in the BDNF system are also associated with neuromechanisms of mood disorders, such as depression and anxiety (Duman & Monteggia, 2006; McEwen et al., 2015). Increased mRNA levels of BDNF in the hippocampus correlate with resilience to chronic mild stress and can block the anhedonic consequences of stress (Bergström et al., 2008; Taliaz et al., 2011). On the other hand, BDNF knockdown animals exhibited depressive-like behavior and a reduction in neurogenesis in the dentate gyrus (Taliaz et al., 2010) and consume higher amounts of alcohol (Hensler, Ladenheim, & Lyons, 2003). In humans, the valine 66 to methionine (Val66Met) polymorphism within the BDNF sequence is associated with psychiatric disorders (Frielingsdorf et al., 2010; Notaras, Hill, & van den Buuse, 2015; Rybakowski, 2008) and in mice the presence of Val68Met polymorphism (a mouse homolog of human Val66Met) has been associated with increased anxiety-like behavior under stressful conditions (Chen et al., 2006). A recent study suggested that female offspring of parents with a history of alcohol dependence exhibit a lower reward dependence score in association with the presence of the Val66Met polymorphism, and this may modulate their vulnerability to depression (Benzerouk et al., 2015). It has also been demonstrated that mice carrying the Val68Met polymorphism have a higher risk of developing uncontrolled and excessive alcohol drinking patterns (Warnault et al., 2016; Pandey, 2016).

A key system in regulating addiction mechanisms is the endogenous opioid system (EOS); the EOS consists of four receptor types (and for each type several subtypes) and their endogenous ligands (Bodnar, 2016). Among these, the involvement of the DYN and the KOP receptor in alcohol addiction and in other psychiatric disorders is well established (Walker, & Koob, 2008; Walker et al., 2012). Following acute alcohol exposure, the positive affective state induced by ethanol appears to be mediated by the μ -opioid (MOP) receptor that is followed by a compensatory negative emotional state due to the activation of KOP receptors (Walker et al., 2012). During chronic alcohol exposure, MOP receptor signaling is attenuated while KOP receptor signaling is increased, thereby inducing a negative affective

state characterizing the alcohol dependent condition (Walker et al., 2012). Several studies reported a correlation between DYN system upregulation and the escalation of alcohol self-administration in dependent animals (D'Addario et al., 2013 a; O'Dell et al., 2004; Walker & Koob, 2008), suggesting a potential therapeutic role of KOP receptor antagonists to attenuate not only the withdrawal-associated negative emotional state but also to reduce alcohol consumption (Walker, Zorrilla, & Koob, 2011). Moreover, in the extended amygdala KOP receptors can regulate other neurotransmitters such as glutamate, γ -aminobutyric acid (GABA) and serotonin (Land et al., 2009; Li et al., 2012; Gilpin et al., 2014), impacting motivational and emotional circuitry. Similarly to alcohol dependence, chronic stress can induce a prolonged activation of KOP receptor signaling in limbic regions leading to depressive and anxious phenotypes, which can be blocked by using KOP receptor antagonists (Knoll, & Carlezon, 2010). In contrast to the negative effects induced by DYN system activation, the N/OFQ system acts as a stress buffer system (Koob, 2015; Witkin et al., 2014). In fact, N/OFQ usually mitigates stress-like responses and induces anxiolytic-like effects in animals (Ciccocioppo et al., 2003; Martin-Fardon et al., 2010). Acting through the N/OFQ peptide (NOP) receptor, N/OFQ can prevent and revert presynaptic GABA release induced by alcohol in the CeA and enhance the decreased GABAergic transmission seen during alcohol dependence (Roberto, Gilpin, & Siggins, 2012; Roberto, & Siggins, 2006).

Both DYN and N/OFQ peptides are tightly related to another neuropeptide involved in stress and addiction, CRF. In the extended amygdala, CRF and DYN are frequently released together inducing similar anxiogenic-like and stressful effects (Kang-Park et al., 2015; Koob, 2015). On the other hand, N/OFQ is considered a functional antagonist of the CRF, since it counteracts CRF actions at the extrahypothalamic level (Ciccocioppo et al., 2003, 2014; Rodi et al., 2008; Uchiyama et al., 2008), similar to what was observed for NPY (Gilpin, 2012; Pleil et al., 2015). Dysregulations of the N/OFQ system were found in the CeA of Marchigian Sardinian alcohol-preferring rats and these alterations could represent an adaptive mechanism to antagonize the increase of CRF signaling responsible for excessive drinking and anxious behaviors of these animals (Economidou et al., 2008).

Additionally, CRF receptor 1 (CRF1) antagonists block anxiety-like behavior during alcohol withdrawal (Baldwin et al., 1991; Funk et al., 2007; Knapp et al., 2004; Overstreet, Knapp, & Breese, 2004), especially when they are infused directly in the CeA (Rassnick et al., 1993). In fact, alcohol withdrawal increases CRF synthesis and release in CeA (Funk et al., 2006; Roberto et al., 2010; Sommer et al., 2008), which in turn can regulate GABAergic transmission (Roberto, Gilpin, & Siggins, 2012). Moreover, alterations in the CRF system induced by stressors, such as maternal separation, are predictive of binge drinking and impulsivity behaviors in adulthood (Gondré-Lewis et al., 2016). The involvement of all these molecules in mediating neurobiological responses induced by both alcoholism and stress disorders are well documented. However, the epigenetic mechanisms responsible for these molecule alterations are still emerging and have been described below.

3. Epigenetic mechanisms of alcohol addiction

3.1. Alcohol and histone modifications

As described above, the transcriptionally active state of the chromatin is characterized by increased acetylation of histone lysine residues. The levels of histone acetylation are regulated by the activity of two enzyme families, the HATs and HDACs, which respectively add and remove the acetyl groups. Animals acutely treated with alcohol exhibit anxiolytic-like behavior and also show a decrease in HDAC activity in the amygdala, which is associated with a global increase in acetylation of histone H3 lysine 9 (H3K9) and histone H4 lysine 8 (H4K8) but not H3K14 in the same area (Moonat et al., 2013; Pandey et al., 2008a). Interestingly, in the amygdala of acute ethanol-treated rats, ethanol induced a significant and specific increase of H3K9 acetylation levels in the prodynorphin (PDYN), pronociceptin, BDNF exon IV and Arc promoters associated with an upregulation in gene transcription (D'Addario et al., 2013 a, b; Moonat et al, 2011, 2013). In another study, it has been shown that HDAC-induced histone modifications (H3K9 and H4K8) and changes in NPY expression in the CeA and MeA are associated with the development of rapid tolerance to anxiolytic effects of ethanol (Sakharkar et al., 2012). Similarly to what was observed in the amygdala, after acute ethanol treatment sensitized mice showed a reduction in global striatal HDAC activity and an increase of H4 acetylation specifically in the core of the nucleus accumbens (NAc) (Botia et al., 2012). Other evidence of HDAC involvement in alcohol addiction showed that the non-specific HDAC inhibitor sodium butyrate (NaB) was able to prevent and reverse behavioral sensitization and gene expression alterations induced by ethanol, such as BDNF mRNA changes in the striatum and prefrontal cortex (PFC) (Legastelois, Botia, & Naassila, 2013).

Different isoforms of HDACs are grouped into four different classes based on sequence similarity and cellular localization. HDAC class I includes HDACs 1, 2, 3 and 8 whereas HDACs 4, 5, 6, 7, 9 and 10 belong to class II; class III, also named sirtuins, is composed of SIRT 1, 2, 3, 4, 5, 6, 7 and finally HDAC 11 is the only HDAC belonging to class IV (de Ruijter, 2003). The involvement of specific HDAC isoforms in alcohol dependence and exposure was investigated in several studies. Recently, results on human primary monocyte-derived dendritic cells, derived from alcohol users, demonstrated that class I HDAC gene expression and protein levels were significantly higher compared to the controls (Agudelo et al., 2016). Another *in vitro* study revealed that alcohol induced a dose-dependent increase of HDAC2 expression (Agudelo et al., 2011). Higher global HDAC activity was observed in the amygdala of P rats as compared to NP rats. This appears to be due to higher HDAC2 but not HDACs 1, 3, 4 and HDAC5 protein levels in the CeA and MeA of P compared to NP rats and accordingly, P animals displayed lower levels of H3K9 acetylation globally and specifically at the BDNF and Arc gene promoters (Moonat et al., 2013). Furthermore, knocking-down HDAC2 in P rats attenuated alcohol drinking and anxiety-like behavior of these animals and also increased BDNF and Arc promoter histone acetylation and their protein levels, (Moonat et al., 2013). Using an HDAC inhibitor selective for class I and II [trichostatin A (TSA)], a reduction in alcohol drinking and anxiety-like behavior and in amygdaloid nuclear HDAC activity was observed in P rats. Moreover, in the CeA and MeA TSA-treated P rats showed lower HDAC2 protein levels (Sakharkar et al., 2014a). All these

studies suggested that HDAC2 could play an important role in the epigenetic alterations observed during the comorbidity of alcohol dependence and anxiety. However, the involvement of each HDAC isoform seems to be tissue specific; following acute alcohol treatment, the protein levels of nuclear HDAC1–3 were significantly decreased in the caudate putamen (CPu), while no changes were detected in the PFC except for the HDAC3 isoform (Caputi et al., 2015). Interestingly, BDNF heterozygous mice, which exhibit a high preference for ethanol consumption (Hansler et al., 2003) and vulnerability to develop alcohol dependence, showed innately low basal levels of nuclear HDAC1–3 in the striatum, which were not altered by acute ethanol treatment (Caputi et al., 2015). In the hippocampus, mice subjected to chronic ethanol treatment exhibited downregulation of HDACs 1, 2 and 5 mRNAs and BDNF levels, and TrkB-mediated neurogenesis in the dentate gyrus was significantly enhanced (Stragier et al., 2015).

During withdrawal from chronic ethanol exposure, animals display increased anxiety-like behavior (Pandey, 2003; Pandey, et al., 2008a,b; You et al., 2014) corresponding to a decrease in H3K9 and H4K8 acetylation levels and an increase in HDAC activity in the amygdala (Pandey et al., 2008a). BDNF and Arc mRNA and protein levels decreased in the CeA and MeA of withdrawn rats and these alterations can be restored by TSA treatment (You et al., 2014). Treatment with TSA was able to attenuate development of anxiety-like behaviors during ethanol withdrawal in rats (Pandey et al., 2008a; You et al., 2014). Interestingly, treatment with HDAC inhibitor, sodium butyrate (NaB) significantly attenuated excessive alcohol intake in dependent rats (Simon-O'Brien et al., 2015). Together, these studies suggest that HDAC inhibitors treatment can restore chromatin remodeling and prevent anxiety-like behaviors and dependent-induced excessive alcohol intake in rats.

During adolescence, epigenetic modifications play an important role in neurodevelopment, and interfering with these mechanisms is a risk factor that can result in developing neuropsychiatric disorders in adult life (Kyzar et al., 2016a). As observed in adult rats, acute alcohol treatment induced anxiolytic effects and the inhibition of HDAC activity in the amygdala of adolescent rats but required higher dose of ethanol as compared with adult rats (Sakharkar et al., 2012, 2014b). However, a lack of tolerance to these ethanol-induced effects was observed during adolescence and this may promote adolescent binge drinking behavior (Sakharkar et al., 2014b). After 24 hours of withdrawal from adolescent intermittent ethanol (AIE) exposure, rats displayed anxiety-like behavior and a simultaneous increase of HDAC activity and protein levels of HDAC2 and HDAC4 in the amygdala (Pandey et al., 2015). Some of these epigenetic changes, such as increased HDAC2 protein levels and deficits in H3K9 acetylation, were persistent into adulthood indicating that adolescent alcohol exposure produced condensed chromatin architecture in amygdaloid structures. Interestingly, AIE exposure enhanced ethanol intake and anxiety-like behaviors in adulthood, which were attenuated by TSA treatment (Pandey et al., 2015).

Long lasting epigenetic consequences of adolescent alcohol exposure were detected at the gene promoter level in the amygdala during adulthood. It was found that AIE treatment decreased the H3K9/14 acetylation levels at BDNF exon I and IV and Arc promoters that were associated with decreased BDNF and Arc expression and dendritic spine density in the CeA and MeA during adulthood (Pandey et al., 2015). Adult rats exposed to the AIE

treatment also exhibited an increase in hippocampal HDAC activity, a decrease in global H3K9 acetylation, and also a decrease in CBP (Sakharkar et al., 2016). Moreover, in the AIE exposed animals, BDNF protein and exon IV mRNA levels were decreased in the CA1 and CA3 regions of the hippocampus. At the same time, the decrease of H3K9/14 acetylation levels of the BDNF exon IV promoter and a reduction in neurogenesis markers were observed, and it has been shown that all these alterations are reversed by TSA treatment (Sakharkar et al., 2016). In addition to the changes observed in histone acetylation, alcohol exposure affects histone methylation mechanisms. For a long time, the methylation of all histone lysine residues was thought to induce chromatin condensation (Peters et al., 2002). Recent evidence demonstrated that chromatin can be more or less condensed depending on the specific lysine residue being methylated (Nowak et al., 2016; Shi Y et al., 2004). For example, a primary culture of rat hepatocytes exposed to ethanol showed a decrease in H3K9 dimethylation levels and an increase of H3K4 dimethylation, corresponding to ethanol-induced upregulation or downregulation of the expression of several genes respectively (Pal-Bhadra et al., 2007).

There are two studies on alcohol dependent patients (post-mortem brain studies) that have identified altered H3K4 trimethylation levels in the hippocampus and cortex, which correspond to gene expression alterations in these regions (Ponomarev et al., 2012; Zhou et al., 2011). Despite the limited evidence available due to a lack of human studies, several researchers have focused on the ethanol-induced alterations of histone methylation levels in animals. Six hours after an acute alcohol injection has been administered, an increase in global and gene specific H3K4 trimethylated levels has been detected in the cerebral cortex of mice (Finegersh & Homanics, 2014). A decrease in trimethylated H3K27 levels was found in the amygdala of ethanol-treated rats, specifically in the promoter regions of PDYN and pronociceptin genes, correlating with increased expression of both genes (D'Addario et al., 2013a,b). In the hippocampus of mice, chronic ethanol consumption increased global H3K4 trimethylation and H3K27 dimethylation, and epigenetic alterations were specifically found at different BDNF exon promoter regions correlating to their expression (Stragier et al., 2015). In fact, the upregulation of BDNF exons II and III was observed with the increase of trimethylated H3K4, just as exon IV upregulation was related to an increase in H3K9 acetylation. On the contrary, BDNF exon VIII expression was downregulated with significant reductions in H3K4 trimethylation and H3K9 acetylation of its promoter (Stragier et al., 2015). Chronic intermittent ethanol (CIE) exposure induced upregulation of the N-methyl-D-aspartate (NMDA) receptor 2B subunit (NR2B), possibly by increasing the acetylation and decreasing the methylation of H3K9 of this gene promoter (Qiang et al., 2011). Also, CIE induced a significant decrease in the expression of nine genes coding for enzymes (G9a, Prmt6, Setdb2, Suv39 h1, Setd1a, Setd1b, Setd4, Setd6 and Setdb1) belonging to the histone methyltransferase (HMT) family (Qiang et al., 2011).

Postnatal exposure to ethanol induced apoptotic neurodegeneration and enhanced the activity of G9a (a histone methyltransferase known also as euchromatic histone-lysine N-methyltransferase 2); as a consequence, an increase in H3K9 and H3K27 dimethylation was observed (Subbanna et al., 2013). In the same paradigm, G9a protein levels were increased by ethanol exposure, consistently with the acetylation of H3K14 of G9a exon1 promoter (Subbanna et al., 2014). Recently, it has been shown that lysine demethylase 1 (LSD1) is

involved in the long-lasting effects of AIE exposure in the amygdaloid brain regions during adulthood. In fact, a decrease in mRNA and protein levels of this enzyme was related to increased global H3K9 but not H3K4 dimethylation in the CeA and MeA of these animals (Kyzar et al., 2016b). An acute ethanol challenge in adulthood can attenuate the anxiety-like behavior, the decrease in Lsd1+8a (a neuron-specific splice variant of LSD1), and reverse the increase of dimethylated H3K9 levels of the BDNF exon IV gene promoter in the amygdala induced by AIE (Kyzar et al., 2016b). Together with adolescent studies on histone acetylation, all these data suggest that alcohol exposure during early life or in adulthood can cause epigenetic alterations leading to changes in gene expression that, in turn, can regulate alcohol-related behaviors (Kyzar et al., 2016a).

3.2. Alcohol and DNA methylation

Aside from histone modifications, alcohol can also modulate DNA methylation mechanisms in the brain (Schuebel et al., 2016). DNA methylation occurs when a methyl group from S-adenosyl methionine (SAM) is transferred to the cytosine residues in the dinucleotide sequence CpG. The enzymes conducting this reaction are two kinds of DNMTs, de novo DNMTs 3a and 3b, and maintenance DNMT1; together interacting with MBDs to regulate gene expression (Holliday, 2006; Klose, & Bird, 2006).

Studies on neural stem cells revealed that alcohol prevents normal DNA methylation programming of several genes deferring cell differentiation, and this mechanism can play an important role in the development of fetal alcohol syndrome (Zhou et al., 2011). Patients with alcoholism showed a significant increase in genomic DNA methylation in the whole blood cell, which was associated with decreased DNMT3b mRNA levels, suggesting a feedback-regulation of this enzyme by increased DNA methylation (Bönsch et al., 2006). Other human studies revealed that alcohol can induce DNA methylation in specific genes. In fact, three of five single-nucleotide polymorphisms (SNPs) in the PDYN gene promoter associated with alcohol dependence (Xuei et al., 2006) form a CpG dinucleotide and in the dorsolateral PFC of postmortem human alcoholics, these PDYN CpG-SNPs were found to be methylated differently (Taqi et al., 2011).

Several studies focused their attention on the DNA methylation alterations induced by alcohol in different animal models. CIE exposed animals showed an upregulation of NR2B gene expression in the adult cortex and cultured fetal cortical neurons, due to decreased methylation of the NR2B gene CpG island (Kumari & Ticku, 1998; Marutha Ravindran & Ticku, 2004). An increase of the tissue plasminogen activator (tPA), a molecule involved in modulating neuronal plasticity, was detected in the primary cortical astrocytes treated with ethanol or with a DNMT inhibitor, 5-aza-2'-deoxycytidine (Zhang et al., 2014). Moreover, ethanol inhibited DNMT activity with a corresponding decrease in DNA methylation levels of the tPA promoter and a reduction in the DNMT3a protein levels, suggesting that this epigenetic mechanism may be involved in astrocyte-mediated inhibition of neuronal plasticity induced by alcohol (Zhang et al., 2014). Excessive alcohol drinking increased DNMT1 levels in the mouse NAc, and this alteration was in line with the effect preventing excessive drinking behavior exerted by 5-azacytidine, which inhibits DNMT activity (Warnault et al., 2013). Accordingly, prolonged abstinence caused an escalation of alcohol

intake, in association with increased DNA methylation and decreased expression of several genes in the medial PFC; the infusion of the DNMT inhibitor RG108 was able to prevent both the escalation of alcohol consumption and the downregulation of the majority of these genes (Barbier et al., 2015). Acute ethanol treatment inhibited DNMT activity in the amygdala and in the bed nucleus of stria terminalis (BNST) of adolescent rats and a lack of tolerance of this effect was observed following a second ethanol challenge (Sakharkar et al., 2014b). Moreover, mRNA of various DNMT isoforms was differently regulated in these two regions, suggesting that different isoforms could play different roles in the alcohol-induced effects during adolescence, as observed for the HDACs (Sakharkar et al., 2014a; Moonat et al., 2013). In contrast to the decreased DNMT activity observed in adolescent rats, combined pre- and post-natal alcohol exposure resulted in a significant increase in DNMT activity and contemporarily, alterations of DNMT1, DNMT3a, and methyl CpG binding protein 2 (MeCP2) gene expression in the hippocampus (Perkins et al., 2013). Although more evidence is still needed, all of these studies underline the crucial involvement of DNA methylation mechanisms in alcohol addiction and suggest that DNMT can serve as a potential epigenetic target to develop a pharmacotherapy for alcoholism (Figure 3).

4. Epigenetics and stress-related disorders

In recent years, a growing body of evidence suggests that epigenetic mechanisms are involved with stress-related disorders similar to alcoholism. As mentioned above, BDNF and related pathways play a crucial role in developing mood disorders, such as depression, anxiety and fear-related disorders (Duman & Monteggia, 2006; Kirtley & Thomas, 2010; McEwen et al, 2015). Particularly, low levels of BDNF in the amygdala compromise fear extinction, but high levels of this neurotrophic factor in the medial PFC or hippocampus can facilitate this process (Karpova, et al., 2011; Peters et al., 2010). It is possible that these changes could be mediated epigenetic mechanisms. For example, the use of the HDAC inhibitor valproic acid (VPA) facilitates fear extinction and at the same time increases the expression of BDNF exon IV mRNA and H4 acetylation on its promoter in the medial PFC (Bredy et al., 2007). Similarly, animals treated with suberoylanilide hydroxamic acid (SAHA), known as vorinostat, showed improvements in fear extinction increasing the acetylation of the NR2B promoter region and, as a consequence, enhancing glutamate signaling in the hippocampus (Fujita et al., 2012), which results in increased BDNF expression (Tabuchi et al., 2000). TSA directly infused into the CeA attenuated anxiety-like behavior induced by elevated corticosteroids, and significantly enhanced global H3K9 acetylation (Tran et al., 2015). Moreover, TSA treatment reversed the reduction of glucocorticoid receptor expression induced by corticosteroids, and, as a consequence, decreased CRF expression in the CeA (Tran et al., 2015). Another study reported that PTSD animals showed greater anxiety-like behavior in the elevated plus-maze (EPM) test compared to controls and increased HDAC activity in the PFC and hippocampus; in these same regions, 30-day treatment with the VPA attenuated symptoms of anxiety and inhibited HDAC activity (Wilson et al., 2014).

Different HDAC isoforms have different roles in regulating stress-related disorders. For example, in rodents HDAC2 negatively regulates fear extinction learning (Guan et al., 2009; Morris et al., 2013). The overexpression of HDAC1 in the hippocampus of adult mice

affected the extinction of contextual fear memories through the involvement of H3K9 deacetylation and the trimethylation of target genes (Bahari-Javan et al., 2012). After chronic social defeat stress, the levels of acetylated H3 were increased in the NAc and at the same time a decrease in HDAC2 and HDAC5 levels was detected (Covington et al., 2009; Renthall et al., 2007). These changes seem to be tissue specific since the same stress paradigm caused a persistent decrease in acetylated H3 in the hippocampus, while a transient increase in the amygdala was also observed (Covington et al., 2011b). However, the infusion of the class I HDAC inhibitor MS-275 in the NAc, amygdala, and medial PFC exerted antidepressant effects (Covington et al., 2009, 2011b, 2015).

The involvement of hippocampal HDAC2 and 5 was also reported in a model of chronic restraint stress. Animals treated with NaB showed a decrease in depressive behavior. Simultaneously, a normalization of CREB phosphorylation, H3 acetylation, HDAC2 and BDNF expression levels was observed (Han et al., 2014). An increase in HDAC5 levels at the promoter of BDNF exon IV and decrease in BDNF expression were observed in the hippocampus in the learned helplessness model of depression in mice. Intraperitoneal injection of HDAC inhibitors or imipramine decreased the occupancy of HDAC5 at BDNF exon IV promoter and reversed BDNF levels in the hippocampus, which was associated with an improvement of depressive symptoms (Su et al., 2016). Another study substantiated the role of class I HDAC isoforms and the antidepressant action of the HDAC inhibitors. By infusing MS-275 in the ventrolateral orbitofrontal cortex, depressive behavior was attenuated and this effect was accompanied with the increase of H3 acetylation along with CREB and BDNF expression in this region (Lin et al., 2012). Regarding histone methylation, an increase in H3K27 dimethylation on BDNF exon III and IV promoter regions, as well as changes in its transcripts III and IV, was detected as a long-lasting consequence in the hippocampus of defeated stress mice (Tsankova et al., 2006). Interestingly, chronic imipramine treatment reversed this downregulation and increased H3 acetylation with the selective downregulation of the HDAC5 (Tsankova et al., 2006). Moreover, DNA methylation plays an important role in stress response. In fact, the hypermethylation of the BDNF gene and decreased BDNF gene expression were detected in the CA1 hippocampal subregion of mice subjected to psychosocial stress (Roth et al., 2011). Female mice resistant to fear extinction showed higher DNA methylation of BDNF exon IV and lower expression in the prefrontal cortex and activation of BDNF signaling via stimulation of trkB prevent the fear in mice (Baker-Andresen et al., 2013).

Epigenetic factors are also involved in stress susceptibility and resilience mechanisms. For example, a mouse strain susceptible to stress showed a difference in the regulation of H3 acetylation and methylation in response to a chronic ultra-mild stress compared to a resilient strain (Uchida et al., 2011). Particularly, the level of H3 methylation in the NAc was similar between mice subjected to chronic defeat stress and those subjected to social defeat stress. Furthermore, chronic imipramine treatment, an antidepressant drug, results in the reversal of decreased repressive H3K9/K27me2 induced by social defeat at several gene promoters (Wilkinson et al., 2009). In the NAc, the reduction of G9a increased the susceptibility to social stress and its overexpression protected mice from the negative consequences of stress exposure (Covington et al., 2011a).

As mentioned above, the exposure to adverse events during pre- and post-natal periods (early life) is considered as a vulnerability factor to developing addiction and mood disorders during adulthood. Maternal separation (MS) is one of the most common ELS animal models and several studies demonstrate that a long period of MS predisposes animals to higher ethanol consumption and/or preference in adulthood (Nylander & Roman, 2013). Rats separated from maternal care for a long period of time exhibited higher mRNA levels of DNMT1 in the pituitary compared to those subjected to a shorter period of MS. Moreover, prolonged maternal separated rats showed a decrease of DNMT1 expression following ethanol consumption compared to a water-drinking group (Todkar et al., 2016).

Differences in ethanol-induced alterations of gene expression between ELS rats and controls were also detected in the NAc. Long-term changes in DNMT1 and MeCP2 were negatively correlated to the voluntary drinking behavior of these rats (Vrettou et al., 2015). Long-term alterations of DNMT1 were also observed in the offspring of animals stressed during pregnancy, which demonstrated behavioral abnormalities similar to what has been observed in schizophrenic patients during adulthood. Particularly, DNMT1 gene expression was increased, while there was a decrease in BDNF mRNA expression, as well as an increase in DNA methylation in its gene promoter; all changes were detected in the hippocampus and frontal cortex (Dong et al., 2015). It is interesting to note that the sex of the animal can affect ELS-induced epigenetic changes (i.e. DNA methylation and histone acetylation) at BDNF specific promoters in the amygdala, hippocampus, and medial PFC of both adult and adolescent animals (Blaze, Asok, & Roth, 2015; Doherty, Forster, & Roth, 2016; Roth et al., 2014). Besides BDNF, ELS can affect the regulation of other molecules, such as CRF. In fact, a negative correlation between CRF mRNA levels and DNA methylation of its promoter region was found in the CeA of stressed rats (van der Doelen et al., 2015). In addition, it has recently been shown that PTSD subjects have higher DNA methylation of BDNF exon 1 promoter region in the blood as compared with those without PTSD. Interestingly, this hyper methylation of BDNF gene is associated with higher alcohol use and combat exposure (Kim et al., 2016). Collectively, these studies suggest that both stress and alcohol have the ability to produce epigenetic reprogramming via histone and DNA modifications that regulate synaptic plasticity associated genes in key brain regions during stress and alcohol use disorders (Figure 3).

5. Conclusion

This review provides a comprehensive overview of the epigenetic mechanisms that may be involved in alcohol dependence and stress related disorders. Among these, BDNF is one of the most heavily investigated molecules since a deficit in the levels of this neurotrophic factor can contribute to the pathophysiology of addiction and mood disorders. Early life stress or alcohol exposure can cause persistent epigenetic reprogramming in key brain regions that regulate adult psychopathology. In the last decade, emerging studies have proven that epigenetic modifiers, such as HDAC or DNMT inhibitors, may represent good modulators of epigenetic alterations observed during alcohol withdrawal in order to attenuate symptoms of anxiety or alcohol drinking behaviors (Krishnan et al., 2014). In addition, these drugs have the ability to normalize behavioral phenotypes related to stress disorders. Particularly, HDACs belonging to class I seem to play a crucial role in stress-

related disorders comorbid with alcohol dependence such as anxiety. Both clinical and preclinical studies suggest that stress can promote drinking (Figure 1) and evidence presented in this review provides the epigenetic basis of this comorbidity (Figure 3). Even though tremendous progress has been made in identifying specific HDAC and DNMT isoforms involved in stress- or alcohol-induced epigenetic regulation of neurobiology, more research is required to improve the inhibitor selectivity to specific isoforms to validate the therapeutic efficiency of these agents in treating alcoholism with or without co-morbid psychiatric disorders.

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Highlights

- Alcohol consumption can temporarily reduce the symptoms of stress-related disorders.
- Several neurotransmitters are involved in mediating the stress process and alcoholism.
- Similar epigenetic alterations are found in alcohol abuse and mood disorders.
- Inhibiting HDACs and DNMTs can represent a promising therapeutic approach.

Fig. 1. Relationship among stress, anxiety, and alcohol consumption

Stress and other psychiatric disorders, such as anxiety, can increase the susceptibility to develop alcohol addiction, as subjects consume alcohol in an attempt to self-medicate and attenuate the negative symptoms of mood disorders.

Fig. 2. Alcohol modulates anxiety through activation of cAMP-responsive element binding (CREB) protein and chromatin remodeling and changes in synaptic plasticity-associated gene expression in amygdaloid circuitry

Acute alcohol exposure activates CREB phosphorylation and increases CREB binding protein (CBP) expression, which may lead to an increase in histone acetylation (relaxed chromatin) and an increase in expression of brain-derived neurotrophic factor (BDNF) and activity regulated cytoskeleton-associated (Arc) protein genes. These actions may be responsible for the anxiolytic response of alcohol. On the other hand, withdrawal after chronic ethanol produces an opposite response on the levels of phosphorylated CREB (pCREB) and CBP. This is associated with a decrease in histone acetylation (condensed chromatin) and BDNF and Arc, and can result in the development of anxiety-like behaviors (Pandey, Ugale, et al., 2008; Pandey, Zhang, et al., 2008). Also, innate deficits in histone acetylation and BDNF and Arc expression in the amygdaloid circuitry has been implicated in anxiety-like and excessive alcohol drinking behaviors (Moonat et al., 2013).

Fig. 3. Regulation of chromatin states by alcohol and stress

The acetylation of histones, as well as low levels of DNA and histone methylation, induce the relaxed state of chromatin promoting gene transcription. Chronic use of alcohol and stress-related disorders can recruit histone deacetylases (HDACs) resulting in the reduction of the acetylation levels of histones. At the same time, other enzymes, such as the DNA methyltransferases (DNMTs), are activated and add methyl groups to cytosines residue of the DNA. These modifications result in a condensed chromatin structure less accessible to transcription factors. Therefore, the transcription of genes that are crucial for synaptic function is reduced. The reduction of synaptic plasticity events contributes to the development and maintenance of alcohol addiction and stress or anxiety can increase the susceptibility to develop alcoholism.