Published in final edited form as:

Addict Biol. 2012 March ; 17(2): 209–223. doi:10.1111/j.1369-1600.2011.00375.x.

Glucocorticoid and polyamine interactions in the plasticity of glutamatergic synapses that contribute to ethanol-associated dependence and neuronal injury

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Abstract

Stress both contributes to the development of ethanol dependence and is a consequence of dependence. However, the complexity of physiological interactions between activation of the hypothalamic-pituitary-adrenal (HPA) axis and ethanol itself is not well delineated. Emerging evidence derived from examination of corticotropin releasing factor systems and glucocorticoid receptor systems in ethanol dependence suggests a role for pharmacological manipulation of the HPA axis in attenuating ethanol intake, though it is not clear how activation of the HPA axis may promote ethanol dependence or contribute to the neuroadaptative changes that accompany the development of dependence and the severity of ethanol withdrawal. This review examines the role that glucocorticoids, in particular, have in promoting ethanol-associated plasticity of glutamatergic synapses by influencing expression of endogenous linear polyamines and polyamine-sensitive polypeptide subunits of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. We provide evidence that interactions among glucocorticoid systems, polyamines and NMDA receptors are highly relevant to both the development of ethanol dependence and to behavioral and neuropathological sequelae associated with ethanol withdrawal. Examination of these issues is likely to be of critical importance not only in further elucidating the neurobiology of HPA axis dysregulation in ethanol dependence, but also with regard to identification of novel therapeutic targets that may be exploited in the treatment of ethanol dependence.

INTRODUCTION

CNS injury is one of the most widely studied consequences of alcohol abuse and dependence, occurring in an estimated 50 to 75% of alcohol-dependent individuals (Eckardt and Martin 1986). Prolonged ethanol (EtOH) dependence in humans is associated with the development of neurological abnormalities and reduced volume of many brain structures, (Fama, Pfefferbaum and Sullivan 2006; Hulse et al., 2005; Moselhy, 2001; Schweinsburg et al., 2001; Sullivan, Rose and Pfefferbaum 2005) and women may be more susceptible than men to development of these volumetric deficits (Homer et al., 2001). Such changes are

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Authors contributions: M.A.P. and P.J.M. were jointly responsible for the organization and content of this manuscript. Both authors critically reviewed the content and approved the final version for publication.

likely the result of multiple biological actions of EtOH. For example, apoptosis is promoted by EtOH, particularly in developing neurons, via EtOH-induced increases in expression of Fas/Apo-1 receptor (Cheema, West and Miranda 2000) and, possibly, acetyldehyde-mediated disruption of microtubule formation (Kannarkat, Tuma and Tuma 2006; Smith et al., 1992). EtOH exposure also attenuates the differentiation of progenitor cells into neurons (Santillano et al., 2005) and inhibits neurogenesis in rodents, though some recovery is observed, a finding that we and others have reported (Nixon and Crews, 2004; Wilkins Jr et al., 2006). Chronic EtOH exposure promotes the proliferation of reactive oxygen species via multiple pathways (Brooks, 2000; Ren et al., 2005) and inhibits the expression or function of neuronal growth factors (Kolb, Trettel and Levine 2005; Walker et al., 1993). EtOH also alters function of many ionotropic transmitter receptors and ion channels (Cook and Moore, 2002; Errico et al., 2002; Harris et al., 2002; Prendergast et al., 2004; Suvarna et al., 2005), effects thought to contribute to acute EtOH intoxication, to the development of EtOH tolerance and physical dependence, and possibly, to neuronal hyperexcitability and toxicity during acute or protracted EtOH withdrawal (Davis and Wu, 2001; Littleton et al., 2001; Zullino et al., 2004). The aim of the present review is to discuss the role that glucocorticoids have in promoting neuroadaptation to EtOH exposure by influencing the plasticity of glutamatergic synapses and their sensitivity to agonist-like effects of the endogenous polyamines (Figure 1).

ETHANOL WITHDRAWAL AS A COMPONENT OF DEPENDENCE

Classic studies of drinking patterns in alcohol dependent individuals indicate that these patterns include prolonged periods of contingency-based EtOH intake separated by regular periods of alcohol withdrawal (Mello and Mendelson, 1972). It is clear that neurotoxicity associated with use of high doses of EtOH may occur during both active drinking and during EtOH withdrawal, possibly including periods of markedly reduced alcohol use. Though the importance of EtOH withdrawal in the pathology of EtOH dependence has been debated, it has increasingly been recognized as a likely neurotoxic event and to induce reinstatement of drinking by promoting anxiety-like affect (Breese et al., 2005; Stockwell, 1994). For example, Paula-Barbosa et al. (1993) demonstrated that hippocampal damage (loss of neurons and dendrites) observed after prolonged EtOH intake was markedly worsened by EtOH withdrawal. Some dendrite re-growth occurred, but only in animals not withdrawn from EtOH. Wilkins et al. (2006) have recently reported similar findings of neurotoxicity produced by EtOH exposure that is markedly worsened during EtOH withdrawal. It is likely that EtOH neurotoxicity is mediated by very different mechanisms during active drinking and withdrawal. Literature indicates adaptive changes in the expression/function of multiple membrane proteins sum to produce neuronal excitability during EtOH withdrawal, leading to entry of high concentrations of Ca^{2+} into neurons (and release from intracellular stores) and excitotoxicity, discussed below.

It is of critical importance to note that EtOH withdrawal neuronal excitability has also been widely suggested to contribute to subjective effects such as anxiety and cue-induced alcohol craving. A wealth of literature exists demonstrating that in both rodents and humans negative affect associated with ethanol withdrawal/abstinence promotes relapse to drinking in both rodents and humans (De Witte, 2004; Läck, Floyd and McCool 2005; reviewed elegantly in Breese et al. 2005; and Heilig et al. 2010). Further, such affect persists for a prolonged period following the cessation of physical symptoms of ethanol withdrawal and likely reflects unique neuroadaptations in the extended amygdala (Koob 2003). Thus, examination of biochemical events that may exacerbate EtOH withdrawal-related neuronal excitability is of clinical importance in understanding not only neuronal injury, but myriad subjective effects of withdrawal that may increase the risk of relapse to drinking. Further,

such studies are likely to be of significance in identifying novel pharmacological targets to be exploited in the treatment of alcohol dependence.

In humans, the alcohol withdrawal syndrome is often marked by anxiety, alcohol craving, tremor, and dysphoria (Puz and Stokes, 2005). Rodent models of EtOH withdrawal, both in vivo and in vitro, support the hypothesis that such symptoms are reflective of EtOH-associated changes in the expression or function of multiple membrane proteins including γ -amino-butyric acid type A (GABA-A) receptors (Brennan, Lewis and Littleton 1989); L-type voltage-sensitive calcium channels (Breese et al., 2005; De Witte, 2004); serotonin type-3 receptors (Lovinger and Zhou, 1998; McBride et al., 2004); *N*-methyl-D-aspartate type glutamate receptors (NMDA) (Harris et al., 2003; Nagy et al., 2004; Rudolph et al., 1997; Suvana, 2005); adenosine receptors (Batistia et al. 2005; Butler et al. 2008, 2009; Jarvis and Becker 1998); purinergic receptors (Davies et al., 2006; Li, Peoples and Weight 1998; Li, Xiong and Weight 2000; Xiong et al., 2000); and nicotinic acetylcholine receptors (Aistrup, Marszalec and Narahshi, 1999; Covernton & Connolly, 1997).

These effects sum to sustain neuronal excitation upon withdrawal leading to behavioral symptoms discussed above and neurotoxicity, primarily in cortical and hippocampal neurons (Becker, Myrick and Veatch 2006; Nagy et al., 2004; Prendergast et al., 2004; Thomas, Monaghan, and Morrisett 1998; Watson and Little, 1999). Indeed, a history of repeated EtOH withdrawal is associated with worse neurological outcome (i.e., cognitive impairment, seizures) in both humans (Ballenger and Post, 1978; Duka et al., 2003) and rodents (Becker et al., 2006; Gonzalez et al., 2001; Veatch and Becker, 2005). These findings clearly demonstrate the development of neurotoxicity with repeated EtOH withdrawals. Currently, the most widely used treatment for acute detoxification from alcohol dependence is enhancement of GABA-A receptor currents via use of allosteric modulators of GABA_A receptors, in conjunction with symptomatic treatments and vitamin supplements (for review see Saitz and O'Malley, 1997). It is entirely unclear if these medications are effective at attenuating the neurotoxicity that likely occurs during alcohol withdrawal. For example, examination of cognitive function in alcoholics, after detoxification, demonstrates a decline in cognitive function regardless of the use of benzodiazepines (or other pharmacological compounds) during acute withdrawal (Duka et al., 2003; Zinn et al., 2003). Further, the well-documented seizure kindling that occurs with repeated drinking and alcohol detoxification cycles develops even if benzodiazepines are used during detoxifications (Ballenger and Post, 1978; Brown et al., 1988). In addition, these compounds possess dependence liabilities and further work is needed to examine the extent to which neuronal injury may persist even in the presence of these medications. The opiate receptor antagonist naltrexone has shown efficacy in the maintenance of abstinence from drinking, but is not indicated in the treatment of acute EtOH withdrawal (Myrick, Brady and Malcolm 2001). Much recent work has examined effects of anticonvulsant medications, such as valproic acid (Lum, Gorman, and Slavick 2006); topiramate (Rustembegovic, Sofic, and Kroyer 2002); and gabapentin (Bonnet et al., 2003) on acute alcohol withdrawal phenomena, though consistent findings of clinical efficacy in most patients have not been observed with these medications.

ETHANOL AND NMDA RECEPTOR SUBUNITS

Glutamatergic receptor systems or more specifically, the widely localized NMDA receptor system(s) participate in (1) adaptation to prolonged EtOH intake/exposure; (2) acute and prolonged EtOH withdrawal effects; and (3) may be potential therapeutic targets in treating alcohol dependence. NMDA receptors are multimeric with each possessing the NR1 subunit and diverse stoichiometry owing to NR1 splice variants and the presence of NR2 (A-D) subunits (for review, see Ron, 2004). NR2 subunits confer sensitivity to the endogenous polyamines putrescine, spermine, and spermidine which function to allosterically promote

channel opening (Williams, 1997). These polyamines are small, aliphatic molecules that affect multiple membrane proteins in mediating cell proliferation, immune responses, and response to injury, both in the CNS and peripheral tissues (Sousa et al., 1998; Williams, 1997). Synthesis of these substance is initiated in neurons primarily by the actions of ornithine decarboxylase on ornithine, an arginine derivative produced as a by-product of the urea cycle (Rock and Macdonald 1995). Polyamines accumulate in high concentrations, both intra- and extracellularly (Shaw 1979) and interestingly, intracellular polyamines appear to exert an inhibitory influence on NMDA receptor channel open probability independently of stoichiometry (Turecek et al. 2004) while extracellular polyamines positively modulate NMDA receptor activity and these effects are influenced by receptor stoichiometry (Butler et al. 2010; Ransom and Stec 1988). Excitatory effects of polyamines on NR subunits of NMDA receptors in oocytes with physiological Mg^{2+} exposure are conferred by NR1A/NR2B stoichiometry and are not observed in NR1 homomers or NR1/NR2A or NR1/NR2C heteromers (Williams et al. 1994). The effects of polyamines at NR2B are presumed to reflect relief of tonic inhibition by protons at an NR1-specific site (Traynelis et al. 1995). Functionally, this relief of proton inhibition confers greater ion channel opening duration (Ran, Miura and Puil 2003); increased channel-ligand binding (Butler et al. 2010; Ransom and Stec 1988); and excitotoxicity (Butler et al. 2010; Kumari and Anji, 2006; Pardridge, Sakiyama, and Judd 1983; Trevisan et al., 1994).

These receptors are critical in CNS plasticity, long-term potentiation and are a focus of medication development for the maintenance of abstinence from drinking alcohol. Indeed, NMDA receptor antagonists, including those that selectively attenuated function of polyamine-sensitive portions of the receptor, have been demonstrated to reduce EtOH intake during active drinking and during alcohol deprivation (Holter et al., 1997; Vengeliene et al. 2005); prevent the development of tolerance to EtOH (Khanna, Morato and Kalant 1993); ameliorate EtOH withdrawal symptoms (Veatch and Becker, 2005); and prevent EtOH withdrawal neurotoxicity (Nagy et al., 2004; Prendergast et al., 2004). Indeed, the beneficial effects of acamprosate use in alcohol-dependent individuals are associated with the ability of this compound to allosterically attenuate activity of NMDA receptors (Spanagel and Kiefer 2008).

NMDA receptor activity is regulated by EtOH intake and changes in receptor activity relevant to withdrawal are highly complex, involving changes in expression of NR subunits, phosphorylation of NR subunits, and/or alterations in trafficking of NR subunits. Acute EtOH exposure inhibits NMDA receptor function (Popp, Licktieg, and Lovinger 1999) with continued exposure promoting increases in function and/or expression of NMDA receptors (for review, see Fada and Rossetti, 1998), though marked variability exists with regard to the relative sensitivity to EtOH's effect of NMDA receptors expressing different polypeptide subunits. Recombinant NMDA receptors expressing either the NR1/NR2A or NR1/2B conformation are more sensitive to inhibition by EtOH than are those including other NR2 subunits (Masood et al. 1993; Mirshahi and Woodward, 1995). NR1 splice variants demonstrate heterogeneity with regard to inhibitory effects of EtOH, with those expressing the NR1-3 or 1-4 subunits demonstrating modest resistance to EtOH inhibition (Jin and Woodward, 2006). The presence of specific NR2 subunits with these NR1 splice variants further influences sensitivity to EtOH effects, suggesting that the specific combinations of NR1 splice variants and NR2 subunits heavily contribute to the heterogeneity in sensitivity to inhibition by EtOH. Multimeric receptors expressing NR1 and NR2A subunits with NR2C subunits also demonstrate reduced sensitivity to EtOH effects (Mirshahi and Woodward, 1995), suggesting that the greatest EtOH sensitivity is conferred specifically by inclusion of NR2A and/or 2B subunits with NR1 and the exclusion of other NR2 subunits. As a whole, these data suggest that the specific compositions of NR1/NR2A and NR1/NR2B

confer the greatest sensitivity to inhibition by EtOH. Thus, it is these receptors that would be expected to adapt to EtOH exposure.

Several laboratories have demonstrated that acute or prolonged EtOH exposure, using rodent models, produces compensatory increases in the expression of mRNA (which may reflect increased mRNA half-life; Kumari and Anji, 2006) or NMDA subunit peptide, largely that for NR1 and NR2B, though increases in NR2A peptide expression have also been reported in cortex and hippocampus of rodents exposed to EtOH (Harris et al., 2002; Hu, Follesa and Ticku 1996; Kalluri, Mehta and Ticku 1998; Trevisan et al., 1994). Others have reported that NMDA receptor function was elevated following prolonged EtOH pre-exposure while NMDA receptor density was not altered (Chandler et al., 1997; Rudolph et al., 1997). It must be noted that in each of these studies radiolabeled MK-801 was used to assay NMDA receptor levels following chronic EtOH pre-exposure. In that this compound labels one discrete binding domain of the NMDA receptor, it is unclear if NR2A or B polypeptide levels were altered in these studies.

Increasing evidence suggests that ethanol-associated homeostatic plasticity of NMDA receptors is more complex than global increases in NMDA receptor number. Indeed, recent studies have demonstrated that chronic ethanol exposure to hippocampal and cortical neurons results in a compensatory trafficking of NMDA receptors containing NR1 and NR2B subunits to synaptic sites without alterations in expression of NMDA receptors in extrasynaptic domains (Carpenter-Hyland et al., 2004; Hendricson et al., 2007; Qiang, Denny, and Ticku 2007). Moreover, a recent study demonstrated an increased expression of NR1 subunits containing the C2' cassette following chronic ethanol treatment of hippocampal neurons (Clapp et al. 2010). Export of alternative spliced C2' domain is accelerated from the endoplasmic reticulum leading to activity-dependent enhanced targeting of NMDA receptors to the synapse (Mu et al., 2003), suggesting that chronic ethanol regulates NMDA receptor surface expression through alternative splicing of the NR1 carboxyl-terminal. This homeostatic increase in synaptic NMDA receptors is associated with and dependent upon a corresponding increase in the localization of the scaffolding protein PSD-95 at the postsynaptic density (PSD) and with an actin-dependent increase in the size of dendritic spines (Carpenter-Hyland and Chandler, 2006). EtOH-induced enlargement of dendritic spines has also been reported in nucleus accumbens (Zhou et al., 2007), a key brain region involved in reward and addiction. Chandler and colleagues hypothesized that ethanol-induced plasticity involves targeting of NR2B-containing NMDA receptors and increases in PSD-95 at the PSD that provides an expanded scaffolding platform for the recruitment and activation of signaling molecules that regulate synaptic plasticity (Carpenter-Hyland and Chandler, 2006; Mulholland and Chandler, 2007).

Ron and colleagues have reported that enhanced hippocampal NMDA receptor function in response to short-term EtOH exposure reflects internalization of NR2A subunits via clathrin-dependent endocytosis (Ron et al. 2004; Suvarna et al. 2005). This internalization is dependent upon EtOH-induced increases in H-Ras activity, subsequent inhibition of Src activity and reduced phosphorylation of NR2A subunits (Suvarna et al., 2005). The remaining NMDA receptors, then, likely reflect a substantial population of those of the NR1/2B conformation. Both acute and prolonged EtOH exposure results in phosphorylation of the polyamine-sensitive NR2B subunits by the Src family tyrosine kinase Fyn (following dissociation from the scaffolding protein RACK1) resulting in increased NMDA receptor channel activity (Miyakawa et al., 1997; Yaka et al., 2003; Yaka, Phamluong, and Ron 2003). Inclusion of the polyamine-sensitive NR2B subunit (and phosphorylation) correlates with a prolonged decay time for agonist-induced currents, as compared to receptors expressing the NR2A subunit (Cull-Candy, Brickley and Farrant 2001; Vicini et al., 1998).

Interestingly, the duration and pattern of EtOH exposure has a critical role in influencing the nature of the plasticity observed. As noted above, short-term EtOH exposure may alter NMDA receptor stoichiometry via internalization of NR2A subunits (Suvarna et al. 2005), while chronic EtOH exposure may more selectively promote trafficking of NR2B subunits to the synapse (Carpenter-Hyland and Chandler 2006). Further, Sheena Rani and Ticku (2006) elegantly demonstrated that chronic intermittent EtOH treatment of cortical neurons produced increases in NR2B expression that persisted during prolonged EtOH withdrawal. Such changes were not observed in neurons exposed chronically to EtOH in the absence of multiple withdrawals. These changes, in kind with those observed in the GABAergic signaling, likely underlie the kindling phenomenon observed in rodents (Becker and Hale 1993; McCown and Breese 1993) and humans (Ballenger and Post 1978) with histories of intermittent EtOH intake.

The summation of these influences of EtOH on NMDA receptor expression and/or function is over activity during acute and prolonged withdrawal from EtOH, producing intracellular Ca^{2+} accumulation and associated excitotoxicity in some brain regions, including the hippocampus and cortex, but not cerebellum (Mulholland et al. 2005). A role for NMDA receptor activity in ethanol withdrawal-associated neurotoxicity has previously been demonstrated with use of in vitro and in vivo rodent models (Mayer et al. 2002; Nagy and Lázló 2002; Prendergast et al. 2004; Thomas, Fleming and Riley 2002; Young, Senglelaub and Steinmetz 2010). Further, a key role for polyamines in promoting this form of neuronal injury has also been demonstrated in vitro and in vivo (Barron et al. 2008; Davidson and Wilce 1998; Gibson et al. 2003; Mayer et al. 2002). Neurons maintain low intracellular Ca^{2+} concentrations by regulating Ca^{2+} efflux, intracellular Ca^{2+} buffering, and intracellular Ca^{2+} storage (Sattler and Tymianski, 2000). Although physiologically relevant amounts of Ca^{2+} are required for competent neuronal function, excessive Ca^{2+} influx, such as that seen during a state of neural hyperexcitability, may quench regulatory mechanisms and initiate cytotoxicity associated with activity of proteases, phospholipases, and endonucleases (Choi, 1995; Sattler and Tymianski, 2000) and alterations in mitochondrial bioenergetics (Szydlowska and Tymianski 2010).

ETHANOL, CORTICOSTEROIDS AND THE NMDA RECEPTOR

The physiological response to stress, including chronic alcohol intake, involves activation of the hypothalamus-pituitary-adrenal (HPA) axis (for review, see Lopez, Akil and Watson 1999). Activation of the HPA axis, beginning with activation of corticotropin releasing factor (CRF) receptors of the paraventricular hypothalamic nucleus, results in the secretion of adrenocorticotrophic hormone from the anterior pituitary gland, and production and secretion of glucocorticoids from the adrenal cortex, into the blood stream where they act at numerous sites and systems within the body. Cortisol is a primary stress-related steroid in humans and is equivalent to corticosterone in rodents. Corticosterone has a 10-fold higher affinity for intracellular mineralocorticoid receptors (MR; $K_D = 0.5 \text{ nM}$) than intracellular glucocorticoid receptors (GR; $K_D = 5 \text{ nM}$) (de Kloet, Oitzl and Joels 1993). Full occupation of MRs occurs at normal, low physiological concentrations of corticosterone ($\sim 10 \text{ ng/ml} = 29 \text{ nM}$), such as those found during the circadian nadir. In contrast, partial occupation of GRs occurs at low physiological concentrations, but optimal occupation occurs under stressful conditions ($\sim 400 \text{ ng/ml} = 1.12 \text{ }\mu\text{M}$), which may include EtOH exposure and withdrawal, and during the circadian peak ($\sim 60 \text{ ng/ml} = \sim 173 \text{ nM}$; McEwen et al., 1987).

In addition to direct cytotoxic effects of EtOH and EtOH withdrawal, alcohol dependence is associated with the presence or later development of secondary pathological conditions such of the hypothalamus-pituitary-adrenal (HPA) axis activation (Adinoff et al. 2003; Rasmussen et al. 2000), in a manner that is clearly relevant to function of NMDA-type glutamate receptors and, more specifically, NR2 subunit function. A significant portion of alcohol

dependent individuals present with a history of major depression, a condition characterized itself by sustained release of CRF, cortisol and attenuated responses to the synthetic glucocorticoid dexamethasone via a presumed downregulation of receptors (Akil et al., 1993). It is evident that depression and other affective disorders may be secondary to alcohol use disorders (Olgati et al. 2007). However, it is also clear that mood disorders may precede alcohol abuse and dependence, suggesting that pre-existing HPA axis dysfunction may contribute the development of alcohol dependence, indeed, many patients report drinking to overcome negative affect (Addolorato et al., 2005). It is also clear that EtOH intake itself may stimulate activity of the mammalian HPA axis. It has been known more than 40 years that acute EtOH intake can promote corticosterone secretion in the rat (Ellis, 1966), an effect requiring activation of the hypothalamic paraventricular nucleus, CRF release and adrenocorticotrophic hormone (ACTH) release (Lee et al., 2004; Rivest and Rivier, 1994; Rivier, Bruhn, and Vale 1984; Rivier, Rivier, and Lee 1996).

Ethanol administration or voluntary intake has myriad effects on CRF signaling that clearly influence development of dependence and withdrawal and appear to be independent of the HPA axis. For example, acute ethanol administration increases hypothalamic heteronuclear RNA for both CRF (Lee et al. 2004) and messenger RNA for the CRF1, but not CRF2, receptor in the paraventricular nucleus of the hypothalamus (Lee and Rivier 1997). Li et al. (2005) demonstrated that in primary cell culture of rodent hypothalamus, acute exposure to a moderate concentration of ethanol markedly increased both CRF mRNA and secretion. Similar effects have been observed in many other brain structures with chronic ethanol exposure and/or withdrawal (Criado et al. 2011; Olive et al. 2002). The relevance of such effects of ethanol to dependence, withdrawal and reinstatement of ethanol intake in rodents is quite clear given evidence that selective pharmacological antagonism of CRF1 receptors reduced intake of high, but not low, doses of ethanol in C57Bl/6J mice in a drinking in the dark paradigm (Sparta et al. 2008); reduced intake of ethanol in dependent Sardinian alcohol-preferring rats (Sabino et al. 2006); and reduced foot shock-induced reinstatement of ethanol intake in abstinent rats (Le et al. 2000). These findings, in particular, suggest that extra-hypothalamic CRF has a critical role in influencing reinstatement and dependence-associated EtOH intake. This literature also demonstrates a specific role for CRF system modulation of affective states in promoting ethanol dependence and is reviewed elegantly elsewhere (Heilig and Koob 2007; Lowery and Thiele 2010; Ryabinin and Weitemier 2006; Silberman et al. 2009).

Acute and chronic intake of EtOH, as well as withdrawal, in both humans and rodents markedly increases plasma glucocorticoid levels and, with chronic use, produces a pattern of HPA axis dysfunction similar to that observed in depressed patients (Adinoff et al., 2003; Esel et al., 2001; Tabakoff, Jafee, and Ritzmann 1978). Esel et al. (2001) have reported that serum concentrations of cortisol reached 0.78 μ M during early alcohol withdrawal in male humans. Rasmussen et al. have reported, using a rodent model, similar concentrations of free corticosterone during early withdrawal from chronic intake of EtOH that produced modest blood alcohol levels (Rasmussen et al., 2000). In agreement with these data, we have observed serum corticosterone concentrations of approximately 0.4 μ M 6 hrs after administration of a 5 g/kg dose of EtOH to adult male rats in a chronic binge model and have demonstrated that once daily injection of the GR antagonist mifepristone (40 mg/kg) for four days prior to withdrawal markedly reduces the severity of ethanol withdrawal (M.A. Prendergast, unpublished observation). It must be noted, however, that a considerable body of literature demonstrates blunting of the HPA axis in ethanol dependent rodents (Richardson et al. 2008; Zorilla, Valdez and Weiss 2001) and humans (Adinoff et al. 2005), even with protracted abstinence. More specifically, one recent study has demonstrated a reduction in basal glucocorticoid levels during the light, but not dark cycle, and a blunted CORT response to an acute EtOH challenge in dependent rats (Richardson et al., 2008).

Nevertheless, acute withdrawal from chronic EtOH may be a potent activator of the HPA axis in rodents and humans resulting in elevated levels of glucocorticoids. As noted by Richardson et al. 2008, further exploration of the reasons for such discrepancies is needed.

Further, a subset of alcohol-dependent individuals displays prolonged elevations in cortisol and symptoms similar to those observed in Cushing's syndrome (Kirkman and Nelson, 1988). Interestingly, Errico et al. (2002) recently reported that poorer cognitive performance in alcohol-dependent individuals was associated with higher serum glucocorticoid levels during a recent withdrawal. We have recently reported that a single administration of the glucocorticoid receptor antagonist mifepristone immediately prior to ethanol withdrawal markedly reduced memory deficits in dependent mice performing object recognition and odor habituation/discrimination tests (Jacquot et al. 2008). The presence of high concentrations of glucocorticoids may also increase the severity of behavioral convulsions in rodents induced by withdrawal from chronic EtOH exposure (Roberts et al., 1991; Roberts, Crabbe, and Keith 1994). It is posited that neuronal damage in alcohol-dependent humans may result from secondary effects, such as HPA axis alterations (Adinoff et al., 2003; Prendergast and Little, 2007; Van and Lester, 1978).

Glucocorticoid receptors (i.e., MR, GR) are predominantly distributed in the cytoplasm of principal neurons, and also localized in the nucleus and the plasma membrane at lower expression levels (Nishi, 2011; Johnson et al., 2005; Orchinik, Murray, and Moore, 1991). After binding with corticosterone, the activated complex translocates into the nucleus where it binds to glucocorticoid response elements of gene promoters and acts as a transcription factor. Elevated levels of glucocorticoid down-regulate GR in cells lines (Okret et al., 1986; Rosewicz et al., 1988), and there is mounting evidence that withdrawal from chronic ethanol alters GR expression levels in rodent models (Figure 2). Chronic ethanol exposure (10 days) significantly reduced GR mRNA levels in hippocampus (Eskay, 1995). In a comprehensive study by Pandey and colleagues, it was demonstrated that a 15 day liquid ethanol diet reduced GR protein levels in cortex, amygdala, paraventricular nucleus of the hypothalamus (PVN), and hippocampus (Roy et al., 2002). During the acute withdrawal phase (24 hr or less), expression of GR remained decreased in cortex, amygdala and PVN. The authors also demonstrated a significant reduction in GRE-DNA binding in cortex and hippocampus after chronic ethanol exposure that remained decreased during acute withdrawal in cortex. A recent study by Little et al (2008) showed a reduction in GR binding and an increase in GR protein in the nuclear fraction prepared from prefrontal cortex 2 weeks after withdrawal from a liquid diet. However, other studies did not observe changes in GR or MR binding levels in hippocampus and cortex after chronic ethanol or during acute and protracted withdrawal (Rachamin et al., 1989; Spencer and McEwen, 1990). Thus, there appears to be a complex relationship between chronic ethanol and withdrawal on GR and MR expression and binding levels that may play a crucial role in ethanol dependence and withdrawal hyperexcitability.

We postulate that two consequences of EtOH-related increases in the release of glucocorticoids may be injurious to the brain. First, evidence described below demonstrates that exposure of rodents to social stress or high concentrations of corticosterone increases the expression of NMDA receptors, particularly in the hippocampus; and (2) elevations in corticosterone concentration promote polyamine synthesis via upregulation of ornithine decarboxylase expression, the rate-limiting enzyme involved in polyamine biosynthesis. Thus, hypercortisolemia may promote EtOH withdrawal excitability by additively or synergistically increasing NMDA receptor expression and polyamine-dependent function of NR2 subunits.

GLUCOCORTICIDS, NMDA RECEPTORS AND DENDRITIC SPINES

Studies suggest that exposure to high concentrations of glucocorticoids, such as those observed in serum of alcohol-dependent individuals, may directly produce neurotoxicity or potentiate subsequent insults, including those characterized as excitotoxic, such as EtOH withdrawal (McEwen, 2001; Mulholland et al., 2005). These effects are related, in part, to activation of intracellular glucocorticoid receptors, rather than plasma membrane-bound receptors (Abraham et al., 2001; Goodman et al., 1996; McIntosh and Sapolsky, 1996a; Mulholland et al., 2005; Mulholland et al., 2004; Sousa et al., 1998), or to corticosteroid-induced increases in the number or function of NMDA receptors (Weiland, Orchinik and Tanapat 1997; Takahashi et al., 2002). Further, glucocorticoid exposure increases extracellular concentrations of glutamate, which may perpetuate synaptic excitability (Abraham et al., 2001). Weiland et al. (1997) reported that implantation of corticosterone pellets in adult male rats, producing plasma concentrations of 1.0 μ M corticosterone, produced increases in mRNA for NR2A and NR2B subunits and binding of the non-competitive NMDA channel blocker [3 H]MK-801. Meyer et al. (Meyer et al., 2004) similarly demonstrated that a very brief exposure to social stress produced a doubling of NR2A immunoreactivity in the pyramidal cell layer of the *Anolis carolinensis* hippocampus and a nearly four-fold increase in NR2B immunoreactivity in this subregion. Corticosterone injection (2 mg/kg/day/2 days) to non-challenged lizards produce robust increases in NR2A and NR2B subunit expression in the pyramidal cell layer. These data suggest that exposure to stress-relevant concentrations of corticosteroids may increase expression of NMDA receptors and polyamine-sensitive NR2 subunits in the hippocampus of mammalian and non-mammalian species. However, a repeated-novel-stressor paradigm reduced [3 H]MK-801 binding and prevented the ethanol-induced increase in [3 H]MK-801 binding in hippocampus (Lei and Tejani-Butt 2010). Further, these authors demonstrate that such effects may be influenced by genetic background in that Wistar Kyoto rats had elevated [3 H]MK-801 binding in multiple brain regions following chronic stress while Wistar rats showed no such changes. It is clear then, that the means by which stress and/or corticosteroids regulate NMDA receptor subunit trafficking or expression is complex and warrants further examination.

Similar to the effect of ethanol on dendrites and dendritic spines (Paula-Barbosa et al. 1993; Carpenter-Hyland & Chandler, 2006), stress or glucocorticoids triggers morphological alterations in hippocampus (Magarinos & McEwen, 1995a,b; Magarinos et al. 2010; Woolley, Gould and McEwen 1990). For example, dendritic atrophy in CA3 and CA1 pyramidal neurons has been reported following chronic unpredictable stress (Sousa et al., 2000), and recent evidence has demonstrated that chronic restraint stress expands the surface area of asymmetric PSDs in CA1 stratum lacunosum-moleculare without changing total asymmetric synapse number (Donohue et al., 2006). It is possible that stress-induced expansion of PSDs could provide a scaffolding platform for recruitment of polyamine-sensitive NR2B subunits to asymmetric synapses. Thus, it will be of importance to examine the role that glucocorticoids may have in mediating the increases in synaptic NR subunit expression and/or function that accompany prolonged EtOH exposure and withdrawal.

GLUCOCORTICIDS, STRESS, AND POLYAMINE EXPRESSION

Selye (1936) first introduced the concept of general adaptation to stress by delineating the behavioral and physiological responses that occur in response to stressors in mammals, including the release of glucocorticoids. There is now abundant evidence of prolonged adaptation to stressors, including EtOH intake, occurring at the cellular level, changes that are maladaptive if persistent. Several authors have postulated that prolonged activation of this “stress response program”, after Gilad and Gilad (2002), may be injurious to multiple organ systems (Wanger and Casero, 2006). We postulate that prolonged activation of this

response during prolonged EtOH exposure and withdrawal is injurious to the CNS, particularly in those areas expressing high concentrations of NMDA receptors (such as the hippocampus) via multiple effects on NR2 subunit function. As stated above, exposure of the brain to high concentrations of glucocorticoids may increase expression of polyamine-sensitive NR2 subunits of NMDA receptors, sensitizing neurons to the potentially excitotoxic effects of EtOH withdrawal and other insults. In addition, exposure to high concentrations of corticosteroids, such as those attained during acute and chronic stress (including alcohol intake and withdrawal) has been shown to induce glucocorticoid receptor-dependent synthesis of NR2 modulating polyamines. For example, while acute exposure to “traumatic stress” produces short-term increases in the expression of ornithine decarboxylase (ODC) (Paschen et al., 1988), the rate-limiting enzyme in polyamine synthesis, prolonged stress increases expression of ODC, as well as, the polyamine degrading enzyme spermidine/spermine N¹-acetyltransferase (SSAT) (Adibhatla et al., 2002; Wanger and Casero, 2006).

Most interestingly, other forms of prolonged stress produce marked elevations in ODC expression and accumulation of high concentrations (~240 μ M) of polyamines (Gibson et al., 2003). We postulate that prolonged physiological stress most closely resembles that observed with prolonged EtOH intake/exposure followed by EtOH withdrawal, particularly given our findings of elevated and increasing polyamine release from 0-18 hrs after the start of EtOH withdrawal in vitro (Gibson et al., 2003). It is apparent that exposure to EtOH for 10 days itself is a stressor but that withdrawal from EtOH is also a marked stressor, producing rises in plasma cortisol and corticosterone levels that reach near μ M concentrations in humans and rats (Grant et al., 2006; Kutscher et al., 2002; Rasmussen et al., 2002). A wealth of data directly links stress-induced increases in glucocorticoid release to elevated ODC expression in brain in that application of exogenous corticosterone produces similar increases in ODC expression in both the CNS and PNS (Cousin, Lando and Moguilewsky 1982; Nsi-Emvo et al., 1996; Wu et al., 2000). The synthetic glucocorticoid dexamethasone produced similar increases in expression of ODC (Vallee et al., 1995). Lastly, RU486, an antagonist of the low-affinity glucocorticoid receptor (GR) blocked stress-induced increases in ODC expression in peripheral tissues (Nsi-Emvo et al., 1996). It must be noted however, that RU486 clearly is a progestin receptor antagonist and progesterone does protect against hippocampal neurotoxicity produced by glutamate exposure (Nilsen and Brinton, 2003). However, progesterone has been shown to be ineffective at increasing ODC expression at least in peripheral tissue (Weiner, Pentecost, and Dias 1990) suggesting that RU486-sensitive increases in ODC expression are, indeed, mediated by GRs.

CONCLUSIONS

In sum, prolonged EtOH intake or exposure produces multiple adaptive responses at the post-synaptic membrane, including increased sensitivity of NMDA receptors because of altered HPA-axis activity; upregulation or altered trafficking of NR2B subunits; and changes in endogenous polyamine synthesis (after Figure 1). These effects contribute to acute/chronic tolerance to EtOH, but also to EtOH withdrawal induced neuronal excitability and neurotoxicity. Alcohol dependence is also often associated with HPA axis abnormalities including hypercortisolemia during active drinking and during EtOH withdrawal. Exposure to high concentrations of corticosteroids may aid in promoting NMDA receptor function during EtOH withdrawal via two means: (1) promoting expression of NR2 subunits and (2) upregulating expression of ODC in a GR-dependent manner, leading to increased synthesis of polyamines. It is intriguing that sex differences in ethanol effects on HPA axis function (reviewed by Witt 2007) and neuroadaptation of glutamatergic systems (reviewed by Devaud, Risinger and Selvage 2006) have been widely reported. It will be of importance to

examine the role that these alterations may have in the “telescoping” phenomenon or rapid progression to dependence and organ damage (Piazza, Vrbka, and Yeager 1989) observed in alcohol-dependent females. Further, the possibility exists that women may suffer from greater brain volumetric deficits (Hommer et al. 2001). However, a recent report (Adinoff et al. 2010) suggests that alcohol-dependent women may not demonstrate HPA axis disruption similar to that observed in alcohol-dependent men. Thus, it is clear that greater exploration of possible sex differences in ethanol effects on HPA axis function is warranted. In both sexes, the summation of these two influences would be expected to produce over activity of a sensitized NMDA receptor system, effects that likely contribute to withdrawal-associated behaviors including cognitive dysfunction, craving and, possibly, neurotoxicity.

Acknowledgments

This work was supported by National Institutes of Health grants AA013561 (M.A.P.), AA013388 (M.A.P.), AA017922 (P.J.M.), AA019362 (P.J.M.) and AA013641 (P.J.M.).

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Figure 1.

Polyamine and glucocorticoid interactions in ethanol-associated plasticity of glutamatergic synapses that contribute to withdrawal hyperexcitability and neurotoxicity. Chronic ethanol increases the expression of NR2B-containing NMDA receptors at synaptic sites and enlarges dendritic spines. This is associated with elevated ornithine decarboxylase (ODC) and HPA axis activity that leads to increases in endogenous polyamine synthesis and hypercortisolemia. These adaptations sum to sustain neuronal excitability and behavioral seizures during ethanol withdrawal and are thought to underlie anxiety and alcohol craving.

Figure 2.

Adaptations of glucocorticoid receptors in pyramidal neurons after chronic ethanol exposure and during acute ethanol withdrawal. Under basal conditions, GR and MR are localized to the cytosol and nucleus of principal neurons (i.e., pyramidal neurons in the hippocampus and cortex) with additional receptors being distributed to the plasma membrane that are associated with non-genomic effect of glucocorticoids. Studies have demonstrated that chronic ethanol reduces binding and expression of GR. During acute withdrawal (24 hr or less), a reduction in GR expression in the nucleus has been reported. The effects of chronic ethanol and withdrawal on membrane-associated GR and MR are unknown.