Alcoholism and neuro-immune-endocrine interactions: physiochemical aspects

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Abstract
The role of alcohol consumption and alcoholism as an addiction in regulating the chemistry of the brain and its physiology has gained a backlog of interest over the past few decades. Besides the notion that alcohol acts as a brain depressant, the molecular mechanisms and neuronal interactions are not well understood. Emerging evidence implicates alcohol as a neurochemical messenger that influences a cross talk amongst the nervous, immune, and endocrine systems. Specifically, alcohol acts as a crucial regulator of the hypothalamic–pituitary–adrenal (HPA) axis, thereby modulating the release of hormones, particularly adrenocorticotropic hormone (ACTH) and corticosterone (CORT). It is the aim of this review, therefore, to investigate current concepts on how alcohol, particularly ethanol, and alcoholism affect neuro-immune-endocrine neurochemical interactions via the regulation of the HPA axis, taking into consideration bio-behavioral and physiochemical aspects.

Keywords: ACTH; Alcohol; CNS; CORT; Cytokine; HPA; Inflammation; Neuro-immune-endocrine system

Alcoholism is a type of drug addiction [1,2]. There is both physical and psychological dependence with this condition [3]. Physical dependence reveals itself by withdrawal symptoms when alcohol intake is interrupted, tolerance to the effects of alcohol, and evidence of alcohol-associated illnesses [3,4]. Alcohol affects the central nervous system (CNS) as a depressant, resulting in a decrease of activity, anxiety, tension, and inhibitions; even a low level of alcohol within the body slows down reactions [3]. Besides, concentration and judgment become impaired and, in excessive amounts, intoxication or poisoning results.

Alcohol also affects other body systems. For instance, irritation of the gastrointestinal tract can occur with erosion of the lining of the stomach, causing nausea and vomiting. Vitamins are not absorbed properly, which can lead to nutritional deficiencies with the long-term use of alcohol [5]. In addition, liver disease, called hepatic cirrhosis, may also develop. The cardiovascular system may be affected by cardiomyopathy and sexual dysfunction can also occur, causing erectile dysfunction in men and cessation of menses in women. In addition, alcohol affects the nervous system and can result in neuropathy and dementia [1]. Chronic alcohol use also
increases the risk of cancer of the larynx, esophagus, liver, and colon [6]. Alcohol consumption during pregnancy can cause problems in the developing fetus known as fetal alcohol syndrome, which may result in mental retardation of the child [7].

The development of dependence upon alcohol may occur over a period of years, following a relatively consistent pattern of progression. At first, a tolerance of alcohol develops—this results in a person being able to consume a greater quantity of alcohol before its adverse effects are noticed. Furthermore, memory lapses relating to drinking episodes may follow tolerance, then a lack of control over drinking occurs and the affected person can no longer discontinue drinking whenever desired [3]. The most severe drinking behavior includes prolonged binges of drinking with associated mental or physical complications. Some people are able to gain control over their dependence in earlier phases before a total lack of control occurs. When a person who is physically dependent on alcohol tries to stop, a withdrawal syndrome develops, with symptoms that may include elevated temperature, increased blood pressure, rapid heart rate, restlessness, anxiety, psychosis, seizures and, rarely, even death [3,6].

There is no definite cause of alcoholism; however, several factors (socio-economic or physiologic) may play a role in its development. A person who has an alcoholic parent is more likely to become an alcoholic than a person without alcoholism in the immediate family [7,8]. The reason for this occurrence is not known, but genetic (and/or environmental) or biochemical abnormalities may be present. Psychological factors may include a need for relief of anxiety, ongoing depression, unresolved conflict within relationships, or low self-esteem. Finally, social factors include availability of alcohol, social acceptance of the use of alcohol, peer pressure, and stressful lifestyles [8–10].

**Alcohol and alcoholism: neurochemical aspects**

Alcohol produces a wide array of effects at the neuro-psychological, neurophysiological, and morphological levels [7,11,12]. The immediate effects of alcohol, which may occur within minutes of consumption, include altered perceptions and emotions, impaired judgment and distorted vision, and hearing and motor skills [1,2]. These physical states occur because alcohol deadens the left side of the brain (the rational, logical side) while the right side of the brain (the creative, impulsive side) becomes more active.

Long-term effects of heavy alcohol consumption may lead to significant brain injury. Ethyl alcohol (ethanol; EtOH) is a CNS depressant [13,14]. EtOH slows the responses of the brain and nervous system, impairing cognitive, perceptual, and motor performance for several hours, especially during the first 2–3 h after drinks are consumed. The degree of impairment is directly related to the blood alcohol concentration. EtOH, even in low concentrations, also affects regions in the brain that control behavior, resulting in the disruption of inhibitions [10]. As the concentration increases, symptoms of intoxication occur, followed by stupor or coma if levels become high enough.

When a person drinks EtOH (henceforth referred to as ‘drinking alcohol’), it is absorbed into the bloodstream. The alcohol is then absorbed by the small intestines and distributed to the entire body through the bloodstream. As the blood passes through the liver, the body tries to fight alcohol toxicity and intoxication. Enzymes in the liver metabolize it to acetaldehyde, a poisonous substance. The enzyme acetaldehyde dehydrogenase (ALDH) then converts acetaldehyde to acetic acid, a chemical that the body can use as a source of energy [15,16]. People who cannot metabolize acetaldehyde to acetic acid are less likely to drink, thus they have very low tolerance. The accumulation of acetaldehyde produces the effects of alcohol intoxication—flushing of the face, increased heart rate, nausea, headache, abdominal pains, difficulty in breathing, impaired coordination of movements, errors in judgment about movements, distance and time, impaired learning and memory, and sedation. Once in the bloodstream, the acetaldehyde can also cross the blood–brain barrier (BBB), a barrier that protects the brain from harmful chemicals, and attack the neuronal cells, which provide the power source for information processing in the body. The neurons of the brain are the most affected by acetaldehyde because it alters the function of the brain by changing communication within and between the neurons by either reducing or increasing the activity of neurotransmitters [3,4]. Neurotransmitters are chemicals that provide a means for neurons to communicate with one another; they are released by one neuron and received by another to pass messages between the brain and other parts of the body. Neurotransmitters work as systems; examples include γ-aminobutyric acid (GABA), glutamate, and adenosine. Some systems increase neurotransmitter activity (excitatory signals) and others decrease activity (inhibitory signals) (see Table 1). Intoxication and other effects of alcohol and alcoholism are caused by disruptions of the balance of excitatory and inhibitory neurotransmitter systems [3,4,7,8]. The specific neurotransmitters affected by alcohol and their specific effects are enlisted in Table 1.

**Signaling pathways in HPA interactions and alcoholism: hypotheses and mechanisms**

*Alcoholism and the HPA axis: role for EtOH*

Following the assumption that stressors play an important part in the etiology and maintenance of disor-
ders related to the CNS, it is necessary to evaluate parameters reflecting stress-related physiological reactions [17]. Results from these examinations may help to deepen the insight into the etiology of disease and to elucidate diagnostic uncertainties [18].

One of the best-known stress-related endocrine reactions is the hormonal release of the hypothalamic–pituitary–adrenal (HPA) axis [3,4]. Dysregulation of this axis is associated with several psychiatric disorders. Profound hyperactivity of the HPA axis, for instance, has been found in melancholic depression, alcoholism, and eating disorders [19]. In this regard, acute exposure to alcohol can stimulate the HPA axis activity [1,2]. EtOH, a highly lipid-soluble compound, appears to exert its effects through interactions with the plasma membrane. Cell membrane alterations indirectly affect the functioning of membrane-associated proteins, which function as channels, carriers, enzymes, and receptors [3,4]. Alcohol-induced alterations of the immune function, therefore, may involve hormones of the HPA axis [4–6,11,14,17–20].

The earliest known report on HPA interactions and the effect of alcohol goes back to several decades [21]. Male rats chronically intoxicated with EtOH, for example, were tolerant to the EtOH-induced stimulation of the HPA axis [19,22]. Chronically intoxicated rats also were tolerant to stimulation of this axis by levorphanol but not by amphetamine or ether [22]. In a similar manner, animals treated with levorphanol were tolerant to stimulation of the HPA axis by either levorphanol or EtOH, thus providing further support for the hypothesis that endogenous opioids may be involved in the development of tolerance to EtOH [23].

The HPA axis and alcoholic patients: role for steroids

The HPA function was investigated in withdrawing alcoholic patients using the dexamethasone (Dex) suppression test (DST) [24]. The measurement of HPA axis function may be a useful marker for endogenous depression in an alcoholic population. Seventy-two patients were enrolled when they had been abstinent from alcohol for 3–6 weeks. Eight patients undergoing detoxification and 79 control subjects were investigated for comparison. Alcoholic patients after a 3- to 6-week abstinence period showed higher prevalence of abnormal DST results (28%) than control subjects (11%). In addition, patients undergoing detoxification showed an even higher prevalence of abnormal DST results (62%). Abnormal DST status was not associated with the presence of depression in these patients but was associated with abnormal liver function [24]. It was proposed that abnormal DST responses in alcoholic patients are not diagnostic of depression but appear to be related to effects of alcohol either on liver metabolism or on the HPA function or both.

Further experiments were reported on DST in chronic alcoholics with and without depression and its relationship to their hepatic status [25]. Fifty admitted male alcoholics (mean age = 42.8 ± 8.5 years) were selected. This study showed that 31/50 chronic alcoholics were severely depressed [Hamilton depression rating scale (HRS) greater than 22]; 12/50 moderately depressed (HRS = 16–22); and 7/50 were not depressed (HRS less than 15). According to DST results, 8/50 patients showed escape from suppression with Dex while 42/50 showed normal suppression, indicating that depression in alcoholics may be of neurotic type or it may be ethanol-induced reactive depression. Furthermore, raised cortisol levels and abnormal DST response showed a definite tendency toward normalization after total abstinence accompanied by clinical improvement of depressive symptomatology. The DST showed improvement of mood and sleep in these patients during total abstinence [25]. An abnormal DST response in chronic alcoholics seems to be state-related and not trait-dependent; it seems to be a non-specific test for depression in alcoholics. Furthermore, hepatic status was affected equally in both suppressors and non-suppressors of DST, suggesting that abnormal DST in alcoholics may be due to the abnormality of the HPA axis and not due to abnormal hepatic function or histological status [25].

In concert, HPA axis function was similarly examined in hospitalized, withdrawing alcoholic patients [26]. Fourteen patients met DSM-III criteria for major depressive disorder (MDD). Elevated basal cortisols were noted in depressed alcoholic patients (50%) and no non-depressed alcoholics. Further, escape from Dex suppression was noted in depressed alcoholics (64%)
and no non-depressed alcoholics [26]. Moreover, a bolus of synthetic ovine corticotropin-releasing factor (CRF) was administered intravenously to non-depressed in-patients suffering from alcohol dependence disorder [27]. The test was performed during withdrawal and after 4 weeks of abstinence. During withdrawal, the plasma cortisol responses of alcoholic patients and control subjects were similar, except for an earlier decrease of cortisol in the former group. However, after 4 weeks of abstinence, the cortisol response was lower in alcoholic patients than in controls [27]. These abnormalities observed during discontinuation of alcohol consumption may reflect adaptive mechanisms of the HPA activity, which may be altered by chronic alcohol intoxication. Basal plasma levels of cortisol and its suppression by Dex were also measured in inpatient alcoholics days after detoxification [28]. HPA system parameters were essentially within normal limits in most patients—those who did not meet criteria for major depressive disorder as well as those who met criteria for MDD per their episode. Basal levels of cortisol below 7 µg/dl distinguished alcoholics without MDD from those who met criteria for MDD per current episodes.

In concert, social isolation of rats immediately after weaning has been shown to result in marked decreases in the cerebrocortical and plasma concentrations of pregnenolone, progesterone, 3α-hydroxy-5α-pregn-20-one (3α,5α-TH PROG), and 3α,5α-tetrahydrodeoxy-corticosterone (3α,5α-TH DOC), as well as a moderate increase in the plasma concentration of corticosterone [29]. This mildly stressful condition has now been shown to increase the sensitivity of rats to the effect of acute ethanol administration on the cerebrocortical and plasma concentrations of neuroactive steroids. The percentage increases in the brain and plasma concentrations of pregnenolone, progesterone, 3α,5α-TH PROG, and 3α,5α-TH DOC, apparent 20 min after a single intraperitoneal injection of ethanol, were thus markedly greater in isolated rats than in group-housed animals. A subcutaneous injection of isoniazid also induced greater percentage increases in the concentrations of these steroids in isolated rats than in group-housed animals. These results suggest that mild chronic stress, such as that induced by social isolation, enhances the steroidogenic effect of ethanol, a drug abused by humans under stress or affected by neuropsychiatric disorders [28]. Social isolation also induced hyper-responsiveness of the HPA axis, as was apparent after reduction of GABA-mediated inhibitory tone by isoniazid administration.

The HPA axis and its hormones in influencing alcoholism

To determine a casual association between HPA regulation and alcoholism, an insulin hypoglycemia test and an adrenocorticotropic hormone (ACTH) stimulation test were performed in 10 chronic alcoholic men, who had been abstinent from alcohol for at least 1 month [29]. Attenuated serum cortisol responses were found in six of the patients, despite a normal ACTH test. Four patients showed normal responses to both the insulin hypoglycemia test and the short ACTH test. No correlation, however, was demonstrated between the cortisol response and the severity of alcoholism, cerebral atrophy, and peripheral neuropathy, indicating that in chronic alcoholism the short ACTH test may fail in disclosing hypofunction of the integrated HPA axis as assessed with the insulin hypoglycemia test [29].

Moreover, investigating the function of the HPA axis during and after withdrawal from alcohol revealed that the rhythms of cortisol were abnormal in that elevated levels were seen throughout the day in patients with moderate to severe, but not mild, withdrawal [30]. This abnormality of circadian secretion of cortisol, which is similar to that seen in Cushing's syndrome and post-operative trauma, returned to normal after a period of 1 week of abstinence on their in-patient ward. Such excessive secretion of cortisol is reported to likely explain some of the complications of chronic alcoholism.

In support of the aforementioned observations, Adinoff et al. [31] assessed the plasma ACTH and cortisol responses to ovine corticotropin-releasing hormone (oCRH) and the cerebrospinal fluid levels of CRH and corticotropin in alcoholics at various durations of abstinence and compared these variables with age-equivalent controls. Alcoholics who were tested at 1 week of abstinence demonstrated a significantly blunted corticotropin response to oCRH compared with their response at 3 weeks of abstinence. Some of these alcoholic patients demonstrated a significantly exaggerated corticotropin response to oCRH, associated with tachycardia, at both 1 and 3 weeks of abstinence. Alcoholics who were abstinent greater than 3 weeks did not differ in their response to oCRH compared with controls [32]. Controls demonstrated a significant inverse correlation between baseline cortisol levels and the cortisol response to oCRH. This correlation was not evident in any of the alcoholic groups, including those patients who were abstinent greater than 6 months. Moreover, there was a positive correlation between cerebrospinal fluid concentrations of CRH and corticotropin in all patient groups, indicating that alcoholics have significantly altered HPA axis functionin up to 3 weeks following the cessation of drinking, with a more subtle impairment present for greater than 6 months following abstinence [9,10,31–34].

Another report discussed the course of plasma cortisol and β-endorphin-like immunoreactivity (β-EP-IR) was determined following a single i.v. administration of naloxone [35]. The test subjects included 20 male alcoholics (medication-free), investigated 1–3 days and
4 weeks after the onset of abstinence, as well as 10 short-time abstinent alcohol abusers and 10 healthy control subjects. The mean baseline values of cortisol and β-EP-IR remained within normal limits in all groups. The significant decrease in the plasma cortisol baseline values in the alcoholics after 4 weeks abstinence may indicate a lower level of the regulation of the HPA under conditions of abstinence. After naloxone administration an increase in plasma cortisol and β-EP-IR was observed in all groups. The multivariate trend analysis showed significant differences in the time course of plasma cortisol between the three groups, however not in the course of β-EP-IR. The changes in the dynamic regulation of the HPA axis, resulting from chronic alcohol consumption, appear to be irrespective of whether the drinking pattern is dependent or abusive. In alcoholics these changes could still be identified following a 4-week abstinence period [35]. These observations were recently reinforced by the observation that ACTH and cortisol modulate responses to a naloxone [36] or naltrexone [37] challenge and increase the risk of alcoholism, correlating a relationship with the family history of alcoholism [38].

Recent studies, furthermore, have demonstrated decreased responsiveness to metyrapone and insulin-induced hypoglycemia in alcoholic subjects. The effect of acute EtOH ingestion on the HPA axis in healthy non-alcoholic men was subsequently followed up. Plasma ACTH/cortisol levels were determined basally and after the ingestion of placebo or EtOH. When the subjects were analyzed as a group, EtOH did not alter ACTH or cortisol levels. However, in two of eight subjects, EtOH ingestion was accompanied by a rise in plasma ACTH [15–17]. Compared to responses to placebo, plasma ACTH responses to oCRH were blunted during the EtOH session. EtOH ingestion also significantly blunted plasma cortisol levels after oCRH compared to placebo treatment. Additionally, EtOH or placebo was ingested and ACTH-(1–24) was administered; cortisol levels were not altered by EtOH administration. In summary, mildly intoxicating doses of EtOH did not stimulate the HPA axis in six of eight subjects. However, mild intoxication significantly impaired oCRH-stimulated ACTH/cortisol secretion. It was speculated that mild intoxication with EtOH might impair the ability of the HPA axis to respond to physiological stressors [39–42].

Alcoholism and the HPA axis: role for the limbic system

Considerable evidence exists that the limbic system and the hypothalamus play an important role in the HPA axis disturbances found in depressive disorders. Evidence also exists that the limbic system plays a role in the modulation of aggressive behavior. Because aggressive behavior has been observed to be extensively correlated with heavy alcohol use, Buydens-Branchey and Branchey [43] explored the HPA function of alcoholics who had had a life-long history of violence (aggressive behavior). Basal cortisol was measured following cessation of drinking in alcoholics with a history of depression, and alcoholics with a history of violent behavior, eight of whom had been incarcerated because of the severity of their violent acts. When compared with alcoholics with no problem in mood or aggression regulation, significant cortisol increases were found in the group of patients who had been incarcerated for violent acts and not in any other group. This increase persisted after cessation of drinking. Of note, a variety of variables, including several measures of alcohol consumption, amounts of benzodiazepines used for detoxification, and liver function tests, failed to show significant associations with cortisol [43]. These results are interpreted as indicating that individuals displaying severe forms of violence could have a dysregulated HPA function revealed by exposure to excessive amounts of alcohol.

Alcoholism and the HPA axis: role for hippocampus

Studies to date provided conflicting views of the relationship between corticosteroids and decreased hippocampal volume in alcoholism [44]. If this was mediated through the HPA axis, enlarged pituitary volumes relative to hippocampal volumes might be expected and be measurable using the hippocampus to pituitary volume (H/P) ratio. Using magnetic resonance imaging (MRI), Beresford et al. [39] performed volumetric analysis of the pituitary and hippocampus on 10 subjects with alcohol dependence (AD) and on 10 normal control subjects. Compared to normal controls, AD subjects demonstrated a trend towards decreased hippocampal volume and increased pituitary volume. More importantly, H/P ratios were significantly smaller in AD subject. This observation persisted even when covaried for age. Reduced H/P ratio fits the hypothesis that EtOH stimulates pituitary corticotrophs, resulting in elevated corticosteroid levels and possible injury to the hippocampus. If replicated, reduced H/P ratio may serve as a clinical measure of reciprocal neuroendocrine changes in chronic heavy EtOH use. Outline of early and CNS-related developments regarding the effect of alcoholism on the HPA axis is depicted in Fig. 1.

Alcoholism and the HPA axis: role for leptin

Leptin has been shown to regulate food intake and energy expenditure. Because leptin acts via regulation of appetite, the hypothesis that suggests leptin might modulate craving for alcohol as well was investigated [45]. Plasma leptin and cortisol elevated at onset of withdrawal, decreasing significantly thereafter. In addition, leptin (and the body-mass corrected ratio leptin/BMI)
was highly correlated with self-rated craving. No correlations of craving with cortisol and BMI were observed, suggesting that leptin may modulate withdrawal-induced craving in alcoholic subjects [46].

Alcoholism and the HPA axis: role for nitric oxide

Prenatal alcohol exposure is known to cause hyperactivity of the mature offspring’s HPA axis. Recently, it has been shown that hypothalamic neurons that produce CRF, the peptide that represents the major ACTH secretagogue, display increased responses to various stimuli in prenatal alcohol-exposed (E), compared to control (C) rats [47]. CRF-producing perikarya are regulated, in part, by nitric oxide (NO), a signaling molecule whose function is also modified by prenatal alcohol exposure. Further experiments were undertaken to test the hypothesis that prenatal alcohol exposure is associated with alterations in NO-stimulated ACTH secretion. Adult male and female Sprague–Dawley rats exposed to alcohol in utero were injected intracerebroventricularly (icv) with the vehicle or the NO donor 3-morpholino-sydnonimine (SIN-1). ACTH levels were measured in blood samples collected from indwelling jugular cannulae following injection. Brains were obtained minute after SIN-1 injection and processed for in situ hybridization.Brains were obtained minute after SIN-1 injection and processed for in situ hybridization. Compared to males, both C and E females exhibited a significantly larger ACTH response to SIN-1. In addition, prenatal alcohol treatment enhanced SIN-1-induced ACTH release in all E animals, but this difference only reached statistical significance in males. This prenatal influence was also observed in the significantly larger SIN-1-induced increase in transcripts for the immediate early gene nerve growth factor-induced protein B (NGFI-B) in the paraventricular nucleus (PVN) of the males’, but not females’, hypothalamus. The ability of increased brain NO levels to release ACTH and stimulate PVN neuronal activity is enhanced in adult male rats exposed to alcohol prenatally [47]. These data support the hypothesis that alterations in HPA axis activity in adult offspring of alcohol-exposed dams may be related to changes in hypothalamic responsiveness to NO.

The mechanisms involved with alcoholism and the HPA axis

What are the mechanisms by which alcohol can affect or influence the HPA axis activity? For instance, activation of the HPA axis by single-dose EtOH administration, which achieved moderately high blood ethanol levels, was explored in naive rats in order to determine the mechanism of EtOH-induced activation of the stress axis [48]. Adult male rats received a single dose of EtOH and the plasma concentrations of immunoreactive ACTH, β-endorphin (BE), and corticosterone (CORT) were determined by radioimmunoassay (RIA), whereas, plasma concentrations of epinephrine (E) and norepinephrine (NE) were quantified following reverse-phase liquid chromatographic separation and amperometric detection. It was reported that EtOH induced maximal plasma ACTH levels within minutes, which declined towards basal levels thereafter, whereas, plasma concentration of CORT rose rapidly and remained elevated. Plasma ACTH and CORT levels in saline-treated control animals did not, however, vary significantly at any time point. Consistent with co-release of ACTH from corticotrophs, the plasma concentration of BE increased fivefold and declined towards basal levels thereafter. In addition, plasma E increased 10- to 20-fold as compared to saline controls or pre-injection levels and returned to pre-injection levels by 90 min, in a manner similar to EtOH-induced changes in proopiomelanocortin-derived peptides and CS. Of note, removal of the adrenal medulla and thus the source of E prior to EtOH administration did not attenuate activation of the HPA axis [48]. Passive immunoneutralization of arginine vasopressin (AVP), furthermore, using a high-titer AVP antiserum and a protocol that was found to block ether-induced ACTH secretion in adult male rats, failed to even partially block EtOH-induced ACTH or CORT secretion, indicating that neither adrenal medulla-derived E nor AVP are significant regulators or co-regulators of corticotrophin secretions following the administration of EtOH [3,49–51].

In concert, EtOH administered to rats has been shown to stimulate the HPA axis. For instance, the effect of chronic exposure (21 days) to EtOH vapors on locomotor response to intracerebroventricular administration of CRF was investigated in male Wistar rats [51]. Responses to CRF were tested during chronic exposure, 1.5 h following removal of EtOH vapors and 2 weeks after withdrawal of EtOH. A greater sensitivity
A related study described alterations in brain CRF neuronal systems that accompanied the voluntary high consumption of ethanol by Wistar rats presented with a free choice between 6% EtOH and tap water [54]. Hypothalamic CRF concentrations (outside median eminence) were increased in animals with a high preference for EtOH whereas concentrations of CRF in neuro-intermediate pituitary and medulla-pons were significantly decreased. No changes of CRF levels, furthermore, were evident in median eminence, frontal cortex, midbrain, thalamus, or cerebellum. Brain CRF concentrations in two strains of mice with genetically determined differential alcohol preference were also measured. In EtOH-naive mice, for example, there were documented differences in CRF concentrations, with an increase in frontal cortex levels, and a decrease in medulla-pons levels in the EtOH-prefering strain (C57BL/6J) compared to the non-prefering strain (C3H/CRGL/2) [54]. Thus, certain brain CRF neuronal systems are preferentially affected by high EtOH consumption and pre-existing differences in these systems may even contribute to the development of a high preference for EtOH.

In separate experiments, 9- and 15-month-old rats were treated with either 6% EtOH or 12% sucrose (to balance caloric intake) in the drinking water to examine the effect of chronic EtOH consumption on the HPA axis of aged rats [13–15]. Adrenal glands were cleaned, quartered, and used to test in vitro responsiveness to ACTH. Anterior pituitary glands from all 15-month-old rats and one half of the 9-month-old rats were collected, frozen, and extracted for measurement of tissue ACTH concentration. The remaining anterior pituitary glands from the 9-month-old rats were challenged with CRH to test in vitro responsiveness. In 9-month-old rats, chronic EtOH consumption decreased plasma ACTH and corticosterone. Pituitary ACTH concentrations were unchanged in treated 9-month-old rats, but the amount of pituitary ACTH released in response to CRH was decreased in rats consuming EtOH. Moreover, in vitro responsiveness of the adrenal gland to ACTH in 9-month-old rats consuming ethanol was unchanged. Plasma ACTH and corticosterone concentrations were also decreased in 15-month-old rats chronically consuming ethanol. No differences, however, were noted in responsiveness of the adrenal gland or in the amount of pituitary ACTH due to ethanol consumption in 15-month-old rats [13–16]. These results indicate that chronic ethanol consumption decreases HPA function in aged rats.

Furthermore, acetaldehyde, a metabolite of ethanol, has been reported to activate the HPA axis in the rat [13–15]. In this respect, cyanamide, a potent inhibitor of ALDH, was used in the treatment of alcoholics. In the presence of EtOH, cyanamide causes an accumulation of acetaldehyde, a highly toxic metabolite of EtOH, with unpleasant side effects. A similar accumulation is seen in some oriental people with low ALDH activity. Kinoshita et al. [15] have investigated the effects of EtOH and cyanamide administration on the activation of the HPA axis using in situ hybridization histochemistry and radioimmunooassay. EtOH plus cyanamide resulted in a significant increase in CRF and arginine vasopressin mRNA in the paraventricular nucleus and pro-opiomelanocortin mRNA in the anterior pituitary. Plasma corticosterone concentrations were also elevated following EtOH plus cyanamide administration. The blood concentration of acetaldehyde in the EtOH plus cyanamide group increased dramatically, suggesting that this derivative of EtOH, induced by blocking EtOH metabolism, is able to activate the HPA axis operating through a central mechanism.

To unravel the molecular mechanisms of abnormal HPA axis during EtOH dependence, a recent study investigated the effect of chronic EtOH treatment (15 days) and its withdrawal (24 h) on the expression of glucocorticoid receptors (GRs) and glucocorticoid response element (GRE)–DNA binding in the rat brain [55]. The effects of chronic mianserin [serotonin (5-HT)(2A/2C)
antagonist] treatment on these parameters in various brain structures of control diet-fed and EtOH-fed rats were also investigated. It was found that ethanol treatment and withdrawal significantly decreased the GR protein levels in various hippocampal structures (CA1, CA2, CA3, and dentate gyrus), but these changes were normalized during EtOH withdrawal. EtOH treatment also decreased GRE-DNA binding in rat cortex and hippocampus, which remained decreased in the cortex but reverted to normal in hippocampus during EtOH withdrawal. Chronic mianserin (alone) treatment had no effect on cortical GRE-DNA binding and GR protein levels in cortical, amygdaloid, or PVN structures but significantly decreased the GR protein expression in various hippocampal structures and GRE-DNA binding in whole hippocampus [55]. However, when administered concurrently with EtOH treatment, mianserin antagonized the reductions in cortical GRE-DNA binding and in GR protein expression in cortical, PVN, and central, but not medial and basolateral, amygdaloid structures during EtOH withdrawal. On the other hand, mianserin treatment along with ethanol administration decreased the hippocampal GRE-DNA binding and GR protein expression in various hippocampal structures during EtOH withdrawal. Furthermore, EtOH treatment and its withdrawal or mianserin treatment had no effect on the neuron-specific nuclear protein levels in the various brain structures. Taken together, these results indicate that interaction of 5-HT(2A/2C) receptors with GRs in cortical, central amygdaloid, and PVN structures may play a role in neural mechanisms of alcohol dependence [55]. It was postulated, thereafter, that decreased GR expression in PVN might be responsible for the abnormal HPA axis during ethanol exposure and withdrawal [1–4].

In addition, the effects of alcoholism on the HPA revealed a molecular interaction with endogenous opioid peptides [20,56,57]. Abnormal baseline HPA axis function and dexamethasone suppressibility seen in withdrawing alcoholics return to normal on abstinence, but some studies report blunting of the ACTH response to CRH persisting during the early abstinence phase. Reduced central levels of endogenous opioid peptides have been postulated to have an aetiological role in alcohol addiction. The alcoholics had a blunted ACTH incremental response to naloxone but the cortisol response was not significantly different. Moreover, the alcoholics also had a blunted ACTH incremental response to oCRH and a significant main effect of group (alcoholic vs control) was seen for the ACTH response to oCRH. There was no difference between the groups in the cortisol incremental response to oCRH. In the control subjects, a negative correlation was found between basal cortisol and the cortisol increment and ACTH increment following oCRH, while in contrast, basal cortisol correlated positively with cortisol increment following naloxone. There was also a trend for basal cortisol to correlate positively with ACTH increment following naloxone in the controls. In the alcoholics, the normal negative effect of basal cortisol on the cortisol increment after oCRH was reversed, with a positive correlation between basal cortisol and cortisol increment. Recently abstinent alcoholics with normal basal HPA axis hormone levels have a blunted ACTH response to naloxone and oCRH. While reduced levels of central endogenous opioid peptides may be a factor in the blunted ACTH response to naloxone in the alcoholics, it is proposed that the alcoholics have reduced pituitary responsiveness to CRH. This may be via a direct pituitary effect of the chronic ethanol exposure or by a reduction in hypothalamic–hypophyseal vasopressin levels [1].

The ability of alcohol to activate the HPA axis has thus far been well documented in investigations based in acute and short-term experimental paradigms. Additionally, the possibility that the prolonged exposure to ethanol concentrations that are initially effective in stimulating corticosteroid secretion might induce alterations in the response of the HPA axis that cannot be evinced by shorter exposures should be addressed. Using conventional histological techniques, immunohistochemistry, and in situ hybridization, Silva and coworkers [58] examined the medial parvocellular division of the paraventricular nucleus (PVNm), and the synthesis and expression of CRH and VP by its constituent neurons, in rats submitted to 6 months of ethanol treatment and to withdrawal (2 months after 6 months of alcohol intake). Ethanol treatment and withdrawal did not produce neuronal loss in the PVNm. However, the total number of CRH- and VP-immunoreactive neurons and the CRH mRNA levels were significantly decreased by ethanol treatment. In withdrawn rats, the number of CRH- and VP-immunostained neurons and the gene expression of CRH were increased relative to ethanol-treated rats and did not differ from those of controls. No significant variations were detected in VP mRNA levels as a result of ethanol treatment or withdrawal [58]. These results show that prolonged alcohol intake blunts the expression of CRH and VP in the parvocellular neurons of the PVN, and that this effect is, partially at least, reversible by withdrawal. They also suggest that the development of tolerance to the effects of ethanol involves changes that take place at the hypothalamic level [56–60].

Alcoholism and the HPA axis: immunologic aspects

Alcohol exposure has been shown to produce hyperresponsiveness of the HPA axis to immune challenges
Because cytokines, which are released in response to immune challenges, are known to activate the HPA axis, altered release of cytokines and their contribution to the HPA hyper-responsiveness to immune challenges have been observed after prenatal alcohol exposure [62]. Pregnant dams were exposed to alcohol vapors between days 7 and 18 of gestation. At post-natal days 45 and 60, control and prenatal alcohol-exposed offspring were subjected to three different types of immune challenges: injections of interleukin (IL)-1β or endotoxin (lipopolysaccharide, LPS), or turpentine-induced tissue injury. Higher plasma ACTH and corticosterone levels were observed in the EtOH-treated group and this HPA hyper-responsiveness was greater in females compared with males. Plasma tumor necrosis factor (TNF)-α or IL-6 responses were comparable in the control and EtOH-treated groups. Moreover, females exhibited higher corticosterone, TNF-α, and IL-6 responses than males. These results unequivocally indicate that: (i) prenatal alcohol exposure produces HPA hyper-responsiveness to immune challenges; (ii) prenatal alcohol treatment does not influence the release of cytokines to immune challenges; and (iii) there are gender differences in the secretory pattern of corticosterone and cytokines to immune challenges [62]. Therefore, these data do not support the hypothesis that cytokines play a role in the hyper-responsiveness of the HPA axis to immune challenges observed after prenatal alcohol exposure.

Further elaborating on the mechanisms, it was reported that altered ACTH and corticosterone responses to IL-1β in male rats exposed to an alcohol diet indicate a possible role of vasopressin and testosterone [64,65]. In addition, blockade of prostaglandin synthesis, but not opiate receptors, modestly interfered with the HPA axis to acute alcohol injection [45]. Pretreatment with a low dose of alcohol, for example, did not significantly modify the ability of cytokines to stimulate ACTH release in intact rats, but higher doses did unless corticosteroid feedback was abolished by ADX. Further, animals exposed to an alcohol diet for 7 days showed a significant blunting of their ACTH response to vasopressin and immune signals. This influence was reversed by blockade of nitric oxide with arginine derivatives, suggesting that this gas participates in the inhibitory action of prolonged alcohol on the HPA axis. Finally, adult rats exposed to the drug prenatally showed the expected enhancement of stress-induced ACTH secretion.

Moreover, neuropeptide Y (NY), which is found in brain tissue, has been shown to enhance the activation of the HPA axis by CRH [53,66]. NY is localized in Fig. 2. Acute central administration of alcohol (ethanol; EtOH) increases plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels. This response is dependent on the delivery of the HPA peptides corticotropin-releasing factor (CRF) and vasopressin (VP) to the pituitary gland. Immune response and the protracted inflammatory mediators reciprocally activate the HPA axis in alcoholic individuals. However, with alcohol tolerance and/or withdrawal, a negative feedback mechanisms is activated which suppresses the HPA axis activity via reduction of CRF and VP release, subsequently shutting down ACTH.
certain catecholamine neurons and to some extent colocalized with somatostatin. Of note, disturbances of the central noradrenergic system may underlie some forms of alcoholism. In this respect, male alcoholics and normal controls on cerebrospinal fluid levels of NY were subsequently examined. There was no significant difference between the two groups for NY; there was also no significant difference for CSF levels of growth hormone releasing hormone (GHRH). However, there were significant positive correlations between CSF levels of NY and CSF levels of CRH, somatostatin, and GHRH [53]. The mechanisms involved in alcohol-induced regulation of the HPA system and their interactions with immune responses are shown in Fig. 2.

Conclusions and future prospects

Alcohol-dependent individuals respond to stress differently than healthy controls [67]. Alcohol affects the CNS as a depressant, resulting in a decrease of activity, anxiety, tension, and inhibitions [68–70]. Difficulties coping with stress result in heightened risk for the development of problem drinking and the precipitation of relapse. Alcohol-related changes in the HPA axis, a critical stress response system, may therefore be an important factor in the addictive process. Administration of alcohol induces dose-related increases in plasma ACTH and CORT levels. This response depends, in part, on the delivery of the hypothalamic peptides CRF and VP to the pituitary. On the other hand, exposure to alcohol can blunt the HPA axis in response to other homeostatic threats, such as mild electroshocks or immune signals. This decreased response is, in part, due to an attenuated ability of VP to increase ACTH secretion. The hypothesis is set forth that the HPA axis activity is crucial in mediating an alcohol-dependent response; thereby, these interactions play a major role in determining the degree of addiction and/or withdrawal potential. Alcoholism and neuro-immune-endocrine interactions [71–76] constitute an interesting arena for the development of anti-depressants and clinically approved approaches for the treatment of alcohol addiction and its debilitating socio-economic and physiologic consequences.

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