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Binge-Like Ethanol Consumption Increases Corticosterone Levels and Neurodegeneration whereas occupancy of Type II Glucocorticoid Receptors with Mifepristone is Neuroprotective

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Abstract

Excessive ethanol (EtOH) use leads to impaired memory and cognition. Using a rat model of binge-like intoxication, we tested whether elevated corticosterone (Cort) levels contribute to the neurotoxic consequences of EtOH exposure. Rats were adrenalectomized (Adx) and implanted with cholesterol pellets, or cholesterol pellets containing basal, medium or high Cort. Intragastric EtOH or an isocaloric control solution was given 3 times daily for 4 days to achieve blood alcohol levels (BALs) ranging between 200-350 mg/dl. Mean 24 hour (24-hr) plasma Cort levels were ~110 ng/ml and ~40 ng/ml in intact EtOH treated and intact control, respectively. Basal Cort replacement in EtOH-treated Adx animals did not exacerbate alcohol-induced neurodegeneration in the hippocampal dentate gyrus (DG) or the entorhinal cortex (EC) as observed by amino-cupric silver staining. In contrast, Cort replacement resulting in levels 2-fold higher (medium) than normal, or higher (high) in Adx-Cort-EtOH animals increased neurodegeneration. In separate experiments, pharmacological blockade of the Type II glucocorticoid (GC) receptor was initiated with mifepristone (RU38486; 0, 5, 15 mg/kg/day, i.p.). At the higher dose, mifepristone decreased the number of degenerating hippocampal DG cells in binge-EtOH treated intact animals, whereas, only a trend for reduction was observed in 15 mg/kg/day mifepristone treated animals in the EC, as determined by Fluoro Jade B staining. These results suggest that Cort in part mediates EtOH-induced neurotoxicity in the brain through activation of Type II GC receptors.

Keywords

ethanol; corticosterone; neurodegeneration; mifepristone. dentate gyrus; Fluoro Jade B; RU38486; entorhinal cortex

INTRODUCTION

Glucocorticoids (GCs) are secreted by the adrenal glands in response to a variety of physical, emotional and chemical stressors. Release of natural GCs (cortisol in primates,

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corticosterone (Cort) in rats and mice) is normally adaptive and results in energy mobilization and redistribution from tissues such as immune and reproductive systems to organ systems that utilize oxygen and glucose to meet short term homeostatic challenges, such as cardiovascular and pulmonary tissues. Prolonged chronic stress or elevated GCs promote insidious tissue pathologies, importantly including neuronal damage in the brain. Neurotoxicity and neuronal death represent deleterious consequences of GC over-secretion (Sapolsky et al., 1986). In rodents, stress and elevated GC concentrations have been shown to suppress adult neurogenesis (Gould and Tanapat, 1999), cause marked loss of pyramidal cells from the CA3 region of the hippocampus (Sapolsky and Pulsinelli, 1985; Watanabe et al., 1992) and hippocampal atrophy in patients with high and persistent GC levels due to Cushing's syndrome (Starkman et al., 1992). One structure particularly vulnerable to GC-mediated neurodegeneration is the hippocampus (Sapolsky and Pulsinelli, 1985), which contains the highest density of CNS GC receptors (McEwen et al., 1986). GCs are thus thought to "endanger" hippocampal neurons, reducing their likelihood of survival under a variety of neurotoxic insults such as excitotoxicity (Stein-Behrens et al., 1992), transient global ischemia (Sapolsky and Pulsinelli, 1985), hypoglycemia or exposure to antimetabolites (Sapolsky, 1985), cholinergic or serotonergic neurotoxins (Hortnagl et al., 1993; Johnson et al., 1989) and oxygen radical generators (McIntosh and Sapolsky, 1996). Accordingly, adrenalectomy (Adx) did protect hippocampal pyramidal cells from age-related loss (Landfield et al., 1981) and hippocampectomy led to increased basal Cort levels (Magarinos et al., 1987), as a result of disrupting the negative feedback loop in which the hippocampus has been persistently implicated. Although to a lesser extent, GCs have also been shown to enhance neurotoxicity in striatum and cortex (Sapolsky and Pulsinelli, 1985).

Chronic alcohol consumption is known to ultimately lead to structural pathologies of the brain followed by learning and memory impairment and other reduced cognitive functions in humans and animals (Obernier et al., 2002b; Bowden and McCarter, 1993; Jarrard, 1993; Oscar-Berman et al., 1992). Animal studies, using a binge-like alcohol exposure model mimicking a single cycle of binge intoxication in human alcoholics (Majchrowicz, 1975), have shown that neurodegeneration can occur after large doses of alcohol administered over 3-4 days. Such a model reliably produces neuronal damage in corticolimbic areas including hippocampal structures such as the dentate gyrus, and the entorhinal cortex (Collins et al., 1996; Zou et al., 1996; Corso et al., 1998; Crews et al., 2000; Cippitelli et al., 2010a; Cippitelli et al., 2010b). Importantly, alcohol is known to activate the hypothalamic-pituitary-adrenal (HPA) axis and to elevate circulating GCs (Rivier et al., 1984; Thiagarajan et al., 1989), suggesting that alcohol-mediated hippocampal neurotoxicity could be due in part to chronically elevated GCs. Furthermore, *in vitro* studies have recently shown that Cort enhances damage associated with excitotoxicity as well as ethanol withdrawal in rat hippocampal slices (Mulholland et al., 2005; Mulholland et al., 2006). Endogenous GCs exert their effects by binding to two distinct intracellular receptor subtypes: the GC receptor Type II (GR) and the mineralocorticoid receptor Type I (MR). Paradoxically, the GR has a lower affinity for GCs than MRs and only becomes highly occupied at high GC concentrations, such as those seen following an alcohol insult (Rivier et al., 1984). In contrast, MRs are rapidly saturated at low GC concentrations (Joels and Dekloet, 1994). This would give rise to the possibility that GRs may play a role in mediating the effects of GCs in alcohol-induced neurotoxicity. In order to better understand EtOH-induced neurotoxicity, animals were subjected to binge-like intoxication under manipulation of circulating GCs or pharmacological blockade of the GR with the selective antagonist mifepristone (RU38486).

We first evaluated the possibility that elevated Cort levels contribute to the neurotoxic effects of a binge-like alcohol exposure, as assessed in EtOH-challenged animals subjected to controlled Cort replacement in Adxed animals. Neuronal cell death was assessed by

counting the number of argyophilic positive hippocampal dentate gyrus granule cells and entorhinal cortical cells, two brain areas previously shown to be sensitive to alcohol-induced neurotoxicity (Collins et al., 1996; Hamelink et al., 2005; Crews et al., 2000). Because we found that supra-normal GC replacement was associated with enhanced EtOH-induced neurotoxicity, we then examined the causal role of EtOH-induced GC-elevations for neurotoxicity in the binge-like intoxication model by examining whether GR blockade with the specific antagonist mifepristone (RU3846) would result in reduced numbers of dead or dying DG and EC cells as detected by Fluoro Jade staining.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Taconic Farms, Rockville, MD) weighing approximately 250 g were maintained in a temperature and humidity-controlled vivarium on a 12-hour light/dark cycle with water and food available *ad libitum*. Upon arrival, all animals were group housed, but individually housed after implantation of chronic indwelling gastric cannulae and Adx. All surgical procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were randomly assigned to either liquid-diet controls with or without EtOH, Adx or Cort pellets and subjected to implantation of chronic indwelling gastric cannula, and Adx or sham operated followed by Cort pellet replacement. Plasma Cort and EtOH levels were monitored by tail knicks in all groups. Forty μ m frozen brain sections were obtained from each animal after infusion and fixation and stained for dead or dying neurons with the amino cupric silver stain, as previously described (De Olmos et al., 1994). In a separate study, groups of intact rats were intubated by gavage with diet alone or with EtOH and given 0, 5 or 15 mg/kg mifepristone with or without EtOH. Frozen brain sections were obtained for analysis with Fluoro Jade B (FJ-B) which stains for dead or dying neurons. Plasma from tail knicks was assayed by radioimmunoassay (RIA) for Cort and by Sigma diagnostic kits or gas chromatography for EtOH in appropriate treatment groups.

Gastric Cannula Implantation and Adrenalectomy

Intragastric catheterization was carried out as previously described (Hamelink et al., 2005). Bilateral adrenalectomy was performed by making a single 3-cm dorsal incision, immediately caudal to the rib cage, followed by bilateral 1.5 incisions through the dorsal abdominal musculature. The adrenal glands were surgically removed and the incisions sutured. The gastric cannula, Adx and Cort-cholesterol surgeries were performed during the same surgical session. Cort-cholesterol pellets were implanted subcutaneously between the scapulae in the basal, medium or high cort groups with 15% Cort 100 mg pellets, 15% Cort 250 mg pellets or 60% Cort 250mg pellets, respectively. The efficacy of pellet implantation and the diffusion of Cort was evaluated by measuring plasma Cort by RIA. Intact animals underwent sham surgery. Following surgeries each animal received an i.p. injection of gentamicin (8.5 mg/kg) and ampicillin (70 mg/kg) and was placed on a heating pad until they became ambulatory, at which time they were returned to their cages and fed *ad lib*.

Binge Alcohol Treatment

Identical to what was reported previously (Hamelink et al., 2005) seven days after cannula implantation, all rats were given *ad lib* access to alcohol-free liquid diet for 3 days formulated to provide 16.9% of calories as protein, 59.2% carbohydrate, and 23.9% fat (Research Diets Inc., Allentown, NJ). The morning of the next day, alcohol administration was begun (day 1 of the 4-day binge). Beginning at lights on (day 1), all rats were given a priming dose of 5g EtOH followed by 12 ml of liquid diet via gastric cannula every 8 h. In the ethanol-treated animals, the 12 ml of liquid diet was modified to contain 10 to 12% less

calories from carbohydrates, which was replaced with an equal number of EtOH calories. Rats were rated for their level of behavioral intoxication at the time of each ethanol feeding and given the appropriate ethanol dose, as reported by (Majchrowicz, 1975) for 4 days. On the morning of the fifth day animals were deeply anesthetized with an i.p. injection of ketamine hydrochloride and xylazine (80:10 mg/kg) and transcardially perfused via gravity flow. Blood was cleared from the animal's vasculature with 200 ml of wash solution (0.8% sodium chloride, 0.4% dextrose, 0.8% sucrose, 0.5% sodium nitrite, 0.023% calcium chloride, and 0.034% sodium cacodylate) followed by 250 ml of fixative solution (4% sucrose, 4% paraformaldehyde, and 1.43% cacodylic acid). Animals destined to be stained with Fluoro Jade B were perfused with 200 ml of normal saline followed by 250 ml of 4% paraformaldehyde in phosphate-buffered saline.

In a follow-up study to the Adx-Cort-replacement experiment, rats were subjected to a 4-day binge intoxication procedure as previously described (Hamelink et al., 2005; Cippitelli et al., 2010a; Cippitelli et al., 2010b). In brief, alcohol (20% v/v) was administered via 18 gauge gavage needles. Alcohol-treated animals (EtOH) were given a priming dose of 5 g/kg. Additional alcohol was administered every 8 h for 4 consecutive days at 7 AM, 3 PM, and 11 PM based on the animal's behavioral intoxication level, as determined using a six-point behavioral intoxication scale (Majchrowicz, 1975). Controls received equal volumes of the vehicle (water with addition of 6% sucrose and 14.7% milk powder). Rats received a daily treatment of either mifepristone (5 or 15 mg/kg) or vehicle 60-min prior to the 3 PM gavage.

Blood EtOH and Plasma Cort Levels

In the intragastric studies blood was obtained daily by tail bleed approximately 2 h after the initial daily ethanol administration for analysis of plasma EtOH and Cort levels. In addition blood was obtained multiple (4) times per day from some animals in order to determine mean 24-hr Cort levels. BALs were determined using a standard alcohol dehydrogenase-based diagnostic kit (Sigma Chemical Co.) or gas chromatography with head-space sampling using a flame ionization detector. Plasma Cort was determined by ¹²⁵I RIA (ICN Biomedicals kit). The sensitivity of the assay was ~1.56 ng Cort per ml plasma.

Amino-Cupric Silver Staining

Neurodegeneration was assessed using the amino-cupric-silver technique of De Olmos as performed by Switzer's Neuroscience Associates group (De Olmos et al., 1994; Switzer, III, 2000). In brief, brains were removed from the skulls 24 h after perfusion and immersed in a solution containing 20% glycerol and 2% dimethyl sulfoxide to prevent freeze artifacts and then embedded in a gelatin matrix in groups of 16. The brain matrices were then sectioned in the coronal plane by sliding microtome (40 µm in thickness). Every eighth section was then stained. Sections were rinsed three times in deionized water and then placed for 4 days in an aqueous mixture of silver and copper nitrate, pyridine, and ethanol. Sections were then transferred through acetone, a diamine-silver solution, reduced in a weak formaldehyde solution, and bleached in a preparation of potassium ferricyanide and sodium borate (to remove unreduced silver). Sections were then mounted, dried, and counter stained with neutral red. Coronal sections from the ventral hippocampus, both left and right side (2 each side), were analyzed for degeneration counts at 6.00 and 6.32 mm posterior to bregma (Paxinos and Watson, 1986). Degenerating cells were defined as dark, argyrophilic neurons with dendrites clearly visible. Dark objects not clearly identified as neurons were not counted. The number of counts was determined from two consecutive circular microscope fields at 20X magnification for each tissue section. Data for hippocampal degeneration are presented as counts per square millimeter by dividing total number of degenerating cells counted in eight microscope fields by the calculated area of the fields counted (1-mm diameter counting field). Two sections from both the left and right side of the entorhinal

cortex were also counted at 7.00 and 7.32 mm posterior to bregma, in one microscope field at 20X magnification.

Fluoro Jade B Staining

Fluoro Jade B was purchased from Histochem, Inc., (Jefferson, AR) and used as a marker of degenerating neurons as previously described (Schmued and Hopkins, 2000). FJ-B staining was used because it is equally sensitive but methodologically simpler than classical previously described amino-cupric silver staining. It has been established that the results obtained by these two methods are highly correlated (Obernier et al., 2002a) and our own personal observation. In brief, 3 hours after the last alcohol gavage, rats were anesthetized and perfused first with normal saline followed immediately with 4% paraformaldehyde in PBS. Horizontal 20 μ m cryosections were obtained, allowing visualization in the same section of both entorhinal cortex and ventral hippocampus containing the dentate gyrus. Sections were mounted directly on gelatin coated slides, and stained for FJ-B according to the manufacturer's protocol. Dried slides were cleared in xylene and cover-slipped with Cytoseal (Richard-Allan Scientific, Kalamazoo, MI). Alternate sets of sections were stained with cresyl violet in order to verify basic histology. Cell density analysis was on a Leica DMLB microscope using an FITC filter set, and BioQuant imaging software (R&M Biometrics, Nashville, TN). Six horizontal sections containing the hippocampus and the entorhinal cortex, both left and right side, were analyzed for degenerating cells between 5.82 to 6.10 mm ventral from bregma (Paxinos and Watson, 1998). Results for EC degeneration are depicted as counts per square mm by dividing the total number of degenerating cells found in 48 examined microscope fields, equivalent to 16.8 square millimeter (single field area was 0.35 square millimeter x 4 fields per side x 2 sides per section x 6 sections per animal, for a total of 16.8 square millimeter) with a 20x microscope objective. Degenerating granule cells of the entire DG were measured using a semiautomatic stereology system (Bioquant). Data for EC and DG degenerating cells are presented as number per square millimeter.

Drug administration

Mifepristone (RU38486, Cayman Chemical Company) was dissolved in propylene glycol by ultrasonication and injected intraperitoneally (i.p.) at the volume of 1 ml/kg. These dosages were selected because previous work demonstrated that the antagonist exert functional effects at these dose levels (Koenig and Olive, 2004).

Statistical Analysis

Data for argyrophilic or Fluoro Jade B positive cells or Cort levels are reported as mean values $\pm$ standard error. The data from each experiment was analyzed by one-way ANOVA and when a statistical significant threshold was reached, ANOVA was followed by Newman-Keuls post hoc test for pairwise comparisons. For all statistical analysis, differences between control and experimental groups were considered significant if $p < 0.05$.

RESULTS

Confirmation of Plasma Cort Levels in Intact and Treated Animals

Plasma Cort levels depicted in Fig. 1A represent mean 24-hr Cort levels obtained from 4 samples per day (7AM, 1PM, 7PM, 1AM) for 4 days in intact EtOH-treated or controls (Fig. 1A ; main effect: $F_{(1, 9)} = 9.39, p < 0.02$). These results serve as a 24-hr index for comparison with the pellet animals (Fig. 1B), since the Adx-pellet animals are devoid of a diurnal Cort rhythm. Fig. 1B Cort levels are the result of samples taken two hours after the first daily dosing over the 4-day binge exposure. Cort levels in intact non-EtOH treated

animals were not significantly different from the adx-basal Cort groups with or without EtOH treatment. Cort levels in the Adx, EtOH-treated medium or high Cort pellet groups were not significantly different from the intact EtOH-treated rats; however, Cort-levels in the medium or high cort group (Fig. 1B) differed significantly from the basal Cort group with or without EtOH (Fig. 1B; main effect: $F_{(3,42)} = 79.77, p < 0.001$)

Elevated Cort Increases Alcohol-Induced Neurodegeneration in the DG and EC

Degenerating neurons were not observed in the hippocampus or entorhinal cortex (EC) of intact or Adx-Cort replaced animals, unless exposed to EtOH. Degenerating cells were evident in the olfactory glomerular layer, the piriform cortex, the dentate gyrus (DG) and the EC. The most intense damage was found in the granule cells of the DG and the layer III pyramidal cells of the lateral EC (Fig. 2 and Fig. 3). Both Adx-Cort replaced medium and high pellet animals treated with EtOH illustrated greater neurodegeneration than the Adx-basal Cort+Control treated animals both in the hippocampal dentate gyrus (Fig. 2; main effect: $F_{(3,29)} = 6.19, p < 0.003$) and the entorhinal cortex (Fig. 3; main effect: $F_{(3,29)} = 7.35, p < 0.001$). *Post-hoc* analysis of EtOH treatment plus basal Cort versus EtOH plus medium or high Cort levels revealed a statistically significant increase in the EC ($p < 0.05$) whereas, only a marginally significant change was seen in the DG.

Mifepristone Treatment Reduces Alcohol-Induced Neurodegeneration

Alcohol treatment induced substantial neuronal cell death in the DG as well as the EC. In the DG neurotoxicity was reduced by daily treatment with mifepristone (Fig. 4; main effect: $F_{(3,29)} = 3.73, p < 0.03$). *Post-hoc* analysis showed that animals treated with the 15 mg/kg dose of mifepristone compared with alcohol only significantly reduced the level of neurodegeneration ($p < 0.05$). Alcohol administration caused a similar neurotoxicity in the EC, in particular in layer III pyramidal cells. However, even though there was a main effect of alcohol similar to that seen in the DG, there was no statistically significant effect of mifepristone (15 mg/kg/day) treatment ($p = 0.078$) on EC neurotoxicity (Fig. 5; main EtOH effect: $F_{(3,29)} = 6.11, p < 0.003$). In areas other than the hippocampal DG granule cells and the EC Layer III pyramidal cells, FJ-B positive cells were too few to be reliably quantified; therefore, it was not possible to observe any neuroprotective effects of mifepristone in other areas of the rat brain. In summary, the FJ-B results indicate that binge-like alcohol exposure produced neuronal cell death both in the DG and EC, and treatment with mifepristone once daily throughout the binge procedure resulted in a neuroprotective effect in the DG, whereas, just a trend to decrease the number of neurodegenerating cells in the EC was observed. It's of interest to note that the observed neuroprotective or blocking effect of mifepristone was apparent even though mifepristone caused a dose-related enhancement of Cort levels on top of the already EtOH elevated levels (Fig. 6).

Mifepristone Enhances Ethanol-Induced Activation of the HPA axis

A robust activation of the hypothalamic-pituitary-adrenal axis occurred in response to binge alcohol exposure. Cort levels were determined from daily plasma samples obtained 2 hours after lights off. ANOVA showed a highly significant main effect of the ethanol treatment [$F_{(3,28)} = 8.20, p < 0.001$]. Mifepristone treatment did not induce a statistically significant increase of Cort levels over EtOH treatment alone (*post-hoc* analysis, $p > 0.05$), although a trend toward increased Cort response was clearly observed following treatment with mifepristone at the 15 mg/kg dose (Fig. 6).

DISCUSSION

Excessive alcohol exposure in both laboratory animals and clinical studies leads to activation of the hypothalamic-pituitary-adrenal axis (stress axis) and site-specific

neurodegeneration in the central nervous system. Memory, learning and other cognitive abilities are compromised in alcoholics and EtOH exposed animals. Even after protracted periods of abstinence certain deficits remain, providing evidence for persistent damage. Morphological and structural studies reveal the hippocampus and cerebellum to be preferentially damaged by excessive alcohol consumption. The availability of a binge-like alcohol intoxication model that produces region-specific damage over a 4-day time frame has enabled a practical analysis of the events responsible for the observed alcohol-induced neurodegeneration.

In numerous non-EtOH studies of brain cytotoxicity, elevated GCs have been shown to energetically endanger or weaken hippocampal neurons, so that in the presence of coincident insults, the observed damage is worsened (Sapolsky et al., 1986). It is envisioned in intoxicating EtOH-exposure studies that the endangering or weakening effects of ethanol act in concert with elevated stress axis GCs. Ethanol alone contributes to cytotoxicity, but the indirect or ethanol-induced secondary effects on humoral (elevated glucocorticoids) and nutritional (energy availability and utilization) events likely act as coincident insults. In light of the clear evidence that the binge-type alcohol model produces ongoing Cort elevation throughout each binge, it was germane to determine the relative contribution of the elevated GC levels versus that of the direct effect of EtOH exposure on cytotoxicity.

We carried out a series of endocrine ablation and replacement studies using classical methodology, aimed at defining the intrinsic importance of normal to elevated Cort levels in the face of EtOH intoxication. Several groups of animals were adrenalectomized and given replacement Cort therapy designed to achieve circulating Cort levels from non-stressed intact animals with basal 24-hr mean Cort levels of approximately 40 ng/ml or less up to 150 ng/ml, while being challenged with intoxicating doses of EtOH in the 4 day binge model (Fig. 1A & 1B). The goal was to achieve a range of circulating Cort that was close to the mean 24-hr basal level in one group (non stressed) through a range of Cort in other groups that would approximate binge-type alcohol-induced levels (stressed). Given that Cort pellets achieve a constant daily circulating concentration without a diurnal rhythm it seemed logical to use mean daily Cort levels as a yardstick of daily Cort exposure. It was not possible to superimpose binge-EtOH treatment on Adx animals without a minimal circulating or permissive level of Cort, because in the absence of minimal replacement, animals do not survive the binge EtOH challenges. In preliminary studies, non-EtOH treated, Adx animals with mean Cort levels clamped from ~ 40-150 ng/ml produced no visible CNS neurodegeneration. In contrast, binge-like EtOH exposure resulted in neurotoxicity in all treatment groups. Furthermore, EtOH-induced neurodegeneration was enhanced at Cort levels ~2-fold higher (medium Cort) than 24-hr mean basal levels, but did not increase further with an additional doubling of plasma Cort (high Cort). Both the alcohol-treated medium and high Cort pellet groups had higher argyrophilic cell counts in the entorhinal cortex and dentate gyrus granule cells than the intact EtOH-treated basal Cort group.

Our neurodegeneration results are consistent with the notion that a narrow range of Cort is required to optimize each Cort-dependent effect or system (Akana et al., 1985). It would appear that mean 24-hr Cort plasma levels of around 75ng/ml or higher are above permissive or optimal replacement concentrations for the brain regions examined, since these Cort levels exacerbated alcohol-induced neurotoxicity in the binge model. In order to directly examine the hypothesis that elevated GCs act as a coincident insult with EtOH to enhance neurotoxicity, a second study was performed in which the important regulatory GR was blocked with increasing concentrations of mifepristone, a specific GR blocker. Daily treatment with mifepristone in the rodent EtOH binge model resulted in a dose-related decrease in the number of degenerating neurons in both the EC and DG granule cells of the hippocampus, although this reduction was statistically significant only in the hippocampus.

Jointly, our data obtained using adrenal gland ablation, Cort replacement and GC receptor blockade provides consistent and compelling evidence that elevated Cort in the rodent in the presence of intoxicating levels of EtOH enhances EtOH-induced neurodegeneration. We have recently shown that preventing EtOH-induced neurotoxicity leads to a rescue of the cognitive impairment it is otherwise associated with (Cippitelli et al., 2010a; Cippitelli et al., 2010b). From a pharmacotherapeutic standpoint, it is therefore likely that keeping GCs low or partially blocking brain GRs might reduce EtOH neurotoxicity and slow the insidious cognitive decline in binge-type alcoholics.

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Figure 1.

(A) Mean 24-hr plasma corticosterone (Cort) levels in intact EtOH-treated and controls.

* $p < 0.05$ difference vs. intact controls. (B) Plasma Cort levels in adrenalectomized (Adx) animals with basal, medium or high Cort pellet replacement and EtOH treatment. Data are given as the mean $\pm$ SEM of ng Cort per ml of plasma. *** $p < 0.001$ Adx-Medium Cort+EtOH and Adx-HighCort+EtOH vs. Adx-Basal Cort+Control. For further details see **Results** and **Materials and Methods**.

Figure 2.

Elevated corticosterone (Cort) exacerbates binge EtOH-induced neurotoxicity in the dentate gyrus (DG) of the hippocampus of adrenalectomized (Adx) rats. Sections were stained by amino-cupric silver staining to visualize neurodegeneration. Panels (A), (B), and (C) show photomicrographs of degeneration in dentate gyrus (DG) of rats without EtOH (A), with EtOH (B) and EtOH-Cort groups (C). Graphical representation of argyrophilic cell quantitation in dentate gyrus (D). Data represent mean values of argyrophilic positive cells/mm² ± SEM. Significant difference from control **p* < 0.05, ***p* < 0.01. For further details see **Results and Materials and Methods**.

Figure 3.

Elevated corticosterone (Cort) exacerbates binge EtOH-induced neurotoxicity in the entorhinal cortex (EC) of adrenalectomized (Adx) rats. Sections were stained by amino-cupric silver staining to visualize neurodegeneration. Panels (A), (B), and (C) show photomicrographs of degeneration in entorhinal cortex (EC) of rats without EtOH (A), with EtOH (B) and EtOH-High Cort groups (C). Graphical representation of argyrophilic cell quantitation in entorhinal cortex (D). Data represent mean values of argyrophilic positive cells/mm² ± SEM. Significant difference from control * $p < 0.05$, ** $p < 0.01$. For further details, see **Results** and **Materials and Methods**.

Figure 4.

Binge EtOH-induced neurotoxicity in intact animals in the dentate gyrus (DG) of the hippocampus, and its reduction by the glucocorticoid Type II receptor antagonist mifepristone (Mif). Sections were stained by fluoro-jade B (FJ-B) to visualize neurodegeneration. Panels (A), (B), and (C) show representative sections visualizing labeled neurons in animals without EtOH (A), with EtOH (B) and EtOH +15 mg Mif/day (C). Panel (D) shows quantitation of histological data, demonstrating EtOH-induced neurodegeneration and its reduction by Mif at 15 mg/kg/day. Data are given as the mean number of FJ-B positive cells/mm² ± SEM. **p* < 0.01, EtOH + 15 mg/kg Mif vs. EtOH + 0 mg/kg Mif. For further details see **Results** and **Materials and Methods**.

Figure 5.

Binge alcohol-induced neurotoxicity in the entorhinal cortex (EC), demonstrates only a neuroprotective trend in the presence of the glucocorticoid Type II receptor antagonist mifepristone (Mif). Sections were stained by fluoro-jade B (FJ-B) to visualize neurodegeneration. Panels (A), (B), and (C) show representative sections visualizing labeled neurons in animals without EtOH (A), with EtOH (B) and EtOH +15mg Mif/day groups (C). Panel (D) shows quantitation of histological data, demonstrating EtOH-induced neurodegeneration. Data are given as the mean number of FJ-B positive cells/mm² ± SEM; #*p* <0.07 EtOH + 15 mg/kg Mif vs. EtOH + 0 mg/kg Mif. For further details see **Results** and **Materials and Methods**.

Figure 6.

Activation of the hypothalamic-pituitary-adrenal axis by binge-like EtOH treatment and enhancement by the glucocorticoid Type II receptor antagonist mifepristone (Mif). Data are given as the mean $\pm$ SEM of ng of corticosterone per ml of plasma. Significant difference from control ** $p < 0.01$, *** $p < 0.001$ For further details see **Results** and **Materials and Methods**.