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Roles of the locus coeruleus and adrenergic receptors in brain-mediated hypothalamic-pituitary-adrenal axis responses to intracerebroventricular alcohol

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Abstract

Background—Alcohol activates the hypothalamic-pituitary-adrenal (HPA) axis through its actions in both the periphery and the CNS. The studies presented here were designed to test the CNS-specific noradrenergic mechanisms by which alcohol stimulates HPA activity in the male rat.

Methods—We used an experimental paradigm in which a small, non-toxic amount (5 microliters) of alcohol was slowly microinfused intracerebroventricularly (icv). Alcohol was administered icv to animals with lesions of the locus coeruleus, or in animals pretreated with alpha- or beta-adrenergic receptor antagonists. Hormonal HPA activation was determined by measuring secretion of the pituitary stress hormone adrenocorticotropin (ACTH). Neuronal activation was determined by quantification of the expression of the transcription factor *c-fos* (Fos).

Results—As expected, icv alcohol stimulated ACTH secretion from the pituitary and Fos expression in the paraventricular nucleus of the hypothalamus (PVN). Bilateral electrolytic locus coeruleus lesions blocked the ability of icv alcohol to stimulate ACTH secretion. Pretreatment with icv propranolol increased basal ACTH secretion levels, but icv alcohol did not increase this effect. Propranolol also blunted icv alcohol-induced PVN Fos expression. A low dose of phenoxybenzamine, an alpha-adrenergic receptor antagonist, did not affect the ability of icv alcohol to stimulate ACTH release. However, a higher dose of the drug was able to block the ACTH response to icv alcohol. Despite this, phenoxybenzamine did not inhibit alcohol-induced Fos expression. Icv pretreatment with corynanthine, a selective alpha-1 adrenergic receptor antagonist, modestly raised basal ACTH levels, and blocked the icv alcohol-induced secretion of this hormone.

Conclusions—These results indicate the LC and NE play important roles in HPA activation caused by icv alcohol administration, but that the specific adrenergic receptor subtypes involved in this phenomenon still need to be identified.

Introduction

Alcohol has long been known to disrupt normal hypothalamic-pituitary-adrenal (HPA) axis function. In addition, the effects of the drug on HPA activity have been linked to both the genesis and maintenance of addiction, although the mechanisms underlying this are uncertain (Lovallo, 2006). Alcohol and its metabolites stimulate the HPA axis by their direct actions on its tissues, and indirectly by altering peripheral physiological parameters that feedback to the hypothalamus and activate the axis. For instance, at the level of the brain,

alcohol administered by intracerebroventricular (icv) injection causes an increase in the release of corticotropin releasing factor (CRF), the main hypothalamic hormone that drives the release of the pituitary stress hormone adrenocorticotropin (ACTH) (Lee et al., 2004). In the periphery, acetaldehyde, the drug's main metabolite, stimulates corticosterone (CORT) secretion at the level of the adrenal glands (Cobb and Van Thiel, 1982). Alcohol can also cause gastrointestinal irritation, induce liver cytokine production, and produce changes in blood pressure and body temperature (El-Mas et al., 2006, Linnoila et al., 1979, Schaffert et al., 2009, Narkiewicz et al., 2000), all of which constitute homeostatic challenges that are perceived at the level of the brain and indirectly stimulate HPA activity. Thus, determining the CNS-specific effects of alcohol on the HPA axis can be difficult, especially when the drug is given systemically.

Several CNS nuclei are hypothesized to be involved in modulating alcohol-induced HPA activation. However, and as discussed above, whether these are stimulated directly by the drug or by its peripheral actions is uncertain. One such brain area is the locus coeruleus (LC) of the pons, which is the main site of norepinephrine (NE) synthesis in the brain (Bender and Abdel-Rahman, 2009). LC neurons send a sparse but important excitatory projection to the paraventricular nucleus of the hypothalamus (PVN) (Dunn et al., 2004, Lee et al., 2011), which is the primary hypothalamic regulator of HPA function. NE released from these neurons activate CRF neurons in the PVN (Dunn et al., 2004), thereby stimulating the HPA hormone secretion cascade (Helmreich et al., 2001). It was previously shown that LC neurons are responsive to systemic alcohol administration and a wide variety of other homeostatic challenges (Sved et al., 2002, Thiele et al., 1998). Importantly for the studies here, the central administration of alcohol rapidly depletes LC-NE content, indicating a potential direct action of the drug at this nucleus (Selvage et al., 2006). It has been hypothesized that NE-induced CRF secretion/HPA activation occurs via the stimulation of α -adrenergic receptors expressed in the PVN. Because of this and other factors, there is interest in the potential use of adrenergic receptor antagonists to decrease anxiety, and even aid with sleep (Southwick et al., 2005, Southwick et al., 1999). Moreover, it has recently been proposed that α -1 adrenergic receptor blockade may help reduce alcohol consumption in persons with a physical dependence for the drug (Simpson et al., 2009, Walker et al., 2008).

We performed the present studies in order to investigate the roles of the LC, NE, and α - and β -adrenergic receptors in alcohol-induced stimulation of the HPA axis directly at the level of the CNS, using the rat as an experimental model. In order to isolate the actions of alcohol to the level of the brain, we used an administration paradigm whereby a small, non-toxic amount of the drug (5 μ L of 100%) was microinjected icv into conscious, freely moving male rats. Icv alcohol rapidly diffuses throughout the brain, and like other low-dose administration paradigms, causes a minor increase in locomotor activity (Larkin and Selvage, unpublished observations). It does not leak to the periphery in measureable amounts, cause neuronal damage, or result in any apparent long-term neural or behavioral changes (Larkin et al., 2010). Further, it has been shown to rapidly trigger CRF mRNA synthesis (Lee et al., 2004) and stimulate a stress-sensitive brain-testes neuronal pathway that bypasses the pituitary to inhibit testosterone production (Lee et al., 2004). Thus, the icv alcohol injection paradigm we employed provides a good model to investigate the CNS-specific actions of the drug that alter HPA function.

In order to assess the role of the LC in icv alcohol-induced stimulation of the HPA axis, we measured the secretion of the pituitary stress hormone adrenocorticotropin (ACTH) in animals with electrolytic lesions of the brainstem area containing the nucleus (LCx). To examine the roles of adrenergic receptors in HPA activation by icv alcohol, animals were pretreated with the nonspecific β -adrenergic receptor antagonist propranolol, the nonspecific

α -adrenergic receptor antagonist phenoxybenzamine, or the selective α -1 adrenergic receptor antagonist corynanthine prior to icv alcohol or vehicle administration. The effect of adrenergic receptor blockade on hormonal HPA activation was determined by measuring ACTH secretion in response to icv alcohol in animals pretreated with propranolol, phenoxybenzamine, or corynanthine. In another set of studies, we examined alcohol-induced Fos expression in the PVN. Fos was chosen as a marker of neural activation in these studies because of the strong link between the stimulation of this transcription factor and CRF synthesis (Yao and Denver, 2007), and because of the overlap between icv alcohol-induced CRF synthesis (Lee et al., 2004) and Fos expression in the medial parvocellular PVN (Larkin et al., 2010).

Taken together, the results below indicate an important role for the LC/NE system in brain-mediated activation of the HPA axis by alcohol. Moreover, the findings suggest a role for *both* α 1- and β -adrenergic receptors in HPA activation by the drug, and give a plausible explanation for the decreased alcohol consumption reported in both rats and humans treated with α - or β -blockers (Simpson et al., 2009, Rasmussen et al., 2009, Grupp et al., 1989).

Materials and Methods

Animals

Adult male Sprague Dawley rats (200–250 g) were held in an AAALAC-approved facility lighted from 0600–1800 hours and kept at a temperature of 70°F. Food and water were available ad libitum. All animals were singly housed following any surgery, and allowed to recover for seven or more days before experimentation unless otherwise noted.

Surgeries

Intracerebroventricular (icv) cannula placement was performed under an anesthetic mixture of acepromazine, ketamine, and xylazine administered subcutaneously. A permanent, indwelling cannula was inserted into the left lateral ventricle using the coordinates from Bregma: anterior-posterior: –0.4 mm, lateral: –1.4 mm, and dorsal-ventral: –3.8 mm. Animals were allowed 7 days to recover from this surgery.

Intravenous (iv) jugular catheterization was performed under an anesthetic mixture of acepromazine, ketamine, and xylazine administered intraperitoneally (ip). A catheter composed of PE 50 and silastic tubing was permanently inserted into the left jugular vein and externalized through the skin on the back of the neck.

Electrolytic lesions of the brainstem area containing the locus coeruleus (LCx) were performed in animals anesthetized with a cocktail of acepromazine, ketamine, and xylazine administered subcutaneously. Briefly, animals were placed into a stereotaxic apparatus, with the electrode arm placed at a 15 degree angle. Lesions were made using the following coordinates from Lambda: anterior-posterior: – 3.4 mm, lateral: $\pm$ 1.2mm, and dorsal-ventral: – 6.8mm. SHAM electrolytic lesions were performed using the same coordinates, but the electrode was only lowered – 5mm dorsal-ventral and no current was passed through the electrode.

Pharmacological Agents

The α - and β -adrenergic antagonists phenoxybenzamine, propranolol, and corynanthine were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in saline. Three or twenty hours prior to experimentation, phenoxybenzamine (1 mg / kg or 10 mg / kg, respectively) was administered iv, whereas propranolol (5 μ g) or corynanthine (2 μ g) were administered icv three hours prior to experimentation.

Day of Experimentation

On the day of experimentation, icv cannula and iv catheters were attached via PE tubing to external locations to allow for the noninvasive icv injection of alcohol or saline (vehicle) and for blood draws. The freely-moving animals were then individually housed in experimental chambers placed in a quiet animal room. They were left to acclimate for 3 hours, allowing the secretion of stress hormones to return to basal levels. 5 μ L of 100% ethyl alcohol or vehicle was then administered to each rat at a rate of 1 μ L/10 seconds. For ACTH experiments, blood draws were taken prior to alcohol injection for a basal value, then at 15, 30, and 60 minutes following icv alcohol. For experiments examining icv alcohol-induced Fos expression, perfusions were performed as noted below.

Immunocytochemistry (ICC)

Transcardial perfusions were done in select experiments 45 minutes following icv alcohol or saline (vehicle). Animals were anesthetized using 35% chloral hydrate and transcardial perfusions performed via the ascending aorta using 0.9% saline followed by 4% paraformaldehyde. Perfused brains were cryoprotected until saturated in a 30% sucrose solution, then quick-frozen in cold methylbutane. All brains were stored at -80°C until transferred to a Leica CM3050 S cryostat where 40 μ m thick PVN brain slices were cut at -21°C . Tissue was processed for Fos immunoreactivity using standard ICC techniques. Fos antibody was purchased from Sigma-Aldrich (St. Louis, MO). Fos immunoreactivity was visualized using cobalt-enhanced diaminobenzidine (DAB), also purchased from Sigma-Aldrich.

Image Analysis

Pictures of brain sections containing the PVN were taken using a Leica DME light microscope with a Motic Image Plus 2.0 camera and image analysis software. Counting of Fos-immunopositive cells in the PVN was accomplished using NIH Image J analysis software.

ACTH Measurement

ACTH ELISAs were performed using kits from MD Biosciences (Baltimore, MD). Colorimetric analysis was performed using a Bio-Tek Synergy HT multi-detection microplate reader with Bio-Tek KC4 microplate data analysis software.

Statistics

One way ANOVA (Fos studies) or one-way ANOVA with repeated measures (ACTH studies) were used to determine significance. Where applicable, Student's T tests were performed using Prism GraphPad Software (La Jolla, CA).

Results

ACTH studies

As can be seen in Figure 1, bilateral lesions of the LC, which have previously been shown to significantly reduce PVN NE levels (Szawka et al., 2005), blocked the ability of icv alcohol to stimulate ACTH secretion when compared to animals receiving sham lesions. In studies testing the role of adrenergic receptors in HPA activation by icv alcohol, pretreatment with a low dose of the irreversible, non-selective α -adrenergic receptor antagonist phenoxybenzamine (1 mg/ kg given i.v.) did not alter the ability of icv alcohol to stimulate ACTH secretion (Figure 2A). However, a higher dose of phenoxybenzamine (10 mg/ kg, i.v.) was able to completely block icv alcohol-induced ACTH secretion (Figure 2B). Pretreatment with the specific α -1 adrenergic antagonist corynanthine (150 μ g, icv)

modestly increased ACTH secretion as compared to controls, and blunted ACTH secretion in response to alcohol as compared to animals receiving sham injections and the drug (Figure 3). Antagonism of β -adrenergic receptors with propranolol also increased basal ACTH secretion; however, icv alcohol was not able to add to this effect (Figure 4).

Fos expression studies

As expected, icv alcohol stimulated PVN Fos expression. Most alcohol-induced Fos expression was in the medial parvocellular PVN, where CRF cells reside. Antagonism of α -adrenergic receptors with phenoxybenzamine at a dose that blocked the ACTH response to icv alcohol (10 mg/kg given i.v.) significantly stimulated PVN Fos expression compared to controls. The icv administration of alcohol in phenoxybenzamine treated animals was still able to significantly increase PVN Fos expression compared to animals receiving phenoxybenzamine and icv vehicle (Figures 5B and 5C). Pretreatment with the non-selective β -adrenergic receptor antagonist propranolol (5 μ g, icv) increased both basal PVN Fos expression as well as ACTH secretion. Icv alcohol also did not increase Fos expression in the PVN above propranolol-stimulated basal levels (Figures 5A and 5C).

Discussion

The studies presented here utilized an icv alcohol administration paradigm to investigate the role of the noradrenergic system in brain-specific HPA axis activation by alcohol. The icv administration route was used in order to obviate the role of alcohol-induced peripheral changes that feedback and activate the axis irrespective of direct actions of the drug on the brain. The effects of this method of alcohol administration on HPA activation have previously been compared to those of systemic administration of the drug (Lee et al., 2004, Selvage et al., 2004). Briefly, alcohol administered icv diffuses rapidly throughout the brain, does not cause neuronal damage, and stimulates a small increase in locomotor activity similar to that seen with other low-dose alcohol administration paradigms. While the icv administration route results in relatively low and transient alcohol levels in the CSF (Larkin et al, 2010), it stimulates smaller but similar HPA responses as those seen following systemic alcohol administration (Lee et al., 2004). For instance, and as noted above, icv alcohol stimulates PVN Fos expression, CRF synthesis and release, and ACTH secretion. Vasopressin release appears to moderately enhance this effect (Lee et al., 2004).

As can be seen in the results section, LCx blocked the ability of icv alcohol to stimulate an ACTH response in male rats. Pretreatment with a low dose of phenoxybenzamine (1 mg/kg) given i.v. caused no alteration in the ability of icv alcohol to induce ACTH secretion. Conversely, a higher dose of i.v. phenoxybenzamine (10 mg/kg) completely blocked the ACTH response to icv alcohol. Despite this, phenoxybenzamine pretreatment did not significantly block the ability of icv alcohol to stimulate Fos expression in the PVN beyond that stimulated by phenoxybenzamine alone, suggesting that phenoxybenzamine may block the ACTH response to alcohol downstream of the PVN. Pretreatment with icv corynanthine also blocked the ability of icv alcohol to stimulate ACTH secretion. This drug also stimulated PVN Fos expression to the point that we determined evaluating the effects of icv alcohol in these animals would not be interpretable. In studies investigating the role of brain β -adrenergic receptors in HPA activation by alcohol, it was found that pretreatment with icv propranolol significantly increased basal ACTH levels. Icv alcohol failed to add to this effect. Additionally, pretreatment with icv propranolol caused a significant increase in basal PVN Fos expression. Icv alcohol did not add to this effect. Collectively, the results presented here suggest that centrally administered alcohol may act through the LC-NE system to stimulate HPA responses via activation of both α - and β -adrenergic receptors in the CNS.

While many studies have shown that CRF release from PVN neurons is driven by α -adrenergic receptors (Chen et al., 2004, Ma and Morilak, 2005), others have indicated a role for β -adrenergic receptors in HPA activation by stressors (McLaughlin et al., 2009, Khan et al., 2004). It is thus quite possible that NE activates the HPA axis via either class of adrenergic receptor, depending on the neural pathways used by various stimuli. This notion is supported by the data here, which show that alcohol's ability to trigger HPA responses directly at the level of the brain is inhibited by both β - and α -adrenergic receptor blockade. Moreover, when the main source of brain NE (the LC) was lesioned, alcohol's ability to stimulate ACTH secretion was significantly reduced.

Perhaps the most interesting result from these studies was that LCx was able to significantly inhibit the ACTH response to alcohol, which has many sites and modes of action in the brain. One possibility is that these lesions blocked the actions of the small, but important direct projection from the LC to the medial parvocellular PVN, where CRF neurons are located. Another possibility is that the lesions blocked LC input to the medial prefrontal cortex (mPFC). It has been hypothesized that the activation of this pathway inhibits inhibitory mPFC input to the PVN. For instance, it was reported by Radley, Williams, and Sawchenko (2008), that ablations of noradrenergic projections from the LC to the mPFC caused resulted in increased activity in that brain area, and thus increased inhibitory input to the PVN. Another possibility is that the electrolytic lesions used in the present studies also destroyed nearby fibers of passage from other the A1 and/or A5 adrenergic areas of the brainstem, which send significant noradrenergic input to the PVN. Regardless, the findings indicate an important role in alcohol-induced HPA responses for either direct or indirect pathways from the brainstem to the PVN. This in turn points to the potential benefit from the use of agents that antagonize adrenergic receptors in treating alcohol-instigated disorders linked to HPA axis disruption.

That either central or peripheral α -adrenergic receptor blockade inhibited both alcohol-induced ACTH secretion and levels of PVN Fos expression (in the case of peripheral phenoxybenzamine administration) is a finding that lends biological support to reports of α -1 receptor antagonism with prazosin reduces alcohol intake in persons addicted to the drug in alcohol preferring rats (Rasmussen et al., 2009). This phenomenon may also occur in humans (Simpson et al., 2009). Since the effects of alcohol are localized to the brain in our drug delivery paradigm, these findings also suggest that the use of α -adrenergic antagonists that cross the blood-brain-barrier may be preferable to those that don't (i.e. yohimbine) in combatting alcohol-induced HPA dysfunction.

One question that arises from the data here is whether adrenergic receptor blockade inhibits the ACTH response to alcohol at the level of the PVN, or downstream from there. Making this distinction more difficult is the ability of adrenergic antagonists to stimulate Fos expression in the PVN despite being given peripherally. For instance, the Fos response seen after pretreatment with icv propranolol could be either due to the drug's effects on peripheral parameters such as changes in blood pressure, or due to it crossing the blood-brain-barrier in sufficient quantities to cause changes in the periphery that alter brain function. This has been suggested to be the case for nicotine (Gadek-Michalska et al., 2002), another addictive substance that activates HPA responses. Given the presence of β 2 adrenergic receptors in the anterior pituitary (Mezey et al., 1983), this is entirely plausible.

In conclusion, regardless of the organ level at which α - or β -adrenergic receptor antagonism affects activation of the HPA axis, the results here indicate that both work to decrease stress responses to the drug that are triggered specifically at the level of the brain. Thus, the data here support the use of antagonists for either receptor in reducing HPA overactivity in

response to alcohol, and potentially to reduce the associated harmful effects of a disrupted HPA axis.

Acknowledgments

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Bibliography

- BENDER TS, ABDEL-RAHMAN AA. Alpha 2A-adrenergic receptor signaling underlies synergistic enhancement of ethanol-induced behavioral impairment by clonidine. *Alcohol Clin Exp Res.* 2009; 33:408–18. [PubMed: 19120051]
- CHEN XQ, DU JZ, WANG YS. Regulation of hypoxia-induced release of corticotropin-releasing factor in the rat hypothalamus by norepinephrine. *Regul Pept.* 2004; 119:221–8. [PubMed: 15120484]
- COBB CF, VAN THIEL DH. Mechanism of ethanol-induced adrenal stimulation. *Alcohol Clin Exp Res.* 1982; 6:202–6. [PubMed: 6285754]
- DUNN AJ, SWIERGIEL AH, PALAMARCHOUK V. Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. *Ann N Y Acad Sci.* 2004; 1018:25–34. [PubMed: 15240349]
- EL-MAS MM, ZHANG J, ABDEL-RAHMAN AA. Upregulation of vascular inducible nitric oxide synthase mediates the hypotensive effect of ethanol in conscious female rats. *J Appl Physiol.* 2006; 100:1011–8. [PubMed: 16293701]
- GADEK-MICALSKA A, BUGAJSKI J, BUGAJSKI AJ, GLOD R. Effect of adrenergic antagonists and cyclooxygenase inhibitors on the nicotine-induced hypothalamic-pituitary-adrenocortical activity. *J Physiol Pharmacol.* 2002; 53:275–87. [PubMed: 12120902]
- GRUPP LA, SNEDDON B, SOLWAY E, PERLANSKI E, STEWART RB. The beta adrenergic agonist isoproterenol suppress voluntary alcohol intake in rats. *Pharmacol Biochem Behav.* 1989; 33:493–5. [PubMed: 2813487]
- HELMREICH DL, ITOI K, LOPEZ-FIGUEROA MO, AKIL H, WATSON SJ. Norepinephrine-induced CRH and AVP gene transcription within the hypothalamus: differential regulation by corticosterone. *Brain Res Mol Brain Res.* 2001; 88:62–73. [PubMed: 11295232]
- KHAN S, LIBERZON I, ABELSON JL. Effects of propranolol on symptom and endocrine responses to pentagastrin. *Psychoneuroendocrinology.* 2004; 29:1163–71. [PubMed: 15219640]
- LARKIN JW, BINKS SL, LI Y, SELVAGE D. The role of oestradiol in sexually dimorphic hypothalamic-pituitary-adrenal axis responses to intracerebroventricular ethanol administration in the rat. *J Neuroendocrinol.* 2010; 22:24–32. [PubMed: 19912475]
- LEE S, CRADDOCK Z, RIVIER C. Brain stem catecholamines circuitry: activation by alcohol and role in the hypothalamic-pituitary-adrenal response to this drug. *J Neuroendocrinol.* 2011; 23:531–41. [PubMed: 21447066]
- LEE S, SELVAGE D, HANSEN K, RIVIER C. Site of action of acute alcohol administration in stimulating the rat hypothalamic-pituitary-adrenal axis: comparison between the effect of systemic and intracerebroventricular injection of this drug on pituitary and hypothalamic responses. *Endocrinology.* 2004; 145:4470–9. [PubMed: 15205375]
- LINNOILA M, MATTILA MJ, KITCHELL BS. Drug interactions with alcohol. *Drugs.* 1979; 18:299–311. [PubMed: 387374]
- LOVALLO WR. Cortisol secretion patterns in addiction and addiction risk. *Int J Psychophysiol.* 2006; 59:195–202. [PubMed: 16434116]
- MA S, MORILAK DA. Chronic intermittent cold stress sensitises the hypothalamic-pituitary-adrenal response to a novel acute stress by enhancing noradrenergic influence in the rat paraventricular nucleus. *J Neuroendocrinol.* 2005; 17:761–9. [PubMed: 16219005]
- MCLAUGHLIN RJ, HILL MN, GORZALKA BB. Monoaminergic neurotransmission contributes to cannabinoid-induced activation of the hypothalamic-pituitary-adrenal axis. *Eur J Pharmacol.* 2009; 624:71–6. [PubMed: 19818759]

- MEZEY E, REISINE TD, PALKOVITS M, BROWNSTEIN MJ, AXELROD J. Direct stimulation of beta 2-adrenergic receptors in rat anterior pituitary induces the release of adrenocorticotropin in vivo. *Proc Natl Acad Sci U S A*. 1983; 80:6728–31. [PubMed: 6314339]
- NARKIEWICZ K, COOLEY RL, SOMERS VK. Alcohol potentiates orthostatic hypotension : implications for alcohol-related syncope. *Circulation*. 2000; 101:398–402. [PubMed: 10653831]
- RASMUSSEN DD, ALEXANDER LL, RASKIND MA, FROEHLICH JC. The alpha1-adrenergic receptor antagonist, prazosin, reduces alcohol drinking in alcohol-preferring (P) rats. *Alcohol Clin Exp Res*. 2009; 33:264–72. [PubMed: 19032582]
- SCHAFFERT CS, DURYEE MJ, HUNTER CD, HAMILTON BC 3RD, DEVENEY AL, HUERTER MM, KLASSEN LW, THIELE GM. Alcohol metabolites and lipopolysaccharide: roles in the development and/or progression of alcoholic liver disease. *World J Gastroenterol*. 2009; 15:1209–18. [PubMed: 19291821]
- SELVAGE DJ, HALES DB, RIVIER CL. Comparison between the influence of the systemic and central injection of alcohol on Leydig cell activity. *Alcohol Clin Exp Res*. 2004; 28:480–8. [PubMed: 15084906]
- SELVAGE DJ, PARSONS L, RIVIER C. Role played by brainstem neurons in regulating testosterone secretion via a direct neural pathway between the hypothalamus and the testes. *Endocrinology*. 2006; 147:3070–5. [PubMed: 16556770]
- SIMPSON TL, SAXON AJ, MEREDITH CW, MALTE CA, MCBRIDE B, FERGUSON LC, GROSS CA, HART KL, RASKIND M. A pilot trial of the alpha-1 adrenergic antagonist, prazosin, for alcohol dependence. *Alcohol Clin Exp Res*. 2009; 33:255–63. [PubMed: 18945226]
- SOUTHWICK SM, BREMNER JD, RASMUSSEN A, MORGAN CA 3RD, ARNSTEN A, CHARNEY DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry*. 1999; 46:1192–204. [PubMed: 10560025]
- SOUTHWICK SM, VYTHILINGAM M, CHARNEY DS. The psychobiology of depression and resilience to stress: implications for prevention and treatment. *Annu Rev Clin Psychol*. 2005; 1:255–91. [PubMed: 17716089]
- SVED AF, CANO G, PASSERIN AM, RABIN BS. The locus coeruleus, Barrington's nucleus, and neural circuits of stress. *Physiol Behav*. 2002; 77:737–42. [PubMed: 12527028]
- SZAWKA RE, HELENA CV, RODOVALHO GV, MONTEIRO PM, FRANCI CR, ANSELMO-FRANCI JA. Locus coeruleus norepinephrine regulates the surge of prolactin during oestrus. *J Neuroendocrinol*. 2005; 17:639–48. [PubMed: 16159376]
- THIELE TE, ROITMAN MF, BERNSTEIN IL. Learned tolerance to ethanol-induced c-Fos expression in rats. *Behav Neurosci*. 1998; 112:193–8. [PubMed: 9517827]
- WALKER BM, RASMUSSEN DD, RASKIND MA, KOOB GF. alpha1-noradrenergic receptor antagonism blocks dependence-induced increases in responding for ethanol. *Alcohol*. 2008; 42:91–7. [PubMed: 18358987]
- YAO M, DENVER RJ. Regulation of vertebrate corticotropin-releasing factor genes. *Gen Comp Endocrinol*. 2007; 153:200–16. [PubMed: 17382944]

Figure 1.

A) Lesions of the brainstem area containing the locus coeruleus (LCx) blocked the ability of icv alcohol (EtOH, 5 μ L of 100%) to stimulate ACTH secretion when compared to sham animal. B) Pictures of a representative LC lesion and the LC of a control animal. Bar height represents the mean $\pm$ SEM. **, $P < 0.01$ versus LCx/alcohol; $N = 8-10$ animals per treatment group.

Figure 2.

A) The icv administration of alcohol (EtOH, 5 μ L of 100%) significantly increased ACTH secretion compared to its vehicle. Pretreatment with a low dose of iv phenoxybenzamine (PBZ, 1 mg/kg in 0.2ml saline) did not affect the ability of icv alcohol to increase ACTH secretion. B) Pretreatment with a high dose of phenoxybenzamine (10 mg/kg in 0.2ml saline) completely inhibited the ability of icv alcohol to induce ACTH secretion. Bar height represents the mean $\pm$ SEM. ***, $P < 0.001$ versus vehicle/vehicle, phenoxybenzamine/vehicle and phenoxybenzamine/alcohol; $N = 6-8$ animals.

Figure 3.

A) The icv administration of alcohol (EtOH, 5 μ L of 100%) significantly increased ACTH secretion compared to its vehicle. Pretreatment with icv corynanthine (150 μ g in 5 μ L of saline) elevated basal ACTH levels, but icv alcohol did not add to this effect. Bar height represents the mean $\pm$ SEM. *, $P < 0.05$ versus vehicle/vehicle; **, $P < 0.01$ versus vehicle/vehicle; a, $P < 0.05$ versus corynanthine/alcohol, $N = 6-8$ animals.

Figure 4.

A) The icv administration of alcohol (EtOH, 5 μ L of 100%) significantly increased ACTH secretion compared to its vehicle. Pretreatment with icv propranolol (5 μ g in 5 μ L saline) elevated basal ACTH levels, but icv alcohol did not add to this effect. B) Cumulative ACTH as a percent of the basal value showed that propranolol significantly inhibited alcohol-induced ACTH secretion *as compared to basal levels*. Cumulative values were derived by adding icv alcohol-stimulated ACTH levels at 15, 30, and 60 minutes after icv injection. Bar height represents the mean $\pm$ SEM. **, P < 0.01 versus vehicle/vehicle; ***, P < 0.001 versus cumulative propranolol/vehicle and propranolol/alcohol; N = 5–8 animals.

Figure 5.

The icv administration alcohol (EtOH, 5 μ L of 100%) significantly increased Fos expression in the paraventricular nucleus of the hypothalamus (PVN) compared to vehicle controls. A) The pretreatment icv propranolol (5 μ g in 5 μ L saline) elevated Fos expression in the PVN. Alcohol given icv did not add to this effect. B) Pretreatment with iv phenoxybenzamine (10 mg/kg in 0.2 ml saline) elevated Fos expression in the PVN, but did NOT affect the ability of icv alcohol to stimulate Fos expression in the PVN. C) Representative 10 $\times$ pictures of Fos expression in the PVN in all treatment groups. Bar height represents the mean $\pm$ SEM. *, $P < 0.05$ versus vehicle/vehicle; **, $P < 0.01$ versus vehicle/vehicle; a, $P < 0.05$ versus vehicle/vehicle; b, $P < 0.05$ versus propranolol/alcohol. $N = 5-7$ animals and 3-4 PVN sections per animal.