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OXIDANT ANTI-OXIDANT IMBALANCE AND EFFECTS OF ETHANOL

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1. ABSTRACT

This article discusses some of the mechanisms through which alcohol, delivered pre- or postnatally, alters the activity of the rodent hypothalamic-pituitary-adrenal (HPA) axis. We show that blockade of prostaglandin synthesis, but not opiate receptors, modestly interfered with the HPA axis to acute alcohol injection. Pretreatment with a low dose of alcohol (0.3 g/kg) did not significantly modify the ability of cytokines to stimulate ACTH release in intact rats, but higher doses ($\geq$2.0 g/kg) did unless corticosteroid feedback was abolished by adrenalectomy. 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.

2. INTRODUCTION

The functional connections between the immune system and the HPA axis are essential for appropriate responses to homeostatic threats due to pathogens [see (1) for ref.]. Briefly stated, this crosstalk is initiated when immune cells are exposed to an antigen and, in response, release cytokines such as interleukins (ILs) into the circulation (Figure 1). Either directly or through intermediates like prostaglandins (PGs), catecholamines and/or NO, these proteins convey to the brain the fact that peripheral immune stimulation has taken place. Upon receipt of this information, the hypothalamus coordinates responses that include changes in behavior (loss of social interaction, decreased appetite, increased sleepiness), increased core temperature, and, through upregulated production of the hypothalamic peptides CRF and VP, release of POMC-like peptides. The ensuing secretion of corticosteroids serves to provide an adequate supply of nutrients, and regulates important changes in cardiovascular and hemodynamic functions. Pertinent to the concept discussed here, is the fact that these steroids lower the number of immune cells and moderate their activity. This latter phenomenon ensures that cytokine production is maintained within limits, so that it does not itself threaten homeostasis. It is also important to note that both CRF (2) and adrenal steroids (3-6) have been reported to promote drug seeking behavior. Their increased release following alcohol consumption may thus play a dual role in alcohol addiction, by participating in the damaging influence of the drug on a variety of bodily functions as well as by promoting drug abuse. Consequently, treatments that will maintain hormones of the HPA axis steroids within normal limits may be useful both to limit drug use, and to prevent the damage this use will cause.

From this brief description, it is easy to understand that conditions that perturb the normal response of the HPA axis to immune signals can result in a number of pathological changes. For example, increased levels of CRF, the secretagogue that represents the essential coordinator of ACTH secretion, can suppress natural killer cell activity (7). This peptide can also inhibit reproductive functions (8), alter the activity of the sympathetic and the cardiovascular systems (9), perturb a number of behavioral responses (10), and induce anxiety (11). Low hypothalamic CRF levels, on the other hand, are associated with psychosis (12, 13). POMC-like peptides also influence a variety of functions, in particular those of immune cells (14-16). Finally, corticosteroids not only alter metabolism [see (17, 18)], they modulate immune functions (19, 20), influence mood (21), and exert profound and potentially damaging effects on brain cells (22), as both hyper- and hypocortisolemia constitute a threat to neuronal integrity (23). Maintenance of appropriate responses of pathways

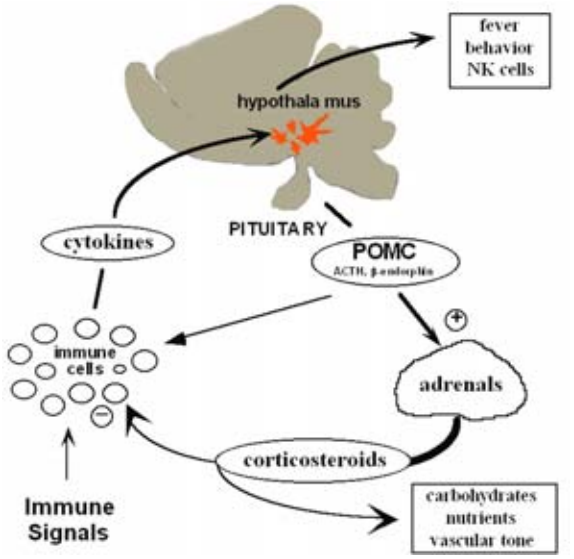

Figure 1. Cartoon of the interactions between the HPA axis and the immune system.

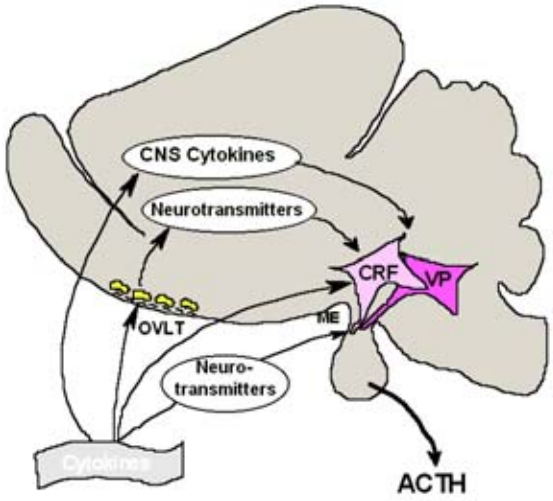

Figure 2. Mechanisms through which blood-borne cytokines may act on hypothalamic CRF production.

that involve CRF, POMC and glucocorticoids, is therefore vital for good health.

Alcohol abuse is well known to be accompanied by an increased incidence of abnormal immune responses, and the steps involved in the direct influence of the drug to impair the activity of immune cells have been extensively studied [see (24-26) for ref.]. The ability of exposure to alcohol during embryonic development to alter immune activity in adult offspring, has also been described (27-30). Though the mechanisms responsible for this particular phenomenon have not yet been clearly delineated, there is little doubt that the activity of immune cells themselves is altered. The hypothesis that we propose is that in addition to these mechanisms, alcohol treatment, whether given to developing embryos or to adults, impairs the function of the immune system by disturbing the normal cross-talk between this system and the HPA axis. The rest of this chapter will be devoted to a discussion of results that substantiate this hypothesis. First, we will present the mechanisms through which cytokines activate the HPA axis. We will then illustrate the ability of alcohol to alter HPA axis function. Specifically we will show how, by acting on structures of this axis that are similar to those altered by cytokines, the drug induces either a blunted or an augmented release of ACTH/corticosteroids, compared to that observed in naive animals. Finally, we will discuss preliminary results suggesting that increased NO production may participate in the damaging influence of alcohol.

3. DISCUSSION

3.1. Influence of cytokines on the HPA axis

The influence of cytokines on the HPA axis has been abundantly reviewed (1, 31-33) and will only be briefly outlined. Several models have been used to study it, the most common being administration of endotoxin or of single ILs. It is usually recognized that blood-borne cytokines first stimulate CRF and possibly VP release from nerve terminals in the median eminence, and do so through intermediates that include PGs and NO (Figure 2).

PGs exert a stimulatory influence on the HPA axis, as indicated by the ability of PG blockers to block CRF (34) and ACTH responses to immune signals (35-38). NO, on the other hand, exerts a restraining influence on pituitary activity because blockade of NO synthase (NOS), the enzyme responsible for NO formation, potentiates the ACTH releasing ability of circulating ILs (39). Cytokines can also stimulate hypothalamic activity, as indicated by increased levels of the immediate early genes c-fos or NGFI-B (40), and upregulated mRNA levels of CRF in the paraventricular nucleus (PVN) of the hypothalamus (41). Though we do not know whether this effect is exerted directly on PVN neurons, there is good evidence that catecholamines and PGs often serve as intermediates. Because it is unlikely that blood-borne cytokines can cross the blood-brain barrier, it is generally believed that these secretagogues are produced by cells (like astrocytes) present within the blood-brain barrier, from which they reach the PVN. An alternate and not mutually exclusive possibility is that cytokines present in the periphery can, through stimulation of C-fibers or other mechanisms, activate astrocytes and/or microglia in the brain, which in turn produce PGs, NO and other intermediates. In summary, regardless of the mechanisms through which circulating cytokines act on CRF and VP pathways, their presence stimulates the synthesis and release of these two hypothalamic peptides, which in turn act on the pituitary corticotrophs to release ACTH.

3.2. Influence of alcohol on the HPA axis

We propose that because alcohol, like cytokines, acts on CRF/VP fibers, it will alter the normal response of these fibers to these proteins. The influence of alcohol on the HPA axis has also been recently reviewed (42) and will only be briefly presented here. The fact that both acute and prolonged alcohol treatment releases ACTH and corticosteroids is well known in humans and laboratory animals. Because of our recent observation that blockade

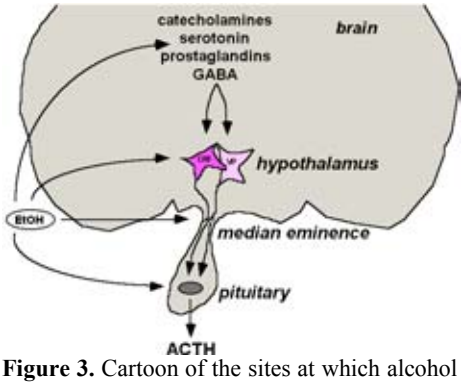

Figure 3. Cartoon of the sites at which alcohol could act to alter the activity of the HPA axis.

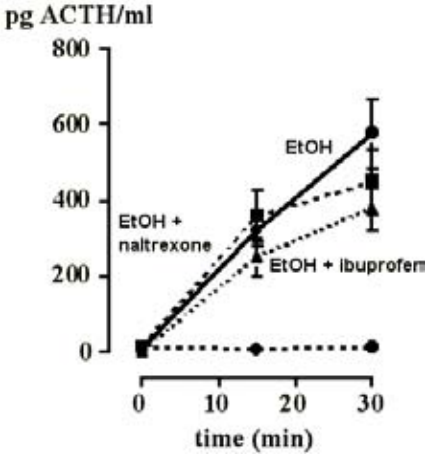

Figure 4. Pretreatment with the PG blocker ibuprofen (10 mg/kg, iv - 15 min) or the opiate antagonist naltrexone (10 mg/kg, iv - 15 min) only modestly alters the ACTH response of intact rats to alcohol (EtOH, 2. 0 g/kg, ip). Each point represents the mean ± SEM of 5-6 animals. *, P<0.05 versus EtOH.

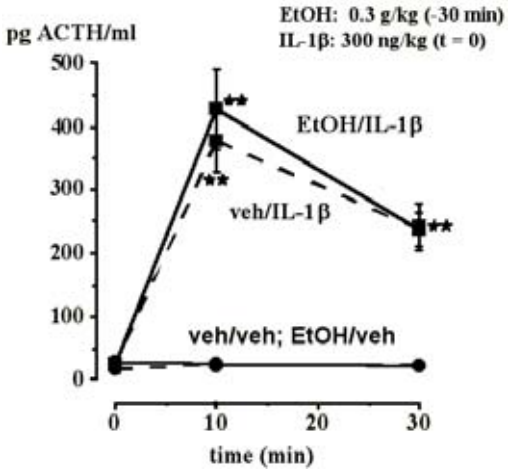

Figure 5. The ip injection of a small dose of alcohol (EtOH, 0.3 g/kg, - 30 min) does not alter the ACTH response of intact rats to the iv injection of IL-1β (300 ng/kg). Each point represents the mean ± SEM of 5-6 animals. **, P<0.01 versus vehicle (veh).

of pituitary, but less so brain CRF receptors interferes with alcohol-induced ACTH secretion (43), we proposed that the initial site of drug action is on nerve terminals in the median eminence. Subsequently, the activity of hypothalamic neurons is stimulated, immediate early genes transcripts are upregulated, and CRF mRNA levels are increased (Figure 3). The participation of intermediate pathways in this effect is not fully understood, but is most probably multifaceted. We have observed that blockade of PG synthesis with ibuprofen produced a relatively modest, but significant decrease in the ACTH response to acute alcohol injection (Figure 4). Blockade of opiate receptors with naltrexone produced no significant change, which suggests that endogenous opiates exert only a subtle, and possibly indirect, modulating role in the response of the HPA axis to the drug. We have also investigated the role played by NO in mediating the acute stimulatory influence of alcohol. We tested this hypothesis by blocking NO formation with L-NAME, a treatment that decreases brain (constitutive) NOS activity by more than 90% and alters ACTH secretion through mechanisms dissociated from changes in blood pressure or altered muscarinic receptors (39, 44-48). We therefore believe that the influence of this compound can indeed be attributed to decreased NO levels. We consistently observed that administration of L-NAME produces modest and inconsistent changes in ACTH release following EtOH treatment (not shown). Collectively, these results show that pathways that depend on activation of PGs and endogenous opiates may play some role in mediating the influence of alcohol on the HPA axis, but that at least during acute treatment, this influence is modest at best.

3.3. Alcohol alters the HPA axis response to immune signals: possible role of NO.

3.3.1. Postnatal alcohol treatment

Pretreatment with low doses of alcohol, administered acutely, does not significantly alter the ability of IL-1beta to increase ACTH levels (Figure 5). Similar results were obtained in rats injected with larger doses of the drug, provided the influence of alcohol-increased corticosterone had been removed by adrenalectomy (Figure 6). This suggests that alcohol does not directly act on the pathways that mediate activation of the HPA axis by IL-1beta (49). However, alcohol will decrease both ACTH secretion and hypothalamic responses when the cytokine is injected into the brain ventricles (49). It is therefore possible that under circumstances of increased cytokine levels [such as exposure to some stresses, peripheral inflammation or central infection] (33), the inhibitory influence of the drug plays a role in pathological responses of the HPA axis to immune stimulation. We then studied the effect of prolonged alcohol treatment. When rats fed an alcohol diet for 7-10 days were systemically injected with immune signals, they released significantly less ACTH and corticosterone than control animals [Figure 7 and (47)].

In searching for mechanisms that might explain this phenomenon, we found that pituitary responsiveness to VP, but interestingly not CRF, was blunted (50). These findings do not rule out, however, the additional possibility that hypothalamic neuronal activation is decreased in rats fed alcohol, a hypothesis for which we recently found evidence (Lee *et al.*, in preparation).

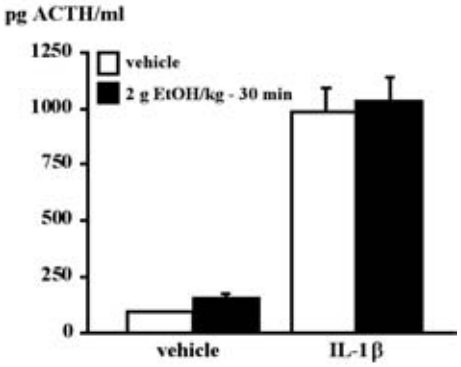

Figure 6. The ip injection of a large dose of alcohol (2.0 g/kg, - 30 min) does not alter the ACTH response of adrenalectomized rats to the iv injection of IL-1β (300 ng/kg). Values represent cumulative ACTH level measured 10 and 30 min after IL-1β. Data are expressed as mean ± SEM of 5-6 animals.

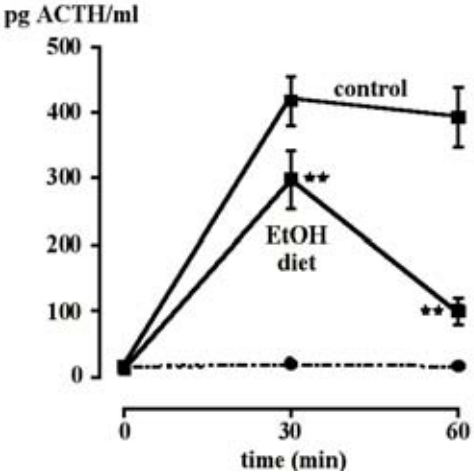

Figure 7. Exposure to an alcohol (EtOH) diet for 7-10 days blunts the ACTH response of intact male rats to the iv injection of IL-1β (300 ng/kg). Each point represents the mean ± SEM of 5-6 animals. **, P<0.01 versus control rats.

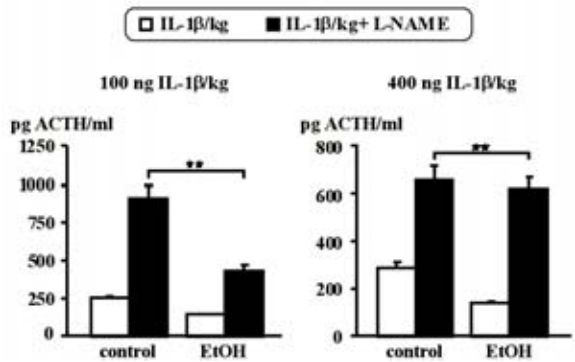

Figure 8. Effect of preinjection with the arginine derivative L-NAME (30 mg/kg, iv - 5 min), which blocks NO formation, on the ACTH response to the iv injection of IL-1β (300 ng/kg) in control rats or rats exposed to an alcohol (EtOH) diet for 7-10 days. Values represent cumulative ACTH level measured 10 and 30 min after IL-1β. Each bar represents the mean ± SEM of 5-6 animals. **, P<0.01.

Does NO play a role in the altered ability of cytokines to induce ACTH secretion? We reasoned that because NO release results from activation of NMDA receptors (51), and because these receptors are upregulated by chronic alcohol consumption (52), overproduction of NO might participate in the blunted ACTH responses described above. We found that L-NAME exerted its expected potentiating influence on the stimulatory effect of IL-1 beta (injected iv) on ACTH release in control animals (Figure 8). Interestingly, it not only also increased corticotrophs' activity in rats fed alcohol, it abolished the difference between animals exposed to alcohol, and naive rats (53). These results suggest that increased NO tone may indeed participate in the inhibitory influence of prolonged alcohol consumption on ACTH secretion. In order to investigate the site(s) at which NO might exert its influence, we determined whether L-NAME would reverse the inhibitory effect of alcohol on VP-induced ACTH secretion. As illustrated in Figure 9, it did so, suggesting that at least part of the mediating role of NO is exerted at the pituitary itself. Whether this is the only mechanism responsible for the decreased ACTH response to circulating cytokines remains to be determined.

3.3.2. Prenatal alcohol treatment

Prenatal alcohol treatment exerts long-term effects on the response of the offspring' HPA axis to a variety of stimuli (54, 55), including IL-1beta [(56, 57) and Figure 10]. When these offspring are studied before puberty, they exhibit significantly blunted ACTH and corticosterone responses to immune signals. In contrast, older animals have increased ACTH/corticosterone levels following IL-1beta or endotoxin injection, as well as during peripheral inflammatory processes (58). We have shown that sex steroids are involved in the qualitative changes of the HPA axis activity (59), but do not know how this influence is exerted. The influence of prenatal alcohol treatment on NO formation in the offspring' brain has not yet been determined, but we hypothesize that prenatal alcohol exposure will alter the HPA axis activity through mechanisms that involve nitrinergic pathways.

4. CONCLUSION

We have shown that adult rats fed an alcohol diet, or immature offspring born to dams exposed to alcohol during gestation, exhibit a blunted ACTH and corticosteroid release in response to immune signals. At least part of this altered response may be caused by pathologically elevated levels of nitric oxide (NO), which result in decreased pituitary responsiveness to vasopressin (VP) and interleukin-1beta. In contrast, adult offspring of alcohol-treated dams have a significantly enhanced ACTH and corticosteroid secretion when challenged with immune signals, a finding that extends those of other investigators who studied the altered ACTH response to other stimuli. Hormones that belong to the hypothalamic-pituitary-adrenal (HPA) axis, in particular corticotropin-releasing factor (CRF), ACTH and glucocorticoids, exert powerful effects on a large spectrum of functions that are essential for good health. Their inadequate secretion can lead to inflammation while abnormally elevated levels of these

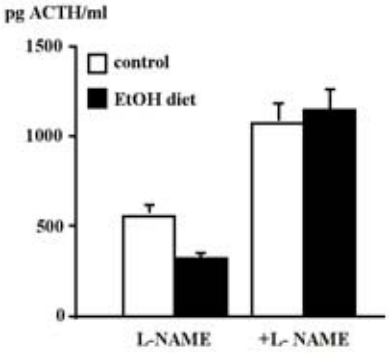

Figure 9. Effect of preinjection with the arginine derivative L-NAME (30 mg/kg, iv - 5 min) on the ACTH response to the iv injection of vasopressin (0.1 μg/kg) in control rats or rats exposed to an alcohol (EtOH) diet for 7-10 days. Samples were obtained 10 min after peptide injection. Each bar represents the mean ± SEM of 5 animals

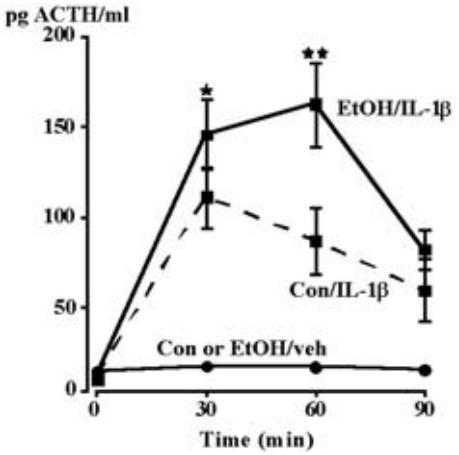

Figure 10. The ACTH response to the iv injection of IL-1β (300 ng/kg) is augmented in intact adult male rats exposed to alcohol (EtOH) prenatally, compared to controls (Con). Each point represents the mean ± SEM of 5-6 animals. *, P<0.05 and **, P<0.01 versus Con.

hormones can facilitate the development of infection. Because CRF and corticosteroids can lead to increased alcohol intake, a better understanding of the mechanisms leading to abnormal HPA axis response to immune signals may provide the basis for the development of therapies aimed at alleviating not only the deleterious influence of the drug, but, under some circumstances, its abuse.

5. ACKNOWLEDGMENTS

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