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Research report

Systemic ethanol administration elevates deoxycorticosterone levels and chronic ethanol exposure attenuates this response

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Abstract

Systemic ethanol administration is known to elevate levels of the GABAergic neuroactive steroid 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC). 3α,5α-THDOC is synthesized from deoxycorticosterone (DOC) by metabolism in adrenals and brain. The present study investigated DOC levels in plasma and brain following ethanol administration to naïve and ethanol-exposed rats. Rats were administered ethanol (2 g/kg, i.p.) or saline and DOC levels were measured in plasma and brain regions by radioimmunoassay. Chronic ethanol-exposed rats were administered an ethanol challenge (2 g/kg, i.p.) following 15 days of ethanol liquid diet consumption. Ethanol administration markedly increased DOC levels in plasma, cerebral cortex, hippocampus, hypothalamus, cerebellum, and olfactory tubercle of naïve rats. Ethanol challenge produced an attenuated elevation of DOC in rat plasma and brain following chronic ethanol consumption for 2 weeks. These findings suggest that acute ethanol increases DOC levels in ethanol naïve rats and chronic ethanol consumption induces tolerance to ethanol-induced increases in DOC levels.

Theme: Neural basis of behavior
Topic: Drugs of abuse: alcohol, barbiturates, and benzodiazepines

Keywords: Neuroactive steroid; Deoxycorticosterone; Ethanol; Chronic ethanol consumption; 3α,5α-THDOC

1. Introduction

Deoxycorticosterone (DOC) metabolite, 3α,5α-THDOC, is a neuroactive steroid with potent agonistic action at $GABA_A$ receptors [30]. Physiologically, neuroactive steroids play a vital role in stress, pregnancy, and CNS neurotransmission [1,4,10,16,32,42]. Neuroactive steroids produce anticonvulsant, anti-anxiety, antidepressant, loss of righting reflex and anesthesia in rats [7,21,25,29,41,43,44,46,47]. Systemic ethanol administration produces neuropharmacological effects similar to GABAergic neuroactive steroids. Recent studies have demonstrated that acute ethanol administration markedly increases plasma and brain levels of 3α,5α-THDOC and 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP) [5,33,35]. Ethanol-induced elevations of GABAergic neuroactive steroids contribute to many behavioral effects of ethanol including its anticonvulsant action [58], sedation and loss of righting reflex [24], impairment of spatial memory [56], anxiolytic [18], antidepressant [17], and reinforcing properties [19]. Although there is emerging evidence that 3α,5α-THDOC and 3α,5α-THP have a role in ethanol action and withdrawal [50], few studies have looked at the role of the precursor DOC.

3α,5α-THDOC is synthesized from DOC, an adrenal steroid, by two sequential A-ring reductions. 5α-reductase isoenzymes first convert DOC to the intermediate 5-dihydrodeoxycorticosterone (DHDOC), which is then further reduced by 3α-hydroxysteroid oxidoreductase to form 3α,5α-THDOC [45]. In contrast to 3α,5α-THP,

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which is present in the brain even after adrenalectomy and gonadectomy, 3α,5α-THDOC appears to be derived exclusively from adrenal sources [35,38,45].

It is hypothesized that these neuroactive steroids are released as a consequence of ethanol-induced increases in adrenocorticotrophic hormone (ACTH) released by the activation of the hypothalamic–pituitary–adrenal (HPA) axis. DOC is an intermediate in aldosterone synthesis in the adrenal zona glomerulosa and itself has weak mineralocorticoid activity, however more DOC is produced in the zona fasciculata, where its synthesis is under the control of ACTH, and its secretion correlates with that of glucocorticoids and not aldosterone [20,55]. In addition to its well-recognized role as a mineralcorticoid precursor, there is substantial evidence that DOC participates in the HPA axis response to acute stress. Since DOC is converted to 3α,5α-THDOC in the body, enhanced DOC availability during acute stress such as ethanol, swimming, foot shock, or carbon dioxide exposure would elicit an increase in 3α,5α-THDOC concentrations in plasma and brain [2,3,38,52,57]. Increased levels of 3α,5α-THDOC, through its actions on $GABA_A$ receptors, likely contribute to neuropharmacological effects of ethanol on CNS function.

A growing body of evidence shows that DOC plays an important physiological role during stress [39,41,45], premenstrual syndrome [51], infantile spasms [40,48], and epilepsy [41,45]. Because ethanol in known to have effects on $GABA_A$ receptors and DOC is an important intermediate in the biosynthesis of the GABAergic neuroactive steroid 3α,5α-THDOC, it is worthwhile to study the interaction between ethanol and DOC. Furthermore, due to the lack of a commercial source for 3α,5α-THDOC antibody, measurement of its precursor steroid (DOC) contributes to our understanding of the interactions between ethanol and 3α,5α-THDOC. In this study, we systematically investigated whether systemic ethanol administration alters plasma and brain levels of DOC in ethanol naïve rats. The effect of acute ethanol challenge on plasma and brain DOC levels was also tested after chronic ethanol consumption for 2 weeks.

2. Materials and methods

2.1. Animals

Experiments were conducted in accordance with National Institutes of Health Guidelines under Institutional Animal Care and Use Committee-approved protocols. Male Sprague–Dawley rats (150–180 g) were purchased from Harlan (Indianapolis, IN) and group housed. Rats were maintained in standard light and dark (lights on, 6:00 A.M. to 6:00 P.M.) conditions with ad libitum access to rat chow and water.

2.2. Chronic liquid diet consumption

Separate groups of rats (N = 32) were housed individually and administered a nutritionally complete liquid diet for the first 3 days (Dextrose diet, ICN Biomedicals, Costa Mesa, CA). Sixteen rats received ethanol (6% v/v in liquid diet) for 7 days followed by ethanol (7.5% v/v in liquid diet) for the duration of study. Control weight-matched rats (n = 16) were pair-fed the identical diet with dextrose substituted for ethanol in an equicaloric manner. Water was available ad libitum. Dietary consumption was monitored daily. The typical daily consumption was 10–12 g/kg. This produces a blood ethanol concentration of 140–200 mg/100 ml [31]. The mean body weight for the controls and pair-fed rats was similar at the termination of the experiment. This procedure reliably results in physical dependence on ethanol [14,31]. To accommodate the circadian variations in steroid levels, all animals were sacrificed between 7:00 A.M. and noon. Rats had free access to liquid diet until the time of sacrifice. All rats were handled and habituated to saline injections and were sacrificed by decapitation.

2.3. Drug administration

To study the effect of ethanol administration on plasma and brain DOC levels in ethanol naïve rats, rats were injected with ethanol (2 g/kg, 20% v/v in saline) or saline and sacrificed after 30 or 60 min. Trunk blood was collected and plasma was separated by centrifugation. The brain was removed from the skull and various brain regions were rapidly dissected over ice. Plasma and brains regions were stored at −80 °C until assayed.

Separate groups of rats that consumed ethanol by liquid diet (or pair-fed diet without ethanol) for 15 days were injected with a challenge dose of ethanol (2 g/kg, 20% v/v in saline) on the 15th day and sacrificed after 60 min. Plasma and brain regions obtained from these rats were assayed for DOC levels.

2.4. Blood alcohol level (BAL)

BALs were determined by using a commercially available kit for the Analox GL-5 (Analox Instruments, USA). For each determination, 5 μl of plasma was injected and the alcohol concentration was expressed as mg/dl.

2.5. Radioimmunoassay (RIA) of plasma and brain DOC

Steroids were extracted from individual plasma aliquots and brain samples using organic solvents. Extraction recovery was monitored by the incorporation of 2000 dpm of [^{3}H] DOC. Plasma samples were extracted two times in 2-ml aliquots of ethyl acetate:hexane (3:2) mixture. Individual brain samples were weighed and suspended in 3 ml of phosphate buffer and homogenized by a sonic dismembrator and extracted three times in 3-ml aliquots of ethyl