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Glucocorticoid receptor antagonism decreases alcohol seeking in alcohol-dependent individuals

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Alcoholism, or alcohol use disorder, is a major public health concern that is a considerable risk factor for morbidity and disability; therefore, effective treatments are urgently needed. Here, we demonstrated that the glucocorticoid receptor (GR) antagonist mifepristone reduces alcohol intake in alcohol-dependent rats but not in nondependent animals. Both systemic delivery and direct administration into the central nucleus of the amygdala, a critical stress-related brain region, were sufficient to reduce alcohol consumption in dependent animals. We also tested the use of mifepristone in 56 alcohol-dependent human subjects as part of a double-blind clinical and laboratory-based study. Relative to placebo, individuals who received mifepristone (600 mg daily taken orally for 1 week) exhibited a substantial reduction in alcohol-cued craving in the laboratory, and naturalistic measures revealed reduced alcohol consumption during the 1-week treatment phase and 1-week post-treatment phase in mifepristone-treated individuals. Mifepristone was well tolerated and improved liver-function markers. Together, these results support further exploration of GR antagonism via mifepristone as a therapeutic strategy for alcoholism.

Introduction

Alcohol misuse accounts for approximately 6% of deaths worldwide and is one of the largest risk factors for morbidity and disability (1). Alcoholism, or alcohol use disorder, is a complex psychiatric condition characterized by craving, compulsive seeking, and excessive drinking of alcohol, as well as the emergence of negative emotional states during withdrawal (2–4). Allostatic changes in brain reward and stress systems are thought to mediate many of these symptoms. We previously reported that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, the principal neuroendocrine stress system, and consequent alterations in brain glucocorticoid receptor (GR) expression accompany compulsive-like alcohol intake in rats (5, 6). Despite these strong preclinical findings and evidence for dysregulation of the HPA axis in alcoholism (7, 8), a role for GR signaling in humans who suffer from alcohol dependence has yet to be determined. Here, employing an integrative conceptual approach to medication development for alcohol use disorder (9), we examined the hypothesis that altered GR signaling mediates compulsive alcohol intake in both rats and humans.

Results and Discussion

We first tested the hypothesis that GRs were activated during alcohol dependence by employing an animal model that produces somatic and motivational signs of alcoholism strongly resembling the human condition (10, 11). The levels of phosphorylated

and total GR were measured in the central amygdala (CeA) and basolateral amygdala (BLA), two brain regions critically implicated in stress, relapse, and the transition to drug addiction (12). Rats were made dependent by exposure to alcohol vapor, with peak blood alcohol levels averaging $237.0 \text{ mg}\% \pm 11.3 \text{ mg}\%$ in the 12th and final week of exposure. Air-exposed rats served as nondependent controls. Brain tissue collection occurred during acute withdrawal (6–8 hours after removal from alcohol vapor) when brain and blood alcohol levels are negligible (13) and withdrawal symptoms — including anxiety and brain reward deficits — are manifest (10, 11, 14). Previous work has established that, under these conditions, nondependent rats will work to obtain and ingest 10% wt/vol alcohol, and dependent rats will escalate their intake sufficient to block withdrawal and show compulsive-like responding for alcohol (6, 10, 15). We found that the levels of GR phosphorylation at Ser²³², a marker of GR nuclear localization and transactivation, were increased in the CeA ($t_{10} = 2.574$, $P = 0.0277$) but not the adjacent BLA ($t_{10} = 0.486$, $P = 0.8087$) in alcohol-dependent rats compared with nondependent rats (Figure 1A). Total GR levels were unchanged, suggesting an increase in physiological GR function, rather than total receptor number in the CeA in the transition to alcohol dependence.

To determine the functional significance of the activation of GRs in alcohol dependence, we tested a GR antagonist, mifepristone, on compulsive-like alcohol intake. Rats were trained to lever press for access to alcohol (10%, wt/vol) or water in an operant task, and half were subsequently made dependent by exposure to alcohol vapor. Dependent and nondependent rats ($n = 11/\text{group}$) were i.p. injected with mifepristone (0, 30, and 60 mg/kg) 90 minutes prior to alcohol self-administration ses-

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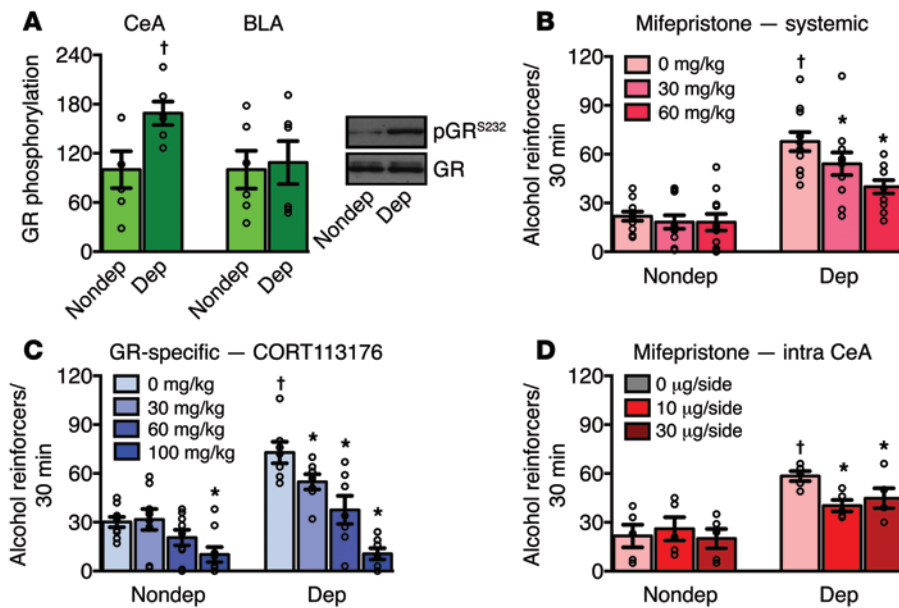


Figure 1. Increased GR function mediates compulsive-like alcohol self-administration in alcohol-dependent rats. (A) The phosphorylation of GR at Ser⁵²² in the CeA (2-tailed Student's *t* test, [†] $P < 0.05$ versus nondep), but not BLA, was increased in dependent (dep) rats compared with nondependent (nondep) rats ($n = 6$ /group). No group differences in total GR levels were observed. Representative Western blots for phosphorylated and total GRs in the CeA are indicated. (B) The blockade of GRs with mifepristone injected systemically ($n = 11$ /group) dose-dependently reduced alcohol intake, specifically in dep rats. (C) The GR-specific antagonist CORT113176 decreased alcohol self-administration ($n = 7$ for dep and $n = 9$ for nondep), with increased effectiveness in dep rats. (D) The blockade of GRs with mifepristone injected directly into the CeA ($n = 5$ /group) reduced alcohol intake, specifically in dep rats. (B–D) 2-way repeated-measure ANOVA followed by Duncan's test (^{*} $P < 0.05$ vs. 0 mg/kg, [†] $P < 0.0001$ vs. nondep). The data represent the mean \pm SEM of the number of lever presses performed by the rats in 30-minute operant self-administration sessions. Drugs were injected 90 minutes prior to self-administration sessions. Every lever press (fixed-ratio 1 schedule of reinforcement) resulted in the delivery of 0.1 ml of alcohol (10%, wt/vol) or water. Dotplots display individual observations for each condition.

sions occurring during acute withdrawal. Dependent rats displayed escalated alcohol intake ($P = 0.0001$), i.e., significantly increased alcohol intake compared with nondependent rats. Systemic injection with mifepristone dose-dependently (dose \times group interaction: $F_{2,40} = 4.675$, $P = 0.0150$) reduced alcohol intake in dependent rats only ($P = 0.0199$, 0 vs. 30 mg/kg; $P = 0.0001$, 0 vs. 60 mg/kg; Figure 1B). These findings provide evidence that acute mifepristone administration, in addition to blocking the development of compulsive-like alcohol drinking (6), significantly decreases alcohol self-administration when dependence has already been established. Our results corroborate and extend previous studies indicating that mifepristone decreases both alcohol drinking under limited-access conditions (16) and the severity of alcohol withdrawal (17).

Because mifepristone potently inhibits both GR and progesterone receptor function, we tested the effect of a selective GR antagonist on alcohol drinking. CORT113176 antagonizes GRs comparably to mifepristone but has no affinity for progesterone receptors (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI79828DS1). CORT113176 dose-dependently ($n = 7$ for dependent; $n = 9$ for nondependent; dose \times group interaction: $F_{3,42} = 5.168$, $P = 0.0039$) decreased alcohol intake (Figure 1C) at doses of 30 mg/kg ($P = 0.0233$) and 60 mg/kg ($P = 0.0001$) compared with vehicle (0 mg/kg) in depen-

dent rats only, while it reduced drinking at 100 mg/kg in both dependent ($P = 0.0001$) and nondependent ($P = 0.0194$) animals. Vehicle-treated dependent rats displayed escalated alcohol intake compared with vehicle-treated nondependent rats ($P = 0.0001$). Additional control experiments indicated that mifepristone and CORT113176 had no effect on water or saccharin intake (Supplemental Figure 1). These results provide clear-cut evidence for a specific role of GRs in the escalation of alcohol drinking that does not generalize to nondrug rewards. This selectivity would predict robust and specific applicability of mifepristone to treat human alcoholism, with a critical new feature not encountered in existing medications, such as naltrexone (18), that act on reward mechanisms.

To confirm a key role of the CeA in the glucocorticoid antagonist effect, dependent and nondependent rats were bilaterally injected with mifepristone (0, 10, and 30 μ g/side) directly into the CeA 90 minutes prior to alcohol self-administration. Vehicle-treated dependent rats displayed escalated alcohol intake compared with vehicle-treated nondependent rats ($P = 0.0007$). Mifepristone ($n = 5$ /group; dose \times group interaction: $F_{2,16} = 4.044$, $P = 0.0379$) at 10 μ g/side ($P = 0.0068$) and 30 μ g/side ($P = 0.0280$), significantly and specifically reduced alcohol intake in dependent rats, when compared with vehicle (0 μ g/side) controls (Figure 1D). Our demonstration of increased GR phosphorylation during dependence and reduction of compulsive-like alcohol drinking by intra-CeA mifepristone injection, combined with previous findings (19), indicate that increased GR signaling in the CeA plays a functional role in alcohol drinking and seeking.

For clinical validation of our findings, we employed a human laboratory model of risk factors for relapse in abstinence in non-treatment-seeking, paid, alcohol-dependent volunteers (20). The subjects (Supplemental Figure 2) were randomly assigned to double-blind dosing with oral mifepristone (600 mg/day, no titration) or placebo for 7 days. Alcohol abstinence was required during the last 3 days of the 7-day dosing period in order to test the effect of mifepristone when motivational signs of early abstinence are manifest. Alcohol cue reactivity manipulations, followed by craving ratings, were conducted in the laboratory at the conclusion of the 7-day dosing/3-day abstinent interval (see Supplemental Table 2). Craving in response to alcohol cues in abstinent alcoholics has been shown to be predictive of subsequent drinking relapse (21), and thus craving severity in response to alcohol cue exposure in the laboratory comprised the primary outcome for this proof-of-concept study. Subjects returned 1 week after drug discontinuation to assess any persisting drug effects on safety indices and on nat-

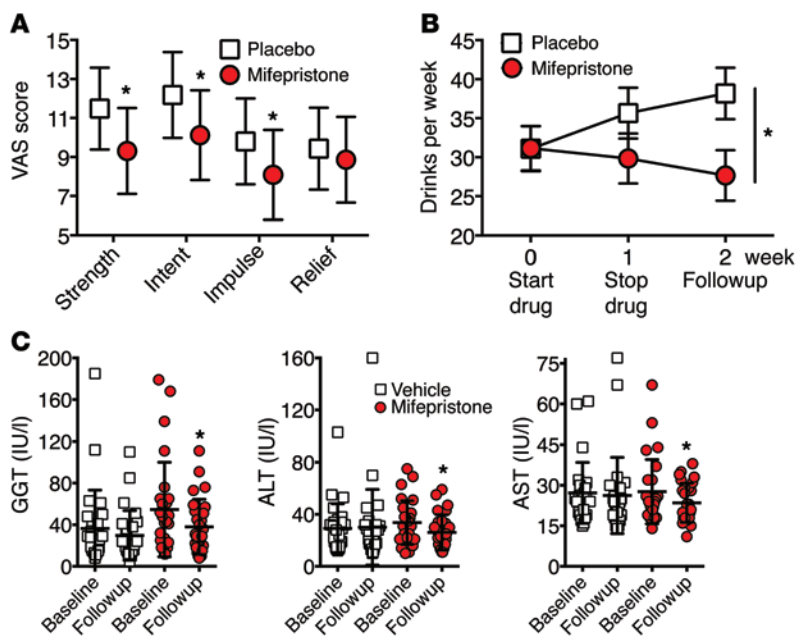


Figure 2. Mifepristone reduces alcohol-cued craving and drinking in alcoholics while improving liver function.

(A) Visual Analog Scale (VAS) scores of craving severity in response to in vivo alcohol cues. Higher scores indicate greater craving severity. Data represent the estimated marginal mean \pm SD. * $P < 0.05$, mifepristone vs. placebo (linear mixed effects modeling). (B) Total number of alcoholic drinks consumed per week. Data represent the estimated marginal mean \pm SEM. * $P < 0.05$, mifepristone vs. placebo (linear mixed effects modeling). (C) Liver enzymes: GGT, ALT, and AST. Data represent the mean \pm SD. Dotplots display individual observations for each condition. * $P < 0.05$ within-group change from baseline (multivariate analysis of covariance). $n = 26$ for placebo and $n = 28$ for mifepristone.

uralistic measures of drinking. Self-reported drinking data were collected on an exploratory basis, as the 3-day abstinent interval constrained complete evaluation of this variable, and drinking data were collected under less controlled conditions than craving in response to in vivo cue exposure. The subjects were 43 male and 13 female non-treatment-seeking volunteers, 21–65 years of age, who met the DSM-IV criteria for current alcohol dependence (see Supplemental Table 3 for baseline subject data). Mifepristone was associated with a significantly greater reduction in alcohol-cued craving in the laboratory relative to placebo after 1 week of treatment (sum score of the four VAS craving items: -6.43 , $t = -3.01$, $P = 0.003$; Figure 2A). Mifepristone was also associated with a significantly greater reduction in the number of drinks per week during treatment and post-treatment followup compared with placebo (-10.58 , $t = -1.99$, $P = 0.05$; Figure 2B). The reduction in alcohol-cued craving in the laboratory significantly predicted a reduction in the number of drinks per drinking day at post-treatment followup ($R^2 = 0.11$, $P = 0.017$ excluding 1 outlier, the full dataset $R^2 = 0.15$, $P = 0.005$; Supplemental Figure 4), thus lending support to the predictive validity of the human laboratory model.

Excessive alcohol drinking is typically associated with elevation in liver-function markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ -glutamyltransferase (GGT). Biochemical tests of liver-function markers indicated significant reductions of GGT, ALT, and AST from baseline to followup in the mifepristone-treated group ($F = 5.46$, $P = 0.005$) but not in the placebo group ($F = 1.60$, $P = 0.218$; Figure 2C). The reductions in these physiological markers in the treated group corroborate the self-reported decreases in drinking and provide evidence of the hepatic safety of mifepristone in alcoholics. Whether mifepristone directly reduces liver-function markers remains to be determined.

No serious or unexpected adverse events were observed, and the groups did not differ in the type or severity of adverse events during treatment (Supplemental Table 4). No effects on measures

of mood were found. The effects of mifepristone did not resemble alcohol or any major classes of abused drugs (Supplemental Figure 3), thus excluding the possibility that drug substitution could explain the effects of mifepristone on alcohol craving and drinking, and mitigating against its abuse potential.

The motivation to drink alcohol is initially driven by its pleasurable/euphorogenic effects via positive reinforcement, and glucocorticoids can facilitate these effects (22). However, during dependence, both the HPA axis and central brain reward systems are dysregulated, and extrahypothalamic stress systems become sensitized, possibly mediated by the GR signaling changes reported herein that putatively drive drinking via negative reinforcement (i.e., stress relief). The molecular mechanisms responsible for mifepristone's ability to reduce compulsive alcohol drinking may involve altered gene expression of stress-related neuropeptide systems (e.g., corticotropin-releasing factor [CRF] and/or vasopressin) (23, 24). For example, acutely, high blood glucocorticoid levels are paralleled by enhanced GR-mediated CRF release in the CeA (25). Chronically, high glucocorticoid levels increase CRF expression in the CeA, whereas glucocorticoids inhibit CRF and vasopressin expression in the paraventricular nucleus of the hypothalamus (PVN) (26).

The rationale for the translational strategy between animal and human experiments in this study is focused on the dual role of glucocorticoids in suppressing HPA axis responses at the same time as sensitizing CeA responses, both of which are adaptive when an organism is chronically stressed. One could speculate that, under chronic stress, from a survival perspective, the blunted HPA response will prevent Cushing's disease-like symptoms, but the sensitized amygdala retains alertness for danger. A similar chronic stress-like profile also characterizes alcoholism. While alcohol can acutely activate the HPA axis via elevated hypothalamic production of CRF, more importantly, glucocorticoids are chronically released during the transition to alcohol dependence and then feedback to shut off the HPA axis and sensitize CRF in

the amygdala. We believe that this CRF overactivity in the amygdala drives negative reinforcement to cause excessive drinking during dependence, as we know that CRF antagonists injected into the amygdala can block dependence-induced drinking and dysregulated cellular plasticity within the amygdala (23, 27). Thus, although activation of the HPA axis may characterize initial drug use within the binge/intoxication stage of addiction, such engagement can also lead to subsequent activation of extrahypothalamic brain stress systems that characterize the withdrawal/negative-affect stage and protracted abstinence in the preoccupation/anticipation stage (2). Our hypothesis is that mifepristone can exhibit its potential therapeutic effects by restabilizing negative feedback along the HPA axis and blocking the sensitization of extrahypothalamic CRF in the amygdala (28). Future investigations at the preclinical and clinical levels should determine the precise stress and reward circuitry mechanisms of GR activity, including the role of epigenetic, genomic, and nongenomic GR signaling (29).

The present studies indicate that attenuation of GR function by mifepristone reduces compulsive-like alcohol intake in alcohol-dependent rats and reduces both excessive drinking and alcohol craving in recently abstinent alcoholics — in addition to improving liver-function markers in subjects with a history of heavy drinking — without any major adverse effects. We suggest that brief (1-week) treatment with mifepristone immediately following acute withdrawal, in conjunction with a course of psychosocial treatment, may offer a novel therapeutic approach for alcohol dependence that optimizes healthcare resources. An adequately powered clinical trial of mifepristone to replicate and extend these findings to treatment seekers with alcohol dependence is indicated.

Methods

Further information can be found in Supplemental Methods, Supplemental Figures 1–4, and Supplemental Tables 1–4.

Statistics. Preclinical data were analyzed using 2-tailed Student's *t* test or using repeated-measures ANOVA followed by Duncan's multiple comparison test. The clinical data were analyzed using 2-tailed Student's *t* test, χ^2 test, repeated-measures ANOVA, Pearson's product moment correlation, linear regression, multivariate analysis of covariance, and linear mixed effects modeling. The accepted level of significance for all of the tests was $P < 0.05$.

Study approval. The preclinical procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Our single-site outpatient study was conducted in accordance with the Declaration of Helsinki. The Scripps Institutional Review Board approved the protocol, which was conducted under an investigator-initiated investigational new drug (IND) granted by the federal Food and Drug Administration. A Certificate of Confidentiality was obtained from the National Institute on Alcohol Abuse and Alcoholism to protect the confidentiality of participants. Written informed consent was obtained from all participants.

Trial registration: NCT01548417 (clinicaltrials.gov).

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