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Publication Date

2021-05-01

DOI

10.1016/j.neuropharm.2021.108510

Peer reviewed

Glucocorticoid receptor modulators decrease alcohol self-administration in male rats

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Abstract

Alcohol use disorder (AUD) is associated with the dysregulation of brain stress and reward systems, including glucocorticoid receptors (GRs). The mixed glucocorticoid/progesterone receptor antagonist mifepristone and selective GR antagonist CORT113176 have been shown to selectively reduce alcohol consumption in alcohol-dependent rats. Mifepristone has also been shown to decrease alcohol consumption and craving for alcohol in humans with AUD. The present study tested the effects of the GR modulators CORT118335, CORT122928, CORT108297, and CORT125134 on alcohol self-administration in nondependent (air-exposed) and alcohol-dependent (alcohol vapor-exposed) adult male rats. Different GR modulators recruit different GR-associated transcriptional cofactors. Thus, we hypothesized that these GR modulators would vary in their effects on alcohol drinking. CORT118335, CORT122928, and CORT125134 significantly reduced alcohol self-administration in both alcohol-dependent and nondependent rats. CORT108297 had no effect on alcohol self-administration in either group. The present results support the potential of GR modulators for the development of treatments for AUD. Future studies that characterize genomic and nongenomic effects of these GR modulators will elucidate potential molecular mechanisms that underlie alcohol drinking in alcohol-dependent and nondependent states.

Keywords

alcohol dependence; alcohol drinking; glucocorticoid; glucocorticoid receptor; addiction

1. Introduction

Alcohol use disorder (AUD) is characterized by heavy alcohol consumption despite negative consequences and the emergence of a negative emotional state when alcohol is unavailable. Alcohol exposure and withdrawal from alcohol both activate the hypothalamic-pituitary-adrenal (HPA) axis, causing the release of corticosteroids. Repeated HPA axis activation is hypothesized to drive cumulative neuroadaptations in brain reward and stress systems that both facilitate the transition to and maintenance of alcohol dependence (Vendruscolo et al. 2012, 2015; Edwards et al. 2015; Somkuwar et al. 2017).

The glucocorticoid receptor (GR) is a steroid hormone-activated transcription factor that is ubiquitously expressed throughout the brain and peripheral tissues (Cintra et al. 1994). Its endogenous ligand is the steroid hormone cortisol in humans and corticosterone in rodents. The GR/progesterone antagonist mifepristone has demonstrated efficacy in reducing alcohol consumption and craving for alcohol in humans with alcohol use disorder (Vendruscolo et al. 2015). Preclinical studies have found that mifepristone reduces alcohol consumption. Chronic, systemic administration of mifepristone prevented development of alcohol dependence-induced escalation of alcohol drinking in male rats (Vendruscolo et al. 2012; Somkuwar et al. 2017) and reduced escalated alcohol drinking in male rats with a history of alcohol dependence during protracted abstinence (Vendruscolo et al. 2012). Acute, systemic treatment with mifepristone and

the selective GR antagonist CORT113176 reduced escalated alcohol drinking in alcohol-dependent male rats during acute withdrawal (Vendruscolo et al. 2015) and mifepristone reduced heavy alcohol drinking in rhesus macaques (Jimenez et al. 2020). In experiments using nondependent animals, mifepristone reduced alcohol consumption in female but not male rats (Logrip and Gainey, 2020), reduced stress-induced reinstatement of alcohol-seeking behavior (Simms et al. 2012), reduced binge-like alcohol drinking in high-drinking male and female mice (Savarese et al. 2020), and prevented an increase in preference for alcohol in low-drinking male and female mice (O'Callaghan et al. 2005). Neither mifepristone nor CORT113176 affected the intake of water or non-alcoholic sweet solutions in rats or mice (Vendruscolo et al., 2015; Savarese et al., 2020), and they did not affect alcohol drinking in nondependent, unstressed male rodents (Fahlke et al. 1995, 1996; Yang et al. 2008; Lowery et al. 2010; Vendruscolo et al. 2012, 2015; Simms et al. 2012; Repunte-Canonigo et al. 2015) or baboons (Holtyn et al. 2019), and mifepristone did not block an alcohol-induced relapse-like behavior in rhesus macaques in early abstinence (Jimenez et al., 2020). These findings suggest preferential effects of mifepristone and CORT113176 in reducing excessive alcohol drinking under multiple conditions, including binge-like drinking, heavy drinking, dependence, and stress. In addition to its effects on alcohol consumption, mifepristone treatment reduced the severity of somatic signs of alcohol withdrawal (Sharrett-Field et al. 2013), reduced hippocampal neurotoxicity following binge-like alcohol exposure in rats (Cippitelli et al.,

2014), and prevented the expression of memory deficits in alcohol-dependent mice during 1-2 weeks of alcohol abstinence (Jacquot et al. 2008).

Several functional characteristics of GRs are known to be altered in alcohol dependence. In humans who were diagnosed with AUD, *NR3C1* methylation was altered in the prefrontal cortex (PFC), resulting in lower GR mRNA and protein levels (Gatta et al. 2019). Gene expression and transcriptional network analyses in brains of alcohol-dependent rats during acute withdrawal (8-24 h) and protracted abstinence (~4 weeks) identified the GR as one of the top transcriptional regulators that contribute to alterations of gene expression profiles in key reward- and stress-related brain regions (Repunte-Canonigo et al. 2015; Vendruscolo et al. 2012). The phosphorylation of GR at serine 232, a site that is associated with higher transcriptional activity, was increased in the central nucleus of the amygdala (CeA) of alcohol-dependent rats during acute withdrawal (Vendruscolo et al. 2015). Glucocorticoid receptor phosphorylation and protein expression in the rat medial PFC was altered during acute withdrawal and protracted abstinence (Somkuwar et al. 2017).

Although side effects of mifepristone that occur through progesterone receptor antagonism are uncommon (Chen et al. 2014), compounds that preferentially and selectively target GRs may have greater efficacy and potency, making them more suitable for chronic administration. Several layers of regulation determine the activity level and transcriptional outcome of GRs. These include the ligand that binds to GRs and composition of

chaperones and cofactor complexes in the cytoplasm and nucleus (Atucha et al. 2015, Desmet et al. 2017). The expression of cofactors differs greatly across GR-expressing cells throughout the brain and periphery. Therefore, the recruitment of various cofactor complexes is a major contributing factor to the diversity of GR-mediated gene expression profiles that can be observed between different tissues and cell types (Meijer et al. 2019). The present study evaluated the effects of four selective GR modulators on alcohol self-administration in alcohol-dependent and nondependent male rats. We hypothesized that these compounds may have differential GR-mediated activity and effects on alcohol drinking in alcohol-dependent and nondependent rats.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (Charles River, Raleigh, NC, USA), at least 8 weeks of age at the beginning of the experiments, were group housed 2-3 per cage in a temperature-controlled (21℃ ± 2℃) vivarium on a 12 h/12 h light/dark cycle (lights on at 8:00 AM), with *ad libitum* access to food and water except during behavioral testing. Behavioral tests were conducted during the dark cycle. Only male rats were used because these experiments were conducted before the National Institutes of Health requirement to include sex as a biological variable. Future studies will test the effects of GR antagonism on alcohol drinking in female rats. All of the animal procedures

adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program and The Scripps Research Institute.

2.2. Operant alcohol self-administration in rats

Self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT, USA). In each experiment, the rats were trained to self-administer 10% (w/v) alcohol and water under a fixed-ratio 1 (FR1) schedule of reinforcement. Each operant response on the alcohol lever or water lever was reinforced with 0.1 ml of solution as previously described (Priddy et al., 2017). The rats that acquired operant alcohol self-administration (i.e., at least 10 lever presses for alcohol in each of the last three 30-min training sessions) were split into two groups that were matched by the average number of lever presses for alcohol in the last three training sessions: alcohol vapor-exposed group (alcohol-dependent) and air-exposed group (nondependent).

2.3. Alcohol vapor exposure

The rats were made alcohol-dependent by chronic, intermittent alcohol vapor exposure as previously described (Vendruscolo et al. 2012, 2015). The rats were exposed to daily cycles of 14 h of alcohol vapor, followed by 10 h of room air, for a minimum of 4 weeks. Blood alcohol levels that were

reached ranged between 150 and 250 mg/dl. Behavioral testing occurred in 2-3 sessions per week, 6-8 h after the alcohol vapor exposure period, a timepoint at which brain and blood alcohol levels are negligible (Gilpin et al. 2009). Nondependent rats were not exposed to alcohol vapor but underwent behavioral testing at the same time as the alcohol-dependent group. The vapor model of alcohol dependence has been shown to produce both somatic and affective symptoms of alcohol dependence, including escalated and compulsive-like alcohol consumption, anxiety-like behavior, and hyperalgesia (Vendruscolo and Roberts, 2014; Edwards et al. 2012).

2.4. Drug treatment

CORT118335, CORT122928, CORT108297, and CORT125134 were provided by Corcept Therapeutics (Menlo Park, CA, USA). The chemical structure of CORT118335 is identified in Hunt et al. 2012. The chemical structure of CORT125134 is identified in Hunt et al. 2017. The chemical structures of the compounds CORT108297, CORT113176, and CORT122928 (compound 13) are identified in Hunt et al. 2015. Separate cohorts of alcohol-dependent and nondependent rats were intraperitoneally injected with CORT118335 (0, 1, 3, and 10 mg/kg), CORT122928 (0, 10, 30, and 60 mg/kg), CORT108297 (0, 5, 10, 15, 30, and 60 mg/kg), or CORT125134 (0, 30, 60, and 100 mg/kg) 90 min before the operant self-administration sessions. The doses of each compound were based on pharmacokinetic and pharmacodynamic data from Corcept Therapeutics. Doses of each compound

were administered in a within-subjects Latin-square design. All of the compounds were prepared with 10% dimethylsulfoxide, 10% Kolliphor EL (Sigma-Aldrich, St. Louis, MO, USA), and 80% saline. The injection volume was 3 ml/kg. Separate cohorts of dependent and nondependent rats were used to test each of the compounds. Sample sizes for each experiment were as follows: CORT118335: nondependent n = 18, alcohol-dependent n = 10; CORT122928: nondependent n = 10, alcohol-dependent n = 10; CORT108297: nondependent n = 8, alcohol-dependent n = 8; CORT125134: nondependent n = 9, alcohol-dependent n = 11. Note that there are differences in group sizes among the compounds that we tested. This was due to difference in cohort sizes, the number of rats that acquired operant self-administration, the number of rats that could have been allocated for each particular experiment, and the amount of drug to be tested.

2.5. Statistical analysis

All statistical analyses were conducted with GraphPad Prism 8 software. Operant alcohol and water self-administration data were analyzed using repeated-measures analysis of variance (ANOVA), with drug treatment as the within-subjects factor and group (alcohol-dependent *vs.* nondependent) as the between-subjects factor. *Post hoc* comparisons were performed using the Holm-Sidak multiple-comparison test (Molutsky 2020). The accepted level of significance for all of the tests was $p < 0.05$. All data are expressed as the mean and SEM.

3. Results

3.1. Effect of CORT118335 on alcohol self-administration

The two-way repeated-measures ANOVA indicated that the alcohol-dependent group self-administered significantly more alcohol than the nondependent group, thus validating our experimental model (main effect of group: $F_{1,26}$ = 17.33, p = 0.0003). CORT118335 significantly reduced alcohol self-administration in both alcohol-dependent and nondependent rats (main effect of dose: $F_{3,78}$ = 12.23, p = 0.0001). The Holm-Sidak *post hoc* test indicated that CORT118335 significantly reduced alcohol self-administration in alcohol-dependent and nondependent rats at doses of 1 mg/kg (p = 0.0477), 3 mg/kg (p = 0.0003), and 10 mg/kg (p < 0.0001; Fig. 1A). CORT118335 treatment exerted a nonsignificant trend toward a reduction of water self-administration in both groups ($F_{3,78}$ = 2.709, p = 0.0508; Table 1).

3.2. Effect of CORT122928 on alcohol self-administration

Alcohol-dependent rats self-administered significantly more alcohol than nondependent rats (main effect of group: $F_{1,18}$ = 19.05, p = 0.0004). The two-way repeated-measures ANOVA indicated that CORT122928 significantly reduced alcohol self-administration in alcohol-dependent and nondependent rats (main effect of dose: $F_{3,54}$ = 8.860, p < 0.0001). The Holm-Sidak *post hoc* test indicated that CORT122928 reduced alcohol self-administration in alcohol-dependent and nondependent rats at doses of 30

mg/kg (p = 0.0001) and 60 mg/kg (p = 0.0003; Fig. 1B). The two-way repeated-measures ANOVA indicated a significant group × dose interaction for water self-administration ($F_{3,54}$ = 3.060, p = 0.0358). The Holm-Sidak *post hoc* test indicated that CORT122928 significantly reduced water self-administration in nondependent rats at the 60 mg/kg dose (p = 0.0251; Table 1).

3.3. Effect of CORT108297 on alcohol self-administration

Alcohol-dependent rats self-administered significantly more alcohol than nondependent rats (main effect of group: $F_{1,14}$ = 22.74, p = 0.0003). The two-way repeated-measures ANOVA indicated that CORT108297 did not significantly affect alcohol self-administration in alcohol-dependent or nondependent rats (main effect of dose: $F_{4,56}$ = 0.9774, p = 0.4273; Fig. 1C), with no effect on water self-administration (main effect of dose: $F_{4,56}$ = 0.3994, p = 0.8082; Table 1).

3.4. Effect of CORT125134 on alcohol self-administration

Alcohol-dependent rats self-administered significantly more alcohol than nondependent rats (main effect of group: $F_{1,18}$ = 21.45, p = 0.0002). CORT125134 significantly reduced alcohol self-administration in alcohol-dependent and nondependent rats (main effect of dose: $F_{3,54}$ = 5.154, p = 0.0033). The Holm-Sidak *post hoc* test indicated that CORT125134 significantly reduced alcohol self-administration in alcohol-dependent and

nondependent rats at the 100 mg/kg dose (p = 0.0012; Fig. 1D), with no effect on water self-administration (main effect of dose: $F_{3,54}$ = 0.4625, p = 0.7096; Table 1).

4. Discussion

Consistent with an extensive literature (reviewed in Vendruscolo and Roberts, 2014), alcohol vapor-exposed (dependent) male rats self-administered significantly more alcohol compared with air-exposed (nondependent) male rats in all experimental cohorts, validating our experimental conditions. We found that the acute administration of CORT118335, CORT122928, and CORT125134 significantly reduced alcohol drinking in both nondependent and alcohol-dependent rats, whereas CORT108297 had no effect. These compounds generally did not significantly disrupt water intake in either alcohol-dependent or nondependent rats (Table 1). Using the same model of alcohol dependence, we previously found that mifepristone and the selective GR antagonist CORT113176 preferentially decreased alcohol self-administration in alcohol-dependent male rats, without affecting the self-administration of water or a non-alcoholic saccharin solution in operant session (Vendruscolo et al. 2015). The mechanisms of action of CORT113176 are largely unknown. *In vitro*, partial antagonism of GRs by CORT113176 was reported (Hunt et al. 2015). *In vivo*, CORT113176 antagonized some, but not all, peripheral effects of cortisone on blood glucose levels (Hunt et al. 2015).

To date, little information is available about the genomic and non-genomic mechanisms of action of the compounds that were tested herein. Perhaps the most characterized of the GR modulators is CORT118335. In the present study, CORT118335 significantly reduced alcohol self-administration, without affecting water self-administration or the consumption of a non-alcoholic saccharin solution (Table 3), indicating that this compound did not suppress consummatory behaviors in general. This compound has no significant affinity for progesterone, estrogen, or androgen receptors, but it has antagonist activity at the mineralocorticoid receptor, with eight-fold lower affinity for mineralocorticoid receptors than GRs (Hunt et al. 2012; Atucha et al. 2015; Table 2). CORT118335 has a co-factor interaction profile that is considered intermediate to the agonist dexamethasone and antagonist mifepristone (Atucha et al. 2015). Similar to mifepristone, CORT118335 significantly interacts with transcription factor 65, a subunit of nuclear factor κB (a proinflammatory transcription factor). CORT118335 and mifepristone both interact with motifs of the transcriptional activators steroid receptor co-activators 1 and 2 (SRC-1 and SRC-2). In contrast to mifepristone, both CORT118335 and dexamethasone do not interact with motifs of the nuclear receptor co-repressors 1 and 2 (NCOR-1 and NCOR-2). These *in vitro* data suggest that CORT118335 exerts GR agonist-like activity (Atucha et al. 2015; Viho et al. 2019). However, mixed GR agonist and antagonist-like activity has been observed *in vivo* (Koorneef et al. 2018). CORT118335 treatment reduced plasma corticosterone levels in rats,

indicating negative feedback on the HPA axis, and therefore agonist-like action of CORT118335 at GRs in the hypothalamus (Atucha et al. 2015; Nguyen et al. 2017). Further, CORT118335 has been shown to exert antagonist activity at GRs in the hippocampus, where it inhibited the GR-induced upregulation of FK506-binding protein 5 and serum- and glucocorticoid-regulated kinase 1 mRNA and inhibited GR-mediated memory consolidation (Atucha et al. 2015). CORT118335 antagonizes the mineralocorticoid receptor, which may play a role in alcohol drinking in dependent rats (Aoun et al. 2017), but with a low affinity that is likely insufficient to produce the behavioral effects that were observed in the present study. The more general effect of CORT118335 on alcohol self-administration compared to the differential effects of mifepristone in alcohol-dependent rats *vs.* nondependent rats may potentially be explained by several differences between these compounds at the molecular level. Mifepristone exhibited strong antagonist activity at the GR in the presence of the splice variant SRC-1e and weaker antagonist activity in the presence of SRC-1a in reporter assays (Meijer et al. 2005), whereas the effect of CORT118335 on the functional interactions between GR and SRCs are unknown. In addition, mifepristone attenuated the expression of Fos, a marker of neuronal activation, in the CeA in stressed rats (Wulsin et al. 2010), whereas CORT118335 did not, suggesting an inability of CORT118335 to repress GR hyperactivity in the CeA (Nguyen et al. 2017).

In the present study, CORT122928 significantly reduced alcohol self-administration in alcohol-dependent and nondependent rats. CORT122928 also reduced water and saccharin self-administration in nondependent rats (Table 3), suggesting that its effects were not alcohol-specific. CORT122928 has no significant affinity for progesterone, mineralocorticoid, androgen, or estrogen receptors (Table 2). CORT122928 exerted antagonist-like activity at the GR *in vitro* by inhibiting GR-dependent prostate cancer cell viability, similar to mifepristone (Isikbay et al. 2014). Glucocorticoid activity may facilitate alcohol drinking behavior in nondependent rats (Fahlke et al. 1995, 1996; Sanna et al. 2016). Accordingly, CORT122928 and CORT118335 may induce an anti-glucocorticoid suppression of alcohol intake via GR–co-factor interactions that interfere with reinforcing properties of alcohol.

CORT108297 had no effect on alcohol or water self-administration (Table 3). This compound had no affinity for progesterone, mineralocorticoid, androgen, or estrogen receptors (Clark et al. 2008; Table 2), whereas it had significant *in vitro* and *in vivo* GR agonist, rather than GR antagonist, activity. CORT108297 interacts with co-factors in a somewhat similar manner to the profile of CORT118335, in that there is overlap with both dexamethasone- and mifepristone-induced co-factor interactions (Atucha et al. 2015). Also similar to CORT118335, CORT108297 does not interact with NCOR-1 (Zalachoras et al. 2013). CORT108297 facilitated memory consolidation similarly to corticosteroids, whereas mifepristone exerted an inhibitory effect, thus indicating GR agonist-like activity of CORT108297. It also

exhibited GR agonist activity on the HPA axis, in which it suppressed stress-induced elevations of plasma corticosteroids (Solomon et al. 2014). CORT108297 also exerted a modest agonist-like effect in its repression of corticotropin-releasing factor (CRF) mRNA expression in the paraventricular nucleus of the hypothalamus, but it did not affect CRF mRNA expression in the CeA (Zalachoras et al. 2013). This differential effect in the paraventricular nucleus of the hypothalamus *vs.* CeA may be attributable to the selective interaction between CORT108297 and the GR co-factor SRC-1a (Zalachoras et al. 2013), which is expressed at a relatively higher level in the paraventricular nucleus of the hypothalamus (Meijer et al. 2000) and facilitates the transcriptional repression of CRF (Zalachoras et al. 2016). Dysregulation of the CRF system is a well-characterized mechanism that underlies alcohol-dependent drinking. The systemic or intra-CeA administration of CRF_1 receptor antagonists reduced alcohol dependence-related behaviors (Funk et al. 2006; Edwards et al. 2012). The lack of an effect of CORT108297 on CRF expression in the CeA is consistent with the absence of a reduction of alcohol self-administration that was observed in the present study.

CORT125134 was developed more recently, and its safety has been evaluated in humans (Hunt et al., 2018). It significantly reduced alcohol self-administration in alcohol-dependent and nondependent rats at the highest dose tested and had no effect on water self-administration. CORT125134 has no affinity for mineralocorticoid, progesterone, androgen, or estrogen

receptors (Hunt et al. 2017; Table 2). CORT125134 exerts GR antagonist effects on corticosteroid-induced insulin resistance in rats at a lower dose than the one that was tested in the present study (15 mg/kg *vs.* 30-100 mg/kg). These data indicate that CORT125134 exerts GR antagonist-like activity in rats.

To our knowledge, there are no published studies that directly compared the brain-penetrance of the compounds tested that were herein and mifepristone and CORT113176 that were tested in our previous study (Vendruscolo et al. 2015). However, based on their physicochemical properties (Wager et al., 2016) and unpublished results (Hunt, personal communication), CORT118335, CORT122928, CORT108297, CORT125134, mifepristone and CORT113176, were predicted to be central nervous system penetrant at the dose-range that we used. However, note that efficacy claims for these compounds based on hypothetical brain penetrance remain speculative.

In the present study, we did not examine the temporal pattern of behavioral responses (we did not collect these data) associated with the decrease in alcohol intake such as cumulative response data that could potentially provide us with more information about the putative therapeutic effects of the present series of compounds on alcohol seeking *versus* taking behavior. Using a similar model of alcohol dependence as the present study, Gilpin et al. (2009) reported that both nondependent and alcohol-dependent rats self-administer the majority of their alcohol within the first 10 minutes of

a 30-min operant session and achieve pharmacologically relevant blood alcohol levels. We expect that the effort required to "seek" alcohol is minimal on an FR1 schedule of reinforcement. Nevertheless, rats had extensive training in operant alcohol (and water) self-administration prior to testing the GR compounds. These environmental stimuli that were associated with alcohol drinking (e.g., the operant chamber, the lever) are expected to have acquired motivational properties, and as they are present during testing, are expected to guide and energize the consumption of alcohol. It thus becomes difficult to delineate the effect of a treatment on "seeking" *versus* "taking" aspects of behavior that naturally occur in concert with one another. In support of the hypothesis that mifepristone decreases drug seeking is that it reduced reinstatement of alcohol seeking under extinction conditions (Simms et al., 2012).

With regard to the potential stressful effects of alcohol-exposure via inhalation, alcohol exposure per se is stressful regardless of the route of administration (Vendruscolo and Koob, 2019). In addition, Mouton et al. (2016) reported that chronic alcohol vapor exposure elicits effects in the liver, lungs, and cardiovascular system that are comparable to those produced by other routes of chronic alcohol administration in rodents. Rodents rarely voluntarily drink enough alcohol to consistently achieve blood alcohol levels that produce alcohol dependence. Implementation of the alcohol vapor model has made it possible for researchers to make significant progress towards understanding the neurobiology of alcohol dependence, as

it produces quantitative and qualitative changes in behavior and brain function (for review see, Vendruscolo and Roberts 2014; Tunstall et al. 2017, 2019). The vapor model may have low face validity in terms of the method of dependence-induction, as inhalation is not the most common route of alcohol consumption in humans and alcohol inhalation in humans appears to be aversive. However, the dependent variable measured in our study was the voluntary self-administration of oral alcohol, providing some face validity. Notably, although vapor exposure in the present study was passive and experimenter controlled, de Guglielmo et al. (2017) demonstrated that rats voluntarily self-administer alcohol vapor in a manner that produces alcohol dependence, suggesting that vaporized alcohol is not an inherently aversive route of administration in rats.

5. Conclusions

The present results support our hypothesis that GR modulators exert heterogeneous behavioral effects. Although little is currently known about the genomic mechanisms of action of these GR modulators or their potential non-genomic effects, they are interesting pharmacological tools to further characterize the complex genomic and non-genomic actions that are hypothesized to drive dysfunction of the HPA axis and central stress circuitries (Dalm et al. 2019). The results of the present study and our previous work indicate that mifepristone and CORT113176 are the most effective GR modulators in decreasing alcohol drinking specifically in alcohol-

dependent rats. Nonetheless, given the efficacy of the compounds that were studied herein in decreasing drinking in both dependent and nondependent rats, further research should consider these compounds for the treatment of AUD.

Acknowledgements

This study was supported by the National Institute on Drug Abuse Intramural Research Program. The authors thank Michael Arends for proofreading the manuscript.

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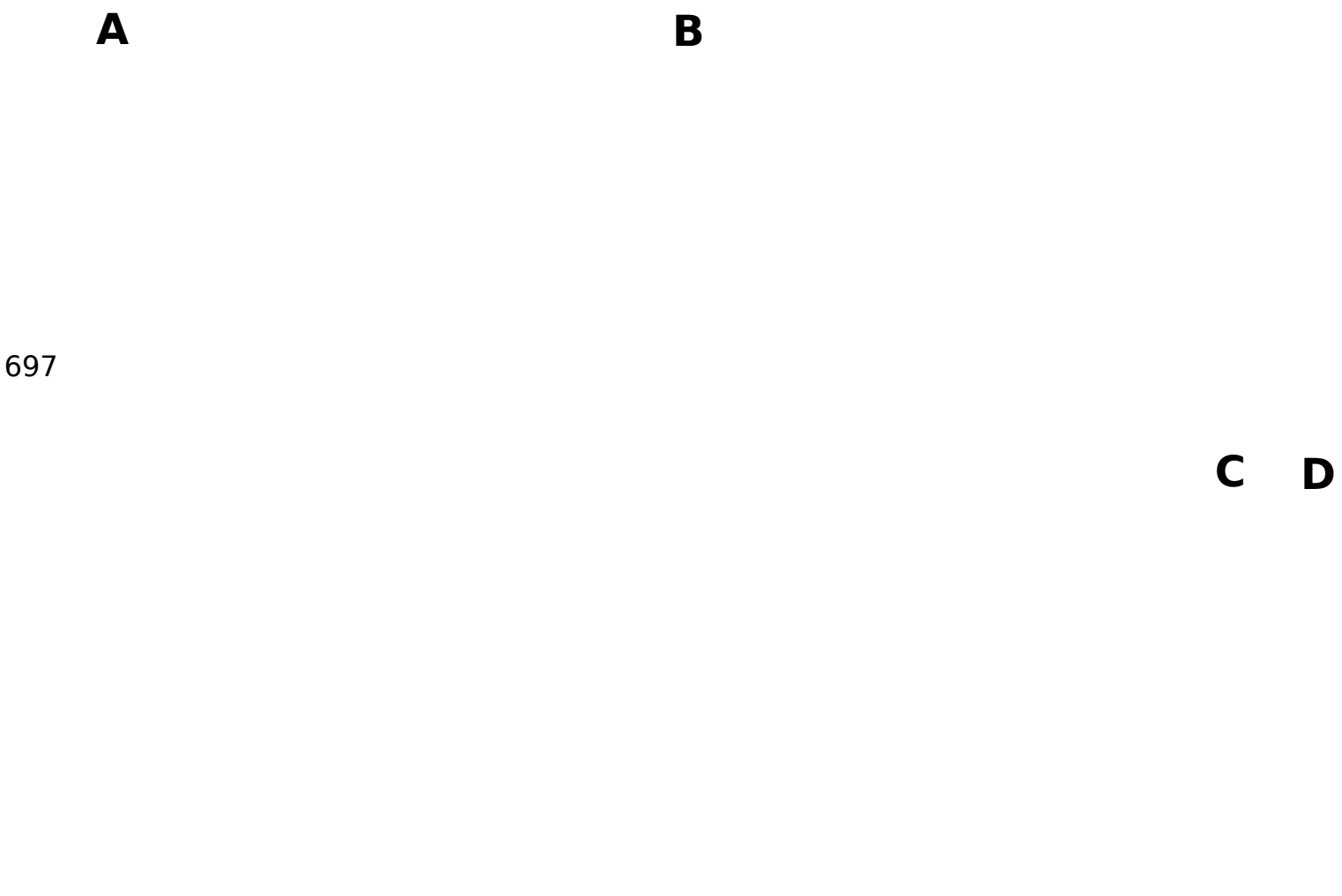

Figure 1. Effects of GR modulators on alcohol self-administration in alcohol-dependent and nondependent male rats. (A) Decrease in alcohol self-administration in nondependent and alcohol-dependent rats 90 min after systemic CORT118335 administration. *p < 0.05, ***p < 0.001, ****p < 0.0001, *vs.* 0 mg/kg, regardless of group (overall dose effect, two-way repeated-measures ANOVA followed by Holm-Sidak *post hoc* test); $^{\#\#\#}p$ < 0.001, nondependent *vs.* alcohol-dependent (overall group effect, two-way repeated-measures ANOVA). NON, nondependent (n = 18); DEP, alcohol-dependent (n = 10). (B) Decrease in alcohol self-administration in

nondependent and alcohol-dependent rats 90 min after systemic CORT122928 administration. ***$p < 0.001$, *vs.* 0 mg/kg, regardless of group (overall dose effect, two-way repeated-measures ANOVA, followed by Holm-Sidak *post hoc* test); $^{###}p < 0.001$, nondependent *vs.* alcohol-dependent (overall group effect, two-way repeated-measures ANOVA). NON, nondependent ($n = 10$); DEP, alcohol-dependent ($n = 10$). (C) Alcohol self-administration did not change in nondependent and alcohol-dependent rats 90 min after systemic CORT108297 administration. $^{###}p < 0.001$, nondependent *vs.* alcohol-dependent (overall group effect, two-way repeated-measures ANOVA). NON, nondependent ($n = 8$); DEP, alcohol-dependent ($n = 8$). (D) Decrease in alcohol self-administration in nondependent and alcohol-dependent rats 90 min after systemic CORT125134 administration. **$p < 0.01$, *vs.* 0 mg/kg, regardless of group (overall dose effect, two-way repeated-measures ANOVA followed by Holm-Sidak *post hoc* test); $^{###}p < 0.001$, nondependent *vs.* alcohol-dependent (overall group effect, two-way repeated-measures ANOVA). NON, nondependent ($n = 9$); DEP, alcohol-dependent ($n = 11$). Separate cohorts of dependent and nondependent rats were used to test each compound.

Table 1. The effects of GR modulators on water self-administration in alcohol-dependent and nondependent male rats.

	Water lever presses	
CORT118335 (mg/kg)	Nondependent	Alcohol-dependent
0	25.1 ± 5.1	36.7 ± 7.7
1	17.9 ± 4.0	27.3 ± 10.3
3	14.4 ± 5.1	29.8 ± 11.1
10	14.4 ± 5.6	21.9 ± 7.1
CORT122928 (mg/kg)		
0	25.6 ± 12.2	13.2 ± 6.7
10	27.1 ± 16.1	8.6 ± 3.6
30	7.1 ± 3.2	17.0 ± 7.2
60	2.7 ± 1.9*	11.9 ± 5.9
CORT108297 (mg/kg)		
0	10.1 ± 3.7	16.3 ± 8.4
5	16.0 ± 6.9	8.6 ± 2.1
15	19.0 ± 11.9	4.8 ± 2.6
30	15.6 ± 7.5	9.5 ± 2.7
60	19.8 ± 9.5	12.4 ± 5.3
CORT125134 (mg/kg)		
0	4.2 ± 2.4	2.8 ± 0.9
30	3.6 ± 2.0	3.4 ± 0.8
60	4.1 ± 2.2	3.5 ± 1.2
100	4.2 ± 1.6	1.6 ± 0.6

Only CORT122928 significantly reduced water self-administration in nondependent rats at the highest dose.
*p = 0.0251, *vs.* 0 mg/kg (two-way repeated-measures ANOVA followed by Holm-Sidak *post hoc* test).

Table 2. Receptor affinity and co-factor interaction characteristics of GR modulators.

Drug	GR binding K_i (nM)	GR antagonism K_i (nM)	MR binding K_i (nM)	PR binding K_i (nM)	ER binding K_i	AR binding K_i	Interaction with NCOR-1	Interaction with SRC-1	Interaction with TF65
CORT118335	1.2	100	8-fold lower than GR	inactive	inactive	inactive	No	Yes	Yes
CORT108297	0.38	34	inactive	inactive	inactive	inactive	No	Yes	?
CORT122928	0.27	18	inactive	inactive	inactive	inactive	?	?	?
CORT125134	0.15	7.2	inactive	inactive	inactive	inactive	?	?	?
Mifepristone	0.09	3	inactive	1	inactive	low	Yes	Yes*	Yes
CORT113176	0.28	12	inactive	inactive	inactive	inactive	?	?	?

GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; ER, estrogen receptor; AR, androgen receptor; NCOR1, nuclear co-repressor 1; SRC-1, steroid receptor co-regulator 1; TF65, transcription factor 65 (a subunit of the proinflammatory transcription factor κB). *Mifepristone interacts with transcriptional co-activator SRC-1 but exhibits strong antagonism of GR in the presence of the SRC-1e isoform and weaker antagonism in the presence of the SRC-1a isoform. Data from Clark et al. (2008), Hunt et al. (2012), Hunt et al. (2015), Hunt et al. (2017), and Corcept Therapeutics (H. Hunt, personal communication).

Table 3. Summary of effects of GR modulators on self-administration behaviors.

Drug	Alcohol self-administration	Water self-administration	Saccharin self-administration
CORT118335	↓ Dependent ↓ Non-dependent	⇔	⇔[#]
CORT108297	⇔	⇔	Not tested
CORT122928	↓ Dependent ↓ Non-dependent	↓ Non-dependent	↓ Non-dependent[#]
CORT125134	↓ Dependent ↓ Non-dependent	⇔	Not tested
Mifepristone	↓ Dependent*	↓ Dependent* ↓ Non-dependent*	⇔*
CORT113176	↓ Dependent* ↓ Non-dependent*	⇔*	⇔*

[#]Data not shown. *Data from Vendruscolo et al. (2015).