UC San Diego

UC San Diego Previously Published Works

Title

Glucocorticoid receptor modulators decrease alcohol self-administration in male rats.

Permalink

<https://escholarship.org/uc/item/28w4t2f1>

Authors

McGinn, M Adrienne
Tunstall, Brendan J
Schlosburg, Joel E
et al.

Publication Date

2021-05-01

DOI

10.1016/j.neuropharm.2021.108510

Peer reviewed

**Glucocorticoid receptor modulators decrease alcohol self-
administration in male rats**

**M. Adrienne McGinn,^{1,*} Brendan J. Tunstall,² Joel E. Schlosburg,³
Adriana Gregory-Flores,⁴ Olivier George,⁵ Giordano de Guglielmo,⁵
Barbara J. Mason,⁶ Hazel J. Hunt,⁷ George F. Koob,¹ and Leandro F.
Vendruscolo¹**

¹ Integrative Neuroscience Research Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA.

² Department of Pharmacology, Addiction Science, and Toxicology, University of Tennessee Health Science Center

³ Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA.

⁴ Institute for Neuroscience, University of Texas at Austin

⁵ Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA, USA.

⁶ Department of Molecular Medicine and Pearson Center for Alcoholism and Addiction Research, The Scripps Research Institute, La Jolla, CA, USA

⁷ Corcept Therapeutics, Menlo Park, CA, USA

*Corresponding author: M. Adrienne McGinn, Ph.D.
NIH/NIDA – IRP/INRB

25 251 Bayview Blvd, BRC Room 08A505
26 Baltimore, MD 21224
27 Phone: +1-443-740-2756
28 E-mail: Adrienne.mcginin@nih.gov

29 **Abstract**

30

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

51

52 **Keywords**

53 alcohol dependence; alcohol drinking; glucocorticoid; glucocorticoid receptor;

54 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

78 the selective GR antagonist CORT113176 reduced escalated alcohol drinking
79 in alcohol-dependent male rats during acute withdrawal (Vendruscolo et al.
80 2015) and mifepristone reduced heavy alcohol drinking in rhesus macaques
81 (Jimenez et al. 2020). In experiments using nondependent animals,
82 mifepristone reduced alcohol consumption in female but not male rats
83 (Logrip and Gainey, 2020), reduced stress-induced reinstatement of alcohol-
84 seeking behavior (Simms et al. 2012), reduced binge-like alcohol drinking in
85 high-drinking male and female mice (Savarese et al. 2020), and prevented
86 an increase in preference for alcohol in low-drinking male and female mice
87 (O'Callaghan et al. 2005). Neither mifepristone nor CORT113176 affected the
88 intake of water or non-alcoholic sweet solutions in rats or mice (Vendruscolo
89 et al., 2015; Savarese et al., 2020), and they did not affect alcohol drinking in
90 nondependent, unstressed male rodents (Fahlke et al. 1995, 1996; Yang et
91 al. 2008; Lowery et al. 2010; Vendruscolo et al. 2012, 2015; Simms et al.
92 2012; Repunte-Canonigo et al. 2015) or baboons (Holtyn et al. 2019), and
93 mifepristone did not block an alcohol-induced relapse-like behavior in rhesus
94 macaques in early abstinence (Jimenez et al., 2020). These findings suggest
95 preferential effects of mifepristone and CORT113176 in reducing excessive
96 alcohol drinking under multiple conditions, including binge-like drinking,
97 heavy drinking, dependence, and stress. In addition to its effects on alcohol
98 consumption, mifepristone treatment reduced the severity of somatic signs
99 of alcohol withdrawal (Sharrett-Field et al. 2013), reduced hippocampal
100 neurotoxicity following binge-like alcohol exposure in rats (Cippitelli et al.,

101 2014), and prevented the expression of memory deficits in alcohol-
102 dependent mice during 1-2 weeks of alcohol abstinence (Jacquot et al. 2008).

103 Several functional characteristics of GRs are known to be altered in
104 alcohol dependence. In humans who were diagnosed with AUD, *NR3C1*
105 methylation was altered in the prefrontal cortex (PFC), resulting in lower GR
106 mRNA and protein levels (Gatta et al. 2019). Gene expression and
107 transcriptional network analyses in brains of alcohol-dependent rats during
108 acute withdrawal (8-24 h) and protracted abstinence (~4 weeks) identified
109 the GR as one of the top transcriptional regulators that contribute to
110 alterations of gene expression profiles in key reward- and stress-related
111 brain regions (Repunte-Canonigo et al. 2015; Vendruscolo et al. 2012). The
112 phosphorylation of GR at serine 232, a site that is associated with higher
113 transcriptional activity, was increased in the central nucleus of the amygdala
114 (CeA) of alcohol-dependent rats during acute withdrawal (Vendruscolo et al.
115 2015). Glucocorticoid receptor phosphorylation and protein expression in the
116 rat medial PFC was altered during acute withdrawal and protracted
117 abstinence (Somkuwar et al. 2017).

118 Although side effects of mifepristone that occur through progesterone
119 receptor antagonism are uncommon (Chen et al. 2014), compounds that
120 preferentially and selectively target GRs may have greater efficacy and
121 potency, making them more suitable for chronic administration. Several
122 layers of regulation determine the activity level and transcriptional outcome
123 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 ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$) 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.

216

217 **3. Results**

218 *3.1. Effect of CORT118335 on alcohol self-administration*

219 The two-way repeated-measures ANOVA indicated that the alcohol-
220 dependent group self-administered significantly more alcohol than the
221 nondependent group, thus validating our experimental model (main effect of
222 group: $F_{1,26} = 17.33$, $p = 0.0003$). CORT118335 significantly reduced alcohol
223 self-administration in both alcohol-dependent and nondependent rats (main
224 effect of dose: $F_{3,78} = 12.23$, $p = 0.0001$). The Holm-Sidak *post hoc* test
225 indicated that CORT118335 significantly reduced alcohol self-administration
226 in alcohol-dependent and nondependent rats at doses of 1 mg/kg ($p =$
227 0.0477), 3 mg/kg ($p = 0.0003$), and 10 mg/kg ($p < 0.0001$; Fig. 1A).
228 CORT118335 treatment exerted a nonsignificant trend toward a reduction of
229 water self-administration in both groups ($F_{3,78} = 2.709$, $p = 0.0508$; Table 1).

230

231 *3.2. Effect of CORT122928 on alcohol self-administration*

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

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

245

246 3.3. Effect of CORT108297 on alcohol self-administration

247 Alcohol-dependent rats self-administered significantly more alcohol
248 than nondependent rats (main effect of group: $F_{1,14} = 22.74$, $p = 0.0003$).
249 The two-way repeated-measures ANOVA indicated that CORT108297 did not
250 significantly affect alcohol self-administration in alcohol-dependent or
251 nondependent rats (main effect of dose: $F_{4,56} = 0.9774$, $p = 0.4273$; Fig. 1C),
252 with no effect on water self-administration (main effect of dose: $F_{4,56} =$
253 0.3994 , $p = 0.8082$; Table 1).

254

255 3.4. Effect of CORT125134 on alcohol self-administration

256 Alcohol-dependent rats self-administered significantly more alcohol
257 than nondependent rats (main effect of group: $F_{1,18} = 21.45$, $p = 0.0002$).
258 CORT125134 significantly reduced alcohol self-administration in alcohol-
259 dependent and nondependent rats (main effect of dose: $F_{3,54} = 5.154$, $p =$
260 0.0033). The Holm-Sidak *post hoc* test indicated that CORT125134
261 significantly reduced alcohol self-administration in alcohol-dependent and

262 nondependent rats at the 100 mg/kg dose ($p = 0.0012$; Fig. 1D), with no
263 effect on water self-administration (main effect of dose: $F_{3,54} = 0.4625$, $p =$
264 0.7096 ; Table 1).

265

266 **4. Discussion**

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

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

308 indicating negative feedback on the HPA axis, and therefore agonist-like
309 action of CORT118335 at GRs in the hypothalamus (Atucha et al. 2015;
310 Nguyen et al. 2017). Further, CORT118335 has been shown to exert
311 antagonist activity at GRs in the hippocampus, where it inhibited the GR-
312 induced upregulation of FK506-binding protein 5 and serum- and
313 glucocorticoid-regulated kinase 1 mRNA and inhibited GR-mediated memory
314 consolidation (Atucha et al. 2015). CORT118335 antagonizes the
315 mineralocorticoid receptor, which may play a role in alcohol drinking in
316 dependent rats (Aoun et al. 2017), but with a low affinity that is likely
317 insufficient to produce the behavioral effects that were observed in the
318 present study. The more general effect of CORT118335 on alcohol self-
319 administration compared to the differential effects of mifepristone in alcohol-
320 dependent rats vs. nondependent rats may potentially be explained by
321 several differences between these compounds at the molecular level.
322 Mifepristone exhibited strong antagonist activity at the GR in the presence of
323 the splice variant SRC-1e and weaker antagonist activity in the presence of
324 SRC-1a in reporter assays (Meijer et al. 2005), whereas the effect of
325 CORT118335 on the functional interactions between GR and SRCs are
326 unknown. In addition, mifepristone attenuated the expression of Fos, a
327 marker of neuronal activation, in the CeA in stressed rats (Wulsin et al.
328 2010), whereas CORT118335 did not, suggesting an inability of CORT118335
329 to repress GR hyperactivity in the CeA (Nguyen et al. 2017).

330 In the present study, CORT122928 significantly reduced alcohol self-
331 administration in alcohol-dependent and nondependent rats. CORT122928
332 also reduced water and saccharin self-administration in nondependent rats
333 (Table 3), suggesting that its effects were not alcohol-specific. CORT122928
334 has no significant affinity for progesterone, mineralocorticoid, androgen, or
335 estrogen receptors (Table 2). CORT122928 exerted antagonist-like activity at
336 the GR *in vitro* by inhibiting GR-dependent prostate cancer cell viability,
337 similar to mifepristone (Isikbay et al. 2014). Glucocorticoid activity may
338 facilitate alcohol drinking behavior in nondependent rats (Fahlke et al. 1995,
339 1996; Sanna et al. 2016). Accordingly, CORT122928 and CORT118335 may
340 induce an anti-glucocorticoid suppression of alcohol intake via GR-co-factor
341 interactions that interfere with reinforcing properties of alcohol.

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

353 exhibited GR agonist activity on the HPA axis, in which it suppressed stress-
354 induced elevations of plasma corticosteroids (Solomon et al. 2014).
355 CORT108297 also exerted a modest agonist-like effect in its repression of
356 corticotropin-releasing factor (CRF) mRNA expression in the paraventricular
357 nucleus of the hypothalamus, but it did not affect CRF mRNA expression in
358 the CeA (Zalachoras et al. 2013). This differential effect in the
359 paraventricular nucleus of the hypothalamus vs. CeA may be attributable to
360 the selective interaction between CORT108297 and the GR co-factor SRC-1a
361 (Zalachoras et al. 2013), which is expressed at a relatively higher level in the
362 paraventricular nucleus of the hypothalamus (Meijer et al. 2000) and
363 facilitates the transcriptional repression of CRF (Zalachoras et al. 2016).
364 Dysregulation of the CRF system is a well-characterized mechanism that
365 underlies alcohol-dependent drinking. The systemic or intra-CeA
366 administration of CRF₁ receptor antagonists reduced alcohol dependence-
367 related behaviors (Funk et al. 2006; Edwards et al. 2012). The lack of an
368 effect of CORT108297 on CRF expression in the CeA is consistent with the
369 absence of a reduction of alcohol self-administration that was observed in
370 the present study.

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

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

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

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

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

412 With regard to the potential stressful effects of alcohol-exposure via
413 inhalation, alcohol exposure per se is stressful regardless of the route of
414 administration (Vendruscolo and Koob, 2019). In addition, Mouton et al.
415 (2016) reported that chronic alcohol vapor exposure elicits effects in the
416 liver, lungs, and cardiovascular system that are comparable to those
417 produced by other routes of chronic alcohol administration in rodents.
418 Rodents rarely voluntarily drink enough alcohol to consistently achieve blood
419 alcohol levels that produce alcohol dependence. Implementation of the
420 alcohol vapor model has made it possible for researchers to make significant
421 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.

References

- Aoun, E. G., Jimenez, V. A., Vendruscolo, L. F., Walter, N. A. R., Barbier, E., Ferrulli, A., Haass-Koffler, C. L., Darakjian, P., Lee, M. R., Addolorato, G., Heilig, M., Hitzemann, R., Koob, G. F., Grant, K. A., Leggio, L., 2018. A relationship between the aldosterone-mineralocorticoid receptor pathway and alcohol drinking: preliminary translational findings across rats, monkeys and humans. *Mol Psychiatry* 23, 1466-1473.
- Atucha, E., Zalachoras, I., van den Heuvel, J. K., van Weert, L. T., Melchers, D., Mol, I. M., Belanoff, J. K., Houtman, R., Hunt, H., Roozendaal, B., Meijer, O. C., 2015. A Mixed Glucocorticoid/Mineralocorticoid Selective Modulator With Dominant Antagonism in the Male Rat Brain. *Endocrinology* 156, 4105-4114.

468 Block, T. S., Kushner, H., Kalin, N., Nelson, C., Belanoff, J., Schatzberg, A.,
 469 2018. Combined Analysis of Mifepristone for Psychotic Depression:
 470 Plasma Levels Associated With Clinical Response. *Biol Psychiatry* 84,
 471 46-54.

472 Chen, J., Wang, J., Shao, J., Gao, Y., Xu, J., Yu, S., Liu, Z., Jia, L., 2014. The
 473 unique pharmacological characteristics of mifepristone (RU486): from
 474 terminating pregnancy to preventing cancer metastasis. *Med Res Rev*
 475 34, 979-1000.

476 Cintra, A., Zoli, M., Rosen, L., Agnati, L. F., Okret, S., Wikstrom, A. C.,
 477 Gustaffsson, J. A., Fuxe, K., 1994. Mapping and computer assisted
 478 morphometry and microdensitometry of glucocorticoid receptor
 479 immunoreactive neurons and glial cells in the rat central nervous
 480 system. *Neuroscience* 62, 843-897.

481 Cippitelli, A., Damadzic, R., Hamelink, C., Brunnquell, M., Thorsell, A., Heilig,
 482 M., Eskay, R.L., 2014. Binge-like ethanol consumption increases
 483 corticosterone levels and neurodegeneration whereas occupancy of type
 484 II glucocorticoid receptors with mifepristone is neuroprotective. *Addict*
 485 Biol 19, 27-36.

486 Clark, R. D., Ray, N. C., Williams, K., Blaney, P., Ward, S., Crackett, P. H.,
 487 Hurley, C., Dyke, H. J., Clark, D. E., Lockey, P., Devos, R., Wong, M.,
 488 Porres, S. S., Bright, C. P., Jenkins, R. E., Belanoff, J., 2008. 1H-
 489 Pyrazolo[3,4-g]hexahydro-isoquinolines as selective glucocorticoid

490 receptor antagonists with high functional activity. *Bioorg Med Chem*
 491 *Lett* 18, 1312-1317.

492 Dalm, S., Karssen, A. M., Meijer, O. C., Belanoff, J. K., de Kloet, E. R., 2019.
 493 Resetting the Stress System with a Mifepristone Challenge. *Cell Mol*
 494 *Neurobiol* 39, 503-522.

495 de Guglielmo, G., Kallupi, M., Cole, M. D., George, O., 2017. Voluntary
 496 induction and maintenance of alcohol dependence in rats using alcohol
 497 vapor self-administration. *Psychopharmacology (Berl)* 234, 2009-2018.

498 Desmet, S. J., Bougarne, N., Van Moortel, L., De Cauwer, L., Thommis, J.,
 499 Vuylsteke, M., Ratman, D., Houtman, R., Tavernier, J., De Bosscher, K.,
 500 2017. Compound A influences gene regulation of the Dexamethasone-
 501 activated glucocorticoid receptor by alternative cofactor recruitment.
 502 *Sci Rep* 7, 8063.

503 Edwards, S., Little, H. J., Richardson, H. N., Vendruscolo, L. F., 2015.
 504 Divergent regulation of distinct glucocorticoid systems in alcohol
 505 dependence. *Alcohol* 49, 811-816.

506 Edwards, S., Vendruscolo, L. F., Schlosburg, J. E., Misra, K. K., Wee, S., Park,
 507 P. E., Schulteis, G., Koob, G. F., 2012. Development of mechanical
 508 hypersensitivity in rats during heroin and ethanol dependence:
 509 alleviation by CRF1) receptor antagonism. *Neuropharmacology* 62,
 510 1142-1151.

511 Fahlke, C., Hard, E., Eriksson, C. J., Engel, J. A., Hansen, S., 1995.
 512 Consequence of long-term exposure to corticosterone or

513 dexamethasone on ethanol consumption in the adrenalectomized rat,
 514 and the effect of type I and type II corticosteroid receptor antagonists.
 515 Psychopharmacology (Berl) 117, 216-224.

516 Fahlke, C., Hard, E., Hansen, S., 1996. Facilitation of ethanol consumption by
 517 intracerebroventricular infusions of corticosterone.
 518 Psychopharmacology (Berl) 127, 133-139.

519 Funk, C. K., O'Dell, L. E., Crawford, E. F., Koob, G. F., 2006. Corticotropin-
 520 releasing factor within the central nucleus of the amygdala mediates
 521 enhanced ethanol self-administration in withdrawn, ethanol-dependent
 522 rats. J Neurosci 26, 11324-11332.

523 Gatta, E., Grayson, D. R., Auta, J., Saudagar, V., Dong, E., Chen, Y., Krishnan,
 524 H. R., Drnevich, J., Pandey, S. C., Guidotti, A., 2019. Genome-wide
 525 methylation in alcohol use disorder subjects: implications for an
 526 epigenetic regulation of the cortico-limbic glucocorticoid receptors
 527 (NR3C1). Mol Psychiatry.

528 Gilpin, N. W., Smith, A. D., Cole, M., Weiss, F., Koob, G. F., Richardson, H. N.,
 529 2009. Operant behavior and alcohol levels in blood and brain of
 530 alcohol-dependent rats. Alcohol Clin Exp Res 33, 2113-2123.

531 Holtyn, A. F., Weerts, E. M., 2019. Evaluation of mifepristone effects on
 532 alcohol-seeking and self-administration in baboons. Exp Clin
 533 Psychopharmacol 27, 227-235.

534 Hunt, H., Donaldson, K., Strem, M., Zann, V., Leung, P., Sweet, S., Connor, A.,
 535 Combs, D., Belanoff, J., 2018. Assessment of Safety, Tolerability,

536 Pharmacokinetics, and Pharmacological Effect of Orally Administered
 537 CORT125134: An Adaptive, Double-Blind, Randomized, Placebo-
 538 Controlled Phase 1 Clinical Study. Clin Pharmacol Drug Dev 7, 408-421.
 539 Hunt, H. J., Belanoff, J. K., Golding, E., Gourdet, B., Phillips, T., Swift, D.,
 540 Thomas, J., Unitt, J. F., Walters, I., 2015. 1H-Pyrazolo[3,4-g]hexahydro-
 541 isoquinolines as potent GR antagonists with reduced hERG inhibition
 542 and an improved pharmacokinetic profile. Bioorg Med Chem Lett 25,
 543 5720-5725.
 544 Hunt, H. J., Belanoff, J. K., Walters, I., Gourdet, B., Thomas, J., Barton, N.,
 545 Unitt, J., Phillips, T., Swift, D., Eaton, E., 2017. Identification of the
 546 Clinical Candidate (R)-(1-(4-Fluorophenyl)-6-((1-methyl-1H-pyrazol-4-
 547 yl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-
 548 yl)(4-(trifluoromethyl)pyridin-2-yl)methanone (CORT125134): A
 549 Selective Glucocorticoid Receptor (GR) Antagonist. J Med Chem 60,
 550 3405-3421.
 551 Hunt, H. J., Ray, N. C., Hynd, G., Sutton, J., Sajad, M., O'Connor, E., Ahmed, S.,
 552 Lockey, P., Daly, S., Buckley, G., Clark, R. D., Roe, R., Blasey, C.,
 553 Belanoff, J., 2012. Discovery of a novel non-steroidal GR antagonist
 554 with in vivo efficacy in the olanzapine-induced weight gain model in
 555 the rat. Bioorg Med Chem Lett 22, 7376-7380.
 556 Isikbay, M., Otto, K., Kregel, S., Kach, J., Cai, Y., Vander Griend, D. J., Conzen,
 557 S. D., Szmulewitz, R. Z., 2014. Glucocorticoid receptor activity

558 contributes to resistance to androgen-targeted therapy in prostate
 559 cancer. *Horm Cancer* 5, 72-89.

560 Koorneef, L. L., van den Heuvel, J. K., Kroon, J., Boon, M. R., t Hoen, P. A. C.,
 561 Hettne, K. M., van de Velde, N. M., Kolenbrander, K. B., Streefland, T. C.
 562 M., Mol, I. M., Sips, H. C. M., Kielbasa, S. M., Mei, H., Belanoff, J. K.,
 563 Pereira, A. M., Oosterveer, M. H., Hunt, H., Rensen, P. C. N., Meijer, O.
 564 C., 2018. Selective Glucocorticoid Receptor Modulation Prevents and
 565 Reverses Nonalcoholic Fatty Liver Disease in Male Mice. *Endocrinology*
 566 159, 3925-3936.

567 Jacquot, C., Croft, A.P., Prendergast, M.A., Mulholland, P., Shaw, S.G., Little,
 568 H.J., 2008. Effects of the glucocorticoid antagonist, mifepristone, on the
 569 consequences of withdrawal from long term alcohol consumption.
 570 *Alcohol Clin Exp Res* 32, 2107-2116.

571 Jimenez, V. A., Walter, N. A. R., Shnitko, T. A., Newman, N., Diem, K.,
 572 Vanderhooft, L., Hunt, H., Grant, K. A., 2020. Mifepristone Decreases
 573 Chronic Voluntary Ethanol Consumption in Rhesus Macaques. *J*
 574 *Pharmacol Exp Ther* 375, 258-267.

575 Logrip, M. L., Gainey, S. C., 2020. Sex differences in the long-term effects of
 576 past stress on alcohol self-administration, glucocorticoid sensitivity and
 577 phosphodiesterase 10A expression. *Neuropharmacology* 164, 107857.

578 Lowery, E. G., Spanos, M., Navarro, M., Lyons, A. M., Hodge, C. W., Thiele, T.
 579 E., 2010. CRF-1 antagonist and CRF-2 agonist decrease binge-like

ethanol drinking in C57BL/6J mice independent of the HPA axis.
 Neuropsychopharmacology 35, 1241-1252.

Meijer, O. C., Buurstedde, J. C., Schaaf, M. J. M., 2019. Corticosteroid Receptors
 in the Brain: Transcriptional Mechanisms for Specificity and Context-
 Dependent Effects. Cell Mol Neurobiol 39, 539-549.

Meijer, O. C., Koorneef, L. L., Kroon, J., 2018. Glucocorticoid receptor
 modulators. Ann Endocrinol (Paris) 79, 107-111.

Meijer, O. C., Kalkhoven, E., van der Laan, S., Steenbergen, P. J., Houtman, S.
 H., Dijkmans, T. F., Pearce, D., de Kloet, E. R., 2005. Steroid receptor
 coactivator-1 splice variants differentially affect corticosteroid receptor
 signaling. Endocrinology 146, 1438-1448.

Meijer, O. C., Steenbergen, P. J., De Kloet, E. R., 2000. Differential expression
 and regional distribution of steroid receptor coactivators SRC-1 and
 SRC-2 in brain and pituitary. Endocrinology 141, 2192-2199.

Motulsky, H. J., 2020. How the Holm-Sidak method works. GraphPad Statistics
 Guide. [http://www.graphpad.com/guides/prism/8/statistics/stat_how_th
 e_holm_method_woks.htm](http://www.graphpad.com/guides/prism/8/statistics/stat_how_the_holm_method_works.htm) (accessed 4 March 2020).

Mouton, A. J., Maxi, J. K., Souza-Smith, F., Bagby, G. J., Gilpin, N. W., Molina,
 P. E., Gardner, J. D., 2016. Alcohol Vapor Inhalation as a Model of
 Alcohol-Induced Organ Disease. Alcohol Clin Exp Res 40, 1671-1678.

Nguyen, E. T., Streicher, J., Berman, S., Caldwell, J. L., Ghisays, V., Estrada, C.
 M., Wulsin, A. C., Solomon, M. B., 2017. A mixed
 glucocorticoid/mineralocorticoid receptor modulator dampens

603 endocrine and hippocampal stress responsivity in male rats. *Physiol*
 604 *Behav* 178, 82-92.

605 O'Callaghan, M. J., Croft, A. P., Jacquot, C., Little, H. J., 2005. The
 606 hypothalamopituitary-adrenal axis and alcohol preference. *Brain Res*
 607 *Bull* 68, 171-178.

608 Peeters, B. W., Tonnaer, J. A., Groen, M. B., Broekkamp, C. L., van der Voort,
 609 H. A., Schoonen, W. G., Smets, R. J., Vanderheyden, P. M., Gebhard, R.,
 610 Ruigt, G. S., 2004. Glucocorticoid receptor antagonists: new tools to
 611 investigate disorders characterized by cortisol hypersecretion. *Stress*
 612 7, 233-241.

613 Priddy, B. M., Carmack, S. A., Thomas, L. C., Vendruscolo, J. C., Koob, G. F.,
 614 Vendruscolo, L. F., 2017. Sex, strain, and estrous cycle influences on
 615 alcohol drinking in rats. *Pharmacol Biochem Behav* 152, 61-67.

616 Repunte-Canonigo, V., Shin, W., Vendruscolo, L. F., Lefebvre, C., van der
 617 Stap, L., Kawamura, T., Schlosburg, J. E., Alvarez, M., Koob, G. F.,
 618 Califano, A., Sanna, P. P., 2015. Identifying candidate drivers of alcohol
 619 dependence-induced excessive drinking by assembly and interrogation
 620 of brain-specific regulatory networks. *Genome Biol* 16, 68.

621 Sanna, P. P., Kawamura, T., Chen, J., Koob, G. F., Roberts, A. J., Vendruscolo,
 622 L. F., Repunte-Canonigo, V., 2016. 11beta-hydroxysteroid
 623 dehydrogenase inhibition as a new potential therapeutic target for
 624 alcohol abuse. *Transl Psychiatry* 6, e760.

625 Savarese, A. M., Ozburn, A. R., Metten, P., Schlumbohm, J. P., Hack, W. R.,
 626 LeMoine, K., Hunt, H., Hausch, F., Bauder, M., Crabbe, J. C., 2020.
 627 Targeting the Glucocorticoid Receptor Reduces Binge-Like Drinking in
 628 High Drinking in the Dark (HDID-1) Mice. *Alcohol Clin Exp Res*.
 629 Sharrett-Field, L., Butler, T.R., Berry, J.N., Reynolds, A.R., Prendergast, M.A.,
 630 2013. Mifepristone pretreatment reduces ethanol withdrawal severity
 631 in vivo. *Alcohol Clin Exp Res* 37, 1417-1423.
 632 Simms, J. A., Haass-Koffler, C. L., Bito-Onon, J., Li, R., Bartlett, S. E., 2012.
 633 Mifepristone in the central nucleus of the amygdala reduces yohimbine
 634 stress-induced reinstatement of ethanol-seeking.
 635 *Neuropsychopharmacology* 37, 906-918.
 636 Solomon, M. B., Wulsin, A. C., Rice, T., Wick, D., Myers, B., McKlveen, J., Flak,
 637 J. N., Ulrich-Lai, Y., Herman, J. P., 2014. The selective glucocorticoid
 638 receptor antagonist CORT 108297 decreases neuroendocrine stress
 639 responses and immobility in the forced swim test. *Horm Behav* 65,
 640 363-371.
 641 Somkuwar, S. S., Vendruscolo, L. F., Fannon, M. J., Schmeichel, B. E., Nguyen,
 642 T. B., Guevara, J., Sidhu, H., Contet, C., Zorrilla, E. P., Mandyam, C. D.,
 643 2017. Abstinence from prolonged ethanol exposure affects plasma
 644 corticosterone, glucocorticoid receptor signaling and stress-related
 645 behaviors. *Psychoneuroendocrinology* 84, 17-31.

646 Tunstall, B. J., Carmack, S. A., Koob, G. F., Vendruscolo, L. F., 2017.
647 Dysregulation of Brain Stress Systems Mediates Compulsive Alcohol
648 Drinking. *Curr Opin Behav Sci* 13, 85-90.

649 Tunstall, B. J., Vendruscolo, L. F., Allen-Worthington, K., 2019. Rat models of
650 alcohol use disorder. *The Laboratory Rat*, 3rd edition, pp. 967-986.

651 Vendruscolo, L. F., Barbier, E., Schlosburg, J. E., Misra, K. K., Whitfield, T. W.,
652 Jr., Logrip, M. L., Rivier, C., Repunte-Canonigo, V., Zorrilla, E. P., Sanna,
653 P. P., Heilig, M., Koob, G. F., 2012. Corticosteroid-dependent plasticity
654 mediates compulsive alcohol drinking in rats. *J Neurosci* 32, 7563-
655 7571.

656 Vendruscolo, L. F., Estey, D., Goodell, V., Macshane, L. G., Logrip, M. L.,
657 Schlosburg, J. E., McGinn, M. A., Zamora-Martinez, E. R., Belanoff, J. K.,
658 Hunt, H. J., Sanna, P. P., George, O., Koob, G. F., Edwards, S., Mason, B.
659 J., 2015. Glucocorticoid receptor antagonism decreases alcohol seeking
660 in alcohol-dependent individuals. *J Clin Invest* 125, 3193-3197.

661 Vendruscolo, L.F., Koob, G.F., 2019. Alcohol dependence conceptualized as a
662 stress disorder. In: Harkness K, Hayden EP (Eds.), *Oxford Handbook of*
663 *Stress and Mental Health*. New York: Oxford University Press, pp. 1-37.

664 Vendruscolo, L. F., Roberts, A. J., 2014. Operant alcohol self-administration in
665 dependent rats: focus on the vapor model. *Alcohol* 48, 277-286.

666 Viho, E. M. G., Buurstede, J. C., Mahfouz, A., Koorneef, L. L., van Weert, L.,
667 Houtman, R., Hunt, H. J., Kroon, J., Meijer, O. C., 2019. Corticosteroid

668 Action in the Brain: The Potential of Selective Receptor Modulation.
669 Neuroendocrinology 109, 266-276.

670 Wager, T. T., Hou, X., Verhoest, P. R., Villalobos, A., 2016. Central Nervous
671 System Multiparameter Optimization Desirability: Application in Drug
672 Discovery. ACS Chem Neurosci 7, 767-775.

673 Wulsin, A. C., Herman, J. P., Solomon, M. B., 2010. Mifepristone decreases
674 depression-like behavior and modulates neuroendocrine and central
675 hypothalamic-pituitary-adrenocortical axis responsiveness to stress.
676 Psychoneuroendocrinology 35, 1100-1112.

677 Yang, X., Wang, S., Rice, K. C., Munro, C. A., Wand, G. S., 2008. Restraint
678 stress and ethanol consumption in two mouse strains. Alcohol Clin Exp
679 Res 32, 840-852.

680 Zalachoras, I., Houtman, R., Atucha, E., Devos, R., Tijssen, A. M., Hu, P.,
681 Lockey, P. M., Datson, N. A., Belanoff, J. K., Lucassen, P. J., Joels, M., de
682 Kloet, E. R., Roozendaal, B., Hunt, H., Meijer, O. C., 2013. Differential
683 targeting of brain stress circuits with a selective glucocorticoid
684 receptor modulator. Proc Natl Acad Sci U S A 110, 7910-7915.

685 Zalachoras, I., Verhoeve, S. L., Toonen, L. J., van Weert, L. T., van Vlodrop, A.
686 M., Mol, I. M., Meelis, W., de Kloet, E. R., Meijer, O. C., 2016. Isoform
687 switching of steroid receptor co-activator-1 attenuates glucocorticoid-
688 induced anxiogenic amygdala CRH expression. Mol Psychiatry 21,
689 1733-1739.

690 Zalachoras, I., Verhoeve, S. L., Toonen, L. J., van Weert, L. T., van Vlodrop, A.
691 M., Mol, I. M., Meelis, W., de Kloet, E. R., Meijer, O. C., 2016. Isoform
692 switching of steroid receptor co-activator-1 attenuates glucocorticoid-
693 induced anxiogenic amygdala CRH expression. Mol Psychiatry 21,
694 1733-1739.
695

A**B****C D**

697

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.

CORT118335 (mg/kg)	Water lever presses	
	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 κ B). *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
CORT11833 5	↓ Dependent ↓ Non-dependent	↔	↔ [#]
CORT10829 7	↔	↔	Not tested
CORT12292 8	↓ Dependent ↓ Non-dependent	↓ Non-dependent	↓ Non-dependent [#]
CORT12513 4	↓ Dependent ↓ Non-dependent	↔	Not tested
Mifepristone	↓ Dependent*	↓ Dependent* ↓ Non-dependent*	↔*
CORT11317 6	↓ Dependent* ↓ Non-dependent*	↔*	↔*

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