

## ORIGINAL ARTICLE

# 11 $\beta$ -hydroxysteroid dehydrogenase inhibition as a new potential therapeutic target for alcohol abuse

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The identification of new and more effective treatments for alcohol abuse remains a priority. Alcohol intake activates glucocorticoids, which have a key role in alcohol's reinforcing properties. Glucocorticoid effects are modulated in part by the activity of 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSD) acting as pre-receptors. Here, we tested the effects on alcohol intake of the 11 $\beta$ -HSD inhibitor carbenoxolone (CBX, 18 $\beta$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate), which has been extensively used in the clinic for the treatment of gastritis and peptic ulcer and is active on both 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 isoforms. We observed that CBX reduces both baseline and excessive drinking in rats and mice. The CBX diastereomer 18 $\alpha$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate ( $\alpha$ CBX), which we found to be selective for 11 $\beta$ -HSD2, was also effective in reducing alcohol drinking in mice. Thus, 11 $\beta$ -HSD inhibitors may be a promising new class of candidate alcohol abuse medications, and existing 11 $\beta$ -HSD inhibitor drugs may be potentially re-purposed for alcohol abuse treatment.

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## INTRODUCTION

Alcohol remains the most prevalent abused substance in the United States, with an estimated 6.8 percent of the population aged 12 or older classified as having alcohol dependence or abuse.<sup>1</sup> Few pharmacotherapies for alcohol abuse are currently available, and these have shown only limited efficacy and compliance.<sup>2–5</sup> Thus, the development of more effective medications for alcohol abuse is a significant unmet medical need.<sup>6</sup>

Alcohol disrupts glucocorticoid regulation in both rodents<sup>7,8</sup> and humans.<sup>9–13</sup> Glucocorticoids have been implicated in alcohol's reinforcing effects,<sup>14</sup> and activation of glucocorticoids by alcohol is involved in the escalation of alcohol intake in dependent rats and alcohol-seeking and drinking during protracted abstinence.<sup>15,16</sup> Both systemic and intracerebral glucocorticoid receptor antagonism with mifepristone blocked compulsive alcohol drinking in rats.<sup>13,15–17</sup> In humans, high adrenal sensitivity (cortisol to corticotropin ratio) in response to stress was found to correlate with greater susceptibility to relapse to heavy drinking,<sup>12</sup> whereas glucocorticoid receptor antagonism with mifepristone significantly reduced alcohol craving and drinking.<sup>13</sup> The effects of glucocorticoids are modulated in target cells by the activity of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) isozymes acting as pre-receptors that contribute to shape the tissue-specific responsiveness to glucocorticoids.<sup>18,19</sup> In particular, 11 $\beta$ -HSD1, which is usually colocalized with the glucocorticoid receptor, converts 11-keto (inert) glucocorticoids such as cortisone in humans and 11 $\beta$ -dehydrocorticosterone in rodents, into 11-hydroxi (active) glucocorticoids such as cortisol in humans and corticosterone in rodents, respectively, to enhance the effects of glucocorticoids.<sup>18,19</sup> The reverse reaction by 11 $\beta$ -HSD2 attenuates local glucocorticoid responses in some mineralocorticoid receptor (MR)-expressing cells, such as classic aldosterone-selective target tissues (distal nephron, colon, sweat gland),

although not in others, such as several MR-expressing brain regions.<sup>20</sup> Given the role for glucocorticoids in mediating the reinforcing effects of alcohol,<sup>14,15</sup> the relevance of 11 $\beta$ -HSD to the modulating effects of glucocorticoids on alcohol drinking is unknown.

Carbenoxolone (CBX, 3 $\beta$ -hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate) is a derivative of glycyrrhetic acid, a molecule present in licorice.<sup>18,19</sup> CBX is a nonselective 11 $\beta$ -HSD inhibitor<sup>21</sup> that has long been used for the treatment of gastritis and peptic ulcer.<sup>22</sup> In addition to its modulatory role on glucocorticoid metabolism in target tissues, CBX also inhibits gap junctional communication, at potencies several orders of magnitude higher.<sup>23</sup>

Here we tested the hypothesis that CBX and its 18 $\alpha$  diastereomer, 18 $\alpha$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate ( $\alpha$ CBX), would reduce alcohol intake in rodents because of their ability to modulate the actions of glucocorticoids. We show that these molecules are capable of reducing alcohol drinking in rodents in both baseline and excessive drinking models, and thus are promising new targets for the treatment of alcohol use disorder. We also show that  $\alpha$ CBX is a selective inhibitor of 11 $\beta$ -HSD2 in the mouse.

## MATERIALS AND METHODS

## Drugs

CBX, 18 $\alpha$ -glycyrrhetic acid and 18 $\beta$ -glycyrrhetic were purchased from Tocris (Bristol, UK);  $\alpha$ CBX was custom synthesized from 18 $\alpha$ -glycyrrhetic acid (Tocris).

## Subjects

Adult male Wistar rats (Charles River, Wilmington, MA, USA), weighing 225–275 g at the beginning of the experiments, were housed in groups of

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two to three per cage. Adult male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) were housed four per cage except during drinking sessions. All the rodents were housed in a temperature-controlled (22 °C) vivarium on a 12 h/12 h light/dark cycle with *ad libitum* access to food and water except during behavioral testing. Operant and limited-access drinking tests were conducted during the dark phase of the light/dark cycle. All the 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.

### Rat operant self-administration

Self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT, USA). The rats were trained to self-administer alcohol as previously reported.<sup>15</sup> First, the rats were given free-choice access to alcohol (10% w/v) and water for 1 day in their home cages to habituate them to the taste of alcohol. Second, the rats were subjected to an overnight session in the operant chambers with access to one lever (right lever) that delivered water in a fixed-ratio 1 schedule where every lever press is reinforced with delivery of 0.1 ml of solution. Food was available *ad libitum* during this training. Third, after 1 day off, the rats were subjected to a 2 h session (fixed-ratio 1) for 1 day and a 1 h session (fixed-ratio 1) the next day, with one lever delivering alcohol (right lever). All the subsequent sessions lasted 30 min, and two levers were available (left lever: water; right lever: alcohol) until stable levels of intake were reached. Upon completion of this procedure, the animals were allowed to self-administer a 10% (w/v) alcohol solution and water on a fixed-ratio 1 schedule of reinforcement.

### Rat alcohol vapor exposure

Rats were made dependent by chronic, intermittent exposure to alcohol vapors as previously described.<sup>15</sup> They underwent cycles of 14 h on (blood alcohol levels during vapor exposure ranged between 150 and 250 mg%) and 10 h off, during which behavioral testing for acute withdrawal occurred (that is, 6–8 h after vapor was turned off when brain and blood alcohol levels are negligible).<sup>24</sup> In this model, rats exhibit motivational and somatic withdrawal signs.<sup>15,17,25,26</sup> Dependent rats were exposed to vapor for at least two months before testing. Non-dependent rats were placed in vapor chambers but were exposed to air for the purpose of control.

A separate cohort of rats was tested for self-administration of saccharin-sweetened water. We used a low saccharin concentration (0.004% w/v) based on previous studies<sup>15</sup> to maintain approximately similar response rates as alcohol. Training for this experiment was identical to, and as described above, for alcohol, except that saccharin solution was used.

To investigate the effect of CBX on alcohol self-administration, we tested rats trained to self-administer alcohol with and without a history of alcohol vapor exposure sufficient to induce dependence and increased alcohol intake. CBX was administered acutely intraperitoneally in saline 90 min before testing at doses of 0, 20 and 40 mg kg<sup>-1</sup>, which are in line with the scientific literature,<sup>27–30</sup> in a within-subject Latin Square design.

### Mouse two-bottle choice and chronic intermittent ethanol exposure

To evaluate the effects of CBX on drinking in nondependent and dependent mice, we used the chronic intermittent ethanol exposure paradigm.<sup>31,32</sup> C57BL/6J mice had access to two bottles, one containing water and the other containing 15% (v/v) ethanol, for 2 h starting 0.5 h before onset of the dark phase. Following acquisition of stable alcohol intake, half of the mice were subjected to repeated bouts of ethanol vapor exposure consisting of 16 h per day for 4 days. Before each exposure to ethanol vapor, mice were intraperitoneally injected with a solution of 1.5 g kg<sup>-1</sup> ethanol and 68.1 mg kg<sup>-1</sup> pyrazole and immediately placed into ethanol vapor chambers (La Jolla Alcohol Research, La Jolla, CA, USA). Tail blood sampling for blood ethanol level determination was carried out every other day. Target blood ethanol levels were 175–250 mg%. Seventy-two hours following removal from the chambers, mice received access to water vs 15% (v/v) ethanol for 2 h, and again over the next 4 days. The following week, the mice were re-exposed to the ethanol vapor/control conditions and again tested for two-bottle choice drinking for 5 days. Three vapor bouts followed by two-bottle choice were carried out. Mice were weighed every 4–6 days throughout the two-bottle choice sessions and daily during the vapor exposure bouts. Food and water were available

*ad libitum* and the mice were group housed except during the ethanol drinking sessions.

### Mouse drinking in the dark

To evaluate the effects of CBX on binge-like drinking, mice were tested in the drinking-in-the-dark (DID) paradigm.<sup>33,34</sup> The DID paradigm capitalizes on the circadian rhythm in drinking of mice utilizing a discrete time of exposure to ethanol to obtain pharmacologically significant ethanol drinking in a 4-day procedure.<sup>33,34</sup> Blood alcohol levels of C57BL/6J mice in DID are reliably over 100 mg dl<sup>-1</sup> (1 mg ml<sup>-1</sup>) following the final drinking bout and produce behavioral intoxication.<sup>34</sup> In the DID procedure, the water bottle is replaced with a bottle containing 20% (v/v) ethanol for 2 h in the home cage 3 h after lights go off. The design involves three daily drinking sessions of 2 h and a fourth of 4 h.<sup>33,34</sup> The effects of CBX and  $\alpha$ CBX were tested in the fourth, 4 h session. Compounds were administered acutely intraperitoneally 90 min before testing at doses of 0, 20 and 40 mg kg<sup>-1</sup>.

### 11 $\beta$ -HSD activity

11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 activities were measured by homogeneous time-resolved fluorescence assays,<sup>35</sup> conducted by SB Drug Discovery (Glasgow, UK) using recombinant human and mouse 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2.

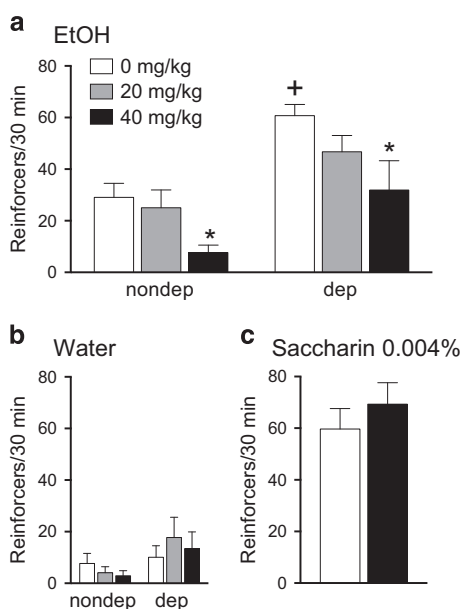
### Statistics

Statistical tests were run and graphs created in GraphPad Prism Version 6 (San Diego, CA, USA). Data are presented as mean  $\pm$  s.e.m. Appropriate statistical analyses were chosen based on experimental design. The specific statistical analysis used is indicated in the text and in each figure caption for all the studies. Bonferroni *post hoc* tests were used for one-way analysis of variance, two-way analysis of variance and repeated-measures analysis of variance. Significance threshold was set to  $P < 0.05$  for all the analyses. Sample sizes, although appropriate for relative studies, were generally too small to test variance; however, in instances where an unpaired two-tailed *t*-test was used, there were no differences in variances. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. Data collection and quantification were performed blinded whenever possible; final analyses were not performed blind to the conditions of the experiments. However, when possible, behavioral analyses and experiments were performed blind to the experimenter.

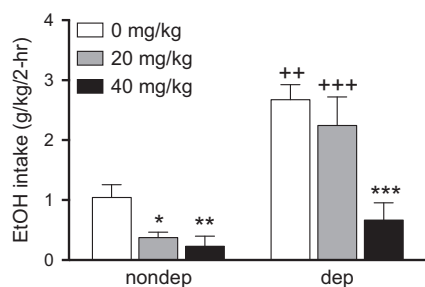
## RESULTS

We first tested the effect of CBX on nondependent and dependent alcohol drinking in rats. An established strategy was used to induce alcohol dependence through chronic exposure to intermittent alcohol vapors.<sup>15,24,26,36–38</sup> Alcohol-dependent rats, as in previous studies, showed increased lever press responding for alcohol compared with nondependent rats (Figure 1a; group effect:  $F_{1,17} = 32.9$ ;  $P < 0.0001$ ). Acute intraperitoneal administration of CBX 90 min before testing dose-dependently reduced responding for alcohol in both dependent and nondependent rats (Figure 1a; dose effect:  $F_{2,34} = 5.0$ ;  $P < 0.05$ ). No significant effects of CBX were found for water responding (Figure 1b; dose effect:  $F_{2,34} = 0.23$ ;  $P = 0.80$ ) or in self-administration of saccharin-sweetened water (Figures 1c;  $t_{(18)} = 0.83$ ;  $P = 0.42$ ). Note that responding levels for saccharin are equivalent to those for alcohol in the dependent group.

We then tested CBX in nondependent mice in a limited-access two-bottle choice paradigm as well as in mice subjected to repeated bouts of ethanol vapors to induce dependence.<sup>31,32</sup> CBX decreased alcohol intake more effectively in nondependent than in dependent mice at a dose of 40 mg kg<sup>-1</sup> (Figure 2). Because 18 $\alpha$ -glycyrrhetic acid has been reported to be a selective inhibitor of human 11 $\beta$ -HSD1,<sup>39</sup> we also synthesized  $\alpha$ CBX, the 18 $\alpha$  diastereomer of CBX (18 $\alpha$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate), to explore its potential isozyme selectivity. We observed that 18 $\alpha$ -glycyrrhetic acid and 18 $\beta$ -glycyrrhetic acid are active both on mouse and human 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 isoforms



**Figure 1.** CBX reduces ethanol intake in rats in an operant self-administration paradigm. (a) Acute, systemic administration of CBX decreases operant alcohol self-administration both in dependent (dep) and nondependent (nondep) rats. (b) CBX did not influence water intake in any group. (c) Acute, systemic administration of CBX does not affect operant self-administration of saccharin-sweetened water. Rats were given CBX (0, 20 and 40 mg kg<sup>-1</sup> or 0 and 40 mg kg<sup>-1</sup>; intraperitoneally) 90 min before alcohol (10%, w/v), water or saccharin (0.004%) self-administration (30 min session; fixed-ratio 1). The data represent means and s.e.m. \* $P < 0.05$ , significant difference from respective vehicle; <sup>+</sup> $P < 0.05$ , significant difference from vehicle (saline)-treated nondependent rats.  $N = 9-10$  per group.



**Figure 2.** CBX reduces ethanol intake in nondependent (nondep) and dependent (dep) mice in a limited-access two-bottle choice paradigm. The mice trained to drink alcohol in a limited-access (2 h) two-bottle choice paradigm were either exposed to alcohol vapor to induce dependence or air for the purpose of control, and tested for the effect of CBX on drinking. CBX reduced ethanol intake in nondependent mice at doses of 20 and 40 mg kg<sup>-1</sup> intraperitoneally (left), and in dependent mice at 40 mg kg<sup>-1</sup>, intraperitoneally (right). The two-way analysis of variance revealed a significant effect of vapor exposure ( $F_{2,35} = 33.38$ ,  $P < 0.0001$ ), dose ( $F_{2,35} = 13.04$ ,  $P < 0.0001$ ) and interaction of vapor exposure and dose ( $F_{2,35} = 3.902$ ,  $P = 0.0295$ ). \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  represent significant difference from the respective vehicle (saline)-treated group; <sup>++</sup> $P < 0.01$  and <sup>+++</sup> $P < 0.001$  represent significant difference from the respective nondependent group.

(Figure 3), whereas  $\alpha$ CBX proved to be a selective inhibitor of mouse 11 $\beta$ -HSD2 with comparable, although slightly lower, potency on mouse HSD2 than CBX (Figure 4).

We then tested the effects of CBX and  $\alpha$ CBX in the 'drinking in the dark' (DID) paradigm of binge-like drinking in mice.<sup>33</sup> We

observed that CBX reduced drinking in the DID paradigm at both 20 and 40 mg kg<sup>-1</sup> (Figures 5a and b).  $\alpha$ CBX showed similar potency as CBX in reducing drinking in the DID paradigm in mice (Figures 5c and d). As 11 $\beta$ -HSD2 in the kidney contributes to blood pressure regulation, we tested the effects of CBX and  $\alpha$ CBX and found that neither compound affected blood pressure in mice (Table 1), consistent with the results of previous studies with CBX in both mice and rats.<sup>40,41</sup>

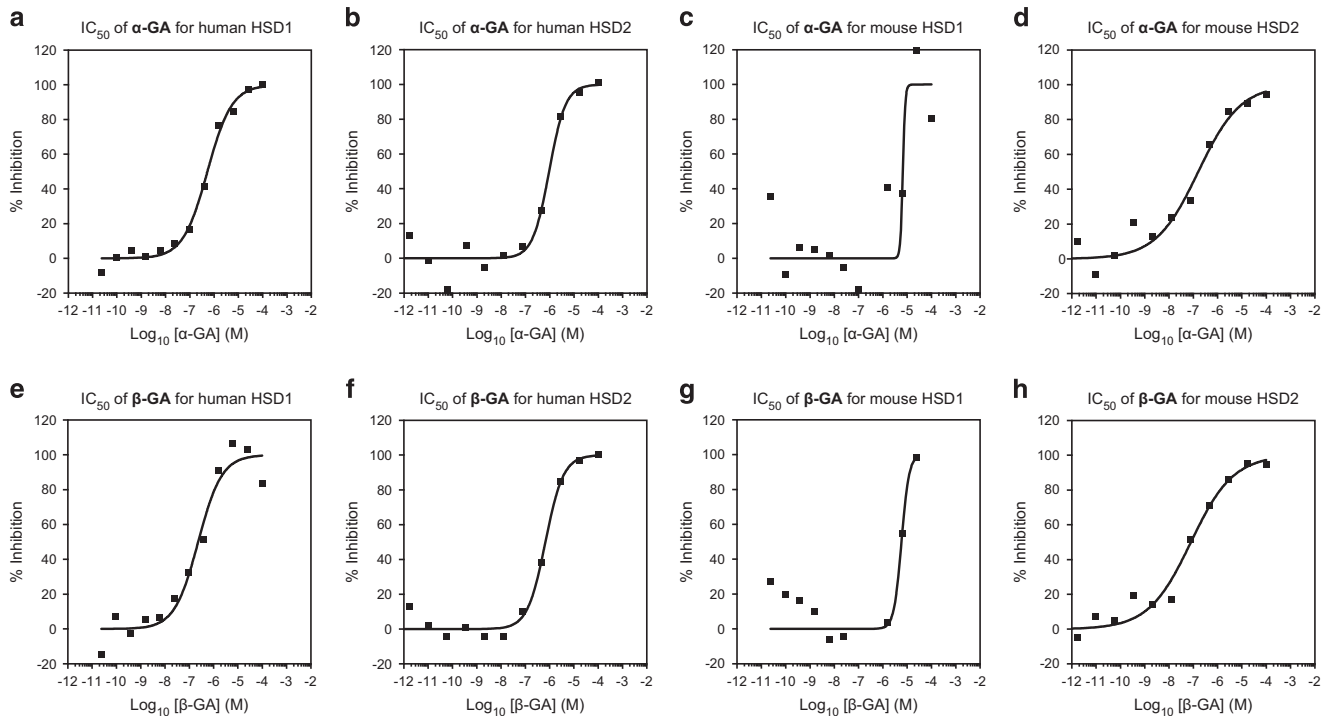
## DISCUSSION

Only a limited number of drugs exist with clinical efficacy for the treatment of alcohol abuse.<sup>6,42</sup> Expansion of therapeutic options is needed to improve treatment success at different stages of disease progression and to bring about individualized therapies based on patient genetic makeup.<sup>6,42</sup>

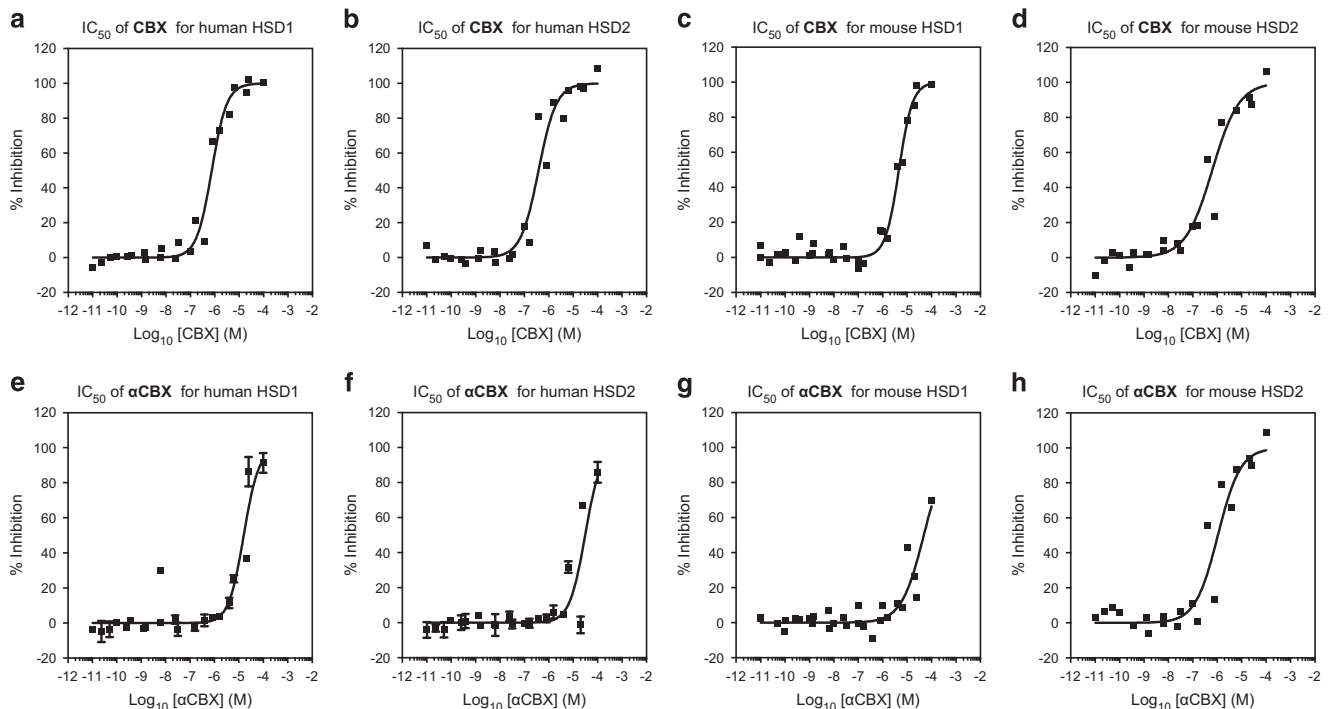
Glucocorticoids facilitate drug seeking, brain stimulation reward, dopamine release and are themselves self-administered.<sup>43,44</sup> The present effects of CBX in reducing alcohol intake could be related to the facilitation of reward mechanisms associated with activation of glucocorticoids.<sup>14,43,45</sup> The present results show that the unselective 11 $\beta$ -HSD inhibitor CBX effectively reduces alcohol intake in both dependent and nondependent rats and mice. This suggests that 11 $\beta$ -HSDs may have a fundamental role in modulating the reinforcing effects of alcohol via their actions in modulating glucocorticoids, and that existing 11 $\beta$ -HSD inhibitor drugs, such as CBX as well as others, for example, see refs 46,47, can potentially be re-purposed for alcohol abuse. Re-purposing of drugs with known safety profiles in humans for diseases other than the ones for which they were originally developed is a potentially fast and effective way to address an unmet medical need.<sup>48</sup>

Glucocorticoid receptors in multiple brain regions have been implicated in the effects of alcohol.<sup>16</sup> 11 $\beta$ -HSD1 is broadly expressed in the adult rat brain, including in brain regions relevant to alcohol reinforcing properties such as the amygdala.<sup>49-51</sup> Thus, the previously shown ability of CBX to inhibit central 11 $\beta$ -HSD1,<sup>52,53</sup> resulting in reduced glucocorticoid signaling, likely contributes to reduced drinking, paralleling the effects of the glucocorticoid receptor inhibitor mifepristone on drinking.<sup>15,16</sup> The comparable effects in the mouse of CBX, which inhibits both mouse 11 $\beta$ -HSD isozymes, and  $\alpha$ CBX, a selective 11 $\beta$ -HSD2 inhibitor in the mouse, point to a potential role also for the latter isozyme in alcohol drinking. In the brain, 11 $\beta$ -HSD2 is expressed primarily in a subpopulation of neurons in the nucleus tractus solitarius.<sup>54-56</sup> This neuronal population, denominated HSD2 neurons, receive inputs from the central nucleus of the amygdala and paraventricular nucleus of the hypothalamus, and project to the ventral BNST, polysynaptically to the nucleus accumbens, and both directly and indirectly to the central nucleus of the amygdala.<sup>54,57,58</sup> Locally, HSD2 neurons are targeted by a group of neurons in the dorsomedial nucleus tractus solitarius that express neurotensin,<sup>59</sup> which has been implicated in the regulation of alcohol intake.<sup>60</sup> Thus, the connectivity of HSD2 neurons in the nucleus tractus solitarius suggests that inhibition of 11 $\beta$ -HSD2 in this neuronal population may result in central effects on alcohol intake. Future studies are needed to explore the relative contributions of 11 $\beta$ -HSD isozymes to alcohol drinking, as well as the contribution of 11 $\beta$ -HSD to the phenotype of genetic models of excessive alcohol drinking, such as alcohol-preferring rodent lines differing in their glucocorticoid regulation.<sup>61,62</sup>

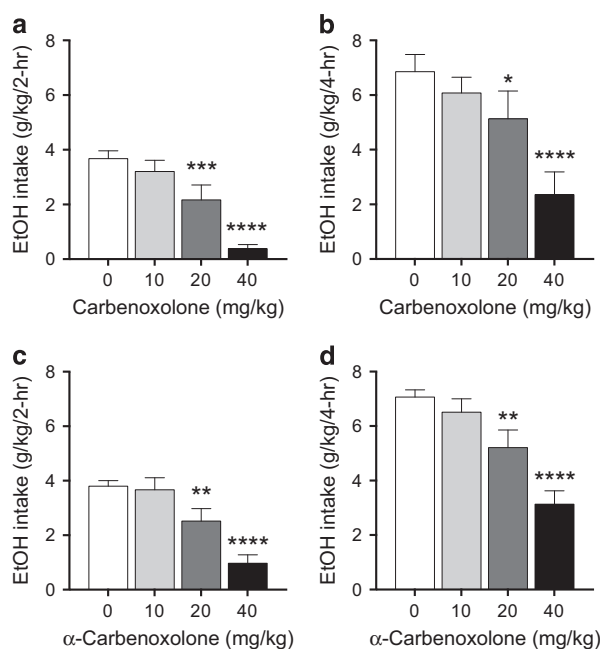
11 $\beta$ -HSD1 inhibitors are being considered as potential cognitive enhancers, as well as for the therapy of type 2 diabetes, metabolic syndrome and obesity.<sup>53,63</sup> 11 $\beta$ -HSD1 knockout mice show resistance to diet-induced obesity, increased glucose tolerance and insulin sensitivity.<sup>63</sup> In addition, studies with 11 $\beta$ -HSD1 null mice and 11 $\beta$ -HSD1-selective inhibitors indicate a role for 11 $\beta$ -HSD1 in the intake of palatable food,<sup>64</sup> but the mechanism behind



**Figure 3.** Activity of 18 $\alpha$ -glycyrrhetic acid and 18 $\beta$ -glycyrrhetic acid on mouse and human 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2. We tested the IC<sub>50</sub> of  $\alpha$ - and  $\beta$ -glycyrrhetic acid (GA) against human and mouse 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 by means of homogeneous time-resolved fluorescence (HTRF) assays. (a and c)  $\alpha$ -GA yielded IC<sub>50</sub> values of 532.1 nM for human 11 $\beta$ -HSD1 and 6.63  $\mu$ M for mouse 11 $\beta$ -HSD1. (b and d)  $\alpha$ -GA yielded IC<sub>50</sub> values of 942.6 nM for human 11 $\beta$ -HSD2 and 159.7 nM for mouse 11 $\beta$ -HSD2. (e and g)  $\beta$ -GA yielded IC<sub>50</sub> values of 232.3 nM for human 11 $\beta$ -HSD1 and 5.85  $\mu$ M for mouse 11 $\beta$ -HSD1. (f and h)  $\beta$ -GA yielded IC<sub>50</sub> values of 674.5 nM for human 11 $\beta$ -HSD2 and 79.7 nM for mouse 11 $\beta$ -HSD2.



**Figure 4.** Activity of carbenoxolone (CBX, 18 $\beta$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate) and  $\alpha$ CBX (18 $\alpha$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate) on mouse and human 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2. We tested the IC<sub>50</sub> of 18 $\alpha$ -18 $\beta$ -glycyrrhetic acid 3 $\beta$ -O-hemisuccinate against human and mouse 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 by means of homogeneous time-resolved fluorescence (HTRF) assays. (a and c) CBX yielded IC<sub>50</sub> values of 753.1 nM for human 11 $\beta$ -HSD1 and 4.62  $\mu$ M for mouse 11 $\beta$ -HSD1. (b and d) CBX yielded IC<sub>50</sub> values of 379.6 nM for human 11 $\beta$ -HSD2 and 628.7 nM for mouse 11 $\beta$ -HSD2. (e and g)  $\alpha$ CBX yielded IC<sub>50</sub> values of 15.92  $\mu$ M for human 11 $\beta$ -HSD1 and 48.25  $\mu$ M for mouse 11 $\beta$ -HSD1. (f and h)  $\alpha$ CBX yielded IC<sub>50</sub> values of 30.67  $\mu$ M for human 11 $\beta$ -HSD2 and 1.06  $\mu$ M for mouse 11 $\beta$ -HSD2. Results for CBX are the average of two to three independent replicates; results for  $\alpha$ CBX are the average of three to four independent replicates.



**Figure 5.** CBX and  $\alpha$ CBX reduce ethanol intake in mice in the drinking-in-the-dark (DID) paradigm. **(a and b)** CBX reduced ethanol intake in the DID binge-like drinking paradigm. **(a)** CBX effect on ethanol intake in the first 2 h of the 4-h drinking session ( $F_{3,30} = 26.00$ ,  $P < 0.0001$ ); **(b)** CBX effect on ethanol intake for the 4-h drinking session ( $F_{3,30} = 10.82$ ,  $P < 0.0001$ ); **(c)**  $\alpha$ CBX effect on ethanol intake in the first 2 h of the 4-h drinking session ( $F_{3,86} = 15.66$ ,  $P < 0.0001$ ); **(d)**  $\alpha$ CBX effect on ethanol intake for the 4-h drinking session ( $F_{3,86} = 18.51$ ,  $P < 0.0001$ ). The effects of CBX and  $\alpha$ CBX were more pronounced in the first 2 h of the session, suggesting an effect on initiation of drinking. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ , significant difference from respective saline vehicle ( $N = 11-15$ ).

**Table 1.** Effects of CBX and  $\alpha$ CBX on blood pressure (mean  $\pm$  s.e.m.)

	BL	SAL	CBX	$\alpha$ CBX
DIA	107.73 $\pm$ 4.14	108.48 $\pm$ 4.66	100.53 $\pm$ 5.66	105.61 $\pm$ 3.10
SYS	139.53 $\pm$ 4.20	140.07 $\pm$ 5.57	133.29 $\pm$ 5.33	138.54 $\pm$ 4.25
MAP	118.00 $\pm$ 4.09	118.68 $\pm$ 4.92	111.09 $\pm$ 5.50	116.27 $\pm$ 3.31

Abbreviations: BL, baseline; CBX, carbenoxolone; DIA, diastolic; SAL, saline; SYS, systolic; MAP, mean arterial pressure.

this effect has been questioned.<sup>65</sup> In the mouse, inhibitors of the CRF1 receptor, which is also responsible for CRF signaling at the pituitary, regulate food and fluid intake as well as alcohol intake in the drinking-in-the-dark model of binge alcohol consumption.<sup>66</sup> Conversely, in the rat, CRF1 antagonists selectively reduce dependent but not nondependent alcohol drinking.<sup>38,67,68</sup> In addition to species differences in terms of neurochemistry underlying motivation for alcohol and sweet solutions in rats and mice, in the present study, the rat studies used operant procedures that involve increased motivation/workload (that is, lever press) for access to the solution, whereas the mouse studies involved voluntary drinking that involves low workload and is more affected by consummatory processes. Therefore, the effect of CBX on alcohol drinking in different models of alcohol consumption, species and animal history of alcohol exposure emphasizes the potential of CBX in decreasing alcohol reinforcement across species and levels of motivation for alcohol.

CBX, which inhibits both 11 $\beta$ -HSD isoforms,<sup>21</sup> has long been used in the clinic for the therapy of gastritis and peptic and duodenal ulcers.<sup>69</sup> However, its use has greatly diminished in favor of other classes of drugs, such as proton pump inhibitors and histamine H2 antagonists. This is in part because of the potential of chronic CBX use to induce pseudohyperaldosteronism,<sup>70,71</sup> which is due to inhibition by CBX of 11 $\beta$ -HSD type 2 isoform (11 $\beta$ -HSD2) in the kidney.<sup>46</sup> By inactivating glucocorticoids in the kidney and other mineralocorticoid target tissues, 11 $\beta$ -HSD2 shields the mineralocorticoid receptor from activation by glucocorticoids.<sup>46</sup> Thus, inhibition of 11 $\beta$ -HSD2 permits glucocorticoids to act on mineralocorticoid receptors.<sup>46</sup> However, this side effect can be managed by combination with an anti-kaliuretic diuretic, such as amiloride, or thiazide diuretics and potassium supplementation.<sup>53,69,72</sup>

The development of new and more effective medications for alcoholism remains a priority.<sup>6</sup> Here we showed that CBX and its diastereomer  $\alpha$ CBX, which is a selective inhibitor of mouse 11 $\beta$ -HSD2, reduce baseline and excessive drinking in rodents. Collectively, the present results suggest that 11 $\beta$ -HSD inhibitors may represent a promising new class of candidate therapeutic targets to treat alcohol use disorders.

### CONFLICT OF INTEREST

PPS is an inventor on a related patent application. The remaining authors declare no conflict of interest.

### ACKNOWLEDGMENTS

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