Severity of drinking as a predictor of efficacy of the combination of ondansetron and topiramate in rat models of ethanol consumption and relapse

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Abstract

Rationale Alcohol dependence is the third leading cause of preventable death in the USA. While single-agent pharmacotherapies have variable efficacy, medication combinations may produce additive effects by modulating multiple neural pathways.

Objectives Here, we examined in animal models of ethanol consumption and relapse the combined effects of ondansetron (a serotonin-3 antagonist) and topiramate (a GABA/glutamate modulator), two medications with demonstrated efficacy for treating alcohol dependence, hypothesizing that their combination would produce a more efficacious response.

Methods The effects of acutely administered ondansetron (0–0.01 mg/kg) and topiramate (0–10 mg/kg) alone and in combination on ethanol consumption were examined in alcohol preferring (P) rats ($N=20$) and in rats from their background strain (Wistars, $N=20$) using a 24-h access free-choice paradigm. Next, we examined their ability to prevent an increase in ethanol consumption following a deprivation period (i.e., an animal model of relapse).

Results Whether administered alone or combined with ondansetron, topiramate produced a similar modest but persistent reduction in ethanol consumption. However, an analysis of efficacy by drinking level revealed that the combination was superior to topiramate alone in heavy-drinking P rats, but was without effect in lighter-drinking P rats and Wistar rats. Both topiramate alone and the combination blocked the alcohol deprivation effect in both

Wistar and P rats with the combination tending to produce a greater decrease than topiramate alone.

Conclusions The combination of ondansetron and topiramate may be a promising treatment for preventing relapse and for treating alcohol dependence in heavy-, but not lighter-drinkers.

Keywords Alcohol consumption · Endophenotype · Ethanol · Ondansetron · Topiramate · Rats · Alcohol · Consumption · Alcohol deprivation effect · Combination treatment

Introduction

Approximately 30% of individuals in the USA are problem drinkers (Hasin et al. 2007). Despite its high prevalence, few efficacious pharmacological treatments exist for alcohol dependence. Even of the more promising medications, efficacy has proven to be variable. For example, ondansetron, a serotonin-3 (5-HT₃) antagonist, has shown promise in treating alcohol dependence, though its efficacy seems to be limited to alcoholics with a genetic predisposition to heavy drinking (i.e., early onset alcoholics; Johnson et al. 2000; 2002). Topiramate, a gamma amino butyric acid (GABA)/glutamate modulator, is another medication that has been reported to improve treatment outcome in alcoholics (for reviews see Johnson 2004a;b;c). Its effects have been best described among heavy drinkers with results showing that although topiramate does not abolish alcohol drinking, it does significantly reduce drinking (Johnson et al. 2003; 2004; 2007). Similar findings have been reported in rats (Hargreaves and McGregor 2007; Knapp et al. 2007) with previous work from our group showing that topiramate effectively reduced ethanol consumption in a line of rats

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genetically predisposed to heavy drinking (i.e., alcohol preferring, P rats), but not in lighter-drinking control rats (i.e., Wistar rats; Breslin et al. 2010). These findings indicate that clinical efficacy may vary between subpopulations of alcoholics according to levels of drinking and they suggest that preclinical studies may be useful in characterizing a pharmac-behavioral response pattern or “finger-print” for the effects of potential treatments as a function of endophenotype (e.g., level of drinking).

Another factor that may increase treatment outcome is the use of medications or medication combinations that target multiple neural pathways in the brain (Johnson 2008). A potential advantage of combination treatments is that the effects of a single medication can be augmented by a second through modulation of additional neuronal networks associated with the reinforcing effects of alcohol. Here, we tested the effects of two medications with demonstrated efficacy as single agents in treating alcoholics to determine whether their combination would result in a greater therapeutic response than either alone. These compounds, ondansetron and topiramate, act through separate neural mechanisms to reduce dopamine levels in the cortico-mesolimbic pathway, a pathway critical for reward and addiction (Koob 1992). For example, ondansetron, by antagonizing 5-HT₃ receptors that are located in mesolimbic brain regions, is believed to produce an overall suppression of dopamine signaling in these areas (Lovinger 1997; De Deurwaerdère et al. 2005; McBride et al. 2004; Mylecharane 1996), with evidence showing that 5-HT₃ antagonism can block acquisition and maintenance of ethanol self-administration (Rodd et al. 2010) and suppresses ethanol-induced dopamine concentration in the nucleus accumbens (Campbell and McBride 1995). Topiramate may result in a suppression of dopamine release via its facilitatory effects on GABA and its inhibitory effects on glutamate in mesolimbic regions (Johnson 2004a; b; 2005). We reasoned that because ondansetron and topiramate manifest their effects through different neuronal processes, both resulting in modulation of cortico-mesolimbic dopamine function, their combination would be more efficacious than either alone in reducing ethanol consumption. Both topiramate and ondansetron, when administered alone, have been reported to decrease ethanol consumption in rat models (Knapp and Pohorecky 1992; Kostowski et al. 1994; Hargreaves and McGregor 2007; Knapp et al. 2007), and as mentioned above, we previously reported that topiramate administered alone reduces consumption in P rats, but not in a control line of lighter-drinking Wistar rats (Breslin et al. 2010). Thus, another goal of this study was to extend these findings to determine whether their efficacy would differ between rats as a function of level of drinking and genetic background (i.e., alcohol preferring P rats as well as in Wistar rats, their control background strain).

The efficacy of the combination was examined in rat models of ethanol consumption and relapse to gain an understanding of the conditions under which the combination of topiramate and ondansetron may exert its anti-drinking effects. Topiramate has been shown to attenuate withdrawal symptoms following chronic ethanol exposure in rats (Cagetti et al. 2004) and may, therefore, be effective at preventing relapse following a period of abstinence. No studies, however, have examined the effects of topiramate alone or combined with ondansetron at preventing relapse to drinking following a period of abstinence in either animals or humans.

Methods

Animals and housing

Male alcohol preferring (P) rats ($N=22$) were obtained from the Indiana Alcohol Research Center's Animal Production Core. Male Wistar rats ($N=20$) were obtained from Charles River Laboratory. The P line of rats has been characterized by numerous studies as a valid animal model of excessive ethanol drinking behavior (for review see Bell et al. 2006). Although average ethanol consumption in this line of rats is greater than 4 g/kg/day, there is considerable variability between animals in terms of total consumption with some animals consuming upwards of 8 g/kg/day (equivalent to approximately 12 standard drinks/day/70 kg person) and others consuming around 2 g/kg/day (equivalent to approximately 3 standard drinks/day/70 kg person; Bell et al. 2006), with the latter amount similar to the amounts obtained by moderate and heavy-drinking Wistar rats not bred for preference for ethanol.

Rats were single-housed in clear, polycarbonate cages with unrestricted access to food and water, in a colony room maintained on a 12:12 light/dark cycle (lights on at 7:00AM). All rats weighed between 350 and 400 g at the start of the experiments, with weights obtained three times per week. Intake of food, ethanol, and water solutions were determined daily with fresh solutions presented several times a week. Sipper tubes contained a ball bearing at the tip to prevent leakage. The effects of topiramate alone were published from some of these animals as part of another study (see Breslin et al. 2010); however, for the current study, one P rat was removed because it had not received ondansetron alone or the combination of ondansetron plus topiramate, and three additional P rats were added for comparison in the present study. Additionally, the data from Wistar rats, which were given access to ethanol under either the two or three-bottle choice paradigm (as described below) were pooled in order to create more variability in consumption for comparison here, as there were no differ-

ences between these two groups with regard to treatment response under any condition. The dose conditions were tested during the same time period and the order of administration was randomly determined between animals. All rats had an average of approximately 3 months with free access to unsweetened ethanol prior to treatment. All protocols were approved by the Animal Care and Use Committee at the University of Virginia and are in accordance with the guidelines set forth by NIH.

Procedure

P rats ($N=22$) and Wistar rats ($N=10$) were trained to consume ethanol using a two-bottle free choice procedure as described previously (Breslin et al. 2010). Briefly, a sucrose-fading procedure was used to induce drinking where ethanol was gradually introduced in 2% increments to a final concentration of 10% v/v and then sucrose was faded completely out in 2% increments. During sucrose fading, the positions of the water and ethanol bottles were switched daily and during the consumption component the bottle positions were switched at random intervals to ensure the animals were tracking the ethanol rather than bottle position. A second group of Wistar rats ($N=10$) were tested using a three-bottle choice procedure (5%, 10%, and water) in order to induce higher levels of drinking without sucrose fading. Intake differences between these two groups of Wistar rats have been published previously (Breslin et al. 2010).

Effect of ondansetron and topiramate alone and combined on ethanol consumption

The effect of topiramate and ondansetron on unsweetened ethanol consumption was examined on a stable baseline (stability was defined as no increasing or decreasing trend in ethanol consumption, with a variation of less than 1 g/kg over three consecutive days) using a within-subject design with compounds administered in random order. On test days, a single treatment of topiramate (5 or 10 mg/kg), ondansetron (0.001 or 0.01 mg/kg), their combination (5/0.001, 5/0.01, 10/0.001, 10/0.01 mg/kg; the dose of topiramate listed first), or an equal volume of saline was administered intraperitoneally during the daily weigh sessions that were conducted between 1:00 PM and 4:00 PM, with water and ethanol consumption measured 24 h after each injection. The doses of ondansetron and topiramate were based on pilot work and on previous work in rats (Breslin et al. 2010; Cagetti et al. 2004; Kostowski et al. 1994; Higgins et al. 1992) and were selected to include doses that are modestly effective and ineffective at reducing the reinforcing effects of ethanol when administered alone. The use of low to moderate doses allowed us to assess

potential additive effects of these drugs combined. All treatments were given once, and a minimum of 5 days of stable consumption separated each test session.

Effect of topiramate alone and combined with ondansetron on the alcohol deprivation effect

In rats, the alcohol deprivation effect (ADE) is one method that has been used to model relapse. With this model, there is a large increase in ethanol consumption following re-exposure to ethanol in animals that have been deprived of ethanol for several days or weeks. Although most of the studies conducted on the ADE have used ethanol-dependent rats or rats bred for high ethanol preference (e.g., ethanol-preferring (P) rats), the ADE also has been shown in non-dependent rats, as well as in outbred rat breeds (e.g., Wistar rats) (Rodd et al. 2004).

Following completion of maintenance intake part of the study, the effect of topiramate alone (0 and 10 mg/kg) and in combination with ondansetron (0.001 mg/kg) on the ADE was examined in a subset of the Wistar rats ($N=10$) and the P rats ($N=6$) using a within-subject design. For this procedure, ethanol was withdrawn for a total of 15 days following three consecutive days of stable ethanol consumption. On day 16, rats received one of the treatments at 3:30 PM and then ethanol was reinstated 30 min later. Food, ethanol, and water consumption were measured 24 h after ethanol was reinstated. A minimum of 2 weeks of ethanol consumption separated each of the deprivation tests with the order of treatment counterbalanced between subjects. The dose and the 30-min pretreatment time were selected based on preliminary findings from the maintenance intake part of the study.

Drugs

Ethanol solutions were prepared from 190 proof absolute ethyl alcohol (Pharmco-Aaper brand, Brookfield, CT, USA) diluted to 10% (v/v) using tap water. Sucrose solutions were prepared as w/v in tap water. Sucrose and topiramate HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA) and ondansetron was purchased from Glaxo Research Development. Both compounds were dissolved in 0.9% sodium chloride in sterile water and administered at a volume of 1 ml/kg.

Statistical analysis

The time course of the effects of topiramate and ondansetron alone and combined on ethanol consumption was examined by comparing percent change from baseline ethanol levels (in order to control for baseline differences and calculated from the day preceding each treatment) on

the day of treatment and for the three (Wistar) or five (P rats) sessions that followed using repeated measures ANOVA. Univariate ANOVA was used to examine the effects of treatment on the day of administration. Posthoc comparisons with vehicle were made using the Dunnett *t* test. The *t* test was used for comparisons between doses using the Bonferroni correction for multiple comparisons. The relationship between baseline consumption and treatment effect on the day of administration was assessed by calculating the Pearson's correlation coefficient using the Bonferroni correction for multiple comparisons. In order to further assess efficacy as a function of baseline consumption, the sample of P rats was divided into two groups based on baseline levels of consumption. Heavy versus lighter drinking was defined as greater versus less than the median intake of 4.8 g/kg/day. The effect of topiramate and ondansetron alone versus their combination on ethanol consumption on the day of administration was then examined in lighter and heavier drinkers using separate univariate ANOVAs. Similar analyses were performed in the overall cohort and within heavy and lighter drinkers for percent preference for ethanol over water and for food and water consumption. In order to examine the relationship between baseline consumption and efficacy of the combination (i.e., percent change from baseline) along a continuum of drinking, we combined Wistar rats and P rats and then categorized them as either light (<1.8 g/kg/day), medium (>1.8<4.8 g/kg/day), or heavy (>4.8 g/kg/day) drinkers based on median drinking levels within Wistar rats (1.8 mg/kg/day) and P rats (4.8 mg/kg/day). The light drinking category was exclusively comprised of lighter-drinking Wistar rats, the middle drinking category was comprised of both heavier-drinking Wistar rats and lighter-drinking P rats, and the heavy-drinking group was exclusively comprised of heavier-drinking P rats with the addition of one heavy-drinking Wistar rat. Three of the P rats developed a skin condition during the course of the study (presumably due to chronic ethanol consumption), and data from these animals were not available for the effects of two or more of the combination treatments. Five of the 20 Wistar rats received only vehicle and the two doses of topiramate.

Similar analyses were used to assess the effects of treatment on the ADE with an additional *t* test comparison of ethanol consumption at baseline versus consumption on the day ethanol was reinstated under vehicle treatment conditions in order to verify increased consumption following the 15-day deprivation period. Baseline was calculated from the day preceding each of the deprivation periods. Seven of the ten Wistar rats received only vehicle and topiramate alone and one Wistar rat did not receive topiramate. With the exception of the Pearson's correlations and *t* test comparison of effective treatments where the

Bonferroni correction was made, the alpha level for statistical significance was 0.05.

Results

Effect of ondansetron and topiramate alone and combined on ethanol consumption

In P rats, whether administered alone (Fig. 1a) or in combination with ondansetron (Fig. 1b), topiramate modestly but persistently decreased ethanol consumption. Results from a repeated measures ANOVA comparing percent change from baseline levels of ethanol intake on the day of administration and for the five sessions that followed revealed significant overall effects of day [$F_{5,895}=4.5$, $p<0.001$] and treatment [$F_{8,179}=2.54$, $p<0.05$]. While posthoc comparison with vehicle across each of these sessions revealed only trends for a difference between topiramate alone at the 10 mg/kg dose ($p=0.09$) and when combined with either the 0.001 mg/kg ($p=0.09$), or the 0.01 mg/kg dose of ondansetron ($p=0.1$), when assessed on just the day of administration, each of these differences were significant ($p's<0.05$). No significant differences were observed for either dose of ondansetron alone, for the 5 mg/kg dose of topiramate, or for the combination of the 5 mg/kg dose of topiramate and either dose of ondansetron. Subsequent analysis within the 10 mg/kg dose of topiramate revealed that intake continued to be decreased on days 1 to 3 following topiramate treatment (Post 1, $t_{42}=4.1$, $p<0.05$; Post 2, $t_{42}=4.3$, $p<0.05$; Post 3, $t_{42}=3.9$, $p<0.05$), but were similar to vehicle levels by day 4. Similarly, following treatment with this dose of topiramate and the 0.01 mg/kg dose of ondansetron, intake was significantly decreased on days 1 and 2 (Post 1, $t_{42}=5.3$, $p<0.01$; Post 2, $t_{42}=3.7$, $p<0.05$) but were similar to vehicle by day 3. The effect of the 10 mg/kg dose of topiramate combined with the 0.001 mg/kg dose of ondansetron was not significantly different from vehicle on any of the sessions following the treatment session. The effects of topiramate alone at the 10 mg/kg dose versus in combination with ondansetron on the day of administration did not differ significantly. This dose of topiramate alone versus the combination also showed a similar time course of effects (see Fig. 1c) with a repeated measures ANOVA comparing the three treatment conditions revealing a non-significant treatment effect. No significant treatment effects were observed for preference for ethanol or for food or water intake (data not shown). Thus, ondansetron alone did not affect ethanol consumption, but when it was combined with the high dose of topiramate, it effectively and persistently reduced consumption. The combination of ondansetron and topiramate was not, however, more

Fig. 1 Time course of the effects of topiramate and ondansetron alone (a) and in combination (b) on mean ($\pm$ SEM) ethanol intake (g/kg) for P rats at baseline (Base), on the day of treatment (0), and for the five sessions that followed (1–5). Although the effects of each compound alone and in combination were analyzed in one model, data are represented in separate graphs for clarity with vehicle represented on both figures. An asterisk indicates a significant difference as compared with vehicle ($p < 0.05$). Time course for the effects of the

10 mg/kg dose of topiramate alone versus combined with ondansetron. Data are plotted as mean ($\pm$ SEM) percent change from baseline levels of ethanol consumption on the day of treatment and for the five sessions that followed. Each data point represents an N of between 19 and 22. Ond-0.001, Ondansetron-0.001 mg/kg; Ond-0.01, Ondansetron-0.01 mg/kg; Top-5, Topiramate-5 mg/kg; Top-10, Topiramate-10 mg/kg

efficacious than topiramate alone in the combined cohort that included both heavy- and lighter-drinking rats.

Notably, when we examined the association of level of drinking and treatment effect on the day of administration, we found that there was a strong negative correlation between these factors following the high-dose combination treatment (see Fig. 2a) but not following treatment with topiramate (5 mg/kg, $r = -0.10$; 10 mg/kg, $r = 0.12$) or ondansetron alone (0.001 mg/kg, $r = -0.431$; 0.01 mg/kg, $r = -0.35$) or following treatment with the combination of ondansetron and the 5 mg/kg dose of topiramate (5/0.001 mg/kg, $r = -0.36$; 5/0.01 mg/kg, $r = -0.52$). A similar relationship was found for this dose of topiramate in combination with the 0.001 mg/kg dose of ondansetron although this relationship did not reach statistical significance at the 0.0056 level ($p = 0.009$; Fig. 2b). Thus, the effect of the combination of ondansetron and topiramate differed by severity of drinking; that is, the greater the drinking at baseline the larger the treatment effect.

This effect is further explored in Fig. 3 by examining treatment response on the day of administration in heavy-

versus lighter-drinking rats (average intake in heavy versus lighter drinking was 5.95 ± 0.10 and 3.47 ± 0.08 g/kg, respectively). Within lighter drinkers, only topiramate at the 10 mg/kg dose appeared to decrease consumption, although the overall effect of treatment was not significant. In contrast, within heavier drinkers, there was a significant overall effect of treatment [$F_{8,90} = 4.05$, $p < 0.001$], and in this case, the combination of the 10 mg/kg dose of topiramate with either the 0.001 or the 0.01 mg/kg dose of ondansetron, but not either compound alone, effectively reduced ethanol consumption as compared to vehicle ($p < 0.01$, $p < 0.001$, respectively). Notably, the effect of the high-dose combination was significantly greater than the effect of topiramate alone at the 10 mg/kg dose ($t_{19} = 3.8$, $p < 0.017$). As in the overall cohort, within heavy drinkers both topiramate alone and in combined with either dose of ondansetron persistently reduced ethanol consumption although the time course for their effects did not differ significantly (data not shown). Also consistent with the overall cohort, none of the treatments significantly affected preference for ethanol or food or water consumption (data

Fig. 2 Association of level of drinking at baseline and the percent change from baseline drinking (marked by dotted line) on the day of treatment with the combination of the 10 mg/kg dose of topiramate and the 0.01 mg/kg (**a**) and the 0.001 mg/kg (**b**) dose of ondansetron. The Pearson's correlation coefficient (r value), as well as regression

line, is shown for both dose combinations and an *asterisk* indicates a significant correlation of drinking level at baseline and percent change from baseline drinking at the 0.0056 level. Ond-0.001, Ondansetron-0.001 mg/kg; Ond-0.01, Ondansetron-0.01 mg/kg; Top-10, Topiramate-10 mg/kg

not shown). Thus, in P rats when the 10 mg/kg dose of topiramate was combined with either dose of ondansetron, it effectively reduced ethanol consumption in heavy-, but not lighter-drinking rats, and when combined with the 0.01 mg/kg dose of ondansetron, it was superior to topiramate alone.

In contrast to the effects observed in P rats in their lighter-drinking control strain (e.g., Wistar rats), neither of the compounds alone nor in combination effectively reduced ethanol consumption. In fact, intake appeared to be increased by many of the treatments particularly following treatment with either dose of ondansetron alone or in combination with topiramate (data not shown). However, the overall ANOVA for the effect of treatment across the four sessions as well as on the day of administration did not reach statistical significance. None of the doses or dose combinations affected preference for ethanol or food or water intake in Wistar rats (data not shown).

Effect of the combination as a function of severity of drinking at baseline

Although the overall effect of treatment was not significant in the lighter-drinking Wistar rats, the tendency for some of the treatments to produce an increase in ethanol intake combined with the findings in P rats showing that efficacy varied between heavy versus lighter-drinking rats suggested that the efficacy of the combination may vary along a continuum of drinking. Thus, in order to further examine the relationship between baseline consumption and efficacy of the combination, we combined Wistar rats and P rats and then categorized rats as either light, medium, or heavy drinkers (see Fig. 4). The results revealed marked differences in the effect of the

combination as a function of drinking group where intake was increased in light drinkers, not affected in medium drinkers, and decreased in heavy drinkers (overall effect of drinking group [$F_{2,158}=37.1$, $p<0.001$]) with subsequent comparison revealing a significant difference between each of the three groups (light versus medium, $p<0.001$; light versus heavy, $p<0.001$; medium versus heavy, $p<0.01$). We also observed a significant effect of treatment [$F_{4,158}=2.8$, $p<0.05$], as well as an interaction of drinking group and treatment [$F_{8,158}=2.8$, $p<0.01$] with subsequent comparison revealing a significant effect of treatment within heavy drinkers [$F_{4,47}=6.0$, $p=0.001$], but not light or medium drinkers. As with the previous analysis in P rats only, within heavy drinkers, ethanol intake was significantly decreased as compared to saline following treatment with the 10 mg/kg dose of topiramate in combination with either the 0.001 or the 0.01 mg/kg dose of ondansetron ($p=0.01$; $p=0.001$; respectively). These findings reveal that the efficacy of the combination varied along a continuum of drinking, and together they indicate greater efficacy of the combination in heavy versus lighter drinkers.

Effect of topiramate alone and in combination with ondansetron on the ADE

As predicted, following a 15-day ethanol deprivation period, P rats showed a transient increase in ethanol consumption from baseline levels (day alcohol was reinstated only; $t_{10}=4.2$, $p<0.05$), and both topiramate alone and in combination with ondansetron blocked this increase (Fig. 5a). In contrast to their effect on consumption, however, treatment under ADE conditions did not produce a persistent decrease in ethanol consumption. An analysis of the percent change from baseline levels of ethanol intake

Fig. 3 Effect of topiramate and ondansetron alone and in combination on mean ($\pm$ SEM) percent change from baseline levels of ethanol consumption on the day of treatment in lighter- (a) versus heavy-drinking (b) P rats. An *asterisk* indicates a significant difference as compared with vehicle ($p<0.05$). A *number sign* indicates a significant difference as compared to topiramate alone at the 10 mg/kg dose. Each data point represents an N of between 8 and 12. Ond-0.001, Ondansetron-0.001 mg/kg; Ond-0.01, Ondansetron-0.01 mg/kg; Top-5, Topiramate-5 mg/kg; Top-10, Topiramate-10 mg/kg

on the day of treatment (and the day that alcohol was reinstated) and for the three sessions that followed treatment revealed a significant interaction of treatment and day [$F_{6,45}=3.5$, $p<0.01$]. While posthoc comparison with vehicle across each of these sessions revealed a significant effect of the combination ($p<0.05$) but only a trend for the effect of topiramate alone ($p=0.08$), when assessed on just the day of administration, both of these differences were significant ($p<0.01$; $p<0.05$, respectively). Although the effects of the combination tended to be greater than topiramate alone on the day of treatment, this difference did not reach statistical significance ($p=0.058$). The effects of topiramate alone and combined with ondansetron were statistically similar to each other and to vehicle on all other days.

Wistar rats also showed a transient increase in ethanol consumption from baseline levels on the day alcohol was reinstated ($t_{18}=5.4$, $p<0.01$), and like P rats, this effect was blocked by both topiramate alone and in combination with ondansetron (Fig. 5b). An analysis of percent change from baseline levels of ethanol intake on the day of administration (and the day that alcohol was reinstated) and for the three sessions that followed treatment revealed a significant interaction of treatment and day [$F_{6,63}=3.8$, $p<0.01$]. While posthoc comparison with vehicle across each of these sessions revealed a trend for a difference following treatment with combination ($p=0.1$), but not topiramate alone, when assessed on just the day of administration, both of these differences were significant ($p<0.01$; $p<0.05$, respectively). Notably, the effect of the combination was

Fig. 4 Effect of the combination of topiramate and ondansetron on mean ($\pm$ SEM) percent change from baseline ethanol consumption in light (<1.8 g/kg/day; $N=7-9$), medium ($>1.8<4.8$ g/kg/day; $N=15-23$), or heavy (>4.8 g/kg/day; $N=8-11$) drinking P rats and Wistar rats. Brackets and asterisks indicate a significant group difference ($p's<0.01$) and an asterisk indicates a significant difference as compared with vehicle ($p<0.05$). Ond-0.001, Ondansetron-0.001 mg/kg; Ond-0.01, Ondansetron-0.01 mg/kg; Top-5, Topiramate-10 mg/kg

significantly greater than topiramate alone on the day of treatment ($t_{14}=4.7$, $p<0.05$), but were statistically similar to each other and to vehicle on all other days. Neither topiramate, the combination, nor deprivation significantly affected food or water consumption in P rats or Wistar rats (data not shown).

Discussion

Two approaches that are likely to increase the efficacy of pharmacotherapy for alcohol dependence are the use of broader pharmacological approaches, and the use of quantitatively measured endophenotypes that help guide the selection of pharmacotherapies depending on the behavioral and/or genetic characteristics of an individual. In this study, the use of these approaches were explored in animal models by examining the combined effects of topiramate and ondansetron, medications shown to be effective in treating alcohol-dependent humans, on ethanol consumption and relapse. Our results demonstrate greater efficacy of the combination of ondansetron and topiramate at the 10 mg/kg dose versus either compound alone to decrease ethanol consumption in heavy-, but not lighter drinkers. The efficacy of the combination to influence ethanol consumption varied along a continuum of drinking with increased consumption in light drinkers, no effect in medium drinkers, and decreased consumption in heavy drinkers. Notably, under the relapse model wherein intake following deprivation is known to be high, both topiramate

Fig. 5 Time course of the effect of topiramate alone and in combination with ondansetron on mean ($\pm$ SEM) ethanol intake (g/kg) following a 15-day deprivation period in P rats (a) and Wistar rats (b). Data are plotted for ethanol intake at baseline (intake on session prior to deprivation; Base), on the day of treatment and the day that ethanol was reinstated (0), and for the three sessions that followed (1–3). A plus sign indicates a significant increase from baseline ($p's<0.05$), an asterisk indicates a significant difference as compared to vehicle ($p's<0.05$), and a number sign indicates a significant difference as compared to topiramate alone ($p's<0.05$). Each data point represents an N of between 6 and 10

and the combination attenuated the ADE in both Wistar and P rats with the combination producing or tending to produce a significantly greater effect than topiramate alone. Thus, we would expect that the combination would be efficacious in preventing relapse and for the treatment of heavy-drinking alcohol-dependent humans, a population that is known to be difficult to treat (Schuckit 2009; Booth et al. 1991).

Although the exact mechanism for the differential effects of the combination between heavy versus light drinkers remains to be determined, our current findings, in conjunction with previous findings on differences in the cortico-mesolimbic reward pathway between alcohol preferring rats and their control strains, provide some insight into this mechanism. It is well established that cortico-mesolimbic

dopamine mediates the reinforcing effects of ethanol, with evidence indicating that this system is deficient in alcohol preferring rats relative to non-preferring rats at baseline (McBride and Li 1998). Both ondansetron and topiramate are believed to modulate dopaminergic signaling (Johnson 2010), and when combined, it is possible that their effects on dopamine depend on basal levels. This interpretation is consistent with our findings that the combination reduced ethanol consumption in animals presumed to have lower basal levels of dopamine (i.e., heavy drinkers and in all rats following alcohol deprivation), but had no effect or increased consumption in animals presumed to have higher basal dopamine levels (i.e., medium and lighter drinkers; see Fig. 4). It is also possible that such differences originate through interactions of serotonergic and/or GABAergic/glutamatergic signaling. For example, serotonin modulates the release of mesolimbic dopamine, and like the dopaminergic system, the serotonergic system is deficient in alcohol preferring rats as compared to non-preferring rats (McBride and Li 1998). Alcohol preferring rats and mice also have a higher density of axon terminals containing GABA in areas of the brain implicated in alcohol dependence (McBride et al. 1990) and show a greater increase in ethanol-induced glutamate concentrations in the NAc following chronic exposure (Kapasova and Szumlanski 2008). Given that the role of glutamate in alcohol dependence appears to become progressively more important following chronic heavy use and during relapse (Vengeliene et al. 2008), medications that influence these pathways may be particularly effective for preventing relapse and for reducing maintenance consumption in heavy drinkers. Further studies are necessary to examine these possibilities and to determine neurochemical differences as a function of severity of drinking.

In the present study, low doses of both ondansetron and topiramate were selected because we were interested in determining additive effects of combined treatments. It is noteworthy that although ondansetron did not decrease consumption on its own in the overall cohort, these doses did reduce ethanol consumption when combined with topiramate. It is also noteworthy that none of the doses or dose combinations tested affected food or water consumption. Thus, it appears that the use of low doses in the present study not only enabled us to observe additive effects, but also had the advantage of producing a selective reduction of ethanol consumption. Although our results show increased consumption in lighter-drinking Wistar rats following treatment with the combination (Fig. 4), it is important to note that this group was included as a control for background, not as a model of lighter-drinking alcoholism. Specifically, most of the Wistar rats drank low, non-physiological levels of alcohol. If a parallel group of humans exists, they would not be considered an

appropriate population to give medication for alcohol dependence treatment.

Psychiatric disorders, particularly with regard to pharmacotherapy, are often considered via a categorical diagnosis rather than an individual formulation. While it is generally accepted that individualized approaches lead to better outcomes in the treatment of medical and psychiatric disorders, the application of this belief has not occurred to a great extent in regard to pharmacotherapy, particularly for treating addictions. The concept of tailored medicine has been well developed in some other fields of medical research, cancer research for example, and is beginning to be applied to more and more areas of medical research (Hobson 2009). For cancer, treatments are selected based on multiple factors, including genetic and behavioral markers of the tumor as well as characteristics of the individual (Curigliano et al. 2004; Hightower 2006). Since addictions are manifest behaviorally, it seems reasonable to select treatments based on not only genetic markers but also behavioral phenotype. The results of the present study suggest drinking level may be one behavioral phenotype to select for in treating alcohol dependence.

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Declaration The experiments described herein comply with the current laws of the United States of America.

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