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Effects of the Triple Monoamine Uptake Inhibitor DOV 102,677 on Alcohol-Motivated Responding and Antidepressant Activity in Alcohol-Preferring (P) Rats

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Abstract

Background—Concurrent inhibitors of dopamine, norepinephrine and serotonin uptake have been proposed as novel antidepressants. Given the high comorbidity between alcoholism and depression, we evaluated the activity of DOV 102,677 (DOV) on alcohol-maintained responding and performance in the forced swim test (FST), a model of antidepressant (AD) activity, using alcohol-preferring (P) rats.

Methods—Following training to lever press for either alcohol (10% v/v) or sucrose (3%, 2%, w/v) on a fixed-ratio four (FR4) schedule, DOV (1.56–50 mg/kg; PO) was given 25 min or 24 h prior to evaluation. The effects of DOV (12.5–50 mg/kg; PO) in the FST were evaluated 25 min post-treatment.

Results—DOV (6.25–50 mg/kg) dose-dependently reduced alcohol-maintained responding by 59–88% at 25 min post-treatment, without significantly altering sucrose responding. The reduction in alcohol responding (44% at 50 mg/kg) was sustained for up to 120 h after a single dose. Administration of a single dose of DOV (25, 50 mg/kg) 24 h before testing suppressed alcohol responding for 48 h by 59–62%. DOV (12.5–50 mg/kg) also dose-dependently reduced immobility of P rats in the FST.

Conclusions—DOV produces both prolonged and selective reductions of alcohol-motivated behaviors in P rats. The elimination kinetics of DOV suggests that its long duration of action may be due to an active metabolite. DOV also produced robust AD-like effects in P rats. We propose that DOV may be useful in treating comorbid alcoholism and depression in humans.

Keywords

Alcohol responding; P rats; monoamine reuptake inhibitor; depression; forced swim test

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INTRODUCTION

Alcohol addiction remains a significant public health issue (Kessler et al., 1997). In recent years, advances have been made in the development of novel therapies to treat alcoholism (Myrick and Anton, 2004; Pettinati and Rabinowitz, 2006; Johnson, 2008). However, a subpopulation of individuals dependent on alcohol also exhibit depression (Cornelius et al., 1997; Kranzler et al., 2006). While both disorders are characterized by low serotonergic activity (Cloninger, 1987), it is well-established that drugs which elevate endogenous 5-HT levels effectively reduce alcohol intake in some alcoholics (Le Fauve et al., 2004; Myrick and Anton, 2004; Johnson, 2008), necessitating new approaches for treating alcoholics with depression (Pettinati and Rabinowitz, 2006).

Substantial evidence suggests that hypofunction of mesocorticolimbic dopamine systems may contribute to the clinical manifestations of both alcoholism (Gonzales et al., 2004; McBride and Li, 1998) and depression (Skolnick and Basile, 2007; Willner, 2000). These pathways play a significant role in core symptoms of major depression (DSM-IV, 2000). Evidence also suggests that depressed patients who exhibit poor responses to serotonin-selective reuptake inhibitors (SSRIs) may benefit from combining therapy with agents that enhance dopaminergic tone (Skolnick and Basile, 2007). Thus, patients suffering from depression, alcohol abuse or the comorbid conditions may benefit from treatment with TUIs that increase synaptic levels of 5-HT and DA.

Pharmacological studies evaluating the role of NE on alcohol intake have been investigated at both preclinical and clinical levels (Lê et al, 2005). While administration of the $\alpha 1$ receptor antagonist prazosin decreases intake in both rats and humans (Rasmussen et al., 2009), recent studies indicate that inhibitors of NE uptake [e.g., desipramine, milnacipran] are highly effective in reducing alcohol intake in dependent and non-dependent rats (Lê et al, 2005, O'Brien et al., 2011). Thus, a therapy elevating synaptic concentrations of NE, 5-HT and DA may have advantages beyond that of increasing synaptic concentrations of just 5-HT and DA in the treatment of chronic alcohol abuse.

Recently, McMillen and colleagues (2007) evaluated the ability of a triple reuptake inhibitor (TUI) of DA, NE and 5-HT, DOV 102,677 (DOV), to attenuate alcohol consumption in the Myers' high ethanol-preferring (mHEP) rat (Myers et al., 1998). They reported that DOV (5, 10, 20 mg/kg, b.i.d.) reduced home-cage alcohol consumption by 48 – 65% of control levels. Moreover, 24 h after administration, the highest doses of DOV continued to suppress alcohol intake by 39 – 55% of control levels. This suppression was selective for alcohol intake, as food intake was not decreased at any of the tested doses on Day 1 or 24 h post-treatment. Based on their findings, McMillen et al. (2007) proposed that triple reuptake inhibitors may have utility in treating alcohol abuse.

DOV antidepressant [AD] activity has also been investigated in the forced swim test [FST] (Popik et al., 2006), a model with high predictive validity for clinically-effective ADs (Cryan et al., 2005). Their results reported that DOV was comparable to imipramine. Because of the need to develop more effective treatments for comorbid alcoholism and depression (Pettinati, 2004), we employed the P rats, an established model of human alcohol abuse to the satisfaction of the alcohol research community (McBride and Li, 1998). The P rat line may represent an optimal animal model to evaluate the DOV treatment in relation to co-morbid alcoholism and depression. Finally, the pharmacokinetic profile of DOV was compared to its duration of action in behavioral studies.

METHODS

Behavioral Assays

Animals—Male P (n = 154) and NP (n = 10) rats were obtained from the Indiana University School of Medicine. Of these, 121 P rats were randomly selected to evaluate the oral effectiveness of DOV on alcohol and sucrose responding. The remaining P rats and 10 NP rats were used to evaluate DOV in the FST. All rats weighed between 420–584 g at the beginning of the experiment and were individually housed. Vivarium conditions were 21 °C, and a normal 12 h light/dark cycle was used. All rats were provided with *ad libitum* access to food and water. However, rats in the operant self-administration studies were fluid-deprived for 23 h daily during the first 5 days of training (June, 2002). All procedures were conducted in adherence with the *NIH Guide for the Care and Use of Laboratory Animals* at the University of Maryland School of Medicine. The pharmacokinetics of DOV were evaluated in male Sprague-Dawley rats (N = 21) (approximately 290 g) obtained from Charles River Laboratories (Raleigh, NC) and maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* at Xenobiotic Laboratories (Plainsboro, NJ). Vivarium lights were set to provide a 12 h light/dark cycle, with an average daily temperature of 19 – 15 °C. Food and water were supplied *ad libitum*.

Drugs and Solutions—DOV 102,677 was obtained from DOV Pharmaceutical, Inc (Somerset, NJ). Imipramine was obtained from Sigma Aldrich (St. Louis, MO). DOV was orally administered (PO) in deionized (DI) water in a volume of 1 mL/kg for all experiments. Imipramine (1 mL/kg) was administered in a similar manner only in the FST study. DI water was administered as the control vehicle (1 mL/kg) in all studies. The oral doses selected were based on preliminary data and from doses employed by McMillen et al. (2007). Alcohol (10% v/v, USP) and sucrose (2% and 3% w/v) (Fisher Scientific) solutions were prepared for the operant chamber (June, 2002).

Apparati

Alcohol and Sucrose-Maintained Responding Studies—Behavioral testing was conducted in 20 standard operant chambers (Coulbourn Instruments, Allentown, PA) equipped with two removable levers and 0.1 mL dipper fluid delivery systems enclosed in sound-attenuated cubicles (June, 2002). All dipper presentations provided 1.5 s access to a dipper, followed by a 3 s timeout. Above each lever three lights (red, green and yellow) were present, and stimulus delivery was indicated by illumination of the green light.

Responses and reinforcements were recorded and controlled using the Graphic State 2.101 (Coulbourn Instruments, Allentown, PA) operant software package. Mean number of responses for a 30 min session was determined.

Modified FST Study—The FST was modeled after that of Lucki and colleagues (Cryan et al., 2005; Detke et al. 1995) and similar to that described by Porsolt et al. (1977), except that water depth was increased to 30 cm (Detke et al., 1995). Swimming sessions were conducted by placing rats individually into clear glass cylinders (46 cm tall, 20 cm in diameter) containing water at 25 ± 0.5 °C. While the enhanced water depth produces lower baseline immobility values, it has been suggested that the behavioral responses to antidepressant (AD) compounds are augmented (Cryan et al., 2005).

Procedures

Alcohol and Sucrose Training—Rats were trained to lever press for alcohol (10% v/v) using a modified sucrose fading technique (June, 2002; June and Eiler, 2007). In brief, rats were water-deprived for 23 h daily for the first five days of training to facilitate lever

pressing. Initially, rats lever pressed for sucrose (3% w/v) under a fixed-ratio (FR) 1 schedule for 5–7 days, and were then divided into sucrose and alcohol reinforcement groups. The sucrose group continued on sucrose responding and was subsequently stabilized on an FR4 schedule on both levers. The alcohol group underwent the modified sucrose fading procedure (June, 2002) wherein they subsequently responded under FR4 for alcohol (10% v/v) on both levers. Stabilization for both reinforcers was defined as having daily responses within $\pm 20\%$ of the average responses for five consecutive days (June, 2002).

Effects of DOV on Alcohol and Sucrose-Maintained Responding

Study 1: Following training, a between-groups design was employed wherein seven (N = 57) and five (N = 28) groups of P rats were randomly assigned to evaluate DOV in a dose-response study [Tables 1A. and 1B, respectively]. To further evaluate the specificity of DOV on alcohol responding, the level of sucrose responding was equated to that of alcohol responding by reducing the sucrose concentration to 2% (w/v) in another cohort of P rats (N = 14). Either DOV or DI water was administered 25 min prior to placement in operant chambers. To control for residual drug effects, 72–96 h was allotted between doses.

Study 2: We evaluated the duration of action of single doses of DOV in reducing both alcohol and sucrose-motivated responding for up to 48 h [6.25–25 mg/kg] [Tables 2A-F], or up to 192 h [50 mg/kg] [Tables 2G-H]. First, a vehicle baseline for each of the 6.25 – 25 mg/kg dosage groups was obtained. Effects of vehicle or DOV treatments on alcohol or sucrose responding were then compared at 25 min, Day 1, and Day 2. Rats in Tables 1A-B are the same as those in Tables 2A-F.

The duration of effect of 50 mg/kg DOV on alcohol responding was studied using two naive cohorts of P rats (N = 12; N = 10) [Tables 2G–H]. Following stabilization, rats were randomly divided into vehicle (N = 12) and DOV (N = 10) groups. Both received DI water as their initial treatment. Three days later, the vehicle group received another administration of DI water, while the DOV group was given 50 mg/kg of DOV. Twenty-five min post-administration, and on days 1–8 post-treatment, alcohol responding was measured in both groups.

Finally, to examine the duration of action of DOV on sucrose responding, P rats (N = 5) from Study 1 [Table 1B] were re-stabilized on 3% (w/v) sucrose [Table 2H]. To control for residual effects of the initial study, a two-week washout period was given. Following stabilization, rats were given DI water, followed by DOV (50 mg/kg) three days later. Twenty-five min post-administration, and on days 1–8 post-treatment, operant responding was measured.

Study 3: In Study 3, we tested the hypothesis that DOV's effects on alcohol-maintained responding were independent from an interaction with alcohol during the day of pretreatment, and could be detectable with intermittent pretreatment doses. Twenty-eight randomly-selected P rats (from the alcohol group of Study 1) [Table 1A] and 12 randomly selected P rats (from the sucrose group of Study 1) [Table 1B] were re-stabilized on their respective reinforcers for 15 days. A three-week period was allocated between studies. Animals were tested during an initial pre-treatment interval (PI) with DI water to assure that residual effects of prior treatment had dissipated, and to confirm similarity in baseline. Following stabilization, 7 of the 28 alcohol-responding P rats were randomly given 6.25, 12.5, 25, or 50 mg/kg DOV while 6 of the 12 sucrose-responding P rats were given 6.25 and 50 mg/kg DOV to reevaluate reinforce-specific effects of the drug treatment.

To evaluate the time course effects of the DOV treatments relative to the initial PI, a mixed ANOVA comprising an administration day [Day 1, 2, 3, 4, PI] X treatment group [6.25,

12.5, 25, or 50 mg/kg] analyses were conducted for the alcohol (Tables 3A). In addition, to evaluate reinforcer-specific effects of the DOV treatment, a mixed ANOVA comprising day [Days 1, 2, 3, PI] X treatment group [12.5, 50 mg/kg DOV] analyses were conducted for the sucrose (Table 3B).

The Forced Swim Test (FST)—The AD activity of DOV in P rats was evaluated in a modified FST (Cryan et al., 2005; Detke et al. 1995). Two swim sessions were conducted, consisting of an initial 15 min pretest followed 24 h later by a 5 min test. The pretest and test sessions were videotaped and analyzed with the Forced Swim Scan System (Cleversys Inc., Reston, VA) to calculate the time a rat spent struggling/escaping/climbing or immobile/floating/passively swimming. The system employs a sampling technique such that the predominant behavior in each 5 s epoch of the 300 s test is recorded (Detke et al. 1995). Following each session, the rats were removed from cylinders, dried, and placed in warm boxes for 10 min before returning to their home cages. Water in the cylinders was changed after every trial.

Effects of DOV in the FST

Study 4—To evaluate the relationship between selective breeding for alcohol preference and immobility, naïve P (N = 11) and NP (N = 10) rats received DI water and were tested 25 min later in the modified FST. The results were evaluated using a between-group ANOVA (Table 4A). The hypothesis that DOV would decrease immobility time in P rats was tested in naïve P rats [N = 22] randomly assigned to groups receiving DOV (12.5 - 50 mg/kg) or imipramine (25 mg/kg) as a positive control (Table 4B). The 11 P rats used in the innate P versus NP comparison study served as the vehicle control animals in the DOV and imipramine pre-treatment study (Table 4B).

Pharmacokinetics of DOV

Study 5—A study of DOV (20 mg/kg) pharmacokinetics was performed by Xenobiotic Laboratories (Plainsboro, NJ). Following a 7 day acclimatization period, rats were dosed orally with 20 mg/kg DOV (free base). The vehicle was sterile water, and the rats were not fasted prior to dosing. At predose (0 h) and 1, 2, 4, 6, 8, and 12 h post-drug administration, the rats were euthanized with an overdose of CO₂, and blood collected via cardiac puncture into heparinized Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ). Three rats were sacrificed at each time point (Table 5). Aliquots (0.2 mL) of plasma containing tranlycypromine (final concentration 6 µM) and 5 ng econazole as the internal standard were extracted by mixing with 5 mL of methyl *t*-butyl ether and 50 µL of ammonium hydroxide. Approximately 1g of brain homogenate was mixed with 10 mL of T-PER® Protein Extraction Reagent containing tranlycypromine, and an aliquot (0.2 mL) extracted in the same way as the plasma. Samples were injected onto a Luna C18 column (2.0 × 50 mm, 5 µm particle size; Phenomenex, Torrance, CA) equilibrated with 85% mobile phase A (1% formic acid in water)/15% mobile phase B (1% formic acid in acetonitrile) at a flow rate of 0.4 mL/min. Multiple reaction monitoring was performed for DOV (*m/z* 228.1 → 187.1), the DOV lactam metabolite (*m/z* 242.1 → 187.1), and the internal standard (*m/z* 381.2 → 125). The scan time was 200 ms. The concentrations of DOV were calculated using a standard curve containing known amounts of DOV. The peak areas of the lactam in plasma and brain homogenate were compared, taking into account the dilution of the brain tissue.

Data Analysis

Data were evaluated using between-group, repeated-measures, or mixed ANOVAs for the mean number of alcohol and sucrose responses. *Post-hoc* analyses were performed using the

Newman-Keuls test. The Stat Most (5.0) programs (Dataxiom Software, Inc.) were employed for all data analyses.

RESULTS

Study 1

Dose-response effects of DOV on alcohol and sucrose-maintained responding

—Rates of responding maintained by alcohol and sucrose in P rats following oral administration of DOV (1.56–50 mg/kg, 25 min post-treatment) are illustrated in Figure 1. Compared to vehicle, DOV markedly suppressed alcohol-maintained responding ($F_{(6,50)} = 9.41$, $p < 0.001$, Figure 1A). Treatment with 6.25, 12.5, 25, and 50 mg/kg reduced responding by 59%, 70%, 73%, and 82% of control levels, respectively, ($p < 0.001$). The MED of 6.25 mg/kg was significantly different from 50 mg/kg ($p < 0.05$), but not 12.5 and 25 mg/kg ($p > 0.05$). However, DOV had no significant effect on responding maintained by 3% sucrose ($F_{(4,21)} = 0.970$, $p > 0.3396$, Figure 1B), although there was a trend towards reduced responding following 50 mg/kg.

Specificity of DOV treatment on alcohol responding was further evaluated wherein levels of sucrose responding approximated those of alcohol. Figure 1C shows that DOV (12.5–50 mg/kg) remained ineffective ($F_{(3,42)} = 0.0387$, $p > 0.989$), although response rates were similar between groups. In addition, 24–48 h post-administration, DOV continued to be ineffective in altering responding of the sucrose groups [$F_{(3,42)} = 0.0287$, $p > 0.886$; $F_{(3,42)} = 0.0399$, $p > 0.973$, respectively].

Study 2

Duration of DOV effects on alcohol and sucrose-maintained responding—Time course effects of 6.25, 12.5, and 25 mg/kg DOV on alcohol responding at 25 min, 24 h, and 48 h after administration are illustrated in Figure 2. These data show that the marked reduction in responding caused by DOV at 25 min post-injection ($F_{(3,27)} = 13.35$, $p < 0.001$, $F_{(3,15)} = 5.77$, $p < 0.007$, $F_{(3,15)} = 11.50$, $p = 0.0004$, respectively) was no longer apparent at 24 and 48 h ($p > 0.05$). Preliminary results revealed that higher doses reduced alcohol-motivated behaviors for longer periods of time. Thus, a protracted time course evaluation was examined with 50 mg/kg of DOV using separate vehicle ($n = 12$) and DOV ($n = 10$) treatment groups (Table 2G). Specifically, employing Post-administration Day [0, 25 min, Day 1, 2, 3, 4, 5, 6, 7, 8] X Group [vehicle, DOV-50 mg/kg] analyses, Figures 3A–B show that, in contrast to the vehicle, 50 mg/kg DOV significantly reduced responding up to 120 h (Day 5) after administration. ANOVA revealed significant group ($F_{(1,20)} = 6.47$, $p < 0.019$), time ($F_{(9,180)} = 3.20$, $p < 0.001$), and group X time interactions ($F_{(9,180)} = 3.82$, $p < 0.00002$). *Post-hoc* testing confirmed that, compared with control, DOV significantly reduced responding up to Day 5 post-injection ($p < 0.05$).

Although DOV significantly reduced alcohol responding 25 min post-administration, it had no effect on responding for 3% sucrose at 25 min, Day 1, or Day 2 post-treatment (Figures 4A, B, and C, [$F_{(3,12)} = 0.2030$, $p > 0.892$, $F_{(3,12)} = 0.505$, $p > 0.688$, $F_{(3,12)} = 3.10$, $p = 0.068$, respectively]). Even 50 mg/kg of DOV failed to alter responding for sucrose (Figure 4D, $F_{(9,36)} = 0.9807$, $p > 0.4722$).

Study 3

Time course of DOV on alcohol and sucrose-maintained responding given 24 h prior to placing animals in the operant chamber—On Day 1, when DOV was administered 24 h before testing, 12.5, 25, and 50 mg/kg doses significantly reduced responding by 44, 83, and 82% of control levels, respectively. At 48 h post-treatment, 25

and 50 mg/kg reduced responding by 59 and 62% of control levels, respectively. At 72 h post-treatment, residual suppressant effects of DOV continued, resulting in significant administration day ($F_{(4, 24)} = 49.42$, $p < 0.0001$), treatment group ($F_{(3, 18)} = 29.91$, $p = 0.0002$), and administration day X treatment group interaction ($F_{(12, 72)} = 9.12$, $p < 0.001$). Post-hoc analyses confirmed that the 4 groups were similar following DI treatment during the PI phase ($p = 0.05$). On Day 1, 12.5–50 mg/kg markedly reduced responding ($p = 0.05$). In addition, post-hoc analyses confirmed that, even at 48 h, 25 and 50 mg/kg continued to markedly suppress responding ($p = 0.01$), with no significant effects at 72 h ($p > 0.05$) (Figure 5A).

In contrast to the suppressant profile seen on alcohol responding, 12.5 mg DOV significantly elevated sucrose responding 24 h post-treatment; however, at 48–72 h, no effects were observed. 50 mg/kg failed to alter responding post-treatment. These data profiles resulted in a significant Administration Day X Treatment Group interaction ($F_{(3, 15)} = 4.69$, $p < 0.017$). Post-hoc analyses confirmed the elevation at Day 1 following treatment with 12.5 mg ($p < 0.01$); however, at 48–72 h, no significant effects were observed ($p > 0.05$) (Figure 5B).

Study 4

Effects of DOV on P rat Performance in the FST—NP rats spent significantly more time immobile than P rats ($F_{(1,19)} = 19.08$, $p < 0.0003$), which spent significantly more time climbing ($F_{(1,19)} = 9.92$, $p < 0.005$) and swimming ($F_{(1,19)} = 15.16$, $p < 0.001$) (Figure 6A). Administration of DOV (12.5–50 mg/kg) dose-dependently reduced immobility of P rats in the FST ($F_{(4, 28)} = 4.36$, $p < 0.0072$, Figure 6B). *Post-hoc* analyses indicated that effects of 25 and 50 mg/kg differed significantly from control ($p < 0.01$). In contrast, 25 mg/kg imipramine (included as a comparator) failed to significantly reduce immobility ($p > 0.05$). DOV dose-dependently and significantly increased climbing behaviors ($F_{(4, 28)} = 5.81$, $p < 0.0015$) above control at all tested doses ($p = 0.05$) while reducing swimming behaviors ($F_{(4, 28)} = 5.34$, $p < 0.0074$). Imipramine produced a similar profile of effects ($p < 0.01$).

Study 5

Pharmacokinetics of DOV—Plasma concentration of DOV in Sprague-Dawley rats administered 20 mg/kg orally peaked (C_{max}) at 1780 ng/mL at 1 h post-administration (T_{max}), declining to approximately 5 ng/mL at 12 h (Figure 7A). Mean residence time ($MRT_{0-\infty}$) of DOV in plasma was 2.6 h, and elimination half-life ($t_{1/2}$) was 1.5 h. C_{max} of DOV in the brain was almost twice as high (3444 ng/g) at 1 h post-administration as in plasma, declining to approximately 10 ng/mL at 12 h, with a mean residence time ($MRT_{0-\infty}$) of 2.6 h and a $t_{1/2}$ of 1.5 h. The primary metabolite of DOV is a lactam, whose T_{max} values in both plasma and brain were observed at 6 h post-administration (Figure 7B). $MRT_{0-\infty}$ of the lactam in brain and plasma were 6.2 and 6.4 h, with a $t_{1/2}$ of 2.5 and 2.7 h, respectively.

DISCUSSION

In an attempt to find an agent active in treating depression and alcoholism, we employed P rats (McBride and Li, 1998; Murphy et al., 2002) to model effectiveness of the TUI, DOV 102,677, on alcohol-motivated behaviors and FST performance. DOV produced robust, prolonged suppression of alcohol-maintained responding, with reductions as large as 59–88% on the day of treatment, and lasted as long as 6 days after treatment with 50 mg/kg. Subsequently, we observed a prolonged suppression of responding (83 and 62% of control levels at 24 and 48 h post-treatment) by DOV (12.5–50 mg/kg) when given 24 h *prior* to evaluation, suggesting that DOV did not interact with alcohol on the day of pre-treatment. Thus, reduction of alcohol drinking by DOV may occur following intermittent treatments.

The exact mechanism by which DOV reduces alcohol-maintained responding is unknown. DOV (20 mg/kg) significantly elevated dialysate levels of all three biogenic amines in the medial prefrontal cortex [mPfc], and dopamine and serotonin levels in the nucleus accumbens, with lower doses (5 mg/kg) preferentially increasing DA and 5-HT levels in the mPfc (Popik et al., 2006). Radioligand binding assays (Popik et al., 2006) reported that DOV was a potent and “balanced” (i.e., approximately equipotent) inhibitor of SERT, NET and DAT transporters at a dose (20 mg/kg) which is highly effective in reducing alcohol responding. Thus, DOV may mediate its effect on alcohol responding via activation of 5-HT, NE, and DA pathways in the mPfc, nucleus accumbens, or other putative alcohol reward loci (McBride and Li, 1998). In addition, DOV may normalize hypodopaminergic and hyposerotonergic tone (McBride and Li, 1998) in putative alcohol reward loci in P rats, thereby reducing alcohol-motivated behaviors.

DOV-induced reductions in alcohol-mediated responding did not result from a generalized reduction of consummatory behaviors, or reduced propensity to work as a reinforcer; DOV did not reduce sucrose-maintained responding either on the day of pretreatment or several days after (Figure 4A-D). Even when the sucrose response rates approximated those produced by alcohol (affected by lowering the sucrose concentration), all the DOV doses failed to alter sucrose-motivated behaviors 25 min (Figure 1C) to 48 h after administration. Both selective and prolonged suppression of alcohol-motivated behaviors by DOV is consistent with previous observations of suppression of alcohol consumption by DOV in mHEP rats (McMillen et al., 2007). The ability of DOV to produce sustained reduction in drinking, and its failure to alter non-alcohol rewarded behaviors may facilitate its effectiveness in human alcoholics.

Potential antidepressant properties of DOV in P rats were examined in a modified FST (Cryan et al., 2005). As previously reported in a “traditional” FST (where only immobility is scored), a negative association was found between immobility and alcohol preference, with decreases in immobility observed in P rats relative to NP rats (Godfrey et al., 1997; Viglinskaya et al., 1995). As rat immobility may model depressive symptoms in humans (Cryan et al., 2005), this suggests an association between depression and alcohol consumption. However, to our knowledge, no evidence exists in the clinical literature for this. Finally, elevations in active behavior measures observed in naïve P versus NP rats may reflect innate alterations in monoaminergic signaling in P rats (McBride and Li, 1998).

Immobile time for naïve P rats in the FST was dose-dependently reduced by DOV administration, with the MED (25 mg) being more effective than imipramine. All doses of DOV, compared to imipramine, significantly elevated climbing. Findings for DOV in the present study partially agree with AD-inhibition of activity of more than one monoamine transporter. Regardless of the predominant mechanism of action, DOV significantly reduces immobility of P and outbred rats in the FST, predictive of clinical AD activity. Together, these data suggest that triple monoamine uptake inhibitors may act as novel ADs (Skolnick and Basile, 2007), even in subjects with comorbid alcoholism.

The long duration of DOV-induced suppression of alcohol-maintained responding following administration may reflect its pharmacokinetic and pharmacodynamic profile as a TUI. This is true particularly when DOV is given 24 h before evaluation [Figure 5A; Study #3]. After oral administration to Sprague-Dawley rats, DOV accumulates in the brain to levels approximately twice that of plasma. Consistent with this, microdialysis studies indicate that extracellular DA, NE, and 5-HT levels in the prefrontal cortex reach maximum 40 min after DOV administration, and are sustained for at least 120 min, after which 5-HT levels decline (Popik et al., 2006). DA and NE levels remain stable or continue to rise throughout the rest of the 240 min observation period. Continued elevations of DA and NE (but not 5-HT)

levels beyond $T_{\max}$ of the parent compound suggest that DOV may be converted to an active metabolite, a lactam which is 165- and 66-fold less potent an inhibitor of the 5-HT and NE transporters, respectively. However, this metabolite inhibits [^3H]DA uptake with an $\text{IC}_{50} \approx 1.9 \mu\text{M}$, so it is possible that brain concentrations may be sufficient to increase synaptic DA levels for at least 24 h after a behaviorally-active dose of DOV (20 mg/kg). Although pharmacokinetic studies were not performed in P rats, elimination $t_{1/2}$ of the lactam metabolite (2.5 h) would have to be 5 times longer in P rats in order to explain the 6 day duration of action of a single, 50 mg/kg dose of DOV. Not even the long $\text{MRT}_{0-\infty}$ of the metabolite can account for this.. Overall, pharmacokinetics of DOV, coupled with the knowledge that alcohol reward is regulated by a number of neurotransmitters (McBride and Li, 1998), suggests that dopaminergic activities of DOV, or of its primary metabolite, are not *solely* responsible for its actions in modulating alcohol-seeking behaviors.

In conclusion, the present study provides compelling evidence that DOV produces both sustained and selective reductions on alcohol-motivated behaviors. The extended $\text{MRT}_{0-\infty}$ of DOV suggests that this may be attributable in part to an active metabolite. This hypothesis merits further investigation. In addition, DOV produced robust effects on measures of AD activity in P rats. We hypothesize that DOV functions to “normalize” monoaminergic neurotransmission in DA and 5-HT deficient P rats (Murphy et al., 2002), thereby reducing alcohol-motivated and depression-like behaviors. Consistent with McMillen et al. (2007), we propose that DOV may be useful in treating comorbid alcoholism and depression in humans.

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Figure 1.

Dose-response curve for the effects of oral DOV given 25 min prior to evaluation on response rate maintained by 10% (v/v) alcohol (**A**), 3% (w/v) sucrose (**B**), or 2% sucrose (w/v) (**C**) in P rats in Study 1. Results represent the mean $\pm$ SEM of the number of responses recorded over a 30 min session ($n = 5-14$ rats/dose). **: Significantly different from vehicle treated controls, 1-way ANOVA followed by Newman-Keuls' test, $p < 0.01$.

Figure 2.

Time course effects of DOV on responding maintained by alcohol in Study 2. DOV was administered orally on Day 1 at a dose of 6.25 mg/kg (**A**), 12.5 mg/kg (**B**), or 25 mg/kg (**C**), and its effect on alcohol-maintained responding tested 25 min – 2 days later. Results represent the mean $\pm$ SEM of the number of responses recorded over a 30 min session ($n = 5-10$ rats/dose). Data for the 25 min post-treatment effect are provided from Figure 1. **: Significantly different from vehicle treated controls, 1- way ANOVA followed by Newman-Keuls' test, $p < 0.01$.

Figure 3.

Time course effects of vehicle (**A**) and 50 mg/kg DOV (**B**) on responding maintained by alcohol in Study 2. Compounds were administered orally on Day 1, and their effects on 10% (v/v) alcohol-maintained responding tested 25 min – 8 days later. Results represent the mean $\pm$ SEM of the number of responses recorded over a 30 min session ($n = 5$ rats/dose). Data for the 25 min post-treatment effect are provided from Figure 1. *, **: Significantly different from vehicle-treated controls, 1-way ANOVA followed by Newman-Keuls' test, $p < 0.05$, 0.01 , respectively.

Figure 4.

Time course effects of DOV on responding maintained by sucrose in Study 2. DOV 102,677 was administered orally on Day 1 at a dose of 6.25 mg/kg (**A**), 12.5 mg/kg (**B**), 25 mg/kg (**C**), or 50 mg/kg (**D**), and its effect on sucrose-maintained responding tested 25 min – 8 days later. Results represent the mean $\pm$ SEM of the number of responses recorded over a 30 min session ($n = 5-10$ rats/dose). Data for the 25 min post-treatment effect are provided from Figure 1. None of the doses of DOV had any significant effect on sucrose responding as evidenced by 1-way ANOVA.

Figure 5.

Time course effects of DOV orally administered 24 h prior to testing on responding-maintained by 10% (v/v) alcohol (**A**) or 3% (w/v) sucrose (**B**) in P rats in Study 3. Testing continued 2, 3, and 4 days post-administration (dosing day inclusive). Results represent the mean $\pm$ SEM of the number of responses recorded over a 30 min session ($n = 6-7$ rats/dose). *, **: Significantly different from vehicle-treated controls, 2-way ANOVA followed by Newman-Keuls' *post-hoc* test, $p < 0.05$, 0.01 .

Figure 6.

Comparison of naïve P versus naïve NP rats on test day 2 in the modified FST for 5 min following oral vehicle administration (**A**) in Study 4. Results represent the mean time $\pm$ SEM in seconds on immobility and active behaviors (e.g., climbing, swimming) ($n = 10\text{--}11$ rats/genotype). **: Significantly different between genotype, 1-way ANOVA followed by Neuman-Keuls' *post-hoc test*, $p < 0.01$. (**B**) Dose-response curve for oral DOV (12.5–50 mg/kg) given 25 min prior to evaluation compared with the positive control, imipramine (25 mg/kg) in Study 4. *, **: Significantly different from vehicle treatment group, 1-way

ANOVA followed by Neuman-Keuls' *post-hoc* test, $p < 0.05$, 0.01 , respectively ($n = 5-10$ rats/treatment group).

Figure 7.

DOV concentrations after a single oral dose of 20 mg/kg. (A) Mean plasma and brain concentrations of DOV in male rats ($n = 3/\text{timepoint}$; total $N = 21$). (B) Relative concentrations of DOV lactam in plasma and brain. Peak areas of the lactam in the plasma and brain were compared, taking into account the homogenization volumes ($n = 3/\text{timepoint}$; Total $N = 21$).

Table 1

Dose response of DOV 102,677 on alcohol or sucrose responding at the 25 min post-injection interval

<i>Study 1</i>	
A.	
<u>10% (v/v) Alcohol</u>	
Between Group Design	
<u>Dose</u>	<u>(n)</u>
Vehicle	8
1.565 mg	8
3.125 mg	8
6.25 mg	10
12.5 mg	7
25 mg	7
50 mg	9
Total N =	57
B.	
<u>3% (w/v) Sucrose</u>	
Between Group Design	
<u>Dose</u>	<u>(n)</u>
Vehicle	6

6.25 mg	5
12.5 mg	5
25 mg	5
50 mg	7
Total N =	28
C.	
<u>2% (w/v) Sucrose</u>	
Repeated-Measure Design	
<u>Dose</u>	<u>(n)</u>
Vehicle	14
6.25 mg	14
25 mg	14
50 mg	14
Total N =	14
----not tested	

Table 2

Time course effects of DOV 102,677 (6.25–25 mg/kg) on alcohol-and sucrose-maintained responding

<i>Study 2</i>	
A.	
<u>Alcohol</u>	
Repeated-Measure Design	
<u>Dose</u>	<u>Interval</u> <u>(n)</u>
Vehicle	0 min 10
6.25 mg	25 min 10
6.25 mg	Day 1 10
6.25 mg	Day 2 10
Total N = 10	
B.	
<u>Alcohol</u>	
Repeated-Measure Design	
<u>Dose</u>	<u>Interval</u> <u>(n)</u>
Vehicle	0 min 7
12.5 mg	25 min 7
12.5 mg	Day 1 7
12.5 mg	Day 2 7
Total N = 7	
C.	
<u>Alcohol</u>	
Repeated-Measure Design	
<u>Dose</u>	<u>Interval</u> <u>(n)</u>
Vehicle	0 min 7
25 mg	25 min 7
25 mg	Day 1 7
25 mg	Day 2 7

C.			
Alcohol			
Repeated-Measure Design			
Dose	Interval	(n)	
Total N =			7
D.			
Sucrose			
Repeated-Measure Design			
Dose	Interval	(n)	
Vehicle	0 min	5	
6.25 mg	25 min	5	
6.25 mg	Day 1	5	
6.25 mg	Day 2	5	
Total N =			5
E.			
Sucrose			
Repeated-Measure Design			
Dose	Interval	(n)	
Vehicle	0 min	5	
12.5 mg	25 min	5	
12.5 mg	Day 1	5	
12.5 mg	Day 2	5	
Total N =			5
F.			
Sucrose			
Repeated-Measure Design			
Dose	Interval	(n)	
Vehicle	0 min	5	
25 mg	25 min	5	
25 mg	Day 1	5	

Sucrose

Repeated-Measure Design

<u>Dose</u>	<u>Interval</u>	<u>(n)</u>
25 mg	Day 2	5
Total N =		5

Time course effects of DOV 102,677 (50 mg/kg) on alcohol-maintained responding

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Vehicle and Alcohol Groups

Mixed Design[†]

<u>Dose</u>	<u>Time</u>	<u>(n)</u>	<u>Dose</u>	<u>Time</u>	<u>(n)</u>
Vehicle	0 min	12	Vehicle	0 min	10
Vehicle	25 min	12	50 mg	25 min	10
Vehicle	Day 1	12	50 mg	Day 1	10
Vehicle	Day 2	12	50 mg	Day 2	10
Vehicle	Day 3	12	50 mg	Day 3	10
Vehicle	Day 4	12	50 mg	Day 4	10
Vehicle	Day 5	12	50 mg	Day 5	10
Vehicle	Day 6	12	50 mg	Day 6	10
Vehicle	Day 7	12	50 mg	Day 7	10
Vehicle	Day 8	12	50 mg	Day 8	10
Total N =		12	Total N =		10

Time course effects of DOV 102,677 (50 mg/kg) on sucrose-maintained responding

H

Sucrose

Repeated-Measure Design

Dose	Time	(n)
Vehicle	0 min	5
50 mg	25 min	5
50 mg	Day 1	5
50 mg	Day 2	5

Time course effects of DOV 102,677 (50 mg/kg) on sucrose-maintained responding

H.

Sucrose		
Repeated-Measure Design		
Dose	Time	(n)
50 mg	Day 3	5
50 mg	Day 4	5
50 mg	Day 5	5
50 mg	Day 6	5
50 mg	Day 7	5
50 mg	Day 8	5
Total N =		5

[†]To evaluate the time course effects of the vehicle and DOV treatment across the post-administration intervals a mixed ANOVA was conducted for group X post-administration treatment days for alcohol responding.

Table 3

Time course of DOV 102,677 on alcohol and sucrose-maintained responding given 24 h prior to placing animals in the operant chamber

<i>Study 3</i>		
A.		
	Alcohol Mixed Design	
Dose	Interval	(n)
6.25 mg	PI, Days 1, 2, 3, 4	7
12.5 mg	PI, Days 1, 2, 3, 4	7
25 mg	PI, Days 1, 2, 3, 4	7
50 mg	PI, Days 1, 2, 3, 4	7
Total N =		28
The mixed design for alcohol comprised a group [6.25, 12.5, 25, 50 mg] X treatment day [PI, Days 1, 2, 3, and 4] design, with the treatment day being the repeating factor.		
B.		
	Sucrose Mixed Design	
Dose	Interval	(n)
6.25mg	PI, Days 1, 2, 3, 4	6
50 mg	PI, Days 1, 2, 3, 4	6
Total N =		12
The mixed design for sucrose comprised a group [6.25, 50 mg] X treatment day [PI, Days 1, 2, 3, and 4] design, with the treatment day being the repeating factor.		

PI = Pretreatment interval. Animals in each group were gavaged with deionized water 24 h prior to the DOV 102, 677 treatment to assure that residual effects of a prior treatment had dissipated, and to confirm similarity in baseline prior to comparison across days. *The time course effects of the DOV treatment given 24 h before placing in the operant chamber were evaluated in a mixed ANOVA design comprising a group X post-administration treatment day analysis for alcohol and sucrose responding.*

Table 4

<i>Study 4</i>		
4A. Innate evaluation of P and NP rats in the forced swim test (FST)		
Between-Group Design		
<u>Rat line</u>	<u>Dose</u>	<u>(n)</u>
P	Vehicle	11
NP	Vehicle	10
Total N =		21
4B. Effects of DOV 102,677 and imipramine on P rat performance in the FST		
Between Group Design		
<u>Dose</u>		<u>(n)</u>
Vehicle		11
12.5 mg	DOV 102 677	6
25 mg	DOV 102 677	6
50 mg	DOV 102 677	5
25 mg	Imipramine	5
Total N =		33

Table 5

Pharmacokinetics of DOV 102,677

<i>Study 5</i>		
<u>Rat line</u>	<u>Interval</u>	<u>(n)</u>
Sprague-Dawley	0, 1, 2, 4, 6, 8, 12	3 rats at each time point
Total N =		21