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Oral and transdermal DL-methylphenidate–ethanol interactions in C57BL/6J mice: Potentiation of locomotor activity with oral delivery

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ABSTRACT

Purpose: Many abusers of DL-methylphenidate co-abuse ethanol. The present animal study examined behavioral effects of oral or transdermal DL-methylphenidate in combination with a high, depressive dose of ethanol to model co-abuse.
Methods: Locomotor activity of C57BL/6J mice was recorded for 3 h following dosing with either oral DL-methylphenidate (7.5 mg/kg) or transdermal DL-methylphenidate (Daytrana®;1/4 of a 12.5 cm^2 patch; mean dose 7.5 mg/kg), with or without oral ethanol (3 g/kg). Brains were enantiospecifically analyzed for the isomers of methylphenidate and the transesterification metabolite ethylphenidate.
Results: An otherwise depressive dose of ethanol significantly potentiated oral DL-methylphenidate induced increases in total distance traveled for the first 100 min ($p<0.05$). Transdermal DL-methylphenidate increased total distance traveled after a latency of 80 min, though this effect was not potentiated by concomitant ethanol. Mean 3 h brain D-methylphenidate concentrations were significantly elevated by ethanol in both the oral (65% increase) and transdermal (88% increase) groups. The corresponding L-ethylphenidate concentrations were 10 ng/g and 130 ng/g.
Conclusions: Stimulant induced motor activity in rodents may correlate with abuse liability. Potentiation of DL-methylphenidate motor effects by concomitant ethanol carries implications regarding increased abuse potential of DL-methylphenidate when combined with ethanol.

1. Introduction

The persistent of attention-deficit/hyperactivity disorder (ADHD) into adulthood has been increasingly recognized over the past few decades (Kessler et al., 2005; Okie, 2006). In a survey, 92% of adult ADHD patients prescribed DL-methylphenidate (MPH) reported concomitant use of ethanol. Further, 100% of individuals who obtained DL-MPH through diversion co-abuse ethanol (Darredeau et al., 2007). The abuse potential of the DL-MPH–ethanol combination is well known in the clinical literature (Jaffe, 1991; Barrett and Pihl, 2002; Teter et al., 2003).

Co-administration of DL-MPH and ethanol results in pharmacokinetic and pharmacodynamic drug–drug interactions in humans (Patrick et al., 2007) and in C57BL/6J (C57) mice (Griffin et al., 2010; Bell et al., 2011). Ethanol elevates biological concentrations of the pharmacologically active D-MPH isomer and yields the metabolic transesterification product ethylphenidate (EPH) (Patrick et al., 2007; Bell et al., 2011). EPH appears to be formed through the actions of carboxylesterase 1 (CES1) (Bourland et al., 1997; Patrick et al., 2007) which exhibits L-MPH substrate enantioselectivity in both the metabolic transesterification and de-esterification pathways (Sun et al., 2004; Williard et al., 2007; Zhu et al., 2008) (Fig. 1). Accordingly, the mean absolute oral bioavailability of L-MPH is limited to only 1–3% compared to approximately 30% for D-MPH (Srinivas et al., 1993). However, dosing with transdermal DL-MPH (Daytrana®) avoids the extensive oral presystemic metabolism and leads to approximately 50 times more L-MPH reaching the systemic circulation when compared with oral dosing (Patrick et al., 2009).

The pharmacological significance of dosing route dependent alterations in the relative bioavailability of D-MPH versus L-MPH was investigated in the present study using a C57 mouse model in the context of ethanol interactions. The C57 mouse has served as a common reference strain in pre-clinical investigations of psychotropic agents, including the study of DL-MPH–ethanol interactions (Williard et al., 2007; Griffin et al., 2010; Bell et al., 2011), as well as for the behavioral characterization of EPH enantiomers (Patrick et al., 2005; Williard et al., 2007) . As with humans, C57 mice enantioselectively transesterify L-MPH to L-EPH (Williard et al., 2007; Bell et al., 2011) (Fig. 1) as well as exhibit a biphasic excitatory-to-depressant activity

Abbreviations: ADHD, Attention-Deficit/Hyperactivity Disorder; C57, C57BL/6J; CES1, Carboxylesterase 1; dH_20, Deionized water; EPH, Ethylphenidate; i.p., intraperitoneal; MPH, Methylphenidate.

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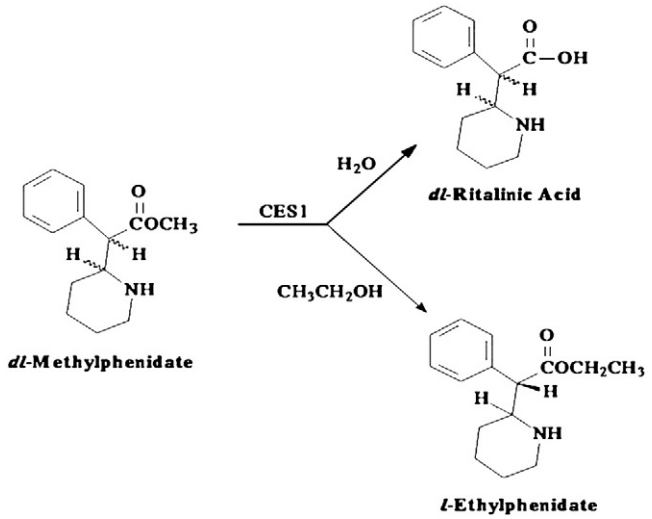

Fig. 1. Enantioselective de-esterification of DL-MPH to ritalinic acid (top right) and transestification to L-ethylphenidate. From Patrick et al., 2007.

profile in response to increasing doses of ethanol (Phillips and Shen, 1996).

A relatively low intraperitoneal (i.p.) dose of ethanol (1.75 g/kg) has been shown to elevate motor activity for 10–15 min in C57 mice (Griffin et al., 2010). However, when this dose of ethanol was combined with a sub-stimulatory dose of DL-MPH (1.25 mg/kg, i.p.), a potentiation of ethanol induced motor activity occurs. As an extension of this low dose DL-MPH–ethanol behavioral study (Griffin et al., 2010), and a C57 mouse dispositional investigation where ethanol was found to elevate blood, brain and urinary D-MPH (Bell et al., 2011), the following investigation examined the pharmacology of a high, otherwise motor depressive dose of ethanol, combined with a high stimulant dose of oral or transdermal DL-MPH. Locomotor activity counts were acquired for 3 h followed by enantiospecific MPH and EPH brain analysis.

The influence of ethanol on the stimulant effects of DL-MPH carries special abuse potential and adverse event liability for patients prescribed DL-MPH to treat ADHD, as well as for individuals obtaining DL-MPH through diversion.

2. Materials and methods

2.1. Materials

Ethanol was from AAPER Alcohol and Chemical Co. (Shelbyville, KY; 95%). DL-MPH·HCl used for oral animal studies was from Sigma-Aldrich (St. Louis, MO; lot # 118K1052) and 12.5 cm^2 transdermal DL-MPH patches (Daytrana®) were from Shire US (Wayne, PA; lot # 2616811; smallest of 4 sizes available). Laboratory tape used to secure transdermal DL-MPH or placebo patch (cut Band-Aid® adhesive which closely resembles the texture, adhesion and thickness of the DL-MPH patch) was from VWR International (white, 12.7 mm). DL-MPH·HCl in methanol (1 mg/mL calculated as free base; Cerilliant, Round Rock, TX) and DL-EPH·HCl in ethanol (1 mg/mL calculated as free base, synthesized in-house (Patrick et al., 2005)) were used as the analytical reference standards. Sodium carbonate (Fischer Scientific, Fair Lawn, NJ), n-butyl chloride (Burdick & Jackson, Muskegon, MI), acetonitrile (Mallinckrodt Inc, Paris, KT), (*S*)-N-(trifluoroacetyl)prolyl choride in dichloromethane (1 M; Sigma-Aldrich, St Louis, MO), were used for extraction and chiral derivatization. Piperidine deuterated DL-MPH·HCl was synthesized in-house (Patrick et al., 1982) and contained approximately 25% of the D_5-isotopolog for SIM monitoring and containing no $D_{0\text{-}1}$-MPH.

2.2. Animals

Male C57 mice aged 8–10 weeks (25–35 g) were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). They were individually housed in a temperature and humidity controlled colony room on a 12 h light/dark cycle (light: 07.00–19.00 h) with free access to food and water. All experiments were approved by and conducted within the guidelines of the Institutional Animal Care and Use Committee at the Medical University of South Carolina and followed the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication no. 80–23, revised 1996).

2.3. Locomotor activity and analysis

2.3.1. Apparatus

Motor activity was assessed with a Digiscan Animal Activity Monitor system, model RXYZCM(8) TAO with a two-animal option (Omnitech Electronics, Columbus, Ohio, USA). Each activity chamber contained 2 arrays of 16 photo beams spaced 5 cm apart, with eight beams located on the x-axis and eight on the y-axis. One array was located 1.5 cm above floor level to capture horizontal activity and the other was located 6.5 cm above the floor to capture vertical activity of the mice. Stereotypic counts were recorded when the same beam was repeatedly interrupted. Photocells were activated when the photo beams on the wall directly opposite to the cells were interrupted. The Versadat analyzer (Version 2.70-137E) recorded the interruption of each beam and provided the total distance (cm) and vertical activity for each animal during testing. Each activity chamber was partitioned into 20×20 cm quadrants with acrylic dividers to allow simultaneous testing of two mice. Four activity chambers allowed testing of eight mice per session. Each of the activity chambers were enclosed in 90×54×35 cm sound-attenuated boxes.

2.3.2. Procedures for locomotor activity assessment

On days 1–3, mice were habituated to the motor activity apparatus for 30 min. On day 4 mice were lightly anesthetized with 5% isofluorane for 8–10 min. The hair was clipped with an electric shaver along the abdomen and back, from shoulders to hips. A placebo patch was placed on the lower left hip and secured by laboratory tape over the patch and around the mouse for one full loop to ensure a constant skin interface and to prevent the mice from disturbing the patch. Mice were then gavaged at a volume of 0.02 mL/g body weight with deionized water (dH_20) and placed in the open-field activity chambers for 3 h. On Day 5, mice were randomly placed into 1 of 6 test groups (all with n = 8): placebo patch + dH_20, placebo patch + ethanol, placebo patch + oral DL-MPH + dH_20, placebo patch + oral DL-MPH + ethanol, transdermal DL-MPH + dH_20, or transdermal DL-MPH + ethanol. Oral DL-MPH was dosed as the HCl salt using 7.5 mg/kg calculated as the free base. This dose was the mean dose absorbed by 1/4 patch as established by drug load difference between an unused versus used mouse patch study (Bell et al., 2011). Each animal was anesthetized and either a placebo patch or 1/4 of 12 cm^2 transdermal patch was placed around the midsection in the same manner as day 4. They were gavaged at a volume of 0.02 mL/g body weight with either dH_20 or ethanol (3.0 g/kg) and placed in the activity apparatus for 3 h. Following the conclusion of the locomotor activity session, animals were sacrificed and brain samples collected.

The order of treatment groups within each week and the particular test chamber used to test the different groups were counterbalanced across the entire experiment to eliminate any contribution of possible differences in activity monitors or days of testing to observed effects on motor activity. Total distance and vertical activity were recorded in 5-min bins for the entire 3 h session.

2.3.3. Locomotor activity data analysis

Locomotor activity data in Figs. 2 and 3 was grouped into 20 bins and analyzed using a mixed factor three-way analysis of variance (ANOVA). Oral DL-MPH data and transdermal DL-MPH data were analyzed separately using a 2(DL-MPH dose)×2(ethanol dose)×9(TimeBin) design. The between groups factors are DL-MPH (dH_20 vs. active dose) and ethanol (dH_20 vs. active dose). The repeated measure is TimeBin. When appropriate, post-hoc comparisons of significant main effects or factor interactions were made using pair-wise comparisons with Bonferroni's correction. Statistical analysis was conducted using PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA).

2.4. Brain MPH and EPH analysis

Enantiospecific analysis of D-, L-MPH and D-, L-EPH was conducted as previously described in a recent DL-MPH–ethanol disposition report (Bell et al., 2011). Briefly, homogenized and alkalinized 1/2 brains were solvent extracted and after chiral derivatization, the samples were injected into a gas chromatograph–mass spectrometer fit with a 5% phenylmethylpolysiloxane column. The trifluoroprolylpiperidyl electron impact fragment ions from analytes and the deuterated DL-MPH internal standard were acquired using selected ion monitoring. A range of spiked blank brain calibrators bracketed all concentrations reported as established by linear regression analysis (r^2>0.99).

2.4.1. Brain concentration data analysis

A two way analysis of variance (ANOVA) followed by pair wise comparisons using the Student's *t*-test method was used in the analysis of all data. Samples were analyzed as independent samples and were assumed to have equal variances. Statistical analysis was conducted using SPSS 12.0 (SPSS I.; Chicago, Illinois, USA).

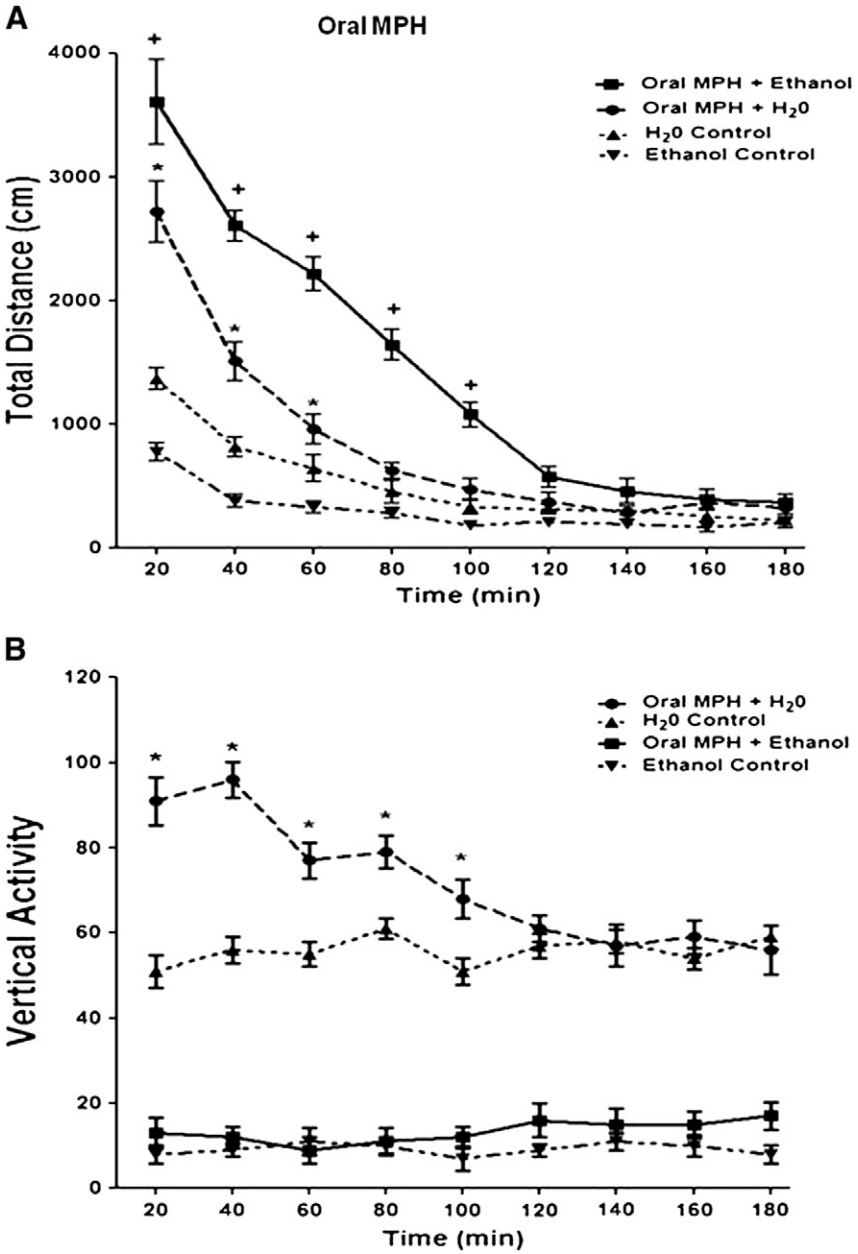

Fig. 2. (A) Oral DL-MPH + dH_20 significantly increased total distance traveled (*, p<0.05) and this effect was potentiated by an otherwise depressive dose of ethanol (+, p<0.05). (B) Oral DL-MPH significantly increased vertical activity of mice over the first 100 min (*, p<0.05).

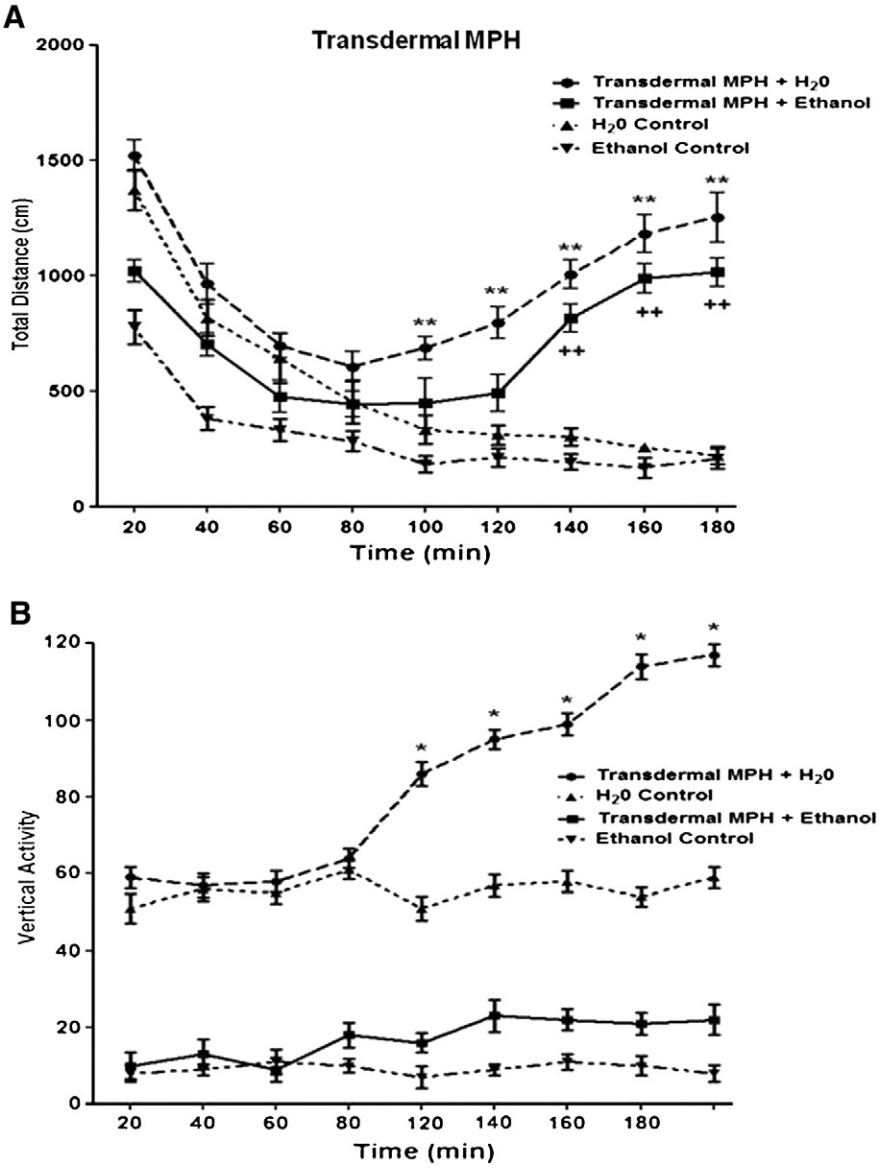

Fig. 3. (A) Transdermal DL-MPH induced locomotor activity after a lag phase of 100 min (**, $p<0.01$). While this effect was not potentiated by ethanol, transdermal DL-MPH + ethanol activity was significantly greater than placebo patch + dH_20 after a lag phase of 140 min (++, $p<0.01$). (B) Transdermal DL-MPH significantly increased vertical activity of mice after 100 min lag time (*, $p<0.05$).

3. Results

3.1. Controls

Mice treated with placebo patches + ethanol (3.0 g/kg) showed significantly less total distance traveled compared to mice treated with placebo patch + dH_20 over the first 100 min (all $p<0.05$) and significantly less vertical activity for the entire 3 h (all $p<0.01$).

3.2. Total distance traveled

3.2.1. Oral DL-MPH

The total distance traveled data was analyzed by examining changes in horizontal activity across time for the different treatment groups and is summarized in Fig. 2A. A significant 3 way interaction was found ($F(8,224)=10.906$, $p<0.001$). Post-hoc analysis indicated a significant increase in total distance traveled for oral DL-MPH + dH_20 compared to the placebo patch + dH_20 for the first 1 h (all $p<0.05$). Further, total distance traveled for the oral DL-MPH + ethanol group was significantly greater than oral DL-MPH + dH_20 group over the first 100 min (all $p<0.5$).

3.2.2. Transdermal DL-MPH

The total distance traveled data was analyzed by examining changes in horizontal activity across time for the different treatment groups and are summarized in Fig. 3A. The 3 way interaction was not significant for the transdermal DL-MPH group. However, the lower level 2 way interactions were significant for TimeBin vs. ethanol ($F(8,224)=5.27$, $p<0.001$) and TimeBin vs. DL-MPH ($F(8,224)=28.07$, $p<0.001$). Post hoc analysis indicated a significant increase in total distance traveled for the transdermal DL-MPH + dH_20 group compared to the placebo patch + dH_20 group over the 100–180 min time period (all $p<0.01$).

3.3. Vertical activity

3.3.1. Oral DL-MPH

The vertical activity data was analyzed by examining changes in activity across time for the different treatment groups and is summarized

in Fig. 2B. A significant 3 way interaction was found ($F(8,224) = 207.747$, $p<0.001$). Post-hoc analysis indicated a significant increase in vertical activity for oral DL-MPH + dH_20 compared to the placebo patch + dH_20 for the first 100 min (all $p<0.05$). Vertical activity data for mice dosed with oral DL-MPH + ethanol and mice dosed with placebo patch + ethanol were significantly decreased compared to the placebo patch + dH_20 and oral DL-MPH + dH_20 for the entire 3 h (all $p<0.001$).

3.3.2. Transdermal DL-MPH

The vertical activity data was analyzed by examining changes in activity across time for the different treatment groups and is summarized in Fig. 3B. A significant 3 way interaction was found ($F(8,224) = 34.935$, $p<0.001$). Post-hoc analysis indicated a significant increase in vertical activity for transdermal DL-MPH + dH_20 compared to the placebo patch + dH_20 100–180 min (all $p<0.01$). Vertical activity data for mice dosed with transdermal DL-MPH + ethanol and mice treated with placebo patch + ethanol were significantly decreased compared to placebo the patch + dH_20 and transdermal DL-MPH + dH_20 groups for the entire 3 h (all $p<0.001$).

3.4. Brain drug and metabolite concentrations

3.4.1. Oral DL-MPH

The brain concentration of D-MPH following oral DL-MPH was significantly greater in the animals dosed with ethanol compared to those given dH_20; increasing from 31 ng/g to 51 ng/g (Fig. 4A; $t = 3.92$, $df = 14$, $p<0.001$). Further, in animals dosed with oral DL-MPH, concentrations of L-MPH were significantly increased from 33 ng/g for animals dosed with dH_20 to 42 ng/g for animals dosed with concomitant ethanol (Fig. 4A; $t = 2.24$, $df = 14$, $p<0.05$). There were no significant differences between the brain concentrations of D-MPH and L-MPH in animals dosed with dH_20 or in animals dosed with ethanol. Only the L-isomer of EPH was detected in animals gavaged with ethanol and was found at a concentration of 10 ng/g (Fig. 4A).

3.4.2. Transdermal DL-MPH

The brain concentration of D-MPH after transdermal dosing was significantly greater in animals dosed with ethanol compared the dH_20 group; increasing from 689 ng/g to 1294 ng/g (Fig. 4B; $t = 7.38$, $df = 14$, $p<0.001$). Further, in animals dosed with transdermal DL-MPH, concentrations of L-MPH were significantly increased by ethanol, rising from 685 ng/g for animals dosed with dH_20 to 1210 ng/g for animals dosed with ethanol (Fig. 4B; $t = 7.689$, $df = 14$, $p<0.001$). There were no significant differences between the brain concentration of D-MPH and L-MPH in animals dosed with dH_20. Only the L-isomer of EPH was detected in animals gavaged with ethanol and was found at a mean concentration of 130 ng/g (Fig. 4B).

4. Discussion

Most oral DL-MPH abusers co-abuse ethanol (Darredeau et al., 2007) and the abuse potential of the DL-MPH–ethanol combination is well known in the clinical literature (Jaffe, 1991; Barrett and Pihl, 2002; Teter et al., 2003). In the present study, C57 mice were used to model pharmacological characteristics of this drug combination to gain insight into the special appeal this drug combination has. The abuse potential of the new transdermal DL-MPH formulation has not been investigated in detail at this time. In industry trials, transdermal DL-MPH been has reported to produce mild euphoria upon application of 3 or 6 of the 25 cm^2 patches. With the 6-patch application group dysphoria was reported in 42% of the test subjects. It is noted that the FDA requested human testing by the contraindicated application of the patch to buccal mucosa. This tissue surface greatly

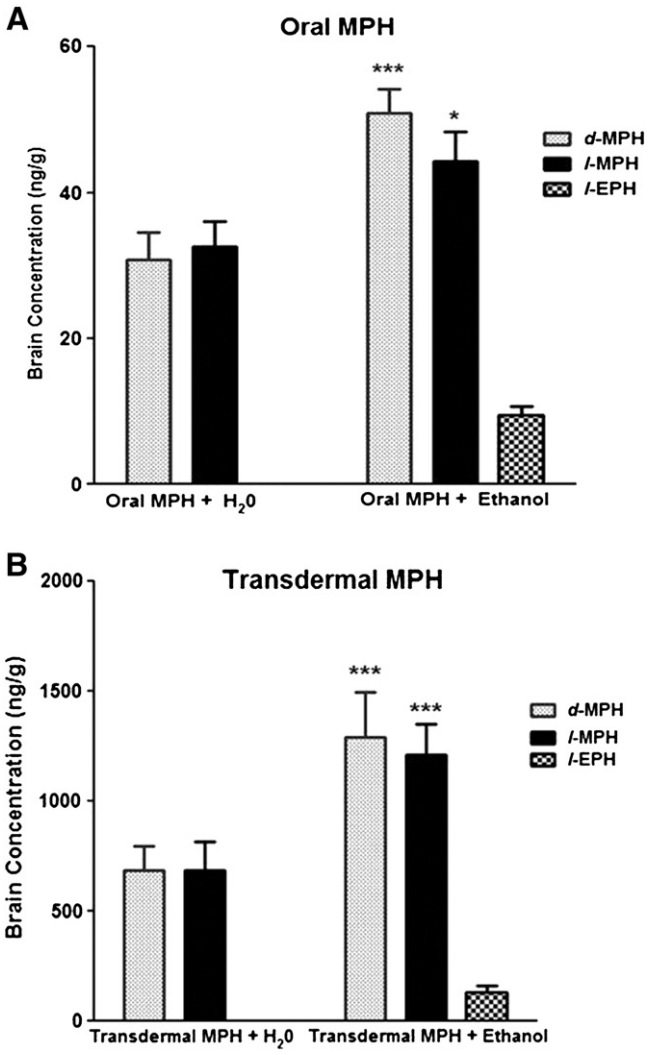

Fig. 4. (A and B) Ethanol significantly increased brain concentrations of D-MPH and L-MPH relative to dH_20 in mice dosed orally or transdermally with DL-MPH (*, $p<0.05$; ***, $p<0.001$).

accelerated DL-MPH absorption relative to the normal hip application. Rather than the mean 36% of the patch DL-MPH content being absorbed during the recommended 9 h wear, 50% was absorbed in 2 h attached in the mouth (see Patrick et al., 2009).

We have previously reported that a sub-stimulatory i.p. dose of DL-MPH in C57 mice potentiates the motor stimulation produced by a low dose of ethanol (Griffin et al., 2010). The present study used stimulatory oral or transdermal doses of DL-MPH (7.5 mg/kg), with or without a depressive dose of ethanol (3 g/kg), to model dosing route dependent behavioral and dispositional drug interactions as may pertain to both the treatment of adult ADHD patients who use or abuse ethanol and the co-abuse pharmacology of diverted DL-MPH and ethanol.

The findings in the present study demonstrate that even a depressive dose of ethanol potentiates a stimulatory dose of oral DL-MPH. This potentiation may result from both pharmacokinetic and pharmacodynamic interactions. The 7.5 mg/kg doses of DL-MPH used in the present study approaches the highest daily doses found in the medical drug abuse literature, e.g., approximately 10 mg/kg/day intranasally (Coetzee et al., 2002) or 29 mg/kg/day intravenously (AtLee, 1972) based on 60 kg total body weight (actual weights were not reported). While these doses were reported as total daily doses and the dosing "regimen" undisclosed, the intranasal route and certainly the intravenous routes are expected to result in higher bioavailability than following oral dosing. For instance, only 19% of an oral DL-MPH

dose reaches the systemic circulation in rats (Wargin et al., 1983) versus approximately 30% in humans (Chan et al., 1983). Further, a transdermal dose of DL-MPH is absorbed in a prolonged fashion analogous to a multiple dose regimen as the abusers above were likely to have used.

When comparing animal doses for potential human extrapolation, body surface area normalization approaches have attempted to indirectly compensate for interspecies physiological differences such as basal metabolic rates, blood volumes and other brain/body relationships. Accordingly, body surface area calculations show a 7.5 mg/kg dose of DL-MPH in mice extrapolating to a 0.6 mg/kg "equivalent dose" for a 60 kg adult (Reagan-Shaw et al., 2008). This would be well within the clinical guidelines for DL-MPH mg/kg dosing (Greenhill et al., 1996). In a previous DL-MPH–ethanol disposition study using C57 mice and the same dosing regimens as in the present study, the mean D-MPH blood concentrations at time of sacrifice were 18 ng/mL 3 h after the oral DL-MPH bolus dose, compared to 360 ng/mL following a 3.25 h transdermal patch wear time. The disparity between the oral versus transdermal D-MPH blood concentrations may be based on the rapid clearance of the oral DL-MPH bolus dose and the expected steady increase in blood concentrations of D-MPH when administered by the transdermal route. In humans, a 0.3 mg/kg oral dose of DL-MPH typically attains a mean maximum D-MPH plasma concentration of 15 ng/mL in 2 h while a comparable maximum plasma D-MPH concentration occurs at 9 h for transdermal DL-MPH following the recommended 9 h wear time (see Patrick et al., 2009).

The ethanol dose of 3 g/kg used in this study corresponds to 10 oz. of 80% vodka in a 70 kg human, well within the range of ethanol consumption associated with binging. The choice of a 3 g/kg dose allowed comparisons with the urinary metabolites, blood and brain concentrations of DL-MPH and DL-EPH found in the previous metabolism study (Bell et al., 2011). Further, this dose allowed us to indirectly gauge probable concentrations of ethanol over the course of the present behavioral studies through comparison with literature values in C57 mice. Haseba et al. (2007) found the peak ethanol concentration to be 322 mg% at 0.5 h, declining to approximately 35 mg% 3 h after oral dosing.

Ethanol has been shown to significantly increase the maximum plasma concentration and total exposure to D-MPH and L-MPH in humans (Patrick et al., 2007), as well as elevate D-MPH and L-MPH blood, brain and urine concentrations in the C57 mouse (Bell et al., 2011). The present brain D-MPH, L-MPH and L-EPH determinations are concordant with our earlier reported brain concentrations (Bell et al., 2011). At the 3 h sacrifice time, the mean D-MPH brain concentration was 23 times higher in the transdermal group than in the oral dosing group without ethanol. Upon co-administration of ethanol, there was an 88% elevation of D-MPH in the transdermal group and 66% elevation following oral DL-MPH at the 3 h time point.

At the pharmacodynamic level, the potentiation of DL-MPH induced behavioral effects may be based on the mutual influence of these drugs on dopamine, i.e., both D-MPH and ethanol have been reported to elevate synaptic dopamine levels. The therapeutic activity of the stimulant DL-MPH in the treatment of ADHD prominently involves the reuptake blockade of impulse released dopamine through binding to the dopamine transporter (Volkow et al., 1998). In our animal model of DL-MPH–ethanol co-abuse, the potentiation of stimulatory effects by concomitant ethanol is consistent with evidence that ethanol releases pre-synaptic dopamine (Tang et al., 2003; Ramachandra et al., 2007) as a consequence of upstream GABAergic modulation (Theile et al., 2011). Hence ethanol may be increasing extracellular dopamine release, whereby D-MPH then blocks a larger dopamine pool from presynaptic reuptake. Further, ethanol-mediated dopamine release significantly increases as the ethanol dose (i.p.) is escalated from 1 g/kg to 2 g/kg to 3 g/kg in C57 mice (Ramachandra et al., 2007); the 3 g/kg of ethanol corresponding to the oral dose used in the present study. In this context, we hypothesize that the increased locomotor activity resulting from concomitant oral DL-MPH and an otherwise depressive dose of ethanol may reflect a synergistic increase in synaptic dopamine, modeling the accentuation of likeability associated with DL-MPH combined with ethanol when compared to DL-MPH alone (Patrick et al., 2007).

In humans, the earliest detection of either MPH isomer in plasma after transdermal DL-MPH application ranges from 1 to 6 h (Shire, 2006 Revised 6/2010), unlike oral DL-MPH which is readily detectable within 30 min or less (Modi et al., 2000; Zhu et al., 2011). The significantly lower total distance traveled of mice dosed with transdermal DL-MPH compared to oral DL-MPH is likely influenced by a lag phase (latency) in transdermal drug absorption during which time locomotor activity of mice inherently decreases as habituation to the activity chamber occurs. We report here that the lag phase between application of the transdermal DL-MPH patch and the onset of pronounced drug-induced motor activity is approximately 100 min in C57 mice. Studies in C57 mice have shown that D- and DL-MPH produce dose-related increases in motor activity, while the L-isomer produces little or no stimulatory effects (Williard et al., 2007). Thus, it is hypothesized that despite significantly higher 3 h brain concentrations of D-MPH following transdermal dosing, the total distance traveled of mice did not reach the same levels of early time points following oral dosing due in part to the mice habituating to the chambers and such low activity was not able to rebound to early activity levels found after oral dosing. A further explanation for the attenuated total distance traveled by the transdermal DL-MPH–ethanol group could pertain to the induction of stereotypic behaviors associated with such high brain D-MPH concentrations, especially in the concomitant ethanol group. This may be supported by the significantly higher vertical activity found at later time points following transdermal dosing. Further, the observation that the stimulant effect of transdermal DL-MPH was not potentiated by co-administration of ethanol may relate to the anticipated lag phase in transdermal drug absorption extending well into the elimination phase of ethanol (see Bell et al., 2011). It is noted that the mean elimination half-life of ethanol in C57 mice has been reported to be approximately 1.3 h to 1.5 h (Haseba et al., 2007; Ramachandra et al., 2007).

The 13-fold greater L-EPH concentration found in the transdermal DL-MPH–ethanol group relative to the oral DL-MPH–ethanol group is unlikely to directly contribute to the neuropharmacology of this drug combination in view of the inactivity of the L-isomer of EPH in vivo or in vitro (Patrick et al., 2005; Williard et al., 2007). However, the L-EPH concentration may indirectly gauge the extent to which DL-MPH and ethanol interact with CES1.

The presence of L-EPH in the C57 mouse brain samples offers further evidence that this transesterification metabolite can serve as a biomarker for concomitant DL-MPH–ethanol exposure (Markowitz et al., 1999). Most importantly, ethanol significantly potentiated oral DL-MPH induced stimulant effects and elevated the brain D-MPH concentrations in this C57 mouse model. These findings could carry implications for increased abuse liability when ethanol is combined with DL-MPH should this model generalize to humans.

Acknowledgments

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