Published in final edited form as:

J Pharm Sci. 2014 December ; 103(12): 3834–3842. doi:10.1002/jps.24202.

Ethylphenidate as a selective dopaminergic agonist and methylphenidate-ethanol transesterification biomarker

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Abstract

We review the pharmaceutical science of ethylphenidate (EPH) in the contexts of drug discovery; drug interactions; biomarker for *dl*-methylphenidate (MPH)-ethanol exposure; potentiation of *dl*-MPH abuse liability; contemporary “designer drug”; pertinence to the newer transdermal and chiral switch MPH formulations; as well as problematic internal standard. *d*-EPH selectively targets the dopamine transporter while *d*-MPH exhibits equipotent actions at dopamine and norepinephrine transporters. This selectivity carries implications for the advancement of tailored attention-deficit/hyperactivity disorder (ADHD) pharmacotherapy in the era of genome-based diagnostics. Abuse of *dl*-MPH often involves ethanol co-abuse. Carboxylesterase 1 enantioselectively transesterifies *l*-MPH with ethanol to yield *l*-EPH accompanied by significantly increased early exposure to *d*-MPH and rapid potentiation of euphoria. The pharmacokinetic component of this drug interaction can largely be avoided using dexmethylphenidate (dexMPH). This notwithstanding, *maximal* potentiated euphoria occurs following dexMPH-ethanol. C57BL/6 mice model *dl*-MPH-ethanol interactions: An otherwise depressive dose of ethanol synergistically increases *dl*-MPH stimulation; A sub-stimulatory dose of *dl*-MPH potentiates a low, stimulatory dose of ethanol; Ethanol elevates blood, brain and urinary *d*-MPH concentrations while forming *l*-EPH. Integration of EPH preclinical neuropharmacology with clinical studies of MPH-ethanol interactions provides a translational approach toward advancement of ADHD personalized medicine and management of comorbid alcohol use disorder.

Keywords

ethylphenidate; methylphenidate; ethanol; dexmethylphenidate; transesterification; drug interaction; pharmacokinetics/pharmacodynamics; metabolism; absorption; bioavailability

Introduction: Methylphenidate-ethanol misuse and co-abuse

The number of attention-deficit/hyperactivity disorder (ADHD) diagnoses has continued to increase in recent years.¹ The stimulant *dl*-methylphenidate (MPH) has long remained the

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most widely prescribed drug to treat ADHD. In adolescents, MPH prescriptions exceed those for all other drugs regardless of therapeutic class.² In addition, alcohol abuse in this age group is on the rise.³ Currently 15% of individuals in the USA ages 16-17 binge with ethanol and this figure increases to 45% by ages 21-25.⁴ The pattern of MPH misuse or abuse usually involves concomitant ethanol.⁵⁻⁷ Further, estimates of alcoholics with comorbid ADHD exceed 70%.⁸ MPH-ethanol misuse and co-abuse contributes to lower educational attainment, higher divorce rates, more arrests, long-term social/psychiatric problems and an increased need for emergency medical care.^{8,9} Ethanol interacts with MPH to elevate blood concentrations of the active *d*-MPH isomer in the course of enantioselectively forming the metabolite *l*-ethylphenidate (*l*-EPH; Fig 1). This pharmacokinetic drug interaction, in addition to compelling evidence of a pharmacodynamic component to MPH-ethanol interactions, results in potentiated stimulant effects and heightened abuse liability of MPH.^{10,11}

The present review chronicles the pharmaceutical literature pertaining to EPH: (1) as a selective dopaminergic agonist; (2) as a candidate agent for personalized ADHD pharmacotherapy in the emerging field of genome-based diagnostics; (3) as a biomarker of concomitant MPH-ethanol exposure; (4) as pertinent to the mechanisms by which ethanol intensifies the abuse liability of MPH; (5) as differentially formed by chiral switch and transdermal MPH formulations; (6) as a historically problematic bioanalytical internal standard; and (7) as a commercially available contemporary “designer drug”.

EPH neuropharmacology

EPH, or ritalinic acid ethyl ester, is the next higher ester homolog of *dl*-MPH, i.e., (2R:2'R, 2S:2'S)- α -phenyl-2-piperidineacetetic acid ethyl ester (Fig 1). It has been chemically characterized as the racemic hydrochloride salt^{12,13} and as its separate enantiomers.¹⁴ As with MPH¹⁵ all reported catecholaminergic activity of racemic EPH resides in the *d*-2R:2'R-isomer. However, the more selective neurochemical actions of EPH^{14,16}, and its greater resistance to metabolic hydrolysis¹⁷, distinguish EPH from MPH. These differences offer the potential for exploitation in psychotherapeutic drug discovery.

Central nervous system activity of EPH was first reported in 1961 when it was found to be 80% as potent as MPH in antagonizing reserpine-induced sedation in mice.¹² The significance of these findings may be of limited value in view of reserpine inhibiting vesicular monoamine transporters, an action which typically abolishes the response to indirect acting catecholaminergic agents such as MPH and EPH.¹⁸ MPH elevates extracellular concentrations of impulse-released dopamine (DA) and norepinephrine (NE). These effects occur through presynaptic transmitter reuptake inhibition at the dopamine transporter (DAT) and norepinephrine transporter (NET).¹⁶ In 1985, Schweri and associates reported that EPH was approximately 50% as potent as MPH in inhibiting tritiated MPH binding to rat striatal synaptosomes.¹⁹ The IC₅₀ values were 440 and 211 nM for EPH and MPH.

Renewed interest in developing MPH ester homologs as candidate therapeutic agents has been prompted by reports that the corresponding ethyl¹⁶ and isopropyl¹⁷ esters exhibit more

selective dopaminergic actions than noradrenergic actions when compared to MPH. These findings were based on experiments using DAT or NET transfected human embryonic kidney cells. Both *d*-MPH (23 nM) and *d*-EPH (27 nM) exhibited low nanomolar DAT IC₅₀ potencies. The DAT binding affinities (K_i) differed by 43%: 161 nM for *d*-MPH and 230 nM for *d*-EPH. However, a much more distinct difference in potencies between MPH and EPH became apparent at the level of the NET, where the IC₅₀ for *d*-MPH again exhibited high potency (39 nM), while *d*-EPH was 7 times less active (290 nM). In addition, the NET K_i values differed by 18-fold: 206 nM for *d*-MPH compared to 3,700 nM for *d*-EPH. These homologs were inactive at the serotonergic transporter.¹⁶

Comparisons of locomotor activity using the neuropharmacological reference strain C57BL/6 mouse demonstrated that both *d*-MPH and *d*-EPH were equipotent using intraperitoneal doses of 2.5 or 10 mg/kg.¹⁶ This range of doses allows comparisons with other literature studies of MPH rodent motor activity data.^{14,20} At the intermediate dose of 5 mg/kg, *d*-MPH induced approximately 25% more stimulatory activity than *d*-EPH;¹⁶ a difference in activity possibly reflecting the reduced influence of EPH on norepinephrine compared to dopamine. Both catecholaminergic systems appear to influence motor activity.²¹

EPH as a drug candidate

A broad range of candidate DNA polymorphisms have been implicated in the heterogeneous neuropathology of ADHD. Much of the genomic literature has focused on gene variants associated with dopaminergic or noradrenergic²²⁻²⁴ neural function as correlating with ADHD symptoms and drug response.²⁵ However, genes expressing products involved in dopaminergic neurobiology have factored most prominently in this literature.^{22,26-28} For instance, MPH efficacy in specific sub-populations of ADHD patients has been associated with gene mutations expressing the DAT. Variable number of tandem repeat DAT polymorphisms have emerged as key candidates for ADHD causation and predictors of gene-drug response to MPH. Increasing favorable responses reportedly are associated with the DAT 10/10 allele > 9/10 > 9/9.²⁸ In this context, development of a more selective DAT inhibitor than MPH, such as EPH, offers the potential to complement the ADHD pharmacological armamentarium, theoretically providing an unmet need in the drug individualization of ADHD patients. As the era of genome-based diagnostics advances through next-generation sequencing²⁹, the current trial-and-error approach to the selection optimal ADHD pharmacotherapy can be envisioned as giving way to rationally tailored selection of patient specific first-line treatments.

Genomic ADHD personalized medicine directed at identifying and ameliorating noradrenergic dysfunction has likewise progressed. Use of the NET selective reuptake inhibitor atomoxetine is contraindicated in ADHD patients who have established loss-of-function *CYP2D6* alleles (unless low dose titration is instituted)³⁰. But more to the point, gene-by-dose and gene-by-drug guidance based on ADHD etiology, rather than metabolic disposition, has begun to make inroads. Variants in genes expressing NET (*SLC6A2* alleles) or α -adrenergic receptors (e.g., *ADR2A*) have significantly been associated with both the incidence of ADHD and response to atomoxetine.^{31,32} In this context, it is noted that the

most recently approved drugs to treat ADHD, guanfacine and clonidine, both target α -adrenergic receptors.

The tailoring of psychotherapeutic drug selection through sculpting of relative monoamine reuptake receptor inhibition finds precedent in the drug individualization of major depression so essential in treatment refractory cases. The tertiary amine tricyclic antidepressants (TCA) were once widely used to target both NET and serotonin transporters (SERT) with the secondary amine TCAs exhibiting more selective for the NET. These early antidepressants have now largely been supplanted by: (a) the better tolerated serotonin selective reuptake inhibitors (SSRIs) fluoxetine/paroxetine/escitalopram; (b) the third generation dual acting (NET and SERT) antidepressants venlafacine/duloxetine; (c) the combined “noradrenalin” and specific serotonergic antidepressant (NaSSA) mirtazapine, and then coming full circle in 2013: (d) the more NET selective inhibitor levomilnacipran.

In a troubling development, however, EPH has recently appeared as a recreational drug of abuse. The legal status of this commercially available (internet) “designer drug” remains ambiguous.³³⁻³⁵ The Federal Analogue Act classifies a congener of an existing schedule I or II (MPH) controlled substances as illicit if the new compound exhibits chemical and pharmacological properties “substantially similar” to the parent controlled substance. The fact that both EPH and MPH are secondary amines, and that EPH can be trivially synthesized from MPH, satisfies two similarity criteria. A third criterion, demonstration that both EPH and MPH produce similar stimulant effects, may be more difficult to prove, especially were this question posed to a lay jury, or prosecutor alike, where the complexities of medicinal chemistry can be inscrutable.^{36,37}

Metabolic formation of EPH and its function as a biomarker

Cocaine serves as a precedent for the metabolic transesterification of ethanol with a drug containing a methyl ester to yield the corresponding ethyl ester: cocaethylene (ethylcocaine or benzoylecgonine ethyl ester).³⁸ This seemingly unlikely biotransformation requires carboxylesterase 1 (CES1) to extract two drugs from the circulation and covalently form a two-drug adduct. CES1 is a serine hydrolase which utilizes a serine hydroxyl group to sequentially acylate the methyl ester of a xenobiotic, followed by base (a histidyl imidazole) catalyzed ethanol transesterification of the acyl intermediate – a “ping-pong” mechanism.³⁹ The toxicological significance of cocaethylene formation is underscored by emergency department reports of plasma cocaethylene concentration, on occasion, exceeding the concentration of the parent drug.⁴⁰ The plasma half-life of cocaethylene is longer and the cardiac toxicity greater than that of cocaine.⁴¹

Cocaine and MPH share a common binding site on the DAT⁴², as well as share a common pharmacophore (ester, amine and phenyl ring) as evidenced by X-ray crystallography.⁴³ Thus, not surprisingly, these two stimulants both serve as substrates for CES1-mediated transesterification with ethanol. This was first confirmed in vitro by Bourland and co-workers (1997) when it was reported that MPH yields EPH upon incubation with deuterium-labeled ethanol in a rat liver homogenate.⁴⁴

Detection of EPH as a human metabolite of MPH and ethanol was first reported by Markowitz et al. (1999) after analyzing two sets of postmortem blood and liver samples obtained from suicides involving high dose MPH and ethanol (Fig 1).⁴⁵ The specific CES isoform involved in the transesterification was subsequently identified as CES1A1⁴⁶, an enzyme highly expressed in liver.⁴⁷ These human findings led to a pilot investigation to determine if EPH formation is pertinent to a clinically relevant dose of MPH and ethanol. Accordingly, a typical therapeutic dose of MPH (20 mg) was administered to six normal human volunteers followed by 0.6 g/kg of ethanol in orange juice. This dose of ethanol approximates a double vodka screwdriver in a 70 kg individual. EPH was detected in both blood and urine samples from all six subjects. This pilot study utilized an achiral chromatographic method and consequently could not address the potential for metabolically formed EPH directly contributing to the pharmacological response to concomitant MPH-ethanol.⁴⁸ Only the *d*-isomer of EPH would be expected to exhibit stimulant actions if the stereospecific pharmacodynamics of MPH generalize to EPH.¹⁵

The presence of this transesterification metabolite also demonstrated that EPH can function as a biomarker for clinical or forensic evidence of concomitant MPH-ethanol exposure.^{10,11,48,49} In the course of validating this utility, an authentic reference standard was synthesized and characterized^{14, 45}, then used for liquid chromatographic-mass spectrometric (LC-MS)^{10,11, 45-48} and gas chromatographic (GC)-MS determinations^{49, 50} from human biological samples. Analyte identification was based on: (a) the molecular specificity of the several MS detectors used in these studies; (b) the linearity of calibration plots from EPH-fortified biological matrices, as well as (c) the identical retention times for metabolically formed *l*-EPH and *d*-EPH compared those from both racemic and enantiomeric reference standards eluting from a range of achiral and chiral chromatographic columns. GC-MS studies have also been extended to animal studies of *dl*-MPH-ethanol metabolic interactions where enantioselective transesterification has again been demonstrated to preferentially form *l*-EPH^{16, 51,52}. In addition to the documented capacity of EPH to serve as a post-mortem toxicological biomarker⁴⁵, an emergency department case study of a non-lethal overdose of *dl*-MPH with wine, van Vulpen et al. (2006)⁵³ reported detection of EPH in the patient's serum. In addition, the discovery of a novel MPH poor metabolizer (*CES1* null allele) singularly fails to form EPH following *dl*-MPH-ethanol not only further demonstrates the role of *CES1* in generating this biomarker, but also offers a unique approach to phenotyping *CES1* null alleles using concomitant *dl*-MPH and ethanol as the probe substrates.⁴⁷

In addition to detecting the metabolite EPH in these 6 subjects, the mean maximum plasma concentration (C_{max}) of MPH was higher than mean C_{max} values reported in larger pharmacokinetic investigations.^{54,55} This preliminary finding raised the question of whether *CES1*-mediated transesterification of MPH with ethanol competitively inhibited hydrolysis of MPH to the inactive⁵⁶ amino acid metabolite ritalinic acid, resulting in elevated plasma *d*-MPH concentrations (Fig 1). It is noted that the facile *CES1*-mediated hydrolysis of MPH limits the oral bioavailability of MPH to approximately 30% for *d*-MPH and 1% for *l*-MPH.^{57,58} Further, rapid metabolic hydrolysis of *dl*-MPH is responsible for the short 2-3 h elimination half-life^{11,55} of *dl*-MPH and the high relative concentration of ritalinic acid in

plasma.⁵⁹ To explore the question of whether ethanol elevates plasma *dl*-MPH levels, more comprehensive studies of MPH-ethanol drug interactions were conducted in larger subject populations, and using enantiospecific analytical methods. Pharmacodynamic interactions were also investigated, including the recording of subjective effects using visual analog subscales designed as surrogates for abuse liability.⁶⁰⁻⁶²

In a normal subject randomized three-way crossover study design, 10 men and 10 women received MPH (0.3 mg/kg) administered 30 min *before* ethanol (0.6 g/kg), 30 min *after* ethanol, or *without* ethanol.¹⁰ The rationale for investigating potential influences of the MPH-ethanol dosing sequence was based on the importance of this parameter in humans administered cocaine and ethanol. Ethanol was reported to elevate plasma cocaine concentrations only when administered before cocaine.⁶³

The transesterification of MPH-ethanol yielded over 10 times more *l*-EPH than *d*-EPH as based on those select plasma samples where *d*-EPH was above the lower limit of quantitation (0.05 ng/ml). Accompanying the formation of *l*-EPH, ethanol significantly elevated the plasma *d*-MPH geometric mean C_{max} and area under the concentration-time curve (AUC) by approximately 40% and 25%, respectively. These values were not influenced by dosing sequence. While mean plasma *l*-MPH remained below 1 ng/ml in all three treatment groups, ethanol did increase *l*-MPH concentrations approximately 3-fold. Importantly, ethanol significantly increased the positive subjective effects of “feeling good” and “feeling high” when compared to MPH dosed alone.

A novel CES1 poor metabolizer was discovered in the course of this study. As consistent with the role of CES1 in mediating the ethanol transesterification pathway, no *l*-EPH was detectable in the plasma¹⁰ or urine⁵⁰ from this individual. Further, plasma *l*-MPH concentrations were approximately 100 times higher (60-70 ng/ml) than the mean values of the other 19 subjects, and unlike the normal metabolizers, *l*-MPH concentrations for this individual were not significantly influenced by ethanol. The *d*-MPH C_{max} concentrations in the poor metabolizer were elevated 2-fold over the mean values of the other subjects. These high drug concentrations correlated with significantly increased hemodynamic responses relative to the mean values of the other study subjects. Two *CES1* gene mutations were identified for this individual, one rare and leading to a loss-of-function protein product, the other reported to be found in 3.7%, 4.3%, 2.0%, and 0% in white, black, Hispanic, and Asian populations.⁴⁷ Genotyping or phenotyping⁵⁰ of CES1 offers the potential to improve ADHD drug individualization as pertains to initiation dose of MPH or drug selection.^{22,64,65}

***dl*-MPH- vs. dexamethylphenidate-ethanol pharmacokinetic interactions and EPH**

The racemic switch product dexamethylphenidate (dexMPH) was approved for the treatment of ADHD in 2001. In the absence of ethanol, the pure *d*-MPH isomer elicits the same pharmacodynamic effects as twice the mg/kg dose of *dl*-MPH⁶⁶; The *l*-isomer has been viewed as a passive component – “isomeric ballast”.¹⁵ However, removal of *l*-MPH from the formulation does eliminate the competitive inhibition of CES1 which is linked to *l*-MPH-ethanol transesterification and elevated *d*-MPH concentrations. The consequence of

avoiding the *l*-MPH-ethanol interaction was investigated in 12 men and 12 women using a 4-way randomized cross-over study design. The treatment groups investigated were *dl*-MPH (0.3 mg/kg) or dexMPH (0.15 mg/kg), with or without ethanol 0.5 h later (0.6 g/kg).¹¹

Administration of the pure isomer dexMPH eliminated the influence of ethanol on the absorption phase of *d*-MPH (Fig 2b). As replicated in the study above¹⁰, combining ethanol with *dl*-MPH inhibited *d*-MPH presystemic metabolism in the course of *l*-EPH formation (Fig 4). The geometric mean ratio (points in the statistical comparisons of treatment groups¹¹, not the average concentrations profiled in Fig 2a) between plasma *d*-MPH concentrations following *dl*-MPH-ethanol versus *dl*-MPH alone reached a 1.97 geometric mean ratio of concentrations one hour after *dl*-MPH dosing compared to 0.96 for the corresponding dexMPH-ethanol versus dexMPH alone, ratios which amount to a two-fold difference in the influence of ethanol on racemic MPH compared to enantiopure dexMPH. The pharmacokinetic interaction of ethanol with dexMPH was largely limited to the drug elimination phase, during which time ethanol elevated plasma *d*-MPH concentrations to a degree comparable to that of the *dl*-MPH-ethanol combination. The increased early exposure to *d*-MPH when combining ethanol with *dl*-MPH correlated with *earlier* significant potentiation of euphoric positive subjective effects where the ratio of “liking the drug”, “feeling good” and “feeling stimulated” with ethanol compared to without ethanol in the *dl*-MPH treatments was 2.46 ($P < 0.000$), 2.07 ($P < 0.01$), and 1.53 ($P < 0.05$), respectively, while not reaching statistical significance ($P < 0.05$) until 1.25 h for the dexMPH treatments, for tabulation see¹¹. Subsequently, however, as the rate of *d*-MPH absorption decreased and the plasma concentrations approached the time to maximum plasma concentration (T_{max}), the dexMPH-ethanol combination induced even more pronounced euphoria than *dl*-MPH-ethanol¹¹ in an apparent pharmacodynamics component to the drug interaction.^{51,67}

An increase in the rate of *d*-MPH absorption has not only been reported to promote euphoria and increased abuse liability^{60,68}, but an increased rate of *d*-MPH absorption has also been suggested to improve stimulant efficacy in the treatment of ADHD⁶⁹⁻⁷³, i.e., the “the ramp or gradient effect”.⁷⁴ In addition, reaching a threshold dose of MPH to induce euphoria has also been demonstrated when escalating the dose of *dl*-MPH from 16 mg to 32 mg (and then to 48 mg).⁷⁵

The *l*-EPH plasma concentration reported in the 2013 *dl*-MPH-ethanol study¹¹ reached a mean C_{max} of 0.53 ng/ml. This concentration exceeded that of the parent drug distomer, *l*-MPH, by 40% (Fig. 3). Subsequently, a more sensitive chiral analytical methodology was developed and applied to residual plasma from one of the above study subjects who received *dl*-MPH-ethanol (Fig. 4).⁷⁶ *d*-EPH reached a quantifiable concentration 1 h following *dl*-MPH dosing (0.028 ng/ml), rising a maximum concentration of 0.032 ng/ml at 1.5 h (0.2% of the *d*-MPH plasma concentration). The corresponding *l*-EPH values were 0.8 and 0.85 ng/ml, as consistent with the enantioselective, rather than enantiospecific, nature of the *dl*-MPH-ethanol transesterification pathway.

A drug interaction study of modified-release formulations of *dl*-MPH and dexMPH, with and without ethanol, is in progress and is using the latter more sensitive analytical method above. It is noted that while some reduction in abuse liability may be associated with

modified-release MPH products relative to immediate-release MPH^{73,77,78}, the abuse liability remains substantial for modified-release MPH.⁷⁹

C57BL/6 mouse models of MPH-ethanol interactions and the formation of *l*-EPH

The MPH-ethanol drug combination in humans appears to involve both pharmacokinetic and pharmacodynamic components of drug interactions, and more to the point, the potentiation of the stimulant actions of MPH.^{10,11} To further mechanistic aspects of these interactions, the neuropharmacological reference strain C57BL/6 mouse has been used as a model system.^{14,16,51,52,80,81} A pharmacodynamic component to the ethanol-induced behavioral potentiation of MPH actions may be based on the release of presynaptic dopamine by ethanol.⁶⁷ Theoretically, this would increase the extracellular pool of dopamine subject to reuptake inhibition by MPH and promote dopaminergic neurotransmission.⁵¹

In C57BL/6 mice: (a) A high depressant dose of ethanol (3 g/kg) dramatically potentiated the stimulant response to MPH⁵¹ (7.5 mg/kg); (b) A lower stimulatory dose of ethanol (1.75 g/kg) potentiated a sub-stimulatory dose of MPH⁸⁰ (1.25 mg/kg); (c) The MPH interaction with ethanol increased ataxia;⁸¹ and; (d) Ethanol elevated blood, brain and urinary *d*-MPH concentrations in the course of enantioselectively forming *l*-EPH.^{16,52}

A transdermal formulation of *dl*-MPH was approved for the treatment of ADHD in 2006. Absorption of *dl*-MPH through the skin avoids the hepatic first-pass metabolism of *dl*-MPH which otherwise so limits the bioavailability of *l*-MPH (*vide supra*). Accordingly, transdermal delivery of *dl*-MPH results in an approximate 50-fold increase in plasma *l*-MPH concentrations when compared to oral *dl*-MPH delivery.⁸² Elevated *l*-MPH raises the prospect that transdermal *dl*-MPH could accentuate the metabolic interaction with ethanol, i.e., more *l*-MPH becomes available for CES1 transesterification which competitively inhibits CES 1 hydrolysis of *d*-MPH. In support of the hypothesis, C57BL/6 mouse models has revealed that transdermal delivery of *dl*-MPH significantly increases systemic concentrations of *l*-MPH, *l*-EPH and *d*-MPH in blood, brain and urine compared to oral delivery.^{51,52} Transdermal *dl*-MPH-ethanol interactions have yet to be studied in humans, though the greatly elevated circulating concentrations of *l*-MPH following this route of administration carries toxicological and abuse liability implications should the animal model generalize to humans.

EPH as an internal standard

Due to the structural similarity of EPH to MPH, EPH has historically been an internal standard of choice, used to fortify biological samples in many MPH pharmacokinetic studies.⁸³⁻⁸⁸ In this capacity, EPH controls for variability in inter-sample extraction efficiency. However, owing to differing steric and electronic effects of a methyl versus an ethyl ester, EPH cannot directly control for potential post-sampling hydrolytic loss. The rates of both chemical^{12, 59} and CES1 catalyzed¹⁷ deesterification occur significantly more rapidly for MPH than EPH. Differential rates of MPH and EPH hydrolysis become an especially important consideration when accelerated by alkalinization^{12, 89} of biological

samples prior to solvent-solvent extraction. With EPH now identified as a MPH-ethanol metabolite, the use of EPH as an internal standard becomes especially problematic. More contemporary analytical methods incorporate piperidyl ⁵⁵ or methyl ⁷⁶ deuterated MPH, or ¹⁸O-labeled MPH ⁹⁰ as an internal standard to provide near ideal analytical control.

Conclusions

EPH has been distinguished from MPH by its greater dopaminergic selectivity relative to noradrenergic actions. This pharmacological profile could potentially be exploited to advance personalized medicine, e.g., improving efficacy over existing agents for ADHD patients whose underlying neuropathology primarily involves dopaminergic dysfunction. However, justifiable societal concerns exist regarding the abuse of EPH as a recreational “designer drug”. For example, EPH abuse may have contributed to a recently documented cardiovascular fatality. The post-mortem femoral blood concentration of EPH was quantified to be 110 ng/ml using reference calibrators; this concentration being an order of magnitude greater than typical therapeutic concentrations of MPH (see Fig. 2). The “illicit” EPH had been purchased on the internet.

Importantly, the metabolic formation of *l*-EPH inhibits CES1 hydrolysis of *d*-MPH. This drug interaction increases the rate (and extent) of *d*-MPH absorption, resulting in an earlier onset, and heightened intensity, of stimulant effects relative to *dl*-MPH alone. The racemic switch product dexMPH reduces the pharmacokinetic interaction with ethanol by eliminating the competitive presystemic *l*-MPH transesterification pathway. However, following the early portion of the absorption phase, a pharmacodynamic interaction between dexMPH-ethanol leads to a more pronounced increase in positive subjective effects than even *dl*-MPH-ethanol.¹¹

The use of EPH as a bioanalytical internal standard became especially problematic following its identification as a metabolite. However, EPH has found a new role as an effective biomarker for concomitant *dl*-MPH-ethanol exposure. The future holds potential for EPH as a more selective DAT-targeted ADHD therapeutic agent than MPH; theoretically better tailored for the individual patient whose underlying neural dysfunction pertains more predominantly to the dopaminergic than the noradrenergic synapse.

C57BL/6 mice model both the pharmacokinetic and pharmacodynamic interactions between *dl*-MPH and ethanol. Findings from these animal models have been integrated with clinical studies as a complementary and translational approach toward elucidating mechanisms by which ethanol so profoundly potentiates the abuse liability of *dl*-MPH and dexMPH.

Acknowledgments

The author very much appreciates the assistance in editing by Jesse McClure, Heather Johnson, Catherine Fu, Maja Djelic, as well as the contribution of Fig. 1 by John Markowitz.

Funding and disclosures Portions of the pharmacology reported in this review were supported by NIH grant R01AA016707 (KSP) with additional support from the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, via use of the Clinical & Translational Research Center, NIH UL1 TR000062, UL1 RR029882, as well as support via the Southeastern Pre-doctoral Training in Clinical Research Program, NIH TL1 RR029881.

K.S. Patrick has received scientific funding support from the National Institutes of Health but has no financial relationship with any organization regarding the content of this manuscript. T.R. Corbin and C.E. Murphy report no financial relationships to the content herein.

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Figure 1.

The absolute configuration of MPH enantiomers and the CES1-mediated enantioselective transesterification and hydrolysis to *l*-EPH and *l*-ritalinic acid, respectively.

Figure 2.

(a) Mean *d*-MPH plasma concentrations ($\pm$ S.D.) in 24 normal human subjects (12M/12W) after administering *dl*-MPH (0.3 mg/kg) alone ($\square$) or dosing with *dl*-MPH (0.3 mg/kg) followed by ethanol (0.6 g/kg) 0.5 h later consumed over 0.25 h ($\blacksquare$); (b) Mean *d*-MPH plasma concentrations after dosing with dexMPH (0.15 mg/kg) alone ($\triangle$) or dosing with dexMPH (0.15 mg/kg) followed by ethanol (0.6 g/kg) 0.5 h later consumed over 0.25 h ($\blacktriangle$) (from¹¹ with permission).

Figure 3.

Mean *l*-MPH (◆) and *l*-EPH (■) plasma concentrations (+/- S.D.) in 24 normal human subjects administrated *dl*-MPH (0.3 mg/kg) followed by ethanol (0.6 g/kg) 0.5 h later consumed over 0.25 h. Mean *l*-MPH (◇) and *l*-EPH (□; not detectable) plasma concentrations in 24 normal human subjects following administration of *dl*-MPH (0.3 mg/kg) alone (from¹¹ with permission).

Figure 4.

Plasma concentration-time profile of *dl*-MPH and *dl*-EPH isomers from a healthy male volunteer administered 0.3 mg/kg *dl*-MPH with or without ethanol (0.6 g/kg; see Figure 1 legend for dosing details) using more recently developed, higher sensitivity technology capable of quantifying *d*-EPH (from⁷¹ with permission).