Carboxylesterase-Mediated Transesterification of Meperidine (Demerol) and Methylphenidate (Ritalin) in the Presence of [$^2H_6$]Ethanol: Preliminary in Vitro Findings Using a Rat Liver Preparation

To the Editor:

Carboxylesterase enzymes hydrolyze many ester-containing xenobiotics to yield carboxylic acids.[1] Certain forms of these enzymes catalyze ethanolic transesterification reactions. A widely publicized and significant transesterification reaction is the conversion of cocaine to cocaethylene (ethylcocaine) in the presence of ethyl alcohol.[2−4] Cocaethylene possesses pharmacological activity nearly identical with cocaine and a longer half-life and greater toxicity than cocaine.[5−7] This new metabolite provides additional concern about ethanol and cocaine coabuse. We have recently shown the ethyl ester exchange between deuterated ethanol and unlabeled cocaethylene.[15]

Meperidine (Demerol) and methylphenidate (Ritalin) are two commonly prescribed compounds, with potential for abuse and coabuse with ethanol, and both are extensively hydrolyzed to their corresponding carboxylic acids, meperidinic and ritalinic acids, respectively.[8−10] To investigate whether or not meperidine and methylphenidate, like cocaine, undergo carboxylesterase-mediated transesterification in the presence of ethanol, an in vitro experimental design was employed.

Excised livers from male Sprague−Dawley rats (250−275 g) were homogenized and the supernatant 9000 (S9) fraction collected. Total protein concentration was determined (BCA Protein Assay, Pierce, Rockford, IL), as was esterase activity by the method of Dean et al.[2]

Meperidine and methylphenidate (50 μM) were separately incubated, in triplicate, with S9 at 37 °C for 4 h with and without [$^2H_6$]ethanol (51.3 mM). The experiments were repeated in buffer and S9 in the presence or absence of specific and nonspecific esterase inhibitors. Samples (100 μL) were collected and extracted using a modified solid-phase extraction procedure designed for cocaine and metabolite extraction.[11] Tropacocaine (25 μM) was used as an internal standard for both assays. The above experiment was repeated using meperidine (50 μM) and unlabeled ethanol (50 mM).

Parent drugs and predicted ethyl ester formation products were assayed via gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring (SIM) mode. The SIM qualifier ions were chosen after examining full scan mass spectra of each analyte. The major ion peaks selected were tropacocaine (*m/z* 82, 124, 245), meperidine (*m/z* 71, 172, 247), [$^2H_5$]meperidine (*m/z* 71, 172, 252), methylphenidate (*m/z* 84, 91,150), and [$^2H_5$]ethylphenidate (*m/z* 84, 91, 169) with retention times of 3.5, 2.0, 2.0, 1.9, and 2.1 min, respectively. Quantitation was accomplished by calculating ion abundance ratios of analyte to internal standard compared to a standard curve of concentrations of meperidine or methylphenidate (3.125, 6.25, 12.5, 25, 50 μM). The quantitation ions selected were meperidine/tropacocaine (*m/z* 247/245), [$^2H_5$]meperidine/tropacocaine (*m/z* 252/245), methylphenidate/tropacocaine (*m/z* 84/82), and [$^2H_5$]ethylphenidate/tropacocaine (*m/z* 84/82).

Pharmacokinetic parameters were calculated using WinNonlin.[12] Statistical analysis of the half-life ($t_{1/2}$) was accomplished using Bonferroni's *t*-test.[13] The harmonic mean and "pseudo" standard deviation of $t_{1/2}$ values were calculated.[14]

The protein concentration of rat S9 was ~40 mg/mL. Esterase activity from freshly thawed rat S9 was 305 ± 4 nmol/min per mg of protein. Esterase activity did not change significantly after 1 and 2 h of incubation (303 ± 14 and 293 ± 13 nmol/min/mg, respectively), but a significant loss of activity (~10%) occurred after 4 h (272 ± 5 nmol/min/mg; $p < 0.05$, Bonferroni's *t*-test).

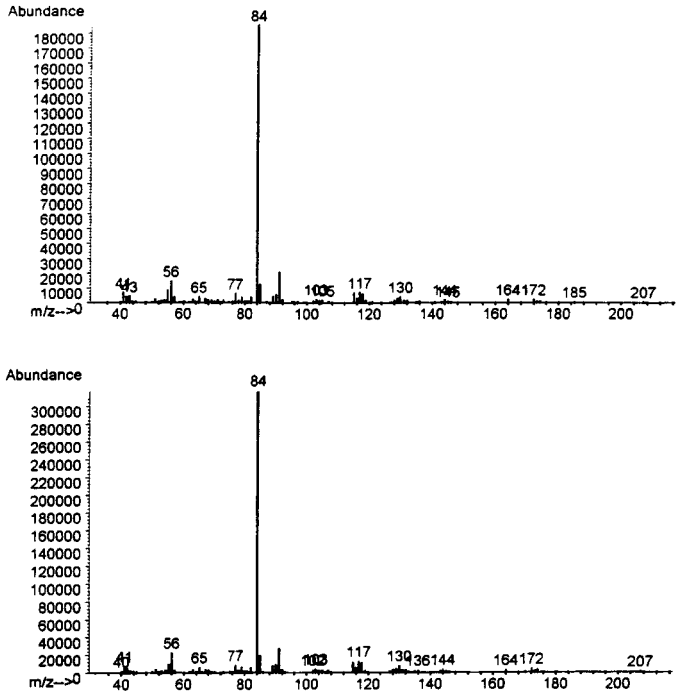

Figure 1—Underivatized ethylphenidate mass spectra (MS). The top MS was produced from an extraction of rat S9 (1 mL) containing methylphenidate (50 μM) and ethanol (50 mM) incubated at 37 °C for 30 min. The bottom spectrum is an injection (2 μL) of ethylphenidate standard (100 ng/mL) in methanol. The retention times for both mass spectra were identical.

Ethylphenidate formation was confirmed by full scan mass spectrometry following the 30 min incubation of methylphenidate and ethanol in rat S9 (Figure 1). This mass spectrum was matched visually and by retention time to a standard of ethylphenidate, conclusively showing the transesterifcation of methylphenidate to ethylphenidate in vitro. [$^2H_5$]Ethylphenidate was formed in vitro when methylphenidate was incubated with [$^2H_6$]ethanol in the rat S9. The concentration−time profiles of unlabeled methylphenidate in the presence of [$^2H_6$]ethanol and the formed [$^2H_5$]ethylphenidate are illustrated in Figure 2. No change in the methylphenidate disappearance rate was seen following addition of [$^2H_6$]ethanol. The methylphenidate profile under control conditions (*i.e.*, no ethanol, Figure 2, open circles) was virtually identical with the profile in the presence of [$^2H_6$]ethanol (Figure 2; solid circles). There were no statistical differences in $t_{1/2}$ (179.9 ± 18.6 min vs 178.1 ± 12.1 min).

The concentration−time profiles of unlabeled meperidine in the absence and presence of [$^2H_6$]ethanol and the formed [$^2H_5$]meperidine are illustrated in Figure 3. These profiles illustrate ethyl ester exchange between unlabeled meperidine and [$^2H_6$]ethanol and that the presence of [$^2H_6$]ethanol does not influence the loss of meperidine. The exchange process begins rapidly ([$^2H_5$]meperidine seen within 5 min; inset). Figure 3 also illustrates (dashed line) total meperidine concentrations (*i.e.*, the sum of labeled and unlabeled meperidine concentrations). That profile is virtually identical with the meperidine profile in the presence of unlabeled ethanol (Figure 4; solid squares in both cases). Similarly, the unla-

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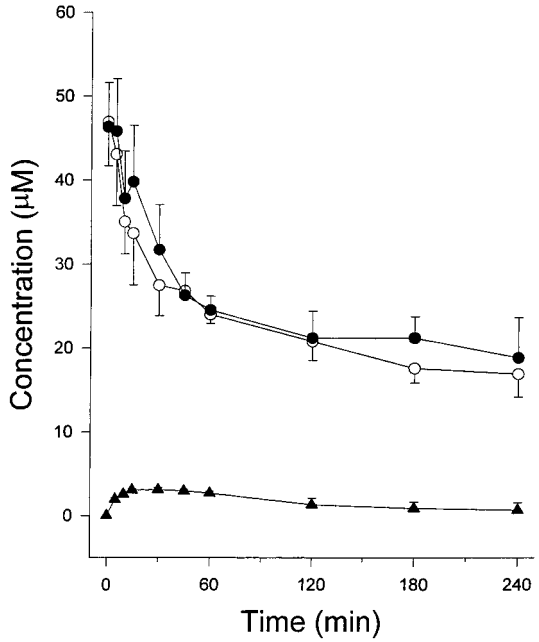

Figure 2—Methylphenidate and [$^2H_5$]ethylphenidate concentrations as a function of time. Methylphenidate (50 μM) was incubated in rat liver 9000*g* supernatant (S9) fraction for 4 h at 37 °C. Unlabeled methylphenidate concentrations as a function of time in the absence (○) [control condition] and in the presence (●) of [$^2H_6$]ethanol (51.3 mM) and the resulting [$^2H_5$]ethylphenidate concentrations (▲). Each value is the mean of three experiments and the crosshatched vertical bars represent the standard deviation of the mean.

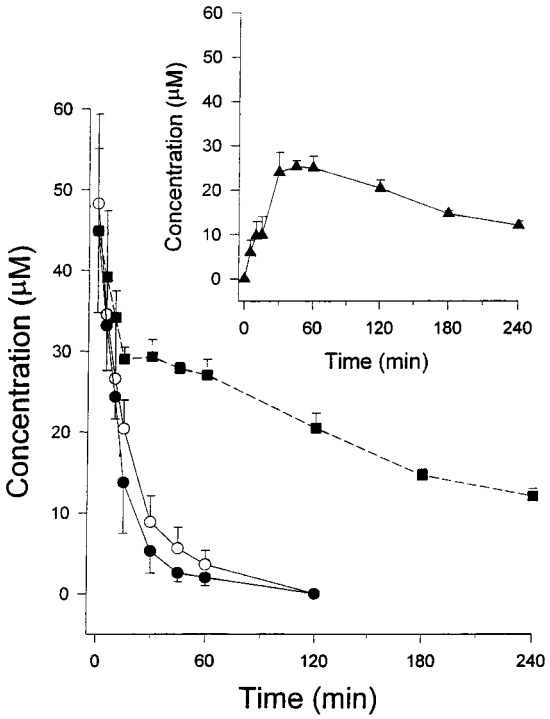

Figure 3—Meperidine and [$^2H_5$]meperidine concentrations as a function of time. Meperidine (50 μM) was incubated in rat liver 9000*g* supernatant (S9) fraction for 4 h at 37 °C. Unlabeled meperidine concentrations in the absence (○) [control condition] and in the presence (●) of [$^2H_6$]ethanol (51.3 mM). Total meperidine concentrations (■) (meperidine + [$^2H_5$]meperidine) in the presence of [$^2H_6$]ethanol are shown with the dashed line. Inset graph: [$^2H_5$]Meperidine concentrations (▲) formed in the presence of [$^2H_6$]ethanol (51.3 mM). Each value is the mean of three experiments and the crosshatched vertical bars represent the standard deviation of the mean.

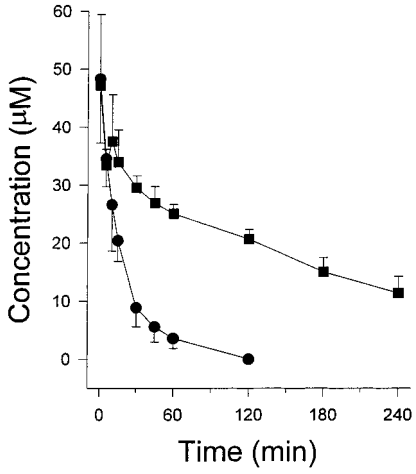

Figure 4—Meperidine concentrations as a function of time. Meperidine (50 μM each) was incubated in 9000*g* supernatant rat liver homogenate preparations for 4 h at 37 °C in the absence (●) and presence of ethanol (50 mM) (■). Each value is the mean of three experiments and the crosshatched vertical bars represent the standard deviation of the mean.

beled form of meperidine under control conditions (*i.e.,* no ethanol) or in the presence of [$^2H_6$]ethanol (Figure 3; open and solid circles, respectively) provides nearly identical concentration−time profiles. There were no significant differences between $t_{1/2}$ values (6.1 ± 3.8 min vs 12.5 ± 2.7 min).

Figure 4 illustrates the in vitro disposition of 50 μM meperidine with and without 50 mM unlabeled ethanol. The $t_{1/2}$ increased by ~9-fold in the presence of ethanol (16.1 ± 3.8 vs 144.9 ± 41.3 min.). The significant increase in $t_{1/2}$ is explained by the ethyl ester formation of additional meperidine. This is corroborated by the extent of formation of [$^2H_5$]meperidine from [$^2H_6$]ethanol (inset, Figure 3). The change in meperidine kinetics in the presence of ethanol (Figure 4) is identical with the change noted when comparing unlabeled meperidine to total meperidine in the presence of labeled ethanol (Figure 3). The $t_{1/2}$ of unlabeled meperidine (12.5 ± 2.7 min) increased to 145.3 ± 19.9 min for total meperidine (labeled + unlabeled) in the presence of [$^2H_6$]ethanol, an ~11-fold increase.

Esterase inhibitors added to the in vitro incubation experiment resulted in an "all or nothing" effect, reported here qualitatively. [$^2H_5$]Ethylphenidate and [$^2H_5$]meperidine formation were completely inhibited by the addition of bis(4-nitrophenyl) phosphate (BNPP) (100 μM), a specific carboxylesterase inhibitor. A 10% solution of saturated NaF completely blocked ethyl ester formation products, while physostigmine (100 μM), a cholinesterase inhibitor, had no effect on the ethanolic transesterification for both methylphenidate and meperidine. No deuterated formation products were detected when samples were incubated in buffer only (no S9) with [$^2H_6$]ethanol.

Both meperidine and methylphenidate were shown to undergo transesterification in the presence of [$^2H_6$]ethanol. Furthermore, the reactions only took place in the presence of viable carboxylesterase enzyme activity. The formation of [$^2H_5$]ethylphenidate conclusively showed ethanolic transesterification of methylphenidate, a previously unreported phenomenon. In the methylphenidate plus ethanol experiment, a new metabolite, ethylphenidate, is formed. Preliminary data in our laboratory show that this phenomenon also occurs in vivo and that ethylphenidate is pharmacologically active in rats. In a clinical setting, coingestion of methylphenidate along with ethanol could result in formation of ethylphenidate. We are pursuing the existence of this phenomenon in humans. Meperidine contains a carboxyl ethyl ester moiety that, when combined with ethanol and carboxy-

lesterase enzyme, will either form additional meperidine before hydrolyzing to meperidinic acid or be hydrolyzed immediately to meperidinic acid. The presence of ethanol provides an alternate pathway and prolongs the ultimate hydrolysis of meperidine, increasing its elimination time. Deuterated ethanol was used to distinguish between transesterified meperidine and meperidine present as starting material. The prolonged $t_{1/2}$ of meperidine in the presence of ethanol is not an indication of altered hepatic clearance due to ethanol interaction with metabolizing enzymes. If ethanol is affecting the rate of carboxylesterase hydrolysis by directly altering the enzyme, then one would expect unlabeled meperidine profiles to differ when [$^2H_6$]ethanol is added. This was not the case; the profiles of meperidine control and unlabeled meperidine in the presence of [$^2H_6$]ethanol were indistinguishable ($t_{1/2}$, 16.1 and 12.5 min, respectively). The increased $t_{1/2}$ of meperidine in the presence of ethanol is more likely explained by a metabolic system with an excess of ethanol providing a substrate pool of an exchangeable ethyl group.

Perhaps of more general importance, however, is the likely possibility that carboxylesterase-mediated transesterification might be a phenomenon applicable to a wide variety of ester-containing compounds. One would expect prolonged elimination and reduced clearance with ethyl ester compounds and newly formed metabolites, potentially active or toxic, with methyl ester compounds. We are currently pursuing the possible generality of this transesterification reaction.

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