

# Binding and Hydrolysis of Meperidine by Human Liver Carboxylesterase hCE-1<sup>1</sup>

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## ABSTRACT

Human liver carboxylesterases catalyze the hydrolysis of apolar drug or xenobiotic esters into more soluble acid and alcohol products for elimination. Two carboxylesterases, hCE-1 and hCE-2, have been purified and characterized with respect to their role in cocaine and heroin hydrolysis. The binding of meperidine (Demerol) and propoxyphene (Darvon) was examined in a competitive binding, spectrophotometric assay. The hCE-1 and hCE-2 bound both drugs, with  $K_i$  values in the 0.4- to 1.3-mM range. Meperidine was hydrolyzed to meperidinic acid and ethanol by hCE-1 but not hCE-2. The  $K_m$  of hCE-1 for

meperidine was 1.9 mM and the  $k_{cat}$  (catalytic rate constant) was  $0.67 \text{ min}^{-1}$ . Hydrolysis of meperidine by hCE-1 was consistent with its specificity for hydrolysis of esters containing simple aliphatic alcohol substituents. Hence, hCE-1 in human liver microsomes may play an important role in meperidine elimination. Propoxyphene was not hydrolyzed by hCE-1 or hCE-2. This observation is consistent with the absence of a major hydrolytic pathway for propoxyphene metabolism in humans.

Carboxylesterases play a major role in the metabolism, detoxification, and elimination of esters encountered in the diet or administered as drugs (Sone and Wang, 1997; Satoh and Hosokawa, 1998). The family of carboxylesterase enzymes has a common mechanism of hydrolysis involving a catalytic triad composed of a serine that is acylated by the substrate ester plus a base (usually histidine) and an acid that activate the catalytic serine (Ollis et al., 1992). These esterases, which include acetylcholinesterases, cholesterolesterases, and lipases, all have a characteristic " $\alpha/\beta$ -carboxylesterase fold" consisting of 8 to 11  $\beta$ -sheet structures (usually parallel) that are connected by  $\alpha$ -helix (or less frequently loop) structures (Ollis et al., 1992). These enzymes catalyze the hydrolysis of various carboxylic ester, thioester, or amide groups. They generally are expressed in high amounts in liver. Their role in xenobiotic metabolism is to convert the apolar esters or amides to the more soluble acid, alcohol, or amine metabolites for elimination (Sone and Wang, 1997; Satoh and Hosokawa, 1998).

Two broad substrate specificity carboxylesterases (hCE-1 and hCE-2) were isolated from human liver, and their activity in the hydrolysis of cocaine and heroin ester groups was characterized (Brzezinski et al., 1994; Kamendulis et al.,

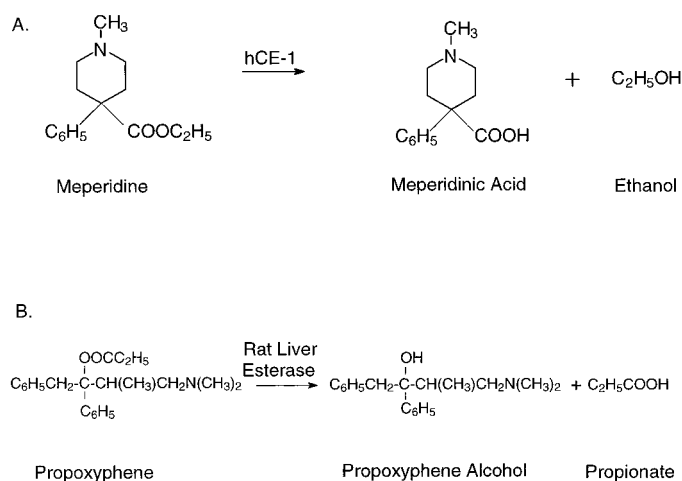
1996; Pindel et al., 1997). hCE-1 is a 180-kDa trimeric enzyme, with a pI of 5.8, that catalyzes the hydrolysis of the methyl ester of cocaine, ethyl transesterification of cocaine with ethanol to form cocaethylene, and hydrolysis of the 3-acetyl group of heroin (Brzezinski et al., 1994; Kamendulis et al., 1996). hCE-2 is a 60-kDa monomeric enzyme, with a pI of 4.9, that catalyzes the hydrolysis of the benzoyl group of cocaine and both the 6- and 3-acetyl groups of heroin (Kamendulis et al., 1996; Pindel et al., 1997). These two enzymes also catalyze the hydrolysis of a wide variety of ester-type drugs, including the angiotensin-converting enzyme inhibitor esters Quinapril, Cilazapril, and Temocapril and the trypsin inhibitor ester camostat mesilate (Takai et al., 1997).

In this study, we examined the binding and hydrolytic activity of hCE-1 and hCE-2 with the synthetic opioid esters meperidine (Demerol) and propoxyphene (Darvon). Both drugs are widely used as analgesics. The major route of metabolism of meperidine is hydrolysis to meperidinic acid and ethanol (Fig. 1A). Meperidine is *N*-demethylated by liver microsomal enzymes to normeperidine to a lesser extent (Reisine and Pasternak, 1996), which may be further hydrolyzed to normeperidinic acid. Only a small amount of meperidine is excreted unchanged. The elimination half-life for meperidine is about 3 h (Reisine and Pasternak, 1996), and the free and conjugated acid metabolites account for >50% of the drug in urine (Plotnikoff et al., 1956). Meperidine carboxylesterase activity has been reported in liver microsomes from the rat

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**ABBREVIATIONS:** hCE-1 and hCE-2, human liver carboxylesterases 1 and 2; GC-MS, gas chromatography mass spectroscopy;  $K_i$ , inhibition constant;  $K_m$ , Michaelis constant;  $k_{cat}$ , catalytic rate constant.



**Fig. 1.** Structures for hydrolysis of meperidine and propoxyphene.

(Yeh, 1982; Luttrell and Castle, 1988), mouse, and human (Lotti et al., 1983; Luttrell and Castle, 1988). Luttrell and Castle (1988) concluded that there are multiple carboxylesterases in liver of different animal species that exhibit different affinity for meperidine. Propoxyphene differs substantially from meperidine in that *N*-demethylation in the liver is the major route of metabolism. McMahon et al. (1971) reported that as much as 20% of propoxyphene is hydrolyzed in the rat to propoxyphene alcohol and propionic acid (Fig. 1B), but this does not occur in humans. In humans, most of the drug appears in urine as norpropoxyphene or the rearranged cyclic dinorpropoxyphene (McMahon et al., 1971; Nash et al., 1975). The serum half-life of propoxyphene is 6 to 12 h (Reisine and Pasternak, 1996). In this study, we examined the possibility of propoxyphene metabolism through hydrolysis by hCE-1 and hCE-2.

## Materials and Methods

Meperidine and propoxyphene were obtained from Eli Lilly and Company (Indianapolis, IN). CHIRALD (propoxyphene alcohol), eicosane, esterase inhibitors, eserine, phenylmethylsulfonyl fluoride, NaF, and dibucane were purchased from Sigma Chemical Co. (St. Louis, MO). Organophosphates, Diazinon, malathion, and methyl parathion were purchased from Supelco, Inc. (Bellefonte, PA). All general chemicals were purchased from Sigma and Fisher Scientific Co. (Pittsburgh, PA). Dithiothreitol was from United States Biochemical Corp. (Cleveland, OH).

**Competitive Inhibition (Binding) Constants for Meperidine and Propoxyphene.** hCE-1 and hCE-2 were purified through the concanavalin A affinity chromatography step from frozen liver tissue obtained at autopsy as previously described (Brzezinski et al., 1997; Pindel et al., 1997). The two enzymes are not cross-contaminated after this chromatography step and have specific activities of about 5 and 40 U/mg with 4-methylumbelliferyl acetate as substrate. Competitive inhibition constants ( $K_i$ ) were determined according to Brzezinski et al. (1997) by use of a spectrophotometric assay with 4-methylumbelliferyl acetate as substrate. The hydrolysis assays were performed in 90 mM  $\text{KH}_2\text{PO}_4$ , 40 mM KCl, pH 7.3, at 37°C, in a total volume of 1.0 ml. One unit of activity is defined as 1  $\mu\text{mol}$  of ester hydrolyzed/min.  $K_i$  values were determined from data sets consisting of 40 initial rate assays generated with four 4-methylumbelliferyl acetate concentrations (0.2, 0.3, 0.4, and 0.5 mM) and five inhibitor (meperidine or propoxyphene) concentrations (0, 0.2, 0.5, 1, and 1.5 mM). The initial rates were simultaneously fit to the steady-state kinetic expression for competitive inhibition:  $v = ([S] \times V_{\text{max}}) /$

$\{[S] + K_m(1 + [I]/K_i)\}$  with nonlinear regression (Grafit; Erithacus Software, Staines, UK), where  $V_{\text{max}}$  is the maximal catalytic activity,  $K_m$  is the Michaelis constant, and  $K_i$  is the competitive inhibition (binding) constant for inhibitor (I) (Cleland, 1979). Protein concentration was determined by the Coomassie dye binding assay (Bio-Rad Labs., Hercules, CA) with BSA as standard (Bradford, 1976).

**Gas Chromatography Mass Spectroscopy (GC-MS) Analysis of Meperidine Hydrolysis to Meperidinic Acid.** The hydrolysis of meperidine to meperidinic acid and ethanol by hCE-1 and hCE-2 was determined by GC-MS analysis according to the procedures of Feng et al. (1994). The enzymes (2 U, 4-methylumbelliferyl acetate assay) were incubated with 5 mM meperidine for 3 h at 37°C in 50 mM  $\text{NaH}_2\text{PO}_4$ , pH 7.0, and the reaction was quenched by 200 mM NaF. This concentration of NaF completely inhibited the hydrolytic activity of hCE-1 and hCE-2. The meperidine reaction mixture was adjusted to pH 9.3 with NaOH/carbonate buffer. The solution was extracted with diethyl ether ( $2 \times 5$  ml). Both the ether and aqueous phases were analyzed by GC-MS. The ether extract was evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted with 45  $\mu\text{l}$  of internal standard (*n*-eicosane, 1 mg/ml) in dichloroethane. A 1- $\mu\text{l}$  aliquot of the solution was injected onto the Hewlett Packard 5890 Series II GC-5971 Series Mass Selective Detector. The column used was a Hewlett Packard Ultra 2 cross-linked 50% phenylmethyl silicone, 12-m  $\times$  0.2-mm  $\times$  0.33-mm film thickness. The total ion chromatogram was monitored. The aqueous phase from the reaction mixture was evaporated to dryness. The residue was refluxed with 15 ml of methanol containing 10% sulfuric acid at 70°C for 5 h. The procedure modified meperidinic acid to its methyl ester. The solution was evaporated to 2 ml at 55°C under reduced pressure, adjusted to pH 9 to 10 with concentrated ammonium hydroxide, extracted with diethyl ether ( $2 \times 15$  ml), and evaporated to dryness. The mixture was further treated with 30  $\mu\text{l}$  of acetic anhydride and 20  $\mu\text{l}$  of pyridine to derivatize any *N*-demethylated meperidine (Feng et al., 1994). The mixture was evaporated and reconstituted in 50  $\mu\text{l}$  of methanol. A 1- $\mu\text{l}$  aliquot of the solution was injected onto the GC-MS, and the total ion chromatogram was monitored.

**$K_m$  for Meperidine Hydrolysis by hCE-1.** The  $K_m$  and  $k_{\text{cat}}$  of hCE-1 for meperidine were determined by analyzing the formation of ethanol with time via GC. Eight concentrations of meperidine ranging from 0.3 to 10 mM were incubated with hCE-1 (2.1 U, 4-methylumbelliferyl acetate assay) for 3 h at 37°C in 50 mM  $\text{NaH}_2\text{PO}_4$ , pH 7.0, 0.1 ml total volume. The reaction was stopped with 200 mM NaF and *n*-propyl alcohol (7.5  $\mu\text{g}$ ) was added as internal standard. Two microliters of the reaction mixture was directly injected into the GC (Hewlett-Packard 5880A series) with a flame ionization detector. The column used was 5% Carbowax (6 m  $\times$  1/8 mm) 60/80 Carbowax (Supelco). The ethanol concentration in the reaction mixture was calculated from a standard curve of five ethanol concentrations (0.1 to 1 mM). The linearity of initial enzyme reaction rates was confirmed by incubation of enzyme with 5 mM meperidine for 1 to 4 h.  $K_m$  values were calculated from the fit of initial rates to:  $v = (V_{\text{max}} \times [S]) / (K_m + [S])$ , where [S] is the meperidine concentration.

**Inhibitors of Meperidine Hydrolase Activity.** The inhibition of meperidine hydrolysis by eserine (physostigmine), phenylmethylsulfonyl fluoride, NaF, dibucaine, Diazinon, malathion, and methyl parathion was examined. Meperidine (5 mM,  $2.6 \times K_m$ ) and hCE-1 (2.2 U, 4-methylumbelliferyl acetate assay) were incubated with 10  $\mu\text{M}$  and 1 mM inhibitors, respectively, for 3 h at 37°C. Ethanol production from meperidine hydrolysis was assayed by GC as described above.

**HPLC Analysis for Hydrolysis of Propoxyphene to Propoxyphene Alcohol.** The hydrolysis of propoxyphene to propoxyphene alcohol and propionic acid was examined with the purified human liver carboxylesterase. Enzyme, 2.2 U of hCE-1, and 2.5 U of hCE-2 (4-methylumbelliferyl acetate assay), was incubated with 5 mM propoxyphene for 3 h in 50 mM  $\text{NaH}_2\text{PO}_4$  at pH 7.0, 37°C. The reaction was stopped with 200 mM NaF and centrifuged. Ten

microliters of the reaction mixture were directly injected onto a Waters C-18  $\mu$ Bondapak column ( $3.9 \times 150$  mm) and eluted with 0.1 M  $\text{KH}_2\text{PO}_4$ , pH 3, with 32%  $\text{CH}_3\text{CN}$ , 0.1% triethylamine. The effluent was monitored at 210 nm. The lowest propoxyphene alcohol standard that gave an easily detectable chromatographic peak was 0.5 mM.

## Results

The binding of the opioid drug esters meperidine and propoxyphene to purified hCE-1 and hCE-2 was examined in a competitive binding assay with 4-methylumbelliferyl acetate as substrate (Brzezinski et al., 1997). The mechanism of inhibition was examined by varying both 4-methylumbelliferyl acetate and the ester inhibitor concentrations. As shown by the reciprocal plot for meperidine inhibition of hCE-1-catalyzed 4-methylumbelliferyl acetate hydrolysis (Fig. 2), the inhibition best fit competitive rather than non-competitive or uncompetitive inhibition. The inhibition of hCE-1 by propoxyphene and the inhibition of hCE-2 by meperidine and propoxyphene were also competitive. The  $K_i$  values of hCE-1 and hCE-2 for propoxyphene (0.44 mM) were somewhat lower than those for meperidine (1.3 and 0.63 mM, respectively), as shown in Table 1.

The formation of meperidinic acid produced from the hydrolysis of meperidine by hCE-1 was verified with GC-MS analysis (Fig. 3). After incubation of hCE-1 with meperidine for 3 h, unhydrolyzed ester was detected in the organic extract by GC-MS analysis (Fig. 3A). Meperidine has a molecular mass of 247 Da (Feng et al., 1994). The hydrolysis product meperidinic acid was detected in the aqueous phase of the incubation mixture after modification by refluxing the extract with methanol and sulfuric acid to produce meperidinic methyl ester (Fig. 3B). Meperidinic methyl ester has a molecular mass of 233 Da (Feng et al., 1994). No meperidinic acid formation was detected in identical incubations with hCE-2.

The  $K_m$  of hCE-1 for meperidine was determined by measuring the rate of formation of ethanol at different meperidine concentrations. Initial reaction rates for ethanol forma-

tion remained linear for 4 h of incubation. The  $K_m$  of hCE-1 for meperidine was  $1.89 \pm 0.31$  mM (Table 1). The  $V_{\max}$  was  $0.0111 \pm 0.0007 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  protein, and this activity corresponds to a  $k_{\text{cat}}$  of  $0.67 \text{ min}^{-1}$ . The catalytic efficiency ( $k_{\text{cat}}/K_m$ ) is  $0.35 \text{ mM}^{-1} \cdot \text{min}^{-1}$  (Table 1).

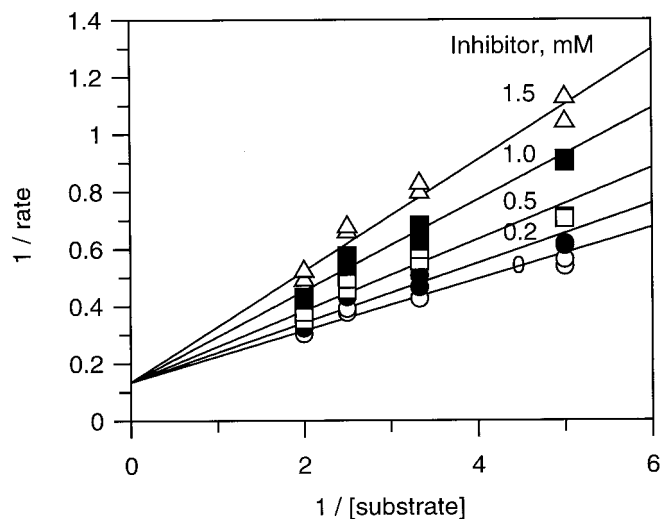
The effect of esterase inhibitors on meperidine hydrolysis by hCE-1 was determined by examining the rate of ethanol formation by GC analysis. The percentage of inhibition for 10  $\mu\text{M}$  and 1 mM inhibitor concentration is shown in Table 2. Eserine, methyl parathion, and Diazinon at 1 mM inhibited hCE-1 substantially. NaF at 10  $\mu\text{M}$  completely inhibited meperidine hydrolysis, and phenylmethylsulfonyl fluoride at 1 mM completely inhibited activity. Dibucaine, a local anesthetic that inhibits cholinesterase, inhibits the esterase activity slightly at 1 mM.

No hydrolysis of propoxyphene to propoxyphene alcohol and propionic acid was detected with purified hCE-1 or hCE-2, as measured by the analysis for propoxyphene alcohol with HPLC. Five millimoles per liter propoxyphene was incubated with enzyme for 3 h at  $37^\circ\text{C}$  in the assay, and the formation of 0.5 mM propoxyphene alcohol would have been readily detected by the HPLC method.

## Discussion

The human carboxylesterases, which are expressed at high levels in liver, are involved in the metabolism and detoxification of various dietary and drug esters (Sone and Wang, 1997; Satoh and Hosokawa, 1998). hCE-1 and hCE-2 have been isolated and purified to homogeneity (Brzezinski et al., 1994; Pindel et al., 1997). The binding of various tropane esters and cocaine metabolites was examined with hCE-1 with a competitive spectrophotometric assay (Brzezinski et al., 1997). In a similar manner, the binding of meperidine (Demerol) and propoxyphene (Darvon) were examined with hCE-1 and hCE-2 in the inhibition assay (Table 1). The kinetics of inhibition for both meperidine (Fig. 2) and propoxyphene obeyed a competitive model. This model applies whether the drugs act as competitive inhibitors or alternative substrates in the assay (Brzezinski et al., 1997). The  $K_i$  values with both enzymes were in the 0.4 to 1.3 mM range, as shown in Table 1. These  $K_i$  values are similar in magnitude to those for cocaethylene, norcocaethylene, heroin, 6-acetylmorphine, and pseudococaine esters with hCE-1 (Brzezinski et al., 1997). The highest-affinity substrate/inhibitor known for hCE-1 is *R*-(-)-cocaine, with a  $K_i$  of 10  $\mu\text{M}$  (Brzezinski et al., 1997).

Meperidine was hydrolyzed to meperidinic acid and ethanol by hCE-1. The hydrolysis of meperidine was identified by GC-MS analysis of meperidinic acid (the methyl ester derivative) as shown in Fig. 3B. The kinetics of hydrolysis were followed by monitoring the formation of ethanol by GC with flame ionization detection. The  $K_m$  of purified hCE-1 for meperidine was 1.9 mM, which is higher than the  $K_m$  of 0.45 mM reported for meperidine carboxylesterase activity in human liver microsomes (Lotti et al., 1983). This may represent the difference in assays with purified hCE-1 versus whole microsomes or there may be more than one carboxylesterase that catalyzes meperidine hydrolysis in microsomes. However, only two major carboxylesterases with broad substrate specificity, hCE-1 and hCE-2, have been characterized from human liver (Pindel et al., 1997; Takai et al., 1997).

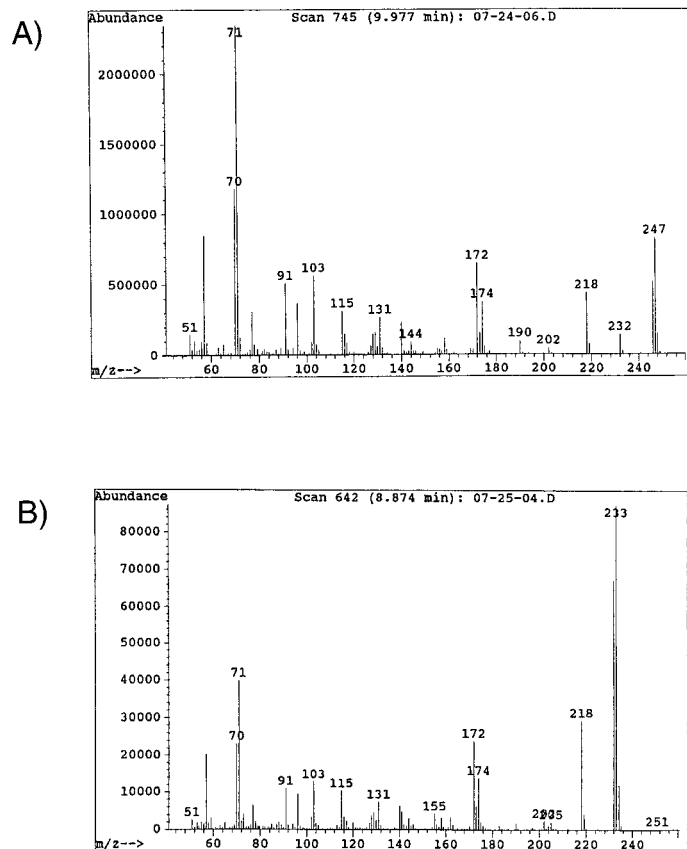


**Fig. 2.** Competitive inhibition of hCE-1 activity by meperidine. Lines represent the fit of data to a competitive inhibition model. The  $K_m$  of 4-methylumbelliferyl acetate is  $0.7 \pm 0.11$ , and the  $K_i$  of meperidine is  $1.3 \pm 0.1$  mM.

TABLE 1

Comparison of  $K_m$ ,  $k_{cat}/K_m$ , and  $K_i$  of hCE-1 and hCE-2 for drug esters $K_m$  and  $k_{cat}/K_m$  data for cocaine and 4-methylumbelliferyl acetate were obtained from Pindel et al. (1997) and heroin and 6-acetylmorphine from Kamendulis et al. (1996). The  $K_i$  data were obtained from the competitive inhibition of 4-methylumbelliferyl acetate hydrolysis.

Substrate	hCE-1			hCE-2		
	$K_m$	$k_{cat}/K_m$	$K_i$	$K_m$	$k_{cat}/K_m$	$K_i$
	mM	$mM^{-1} \cdot min^{-1}$	mM	mM	$mM^{-1} \cdot min^{-1}$	mM
Meperidine	$1.9 \pm 0.3$	$0.35 \pm 0.02$			0	
Cocaine	0.12	0.5		0.39	18.4	
Heroin	6.3	69.0		6.8	314.0	
6-Acetylmorphine	8.3	0.024		0.13	22.0	
4-Methylumbelliferyl acetate	0.8	2,000		0.15	60,000	
Inhibitor						
Meperidine			$1.30 \pm 0.12$			$0.63 \pm 0.06$
Propoxyphene			$0.44 \pm 0.03$			$0.44 \pm 0.07$



**Fig. 3.** GC-MS total ion chromatograms of meperidine and meperidinic acid. A, the organic phase extract of meperidine from the hCE-1 incubation. The molecular ion at 247 corresponds to meperidine. B, the aqueous phase from the incubation mixture. The molecular ion at 233 corresponds to meperidinic acid methyl ester.

The hydrolysis of meperidine by hCE-1 was subject to inhibition by various esterase inhibitors, as shown in Table 2. The complete inhibition of hCE-1 by NaF agreed with the results of Dean et al. (1991), where 40 mM NaF was used as an inhibitor of cocaine methyl esterase and benzoyl esterase. The inhibition of hCE-1 by organophosphates (e.g., eserine, Diazinon, and methyl parathion) may be important in the toxicology of these compounds.

The substrate specificity of hCE-1 and hCE-2 for meperidine is consistent with the specificity of the enzymes for cocaine, heroin, and other drug esters. hCE-1 hydrolyzes the

TABLE 2

Inhibition of meperidine hydrolysis by hCE-1

Percent inhibition was normalized to the activity without inhibitors.

Inhibitors	Inhibition	
	10 $\mu$ M	1 mM
	%	
NaF	100	100
Phenylmethylsulfonyl fluoride	17	100
Eserine	16	47
Dibucaine	0	6
Diazinon	6	42
Malathion	0	10
Methyl parathion	4	78

ethyl ester of meperidine, forming ethanol and meperidinic acid (Fig. 1A and Table 1). It also hydrolyzes the methyl ester group of cocaine, forming methanol and benzoyl ecgonine. hCE-2 does not catalyze either reaction (Dean et al., 1991; Pindel et al., 1997). Takai et al. (1997) showed that hCE-1 hydrolyzes the ethyl ester of the family of antihypertensive angiotensin-converting enzyme inhibitors based around N-substituted 4-phenyl-2-aminobutanoic acid ethyl ester structures (e.g., Quinapril, Cilazapril, Temocapril, and Delapril). Whereas each member of this group was hydrolyzed to ethanol and the substituted butanoic acid by hCE-1, it was hydrolyzed at least three times less efficiently, if at all, by hCE-2. Hence, hCE-1 hydrolyzes esters with small aliphatic alcohol substituents such as the ethoxy group of meperidine much more efficiently than hCE-2.

Meperidine is mainly metabolized in the liver by hydrolysis to meperidinic acid and by *N*-demethylation to normeperidine, which is subsequently hydrolyzed to normeperidinic acid (Clark et al., 1995). The free and conjugated acid metabolites of meperidine and normeperidine in urine account for more than half of the dose (Plotnikoff et al., 1956). The  $K_m$  of the purified microsomal hCE-1 for meperidine, 1.9 mM in Table 1, is several orders of magnitude higher than the pharmacological meperidine concentration of 2  $\mu$ M in blood (Clark et al., 1995). Hence, hCE-1 would exhibit first-order kinetics in the hydrolysis of meperidine at pharmacological conditions.

Yeh (1982) showed that a rat microsomal meperidine carboxylesterase was significantly inhibited by ethanol. The presence of 0.5% ethanol in the incubation of the rat microsomal enzyme and meperidine caused a reduction in meperidinic acid formation by 63%. In a more recent study (Bourland et al., 1997), it was reported that a rat liver microsomal

extract catalyzed the ethyltransesterification of meperidine with deuterated ethanol as substrate. Rat hydrolase A catalyzes the ethyltransesterification of cocaine to form cocaethylene (Morgan et al., 1994). The metabolic transesterification catalyzed by rat hydrolase A could account for the 9-fold increase in  $T_{1/2}$  of meperidine in the presence of ethanol (Bourland et al., 1997). The homolog of hCE-1 in rat is called hydrolase A (Brzezinski et al., 1994; Morgan et al., 1994). Studies on the effects of ethanol on meperidine metabolism in rats by hydrolase A suggest that similar effects of ethanol on hCE-1-mediated meperidine metabolism may occur in humans.

Whereas propoxyphene bound to hCE-1 and hCE-2, it was not hydrolyzed by either enzyme. The inability of hCE-2 to hydrolyze the propionyl ester group of propoxyphene is surprising in view of the fact that it efficiently hydrolyzes the benzoyl group of cocaine (Dean et al., 1991; Pindel et al., 1997) and the 3- or 6-acetyl group of heroin (Kamendulis et al., 1996). We suspect that the bulky tertiary alcohol (propoxyphene alcohol in Fig. 1B) prevents proper orientation of the ester in the active site to facilitate enzymatic hydrolysis. A similar restriction in specificity for esters with secondary alcohols was reported for the coronary vasodilator diltiazem, which has the acetyloxy group bound to a hindered secondary alcohol (Takai et al., 1997). In humans, most of propoxyphene is eliminated after N-demethylation (McMahon et al., 1971; Girre et al., 1991). In rats, hydrolysis to propionic acid and propoxyphene alcohol (Fig. 1) is a minor metabolic pathway (McMahon et al., 1971). However, there is no evidence of significant hydrolysis of propoxyphene in humans (McMahon et al., 1971). The major metabolites in urine are norpropoxyphene and cyclic dinorpropoxyphene (McMahon et al., 1971; Nash et al., 1975). This lack of hydrolysis in humans is consistent with the inability of hCE-1 and hCE-2 to hydrolyze propoxyphene. The difference between human and rat metabolism may represent the species difference in expression of carboxylesterases. Up to four different carboxylesterase genes have been identified in the rat (Morgan et al., 1994; Yan et al., 1995a,b) but only two in humans (Brzezinski et al., 1994; Pindel et al., 1997).

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