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Stereospecific hydrolysis of the optimal isomers of *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonate by liver microsomes*

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The stereospecificity of an enzymatic reaction involving a substrate or inhibitor is generally attributed to the asymmetrical nature of the enzyme surface. Phosphonates may have four different substituents arranged around the phosphorus atom, resulting in two optically active forms. Studies with tabun, ethyl-*N*-*N*-dimethylphosphoroamidocyanidate [1, 2], showed a high degree of specificity with regard to the rate of hydrolysis of the isomers. The *l*-isomer of *O*-ethyl-*S*-(2-ethyl mercaptoethyl) ethylphosphonothiolate reacted ten to twenty times faster than the *d*-isomer in the inhibition of cholinesterase from four different sources [3], while stereoselectivity in the metabolism of cyanofenphos, *O*-ethyl *O*-*p*-cyanophenyl phenylphosphonothionate, in the rat appeared to be mainly due to selective hydrolysis of the (−)-oxon analog by an arylhydrolase [4].

The present study was undertaken to compare the rates of hydrolysis of the optical isomers of EPNO, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonate, by rat, mouse and rabbit liver microsomes. Studies were also conducted on the activation and inhibition of such hydrolysis reactions by various factors.

Chemicals. The optical isomers of EPNO were prepared by oxidation of the optical isomers of EPN, (O-ethyl O-*p*-nitrophenyl phenylphosphonothionate) as described previously [5]. $CaCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$ were purchased from the Fisher Scientific Co., Pittsburgh, PA, $HgCl_2$ from Reagent, Inc., Middlesex, NB, $BaCl_2 \cdot 2H_2O$ from the J. T. Baker Chemical Co., Phillipsburg, NJ, and EDTA from Matheson, Coleman & Bell, Cincinnati, OH.

Enzyme preparation. Male mice (20–25 g) (Dublin ICR strain), male rats (250–300 g) (Sprague–Dawley strain), and a male rabbit (3700 g) (New Zealand White strain) were decapitated and their livers removed and homogenized as 20% homogenates in 0.05 M Tris–HCl buffer, pH 7.4, at 0–4%. Differential centrifugation was carried out as described previously [6]. The microsomal fractions were washed, suspended in the same buffer and centrifuged at 100,000 *g* for 1 hr at 4°. The pellets were suspended in the same buffer and used as a source of the hydrolase.

Reaction. The reaction mixture consisted of a microsomal preparation equivalent to 300 mg liver in a total of 3 ml of 0.05 M Tris–HCl buffer, pH 7.4. The reaction and control cuvettes were equilibrated at 37° for 3 min before adding the appropriate EPNO isomer to the reaction cuvette in 5 μl acetone. The final concentration of the isomer was 0.05 mM.

The hydrolytic reaction was followed by measuring at 405 nm the *p*-nitrophenol formed as a result of the hydrolysis of EPNO, using an AMINCO DW-2 UV-Visible Spectrophotometer. The control cuvettes were prepared in the same manner except that EPNO was not added.

The effects of a number of compounds on inhibition and activation were studied by the addition of the corresponding

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Table 1. Hydrolysis of EPNO isomers by liver microsomes

Isomer	EPNO* (nmoles hydrolyzed/g liver/min) Rat	Mouse	Rabbit
(−)-EPNO	16.04 ± 0.52	11.13 ± 0.45	8.89 ± 0.2
(+)-EPNO	10.61 ± 0.3	7.16 ± 0.21	5.80 ± 0.11
(±)-EPNO	13.30 ± 0.6	8.52 ± 0.23	7.90 ± 0.15

* The results are the averages of four replicates from pooled samples ± S.D.

Table 2. Effects of EDTA and various divalent cations on the hydrolysis of (±)-EPNO by liver microsomes

Modulator (concn)	EPNO activity* (nmoles hydrolyzed/g liver/min) Rat		Mouse		Rabbit	
Ca^{2+} (1.0 mM)	36.10 ± 0.29	(+271)†	16.26 ± 0.43	(+190)†	10.30 ± 0.12	(+130)†
EDTA (0.5 mM)	1.93 ± 0.04	(+85)	1.68 ± 0.15	(−80)	2.53 ± 0.03	(−68)
Ba^{2+} (1.0 mM)	1.77 ± 0.05	(−87)	1.18 ± 0.6	(−86)	2.02 ± 0.06	(−74)
Mn^{2+} (1.0 mM)	1.35 ± 0.08	(−90)	1.29 ± 0.24	(−85)	1.40 ± 0.03	(−82)
Hg^{2+} (1.0 mM)	0.60 ± 0.02	(−95)	0.97 ± 0.01	(−89)	1.30 ± 0.62	(−84)
Co^{2+} (1.0 mM)	0.53 ± 0.01	(−96)	0.47 ± 0.12	(−94)	0.72 ± 0.46	(−91)

* Activities in the absence of activators or inhibitors are presented in Table 1.
† Per cent activation or inhibition.

compound to both the reaction and the control cuvettes before temperature equilibration. The reactions were linear for at least 5 min. All results were corrected for non-enzymatic hydrolysis.

The data in Table 1 indicate that the (−)-EPNO isomer was hydrolyzed at a higher rate than the (+)-isomer by liver microsomes isolated either from rat, mouse or rabbit. The preference for the (−)-isomer is similar to that reported for cyanofenphos oxon and a rat microsomal enzyme [4]. The explanation offered by these authors of a single enzyme being responsible for the hydrolysis seems equally plausible in our case, since cyanofenphos oxon and EPNO are structurally similar. Further, the hydrolysis ratios of the (−)-and the (+) isomers are almost the same (≃1.5) with the microsomes from the three species. This supports the hypothesis that the hydrolytic enzyme(s) in the liver microsomes prepared from rat, mouse and rabbit are similar at least from the standpoint of stereospecificity [1, 2]. Microsomes from all three sources hydrolyzed the racemic compound at a rate intermediate between the (+) and (−)-isomers. The same preparations do not hydrolyze the optical isomers of EPN (unpublished observations). Based on the nmoles hydrolyzed/g of liver/min, the rat has the highest hydrolytic activity, followed by the mouse, while the rabbit has the lowest (Table 1). Other studies indicate the presence of organophosphate-hydrolysing activity in different mammalian tissues [7–15].

Results in Table 2 show that Ca^{2+} stimulates the hydrolytic activity of liver microsomes from the three different animals. The activation decreased in the following order: > rat > mouse > rabbit. EDTA, Ba^{2+}, Mn^{2+}, Hg^{2+} and Co^{2+} all inhibited the hydrolysis of EPNO by liver microsomes from the three sources (Table 2). However, the rabbit enzyme was less sensitive to activation and inhibition by divalent cations than the mouse or the rat enzyme. Similar effects of activation and inhibition have been reported in the hydrolysis of other organophosphorus compounds [9, 13, 16].

We conclude that EPNO-hydrolyzing enzyme(s) located in microsomes prepared from rat, mouse or rabbit liver are generally similar in their stereospecificity and in the manner in which they are affected by divalent cations. The same system was also found to be closely related to other organophosphorus-hydrolyzing enzyme(s) found in other mammalian tissues.

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