Inhibition of liver microsomal epoxide hydrase by cyproheptadine epoxide

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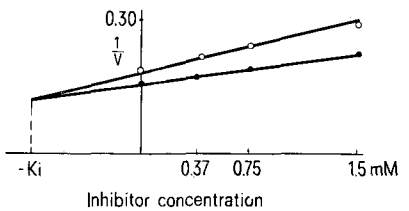
Summary. Cyproheptadine epoxide is a competitive inhibitor of rat liver microsomal epoxide hydrase with an apparent K_i-value of 0.75 mM. Cyclobenzaprine and its epoxide stimulate in vitro the activity of this enzyme, whereas cyproheptadine, carbamazepine and carbamazepine epoxide have no effect.

There is increasing evidence that labile epoxides formed during metabolism of many chemicals may play an important role in carcinogenesis[3-5]. The concentration of these epoxides in the body is the result of a balance between the amount formed and the amount metabolized through various metabolic processes[6,7]. A key enzyme for catabolism of epoxides is the epoxide hydrase (EC 4.2.1. 64). Therefore, it is obviously of interest to know which factors may affect the activity of this enzyme. It has been shown that some epoxides, e.g. cyclo-hexane epoxide and trichloropropene epoxide, are able to decrease the activity of epoxide hydrase[8]; however, the effect of other epoxides, and in particular those formed during the metabolism of some drugs, has not been studied. In previous studies we have found that several tricyclic drugs including carbamazepine, cyproheptadine and cyclobenzaprine are metabolized to form 10, 11-epoxides, which can be detected in blood and urine[9,10]. This communication summarizes our recent findings concerning the effects of these epoxides as well as their parent compounds on the activity of epoxide hydrase in rat liver microsomes.

Effect of some tricyclic drugs and their epoxides on epoxide hydrase activity

Drug added	Concentration of the drugs (mM)	Specific activity (nmoles phenylethylene glycol mg protein^{-1} min^{-1})	Activity %
None	–	5.42 ± 0.22	100
Cyproheptadine	1	5.63 ± 0.15	104
Cyproheptadine epoxide	0.75	4.10 ± 0.10*	76
Carbamazepine	1	5.20 ± 0.05	96
Carbamazepine epoxide	1	5.53 ± 0.08	102
Cyclobenzaprine	1	6.30 ± 0.28**	116
Cyclobenzaprine epoxide	1	6.13 ± 0.29**	113

The substrate (styrene epoxide) concentration was always 0.5 mM. The figures represent the mean value ± SE of 4 individual experiments. *$p < 0.01$; **$p < 0.05$.



Dixon plot of the inhibitory effect of cycloheptadine epoxide on the activity of epoxide hydrase. The concentration of the substrate (styrene epoxide) utilized were 0.5 mM (○—○) and 0.75 mM (●—●). V is given as nmoles of phenylethylene glycol formed mg protein^{-1} min^{-1}.

Materials and methods. The liver microsomes were prepared from Charles River CD male rats (body weight 150–180 g) according to the method of Kato[11], with slight modification in the composition of the solution utilized for liver homogenization which consisted of Tris-HCl buffer 0.05 M, pH 7.4 containing NaCl 0.15 M and $MgCl_2$ 5 mM. Carbamazepine was a kind gift of Ciba-Geigy, carbamazepine epoxide was donated by Italseber, Milano, cyproheptadine and cyclobenzaprine were obtained by Merck Sharp and Dohme, while their epoxides were synthesized as previously described[10,12]. The activity of epoxide hydrase was determined by utilizing styrene epoxide as a substrate. The enzymatic reaction was carried out as follows: 4.3 ml buffer was added to 0.1 ml methanol solution of the compound in order to obtain a final concentration ranging from 0.35 to 2 mM. At the same time 0.5 ml of a liver microsomal suspension containing about 5 mg of protein were added. After 15 min of preincubation at 37°C, 0.1 ml of an acetone solution of styrene epoxide (Merck) was added to obtain a final substrate concentration of 0.5 mM or 0.75 mM. The reaction was stopped after 5 min by the addition of 1 ml of 0.6 M NaOH. The samples were immediately extracted twice with 5 ml of ethyl acetate. The combined extracts were dried under vacuum at room temperature. In order to measure phenylethylene glycol, the diol formed during the enzymatic reaction, a simple gas chromatographic method was developed. The diol was quantitatively determined through an esterification process with butylboronic acid[13]. 5 µl of butylboronic acid (100 mg/ml in dimethylformamide) were added to the sample residue dissolved in 200 µl of an acetone solution of m-dinitrobenzene (0.5 µg/µl) used as an internal standard. 1 µl of this solution was injected in a Model GI gas chromatograph apparatus (Carlo Erba, Milano) equipped with a hydrogen flame ionization detector. The stationary phase was OV-17 3% on Gas Chrom Q (100–200 mesh) packed into a 2.5 m glass column (internal diameter 2 mm, external diameter 4 mm). The flow rate of the carrier gas (nitrogen) was 30 ml/min and the column temperature was 170°C. The recovery of phenylethylene glycol was

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more than 90%. To confirm the nature of the derivative of phenylethylene glycol with butylboronic acid, mass spectra were carried carried out by using an LKB 9000 instrument at the following conditions: ion source temperature 290°C; ionization energy 70 eV; trap current 60 μA. Sample introduction was carried out either by direct inlet system (DIS) or by GC at the conditions described above, except for the use of helium instead of nitrogen as carrier gas. The 2 mass spectra were comparable. The enzymic activity of epoxide hydrase was expressed in nmoles of diol formed per min of incubation per mg of microsomal protein. The spontaneous hydration of styrene epoxide occurring in our conditions was lower than 5% with respect to the enzymatic and it was substracted for the calculation of enzymatic activity. Protein concentration was determined by the method of Lowry[14].

Results and discussion. The table summarizes the effects of the tricyclic drugs and their epoxides on the activity of epoxide hydrase. Only cyproheptadine epoxide had a marked inhibitory effect on the enzyme, while cylobenzaprine and its epoxide showed a slight stimulatory effect. On the other hand, cyproheptadine, carbamazepine and carbamazepine epoxide did not show any influence on the activity of the enzyme. As shown in the figure, cyproheptadine epoxide is a competitive inhibitor of epoxide hydrase; the apparent K_i-value is 0.75 mM. It may be suggested that styrene epoxide and cyproheptadine epoxide are substrates for the same enzymes, although to date there is no evidence that cyproheptadine epoxide is metabolized to the correspondant diol[15].

The significance of these in vivo findings remains to be established. However, the hypothesis may be advanced that cyproheptadine epoxide could interfere with the biotransformation process of some carcinogenic agents by affecting the degradation of their epoxides into metabolites devoid of toxic effects. Conversely, it may be suggested on the basis of data from studies in progress that tricyclic agents may also affect the formation of the epoxides of carcinogenic agents. Therefore, it seems possible to modulate the accumulation of epoxides from such agents, permitting us to assess the significance of epoxide formation in chemical carcinogenesis.

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A new approach to preparation and purification of colostrokinin from bovine colostrum*

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Summary. A simple and rapid method is reported for preparation and partial purification of colostrokinin from bovine colostral whey. The active substance obtained at the described stage of purification appears to be a basic polypeptide of low molecular weight. This peptide proved to be undestroyed by pepsin, only partially by trypsin and completely by papain.

The first report about some properties of colostrokinin dates from 1959 by Guth[1]. The crude substance was obtained by incubating kallikrein from urine or saliva with colostrum[2,3]. A method for colostrokinin preparation with a 15–20fold purification was later performed by Werle and Trautschold[4], who found some biological and biochemical differences between colostrokinin and plasmakinins, but concluded that the 2 types of kinins were chemically and pharmacologically very closely related. About 10 years later, this subject of research drew the attention of some Japanese investigators, who proposed a method for isolation and purification of colostrokinin

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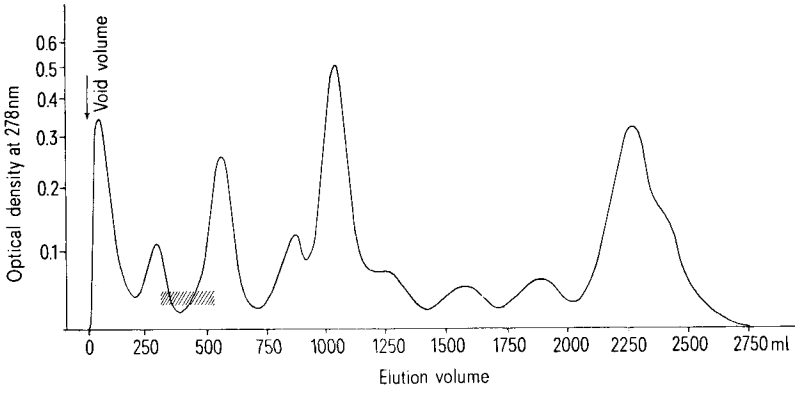

Fig. 1. Gel filtration pattern of crude colostrokinin (step 3), prepared from 2000 ml of natural bovine colostrum, on a Sephadex G-25 fine column (5.5 cm × 100 cm), equilibrated with 0.1 M ammonium acetate buffer (ph 5). Flow rate 125 ml/h. Fraction size 25 ml. Void volume measured with Blue Dextran 2000. Shaded area represents the presence of biological activity. The separation pattern was recorded at 278 nm.