Possible Mechanisms Responsible for the Effect of Dimedrol on Nonsynthetic Biotransformation of Xenobiotics in Rat Liver

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The ability of dimedrol to inhibit o-demethylation of paranitroanisole, which in rat liver is catalyzed by cytochrome P-450, is demonstrated *in vivo* and *in vitro*. The affinity of dimedrol for cytochrome P-450 is higher than that of other xenobiotics. It is suggested that the effect of dimedrol on the metabolism of paranitroanisole and some other xenobiotics is realized during formation of the enzyme—substrate complexes between these substances and cytochrome P-450.

Key Words: *cytochrome P-450; dimedrol; paranitroanisole; demethylase activity of microsomes*

It was hypothesized that the ability of dimedrol to potentiate pharmacological effects of some compounds (hexobarbital, morphine derivative, etc.) is associated with inhibition of enzymes involved in biotransformation of these xenobiotics in the liver endoplasmic reticulum [2,7]. In order to check up this hypothesis we studied the interactions between dimedrol and cytochrome P-450 (CC), a major enzyme of microsomal oxidation in the liver. This enzyme catalyzes nonsynthetic reactions of the first phase of biotransformation of numerous exogenous substrates, including barbiturates and morphine derivatives.

MATERIALS AND METHODS

Male Wistar rats weighing 150-180 g were used. They were maintained on the standard vivarium diet and were deprived of food for 24 h before the experiment. The pharmacopeial preparation dimedrol (β-dimethylaminoethyl ester benzhydrol hydrochloride) was injected intraperitoneally in a dose of 30 mg/kg body weight. Liver microsomes were isolated by differential centrifugation [4]. Their paranitroanisole (PNA)-o-demethylase activity was determined by measuring the rate of paranitrophenol (PNP) formation as described [5]. The incubation medium (total volume 1.7 ml) contained 50 mM Tris-HCl buffer (pH 7.4 at 37°C), 1.33 mg/ml microsomal protein, 0.02-0.4 mM dimedrol, 12 mM $MgSO_4$, and 1 mM NADPH. The amount of formaldehyde was measured after a 10-min incubation at 37°C [6]. The apparent Michaelis constant (K_m) and the maximum rate (V_{max}) of dimedrol and PNP demethylation were determined in the Lineweaver—Burk plots. The CC content in the microsomal fraction was measured spectrophotometrically [9]. The apparent spectral dissociation constant (K_s^{app}) for the CC—dimedrol complex was determined from the amplitude of the light absorbance changes ($\Delta D_{390-420}$) induced in the microsomal fraction by varied concentrations of dimedrol [8]. The differences in the number of ligands bound to CC in microsomal fractions isolated from experimental and control rats was determined by the method of "paired cuvettes." The compared preparations of microsomes (protein concentration about 2 mg/ml) were adjusted for the same CC content and transferred into two pairs of 0.2-cm^3 spectrophotometrical cuvettes (0.2 ml in each). Then the four cuvettes in pairs (each

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pair consisting of experimental and control cuvette) were placed in a thermostat-controlled (20°C) Spekord-M40 spectrophotometer, and the differential light absorbance spectrum in the 350-500 nm range was recorded after a 5-min stabilization period. Then the contents of one pair of the cuvettes were mixed, and the differential light absorbance spectrum was recorded after 5 min. The degree of CC saturation with a ligand was determined by analysis of the first and second spectra.

RESULTS

Analysis of the differential light absorbance spectra (microsomes+dimedrol)—microsomes recorded after the addition of dimedrol to the microsome suspension showed that similar to type I CC substrates, dimedrol binds to CC, forming an optically active complex with a maximum at 390 nm and a minimum at 420 nm (Fig. 1, *a*).

The calculated K_s^{app} of this complex is 3.2±0.9 μM, which is 1-2 orders of magnitude lower than K_s^{app} typical of CC complexes with the majority of substrates (58 and 250 μM for hexobarbital and ethylmorphine, respectively) and is comparable to that for the CC complex with SKF525A (K_s^{app}=0.5 μM), a potent inhibitor of CC [1].

As other type I CC substrates, in the presence of NADPH dimedrol undergoes oxidative demethylation, which is confirmed by the formation of formaldehyde in the incubation medium. K_m and V_{max} for this reaction (N-demethylation) calculated from the dependence of formaldehyde formation on dimedrol concentration were, respectively, 37±2 μM and 5.9± 0.3 nmol/mg protein/min.

It was found that dimedrol inhibits demethylation of PNA in rat microsomes (Fig. 2). In a concentration of 10 μM, which is comparable to K_s^{app} for the CC—dimedrol complex, dimedrol modified the rate of PNP formation (PNP is the final product of this reaction). It increased K_m almost 2-fold (from 36±1 to 71±3 μM) without changing V_{max} (1.3±0.1 nmol/mg protein/min) (Fig. 2). It is obvious that in rat liver microsomes the suppression of PNP formation (PNA-o-demethylation) by dimedrol is of the competitive inhibition nature.

Similar changes of the parameters of PNA-o-demethylase activity (an increase of K_m from 36±1 to 170±15 μM with no changes in V_{max}) were observed in liver microsomes obtained from rats sacrificed 15 min after administration of 30 mg/kg dimedrol (Fig. 2).

Using the method of paired cuvettes we have found that microsomes of dimedrol-treated rats differed from those of intact rats in the levels of CC-bound substrates. In a mixture of microsomes with different degree of CC saturation with a ligand, the ligand is redistributed between microsomes until a new equilibrium state for the CC—ligand complex is reached. This is reflected by the emergence of typical changes in differential light absorbance spectra in the (control+experiment)—control+ experiment system. The mechanism of these changes is as follows: under the conditions of free redistribution of substrate between membranes upon linear dilution of one microsomal preparation by another the concentration of substrate decreases proportionally to dilution, while the shift of thermodynamic balance of the spin states of CC heme is of hyperbolic nature [3].

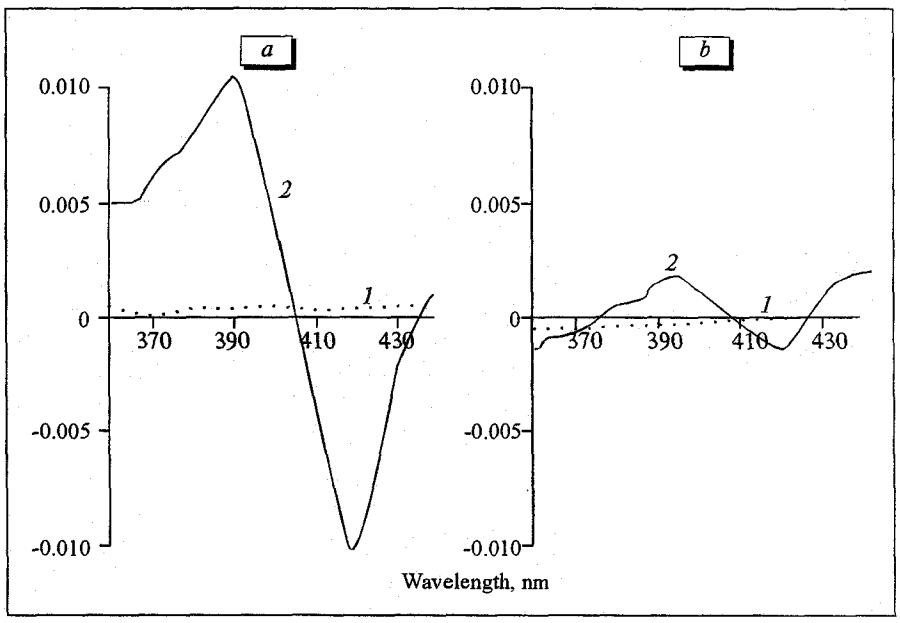

Fig. 1. Effect of dimedrol on differential light absorbance spectra of rat liver microsomes. *a*) before (*1*) and after (*2*) addition of dimedrol (0.3 mM) to liver microsomes of control rats; *b*) before (*1*) and after (*2*) mixing microsomes from control and dimedrol-treated (30 mg/kg) rats. A scheme of arrangement of cuvettes in a spectrophotometer (left pairs are cuvettes with microsomes from dimedrol-treated rats, right pairs are cuvettes with microsomes from control rats).

1 ● ● *2* * ●
○ ○ * ○

where:

○ microsomes of control rats;

● microsomes of dimedrol-treated rats;

* mixture of microsomal preparations.

Ordinate: optical density units.

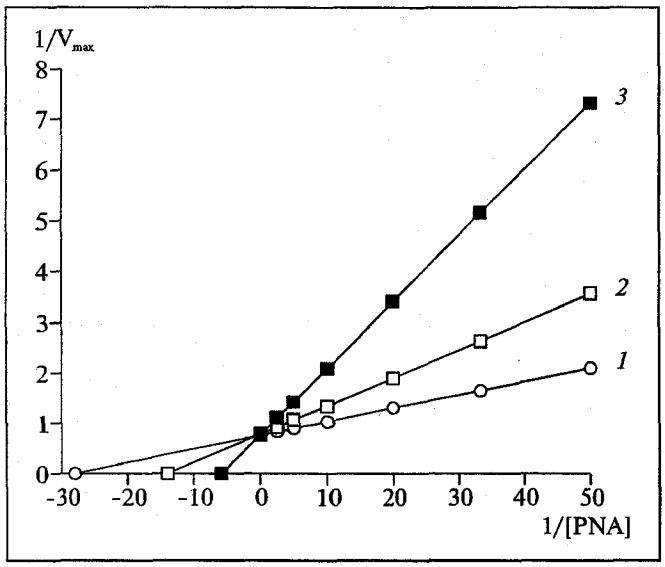

Fig. 2. Effect of dimedrol on the rate of PNP formation in the reaction of PNA-o-demethylation in rat microsomes (double reciprocal plots). *1*) microsomes from control rats; *2*) control+ dimedrol (10 μM); *3*) microsomes isolated from rat liver 15 min after administration of dimedrol (30 mg/kg).

A similar effect was observed after mixing microsomes from control and dimedrol-treated rats. Figure 1, *b* shows light absorbance spectra recorded from two pairs of cuvettes with experimental and control microsomes before and after mixing their contents. The 392 nm maximum and the 419 nm minimum point to the difference in the amounts of type I substrates bound to CC in the microsomal preparations.

It is likely that the differences in the amount of bound ligand are caused predominantly by dimedrol forming a tight complex with CC which is preserved after isolation of microsomes. The possibility that the saturation of CC with some endogenous substrates is different in microsomal preparations from control and dimedrol-treated rats cannot be ruled out. This is confirmed by a considerably higher (2.5-fold) effect of dimedrol on K_m for o-demethylation of PNA *in vivo* compared with that elicited in a microsomal preparation.

Our results suggest that a decrease in the PNA-o-demethylase activity of liver microsomes after the administration of dimedrol is caused by an increase in liver content of the CC—ligand (dimedrol and endogenous substrates) complex, which suppresses the formation of the PNA—CC complex by competitive inhibition. Dimedrol may modify nonsynthetic biotransformation of other xenobiotics in the liver, specifically, microsomal oxidation of barbiturates and morphine derivatives.

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