Biochemical Pharmacology, Vol. 36, No. 23, pp. 4167–4168, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

SHORT COMMUNICATIONS

Physical partitioning as the major source of metoprolol uptake by hepatic microsomes

(*Received* 26 *January* 1987; *accepted* 7 *May* 1987)

Metoprolol, a lipophilic beta-blocker, undergoes oxidative metabolism to the extent of 80% in the liver [1]. Involvement of cytochrome P-450-dependent monooxygenases in this oxidative breakdown is supported by *in vitro* studies [2, 3] which have demonstrated the ability of rat liver microsomes to catalyze the aliphatic hydroxylation and *O*-demethylation of metoprolol. In the present partition equilibrium study [4, 5] we show that such chemical phenomena contribute negligibly to microsomal uptake of metoprolol, which is dominated by direct drug partitioning into lipophilic regions of hepatic microsomes from rat and human sources.

Materials and methods

Microsomes were prepared by the Guengerich [6] method from freshly excised liver, as detailed elsewhere [7]. The current batch of rat liver microsomes had a cytochrome P-450 content of 0.9 nmol/mg protein, whereas that of the human liver microsomal preparation was 0.23 nmol/mg protein. Metoprolol tartrate was provided by Astra Pharmaceuticals, and [^{3}H]metoprolol (1-[4-(2-methoxyethyl)phenoxyl] - 3 - isopropylamino-2-^{3}H-propan-2-ol with a specific radioactivity of 527 Ci/mol and a radiochemical purity of 97% by TLC) was a gift from Dr K.-J. Hoffman, Hässle, Mölndal, Sweden. Cimetidine was donated by Smith Kline & French (Australia).

In partition equilibrium studies [4, 5] of the interaction between metoprolol and rat liver microsomes, reaction mixtures (170 μl) containing microsomal suspension (6 μM cytochrome P-450) and metoprolol (0.9–90 μM, suitably supplemented with trace quantities of [^{3}H]metoprolol in 0.067 M sodium phosphate buffer, pH 7.9 (0.005 M NaH_2PO_4–0.062 M Na_2HPO_4), were allowed to equilibrate for 25 min at room temperature (25–28°). Mixtures were then centrifuged for 10 min at 178,000 *g* in a Beckman Airfuge to yield samples of the supernatant liquid for the determination of free metoprolol concentration by scintillation counting in a Packard model C2425 spectrometer [4, 5]. Equivalent studies with the human liver microsomal preparation employed 2 μM cytochrome P-450 and metoprolol concentrations in the range 0.8–80 μM.

To obtain an estimate of the apparent partial specific volume, $\bar{v}_m^{app}$, of rat liver microsomes, an Anton Paar DMA 60/602 density meter was used to compare the density (ρ) of a mirosomal suspension having a protein concentration (c_P) of 14.4 mg/ml with that (ρ_0) of the buffer (0.067 M sodium phosphate buffer, pH 7.9). Substitution of these densities into the expression $\bar{v}_m^{app} = (\rho - \rho_0)/c_P$ yielded a value of 0.85 ml/g protein for the apparent partial specific volume expressed in this manner.

Results and discussion

Results of partition equilibrium studies of the interaction between metoprolol and rat liver microsomes are summarized (●) in Fig. 1(a), their most characteristic feature being the essentially linear form (correlation coefficient 0.98) of the dependence of the binding function, *r* (moles of drug bound/mole cytochrome P-450), upon free metoprolol concentration. Although this behaviour could reflect rectangular hyperbolic dependence for a system governed by an extremely large dissociation constant, such interpretation seems precluded in the light that a Michaelis constant of 18 μM emanates from kinetic studies of monooxygenase-catalyzed oxidation of metoprolol by an equivalent rat liver microsomal preparation [3]. Furthermore, the extremely high values of *r* attained (greater than 4 for a free metoprolol concentration of 100 μM) would require the existence of a large number of metoprolol-binding sites per mole of haemoprotein; in which case the interaction would certainly not be restricted to the single haem moiety, as required for monooxygenase catalysis. In this regard the extent of linear concentration dependence of *r* is even greater for the interaction of metoprolol with the human liver microsomal preparation (▲ in Fig. 1a: regression coefficient 0.97). However, re-expression of the binding function on a protein basis largely eliminates this disparity (Fig. 1b), an observation which adds weight to the above contention that drug-binding by cytochrome P-450 must represent a relatively minor contribution to metoprolol uptake by microsomes. In view of metoprolol's hydrophobic nature, a much more likely explanation of the observed phenomenon, frequently described erroneously as "nonspecific binding", is that the results presented in Fig. 1 are dominated by partition of metoprolol into the microsomal membranes. On the basis of the pK_a of 9.8 for metoprolol, the extent of ionization of its single amine nitrogen is only 1% under present conditions and even less at physiological pH.

Any rectangular hyperbolic concentration dependence of *r* emanating from strong interaction of metoprolol with the haem group of microsomal cytochrome P-450 is clearly masked in Fig. 1. However, the inclusion of 1 mM cimetidine, a competitive inhibitor of metoprolol oxidation [3], did lead to a slight decrease in *r* values for the rat liver microsomal system (○ in Fig. 1), without effect on the linear form (correlation coefficient 0.98). This observation verifies the existence of a metoprolol-binding contribution to the essentially linear binding curve (●); and also the existence of competition between metoprolol and cimetidine for at least some microsomal sites. In this regard it is first noted that the relative inability of cimetidine to partition into lipophilic regions [8] renders unlikely the interpretation that the decreased extent of metoprolol partitioning is the result of cimetidine incorporation into the microsomal phase. Secondly, the cimeditine concentration employed (1 mM) should suffice to saturate effectively all microsomal sites with affinity for this drug; and thus eliminate metoprolol-binding to those cytochrome P-450 isoenzymes. On that basis the slope of the line drawn through the results obtained in the presence of cimetidine (– – –, Fig. 1) provides an upper-limiting estimate of the coefficient describing partition of metoprolol between aqueous and microsomal phases.

For the partitioning of drug (*D*) between aqueous (a) and microsomal (m) phases with volumes V_a and V_m respectively, two types of coefficient seem pertinent. First, there is a partition coefficient describing the ratio of drug concentrations in the two phases (Eqn 1a); and secondly, there is a coefficient for the ratio of the amounts of drug in the two phases (Eqn 1b).

$$P = [D]_m/[D]_a \quad (1a)$$

$$R = P(V_m/V_a) \quad (1b)$$

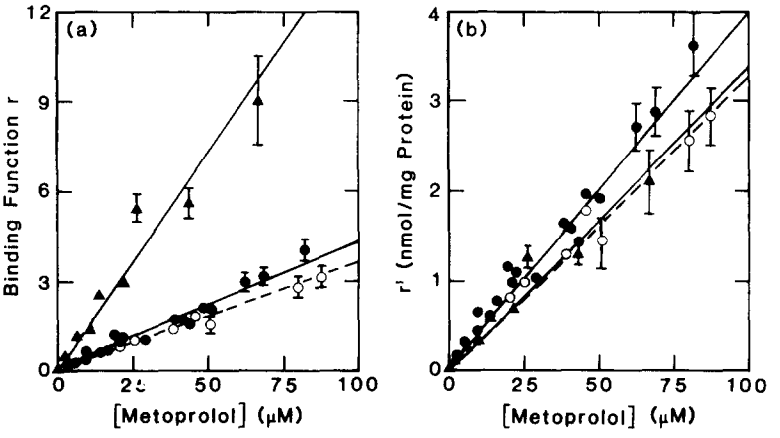

Fig. 1. Partition equilibrium results for the interaction of metoprolol with rat liver microsomes (●) and human liver microsomes (▲) in 0.067 M sodium phosphate buffer, pH 7.9, the results being expressed on the bases of (a) the cytochrome P-450 and (b) the protein contents of the microsomal preparations: ○, results obtained for rat liver microsomes in the presence of 1 mM cimetidine. The straight lines denote the relationships obtained by least-squares calculations on the appropriate sets of results; and the error bars the uncertainty inherent in quadruplicate estimations of r for the same batch of microsomes.

The coefficient that is independent of phase volumes, P, may be obtained from the slope of the broken line in Fig. 1(a), since, from the definition of the binding function, it follows that $P = [\text{P-450}](dr/d[D]_a)$, where [P-450] is the molar concentration of cytochrome P-450 in the microsomal suspension. The partition coefficient of 40 that is obtained by this means may be compared with a value of 93 for the corresponding distribution of metoprolol between octanol and water [9]: a comparison that may reflect the extent to which microsomes are comprised of lipophilic membranes.

From density measurement the apparent partial specific volume ($\bar{v}_m^{app}$) of rat liver microsomes was found to be 0.85 ml/g microsomal protein. In a 1-ml reaction mixture containing 6 mg/ml of microsomal protein suspension (the operative concentration in Fig. 1 (●)) the volumes of microsomal (V_m) and aqueous (V_a) phases are thus 5 μl and 995 μl respectively. Substitution of these quantities with the value of 40 for P into Eqn (1b) gives a value of 0.2 for R, the ratio of the amount of metoprolol in the microsomal phase to that in the aqueous phase. For this relatively dilute microsomal suspension the concentration of metoprolol in the aqueous phase thus accounts for only 83% of the free drug present: in the physiological situation the discrepancy would be much greater. From Table III of Fleischer and Kervina [10] the concentration of microsomal protein in a 5:1 homogenate of rat liver is approximately 18 mg/ml, which implies a value of 108 mg/g wet weight for the protein content of rat liver microsomes. On the basis of an assumed density of 1 g/ml for wet liver, and by reasoning analogous to that adopted above, $V_m = 0.092$ ml and $V_a = 0.908$ ml, whereupon $R = 4.1$. A measure of the metoprolol concentration in the systemic circulation (as indicative of the amount in the liver) would thus reflect only one-fifth of the total free drug present in the hepatic microsomal tissue.

Another consequence of the above calculation is that a fivefold difference in the magnitude of the dissociation constant for any metoprolol interaction with microsomal cytochrome P-450 would exist depending on the manner used to express free drug concentration [aqueous or total (aqueous plus microsomal) concentration]. Either description has equal thermodynamic validity, but that based on the aqueous concentration of metoprolol would be of greater clinical relevance inasmuch as the quantity most readily monitored is the concentration of drug in the systemic circulation. By indicating the existence of alternative thermodynamic descriptions of the same microsomal drug interaction, the present investigation draws attention to the fact that for lipophilic drugs there may be need for distinction between total free ligand (substrate) concentration, the quantity conventionally used in *in vitro* studies, and its aqueous counterpart, which would correspond to the systemic concentration of drug in the liver.

Though unsuccessful in its initial aim to provide further information on the binding of metoprolol to microsomes, this partition equilibrium study has served to highlight the need for consideration of an equally fascinating and pharmacologically important phenomenon—the microsomal uptake of lipophilic drugs by partition.

Acknowledgement—The support of this investigation by a Special Project Grant from the University of Queensland is gratefully acknowledged.

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