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Prediction of mescaline clearance by rabbit lung and liver from enzyme kinetic data

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Although many organs metabolize drugs and other xenobiotic agents, the liver is commonly accepted as the primary organ of drug metabolism *in vivo* since hepatic enzyme content is usually much greater than that of other organs. It has become evident, however, that enzyme content is not the sole determinant of drug clearance. Substrate delivery to a clearing organ, determined in part by the blood flow to the organ and by the extent of binding of the drug to plasma proteins, is also important [1].

In the rabbit, mescaline clearance is due to metabolism by an amine oxidase, the amount of which is much greater in liver than in other organs [2]. Although the lung contains less enzyme than liver, it receives the entire cardiac output while liver blood flow is only one-fourth of this value [3–6]. The higher blood flow to the lungs and, hence, greater substrate delivery may permit the lungs to contribute significantly to the clearance of mescaline *in vivo*.

In the isolated, perfused liver [7] as well as *in vivo* [5,6,8], clearance of drugs which are eliminated by hepatic metabolism has been shown to depend upon hepatic blood flow in the following manner:

$$Cl = \frac{Qf_B Cl_{int}}{Q + f_B Cl_{int}}, \quad (1)$$

where Q in the blood flow to the eliminating organ, f_B is the fraction of drug unbound in the plasma, and Cl_{int} is the intrinsic metabolic clearance. At low flows, clearance, as defined in equation (1), is proportional to flow; that is, substrate delivery limits clearance. At high flows, clearance approaches the intrinsic metabolic clearance, which is the maximum clearance of which an organ is capable when drug delivery is not limiting. Rane *et al.* [7] have shown that the intrinsic clearance of drugs eliminated by hepatic metabolism can be predicted from enzyme kinetic parameters using the following relationship:

$$Cl_{int} = \frac{V_{max}}{K_m}, \quad (2)$$

where V_{max} is the maximum rate of metabolism and K_m is the apparent Michaelis–Menten constant of the enzyme responsible for disposition.

We have extended the above model to predict pulmonary drug clearance. Since mescaline is cleared rapidly by isolated rabbit lungs perfused at low flows [2], it was of interest to predict the role of lung relative to liver in the clearance of mescaline *in vivo*. In the present study we have determined the kinetic constants for mescaline oxidase activity *in vitro* and have used the model of Rane *et al.* [7] to predict the relative clearance by rabbit lung and liver *in vivo*.

Female rabbits (3–4.5 kg) were given 5000 units of heparin i.v. and anesthetized by subsequent administration of pentobarbital (30 mg/kg, i.v.); the organs were cleared of blood as described previously [2]. The liver and lungs were then removed, weighed and homogenized in 0.1 M phosphate buffer containing 0.25 M sucrose (pH 7.4) in a Waring Blendor. Lungs and livers were homogenized in 12 and 6 vol. of buffer, respectively, and the homogenates were centrifuged for 10 min at 600 *g*. The resulting supernatant fractions were used in determining mescaline metabolism. Protein was analyzed by the method of Lowry *et al.* [9].

Determination of mescaline oxidase activity has been described previously [2]. Briefly, reaction mixtures contained 1 μM [8–^{14}C]mescaline hydrochloride (22.8 mCi/mmole, New England Nuclear, Boston, MA), sufficient cold mescaline hydrochloride (Aldrich Chemical Co., Milwaukee, WI) to achieve concentrations of 25, 50, 75, 100, 125 or 200 μM, 0.8 ml of phosphate buffer and 0.2 ml of the 600 *g* supernatant fraction of liver or lung homogenates. Reaction mixtures were incubated for 10 min at 37°. The reaction was stopped by the addition of 0.2 ml of 0.2 M $ZnSO_4$ followed by 0.2 ml of 0.2 M $Ba(OH)_2$. The

Table 1. Mescaline oxidase kinetics in 600 *g* supernatant fractions of rabbit lung and liver homogenates

Organ	K_m* (μM)	V_{max}* (nmole/min) (per g protein)	(per g organ)	(per organ)	Predicted Cl_{int}† (ml/min)
Lung	41.1 ± 10.9	739 ± 193	142 ± 36	1847 ± 432	56 ± 17
Liver	48.7 ± 6.1	351 ± 9	144 ± 8	13,750 ± 1,784	282 ± 56
Lung/liver	0.84	2.11	0.99	0.13	0.20

* Values represent means ± S.E.M. for five animals. Mescaline oxidase kinetic constants were determined as described in the text.

† Intrinsic metabolic clearance was calculated as $Cl_{int} = V_{max}/K_m$.

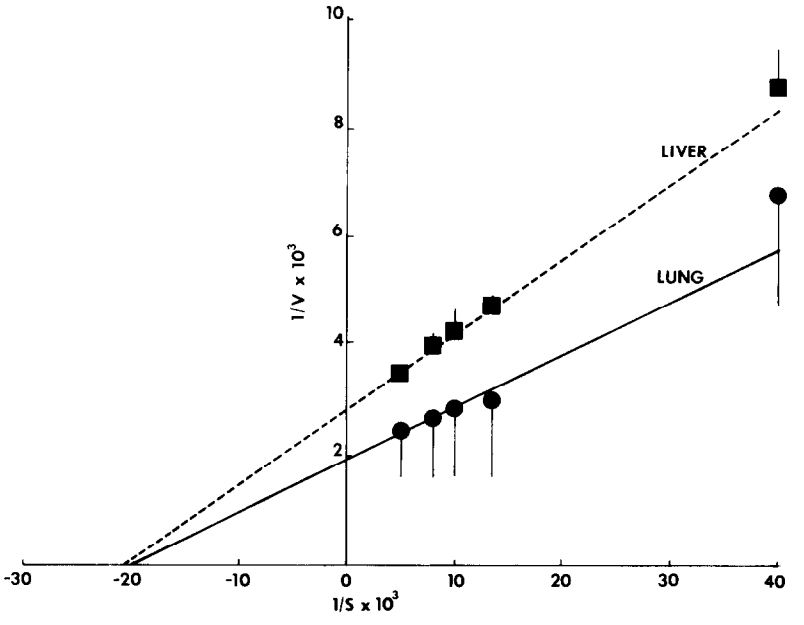

Fig. 1. Double-reciprocal plot (Lineweaver-Burk) of mescaline metabolism in 600 *g* supernatant fractions of rabbit lung and liver homogenates. Velocity is expressed as nmoles mescaline oxidized/min and substrate concentration as μM. Points represent means ± S.E.M., N = 5.

precipitate formed was removed by centrifugation at 3000 *g* for 10 min. The amine and its deaminated metabolite(s) were separated by applying aliquots of the resulting supernatant fraction to columns (0.5 × 1 cm) of Bio-Rex 70 (sodium form, pH 6.0) cation exchange resin (Bio-Rad Laboratories, Richmond, CA). Previous studies have shown that 3,4,5-trimethoxyphenylacetic acid is the only major metabolite produced by rabbit tissues *in vitro* [2]. The metabolite was removed with water, and then the amine was eluted with 0.2 N HCl. Radioactivity was determined by liquid scintillation spectrometry, and the concentrations of amine and metabolite were calculated from these values. The K_m and V_{max} values were calculated from the nmoles of mescaline metabolized per min by the method of Wilkinson [10]. Means were compared statistically using Student's *t*-test with $P < 0.05$ as the criterion for significance.

Mescaline binding to plasma protein was determined by equilibrium dialysis of rabbit plasma, rat plasma, or 4.3% bovine serum albumin (Pentex Fraction V, Miles Laboratories, Inc., Elkhart, IN) against a 0.01 M phosphate buffer in 0.15 M saline (pH 7.4) containing [^{14}C]mescaline hydrochloride (1 μM). The rabbit plasma was treated with semicarbazide (10^{-3}M) to inhibit plasma amine oxidase activity. Dialysis was performed with continual agitation for 24 hr at 4°. Radioactivity of mescaline was measured in the dialysis bags (plasma) and surrounding buffer. After correcting for volume changes during dialysis the fraction of mescaline bound to plasma proteins was calculated as:

$$\% \text{ bound} = \left(1 - \frac{\text{buffer d.p.m./ml}}{\text{plasma d.p.m./ml}}\right) \times 100. \quad (3)$$

The results of mescaline oxidase kinetic studies in 600 *g* supernatant fractions of liver and lung are presented in Fig. 1. The kinetic constants obtained from these experiments are summarized in Table 1. There was no difference between the K_m values for lung and liver. The specific activity (activity/g of protein) of pulmonary mescaline oxidase was twice that of liver; however, when expressed on a whole organ basis the liver had more than seven times the metabolic capacity of the lung. This was due primarily to the greater mass of the liver.

Equilibrium dialysis studies showed little binding (< 3 per cent) of mescaline to rabbit plasma, rat plasma or 4.3 per cent bovine serum albumin. Therefore, subsequent calculations were performed using $f_B = 1.0$ (i.e. no binding).

The intrinsic metabolic clearance (Cl_{int}) was estimated according to the model of Rane *et al.* [7] from the kinetic parameters (Table 1), and the relation between clearance and blood flow was predicted by substitution of these values into equation (1). These results are presented in Fig. 2. As mentioned above, the lung receives the entire cardiac output while liver blood flow is about one-fourth of this value [3–6]. Cardiac output in the rabbit is approximately 350 ml/min [11,12]. The predicted clearance of each organ at its respective blood flow *in vivo* is summarized in Table 2. The results predict that the rabbit lung is capable of clearing mescaline *in vivo* at a rate 72 per cent of that of liver.

Implicit in this model is the assumption that the rate of drug entry into the cells and transport to the active site of the enzyme do not limit clearance. This is consistent with the assumption of the model that drug in the tissue is equal to and in equilibrium with drug in the effluent perfusion

Table 2. Predicted clearance of mescaline *in vivo*

Organ	Blood flow* (ml/min)	Predicted Cl† (ml/min)
Lung	350	48
Liver	88	67
Lung/liver	4	0.72

* Rabbit cardiac output was approximated from Korner and Edwards [11] and from Boerboom and Boelkins [12]. Liver flow was approximated as one-quarter of cardiac output.

† Clearance was predicted according to the model of Rane *et al.* [7], as described in text.

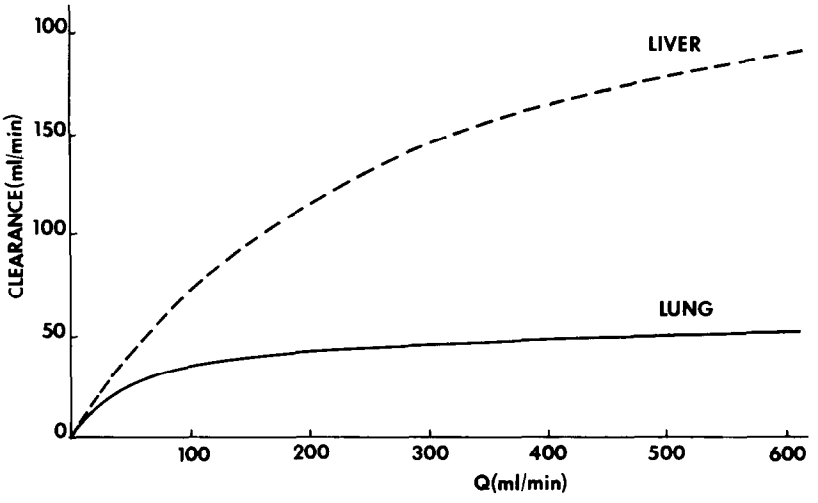

Fig. 2. Predicted relation between mescaline clearance and blood flow in rabbit lung and liver. Curves were predicted from equation (1) and from kinetic data as described in the text.

medium [13]. Thus, for drugs dependent upon a cellular transport mechanism to reach the active site of an enzyme, this model may fail to yield a reasonable prediction since the intrinsic clearance is predicted solely from kinetics of the enzyme involved in disposition. However, previous studies suggest that mescaline enters lung tissue by passive diffusion [2,14] and not by carrier-mediated transport.

In a previous study of pulmonary mescaline clearance, isolated rabbit lungs were perfused at a flow of 20 ml/min [2]. To test the validity of our predictions of lung clearance, the clearance at this flow was predicted as described above and compared statistically to the clearance values reported in Ref. 2. The predicted clearance and the clearance observed in the perfused lung [2] were not significantly different ($P > 0.10$, Student's t-test). This suggests that the model of Rane *et al.* [7] predicts adequately mescaline clearance by intact lung.

Thus, despite the fact that the liver has seven times the mescaline oxidizing capacity of the lung, the lung may contribute substantially to the total body clearance of mescaline. While these results need to be confirmed in intact organ preparations perfused at relevant flows, they suggest that the lung may be more important than has been recognized in the total body clearance of certain xenobiotic agents.

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