DISPOSITION OF PROPRANOLOL ISOMERS IN MICE

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A. LEVY, S.H. NGAI, A.D. FINCK, K. KAWASHIMA and S. SPECTOR, *Disposition of propranolol isomers in mice*, European J. Pharmacol. 40 (1976) 93—100.

l-Propranolol was found to protect mice from hyperbaric oxygen-induced seizures. The disposition of effective doses of propranolol isomers in mice was followed using stereospecific antibodies with a radioimmunoassay procedure. Serum and tissue concentrations were determined and correlated with the protective effect. Following racemic administration, there were no differences in serum disposition of d- and l-propranolol, although there was initially a preferential uptake of the l-isomer both into cardiac and brain tissue. The d-isomer exerted synergistic action on the l-isomer protective effect.

Propranolol disposition   Propranolol isomers   Radioimmunoassay   Synergism
Stereospecific uptake   Hyperbaric oxygen

1. Introduction

Ngai and colleagues have reported (1976) that seizures ensue in mice when exposed to hyperbaric oxygen (HPO) in the dark. The incidence of seizures was markedly reduced, and the time at which 50% of the mice convulsed (CT_{50}) prolonged when experiments were conducted in the light. It was reported that in the rat circadian rhythm of biochemical events in the pineal gland are controlled by stimulation of a β-adrenoreceptor (Brownstein et al., 1973). Ngai also showed that if central norepinephrine concentration was reduced by either a dopamine β-hydroxylase inhibitor (FLA-63) or by the combined treat-

ment with l-α-methyldopahydrazine and 6-hydroxydopa, the incidence of HPO-induced seizures decreased and the onset of seizures as measured by CT_{50} was delayed. The β-adrenergic blocking agent propranolol had the same protective effect in Swiss-Webster mice, however, only the l-isomer exhibited the protective action.

Propranolol disposition has been studied by many investigators in various species (Shand et al., 1970, 1971, 1972a,b); Hayes and Cooper, 1971; Evans et al., 1973). While some have reported differences in the kinetics of the d- and l-isomers (George et al., 1972; Nies et al., 1973; Mayers et al., 1974), they were unable to follow each isomer's disposition in the presence of the other because of the limitations imposed by analytical procedures. Since each isomer has different effects on biological tissue sites and as a consequence elicit different pharmacological effects, it is possible that the disposition of one isomer could be influenced by the presence of the other. Hemodynamic changes, caused by l-

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propranolol, can affect greatly the rate of metabolism.

The present studies were done in order to relate tissue concentrations of the propranolol stereoisomers to the protective effect on HPO seizures, using stereospecific antibodies in a radioimmunoassay procedure (Kawashima et al., 1976).

2. Materials and methods

d,l-Propranolol, as well as the stereoisomers (99.56% pure) were supplied by Ayerst Laboratories, Montreal, Canada. Swiss-Webster, male mice weighing 20—25 g were used in these studies. Propranolol in doses ranging from 4 to 16 mg/kg, was injected through the tail vein. Groups of 5 or more mice were killed at intervals following the injection. Blood was collected by decapitation and serum separated after the clotting. Brain, heart, liver or lungs were removed rapidly, and frozen until analysed. Serum samples were diluted in phosphate buffer saline (Dulbeccos' PBS by Gibco, Grand Island, N.Y.) and assayed by the radioimmunoassay procedure previously described (Kawashima et al., 1976). The antibodies principally bound the parent compound while the major metabolite, 4-hydroxypropranolol, required about 50 times the concentration of propranolol to produce the same degree of binding to the antibody. Hearts, lungs and livers were homogenized in 0.05 N HCl in icewater bath and centrifuged at 5,000 × g for 15 min. The supernatant was then diluted in PBS and assayed. Brain tissue was homogenized as above, a 100 μl aliquot was added into a small test tube (12 × 75 mm) and the solution made basic by the addition of 50 μl of 0.5 N NaOH. Propranolol was then extracted into 1.2 ml of petroleum ether containing 10% isopropyl alcohol, by vortexing the mixture for 30 sec. The tubes were centrifuged at 4°C and 1,500 × g for 15 min. The lower aqueous layer was frozen in an acetone-dry ice bath, and the upper organic layer decanted and evaporated under a stream of air. The

residue was dissolved in distilled water containing 25% isopropyl alcohol. This solution was then assayed, after being diluted to fit the range of the radioimmunoassay standard concentration curve.

Recovery of propranolol through the procedure was determined by homogenizing tissues from control animals in the presence of known amounts of d,l-propranolol. Recovery values were 97 ± 4% for hearts, lungs and livers, and 80 ± 3% for the extracted brains. Drug concentrations in the brain were corrected for recovery. Each sample was assayed in duplicate by both d,l- and l-propranolol antibodies. In each assay a standard curve was run in the presence of control serum or a control tissue, processed together with the sample tissues. All samples were counted twice in the liquid scintillation counter for 5 min. Results were then calculated separately for each set, and averages were taken. The d,l-antibody (d,l-Ab) recognizes total amount of d- plus l-isomers, and recognizes equally each of the isomers. The antibody directed against the l-isomer can bind the d-isomer, however, it requires 11 times more of the d-isomer to produce the same degree of inhibition as the l-isomer. Thus, if one injects the racemic mixture, the results obtained by using l-antibody (l-Ab) represent l-concentration plus 1/11 of the concentration of the d-form.

(a) d,l-Ab result = {l-isomer} + {d-isomer}
(b) l-Ab result = {l-isomer} + 1/11 {d-isomer}

Multiplying equation (a) by —1 and equation (b) by 11, one gets the following equation for the corrected l-isomer concentration.

{l-isomer} = 11[(l-Ab result) —(dl-Ab result)] / 10

3. Results

It has been shown (Ngai et al., 1976) that the administration of 8—16 mg/kg of l-propranolol or 16 mg/kg d,l-propranolol protected Swiss-Webster mice from HPO-induced seizures. Serum concentrations for d- and l-propranolol at various time intervals follow-

TABLE 1

Serum concentrations (ng/ml) at different intervals following i.v. administration of propranol isomers.

Time (min)	Dose (mg/kg)					
	16 (d, l)		16 (l)	8 (l)	8 (d)	4 (l)
	d-isomer	l-isomer				
5	830 ± 100 [1]	880 ± 50	1.510 ± 130	880 ± 50	1.390 ± 110	590 ± 60
15	540 ± 90	560 ± 50	770 ± 30	550 ± 50	1.050 ± 100	400 ± 50
30	420 ± 60	430 ± 30	550 ± 40	380 ± 40	520 ± 50	240 ± 20
60	260 ± 50	200 ± 20	260 ± 30	190 ± 20	320 ± 30	120 ± 10
90 [2]	150 ± 30	130 ± 20	150 ± 10	140 ± 20	180 ± 30	75 ± 10
120	80 ± 10	80 ± 10	75 ± 10	80 ± 10	90 ± 10	50 ± 5
180	26 ± 4	24 ± 3				
240	15 ± 2	16 ± 2				
360	7 ± 1	8 ± 1				

[1] Values at each time period represent mean ± S.E. from 4—5 Swiss-Webster mice. The standard error in a single determination was 0.7—2.5% of the mean. The standard error for a group of mice was 8—12% of the mean value.
[2] The 90 min figures represent blood concentrations at the time when the mice were exposed to hyperbaric oxygen.

ing the i.v. injection of different doses in this range are given in table 1.

Fig. 1 shows drug concentrations in the heart following the injection of 16 mg/kg of
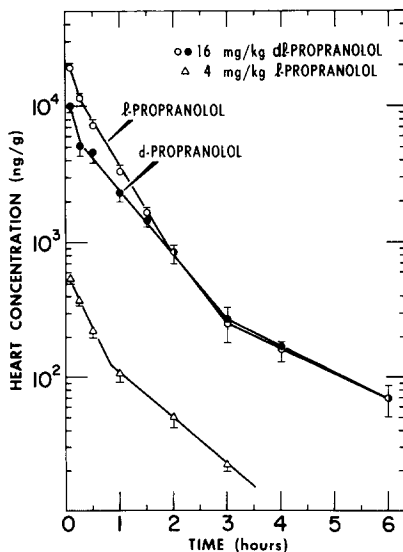

Fig. 1. Propranolol concentrations in the heart, following i.v. injection of 16 mg/kg d,l-propranolol (results given for d- and l-isomers separately), and following i.v. injection of 4 mg/kg l-propranolol.

d,l-propranolol. The figure shows corrected concentrations for l-propranolol, as derived by the empirical equation. The concentrations for d-propranolol were obtained by subtracting the l-isomer concentrations from the results obtained by the assay using d,l-antibody. The third curve on this figure shows the drug concentrations in the heart following the injection of 4 mg/kg of l-propranolol.

Fig. 1 shows a preferential initial uptake of l-propranolol over the d-isomer. In the first 15 min twice as much l-propranolol was found in the heart as the d-form.

Plotting the ratio of heart to blood total drug concentrations (fig. 2), it is apparent that at the higher dose of propranolol the drug accumulated initially in the heart to a much greater extent than with the lower dose, but after 2 h tissue to blood ratios were the same with both doses (10 ± 0.3).

Fig. 3A, 3B and 3C show the concentrations of d,l- and l-propranolol in the lungs, liver and brain, following the i.v. injection of 16 mg/kg d,l-racemate. Results of the l-isomer were corrected as explained above. In fig. 3D brain concentrations are followed for two different doses of l-propranolol.
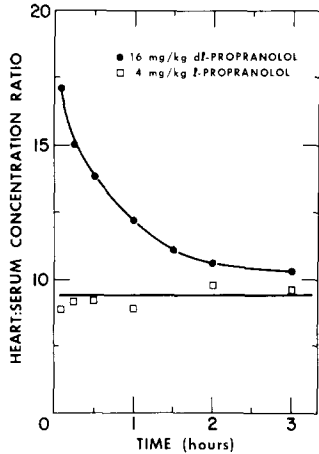
Fig. 2. Ratio of total propranolol concentration in the heart to propranolol concentration in the serum, at intervals following i.v. injection of 16 mg/kg d,l-propranolol and 4 mg/kg l-propranolol.

Since mice were exposed to HPO 90 min following the injection of propranolol, brain concentrations at this time point were plotted in fig. 4 against the doses. This curve shows a linear relationship between the drug concentrations in the brain and the dose administered. On the same curve results for experiments conducted in the dark are reported, showing that there was no effect of light on the concentration of propranolol in the brain.

The ratio of brain to blood concentrations is plotted in fig. 5 against time, for two of the doses administered. A rapid accumulation of propranolol into the brain is shown, with the brain to blood ratio reaching a high level of 48 ± 3. The accumulation with the higher dose was faster than the one recorded for the
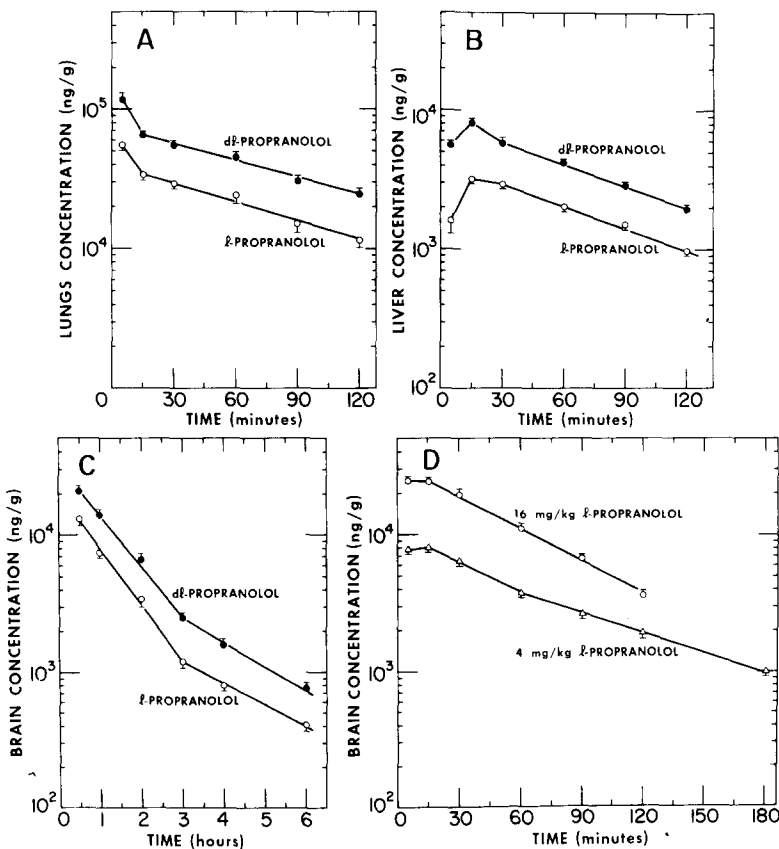

Fig. 3. Propranolol concentrations in the lungs (A), liver (B) and brain (C), following i.v. injection of 16 mg/kg d,l-propranolol. Results are given for d,l- and l-propranolol concentrations in each case. Propranolol concentrations in the brain (D) after two other doses of l-propranolol (4 and 16 mg/kg).
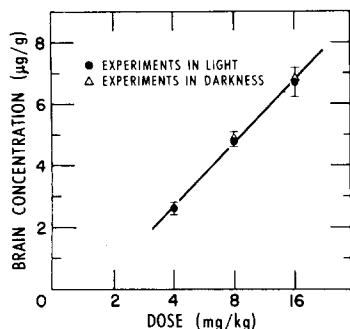
Fig. 4. Propranolol concentrations in the brain 90 min following i.v. injection of 4, 8 and 16 mg/kg l-propranolol.
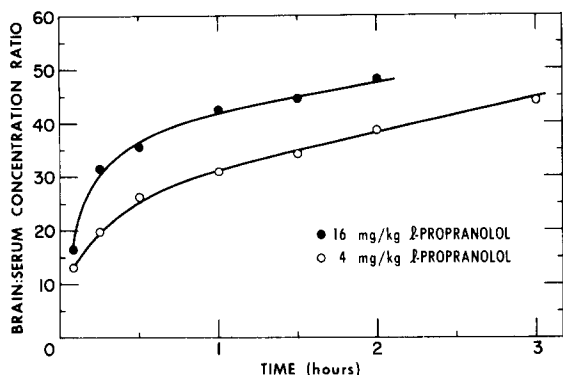

Fig. 5. Ratio of propranolol concentration in the brain to propranolol concentration in the serum, following i.v. injection of 4 and 16 mg/kg l-propranolol.

TABLE 2

Brain propranolol concentrations vs. protection against HPO-induced seizures.

Dose (mg/kg)	Brain concentration (ng/g) 90 min following injection	Protection[1]
4 (l)	2,600	—
8 (l)	4,800	+
8 (d)	3,800	—
4 (l) + 8 (d)	2,600 (l) 3,800 (d)	+
16 (l)	6,700	+
16 (d)	5,800	—

[1] Report of experimental conditions and detailed results are to be published.

lower dose, although finally they achieved a similar plateau level.

Table 2 relates the protective effect of propranolol and brain concentration in Swiss-Webster mice, 90 min post-injection of the drug, which was the time the animals were introduced into the hyperbaric oxygen chamber. Protection against HPO-induced seizures was demonstrated by both a decrease in the incidence of seizures from around 70% to 30—40%, and by increase of CT_{50} from 17 min to around 30 min (Ngai et al., to be published).

It was found that the protective dose using a different strain of mice (CF1) was lower (4 mg/kg l-propranolol) than the one needed for Swiss-Websters (8 mg/kg). Therefore, the disposition of l-propranolol was followed in the CF1 strain of mice. In fig. 6, blood and brain concentrations for two l-propranolol doses injected to CF1 mice were plotted against the time following injection. Concentrations are significantly higher, especially those found in the brain. This observation
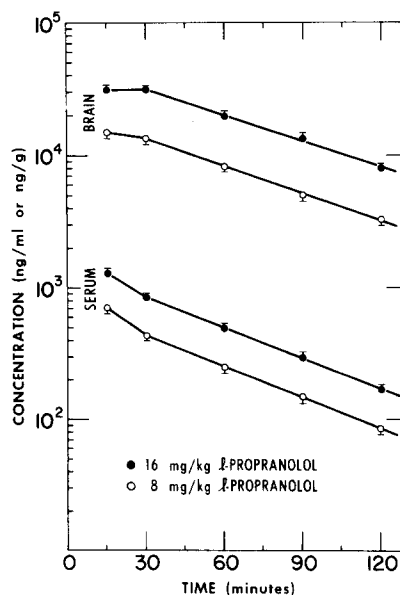

Fig. 6. Propranolol concentrations in the brain and in the serum following i.v. injection of 8 and 16 mg/kg l-propranolol using CF1 mice.

may explain the fact that CF1 mice are also less tolerant to propranolol than the Swiss-Webster strain.

4. Discussion

Exploiting the sensitivity and specificity of the propranolol antibodies, the disposition of propranolol was followed in various tissues of mice by the radioimmunoassay, with the thought of correlating the protective effects of propranolol against HPO-induced seizures and concentrations of drug in tissues.

Propranolol exhibits a very rapid initial decay curve, which reflects the distribution of the drug into various tissues followed by an extensive metabolism by the liver (Shand et al., 1971; Walle and Gaffney, 1972). As discussed previously (Wilkinson and Shand, 1975), propranolol hepatic clearance is blood flow-limited. In the present investigation, serum levels, and not blood levels were determined. Since dose-dependent changes in the blood-to-serum concentration ratio may have occurred, it is difficult to evaluate extraction of the drug from the blood by the liver. Also, the drug itself induces changes in organ blood flow rates, which have not been evaluated.

Serum concentrations were determined up to 120 min post-injection in conjunction with the time table of the HPO experiments. Detailed pharmacokinetic analysis of these data in terms of two-compartment models is equivocal as we cannot be sure that the terminal exponential phase was reached. Therefore, determination of rigorous half-lives for the two phases was avoided.

These studies show that (a) no difference was recorded in serum disposition of d- and l-isomers following racemic administration, (b) preferential uptake of the l-isomer was noted both in the heart and the brain, (c) synergistic action of the d-isomer on the l-propranolol protective effect was found.

Following the i.v. administration of 16 mg/kg d,l-propranolol, elimination rates of both the d- and l-isomers from the serum appear to be the same (table 1 under 16 (d,l)). This present study shows no significant difference in serum levels of the isomers of propranolol in mice. This is in contrast to reports of other investigators (George et al., 1972; Nies et al., 1973; Mayers et al., 1974) who did show differences in other species. Another possible explanation for the difference is that in other studies propranolol isomers were administered separately, and only the l-isomer affects hepatic blood flow thereby decreasing hepatic clearance.

We are able to measure one isomer in the presence of the other following the administration of a racemic mixture. The concentrations of propranolol found in the heart following the injection of 16 mg/kg d,l-propranolol showed a preferential uptake of the l-isomer. The initial ratio of (l/d) drops gradually from a ratio of 2 in the first 15 min to 1.0 90 min after the injection. This could be a result of a rapid specific uptake of the l-isomer which prevails initially. Since we are administering a high dose of d,l-propranolol, a slower non-specific uptake process could account for the later accumulation of the d-isomer. In a previous report (Kawashima et al., 1976) we showed that in rats l-propranolol was selectively taken by the heart following i.v. injection of 1 mg/kg of the racemic mixture. The present study differs in that high doses of propranolol were given to mice. Thus, while diffusion as well as an active uptake always play a role in the uptake of a drug into tissues, following administration of high doses of drug the role of non-specific diffusion may be more pronounced.

The very high heart-to-blood concentration ratio following 16 mg/kg of d,l-propranolol (fig. 2) can also be attributed to the high dose, and with time (approximately 3 h) the same ratio was achieved as obtained immediately with a lower dose. Our emphasis is on dose rather than isomeric form influencing this ratio, as the concentration of the l-isomer in the heart is greater than the d-isomer during this time period. Therefore, the high ratio observed could not be explained by

high concentration of d-propranolol, following the injection of the racemic mixture. 4 h after injection the heart and the blood seem to reach a complete equilibrium, with similar slopes of the decay curves.

The lung and liver achieved equal concentrations of d- and l-isomers 30 min post-injection. In the brain, however, there seems to be a preferential initial uptake of l-propranolol, similar to that of the heart. At 30 min following injection the ratio of (l/d) is 1.6, then falling to 1.0 from 60 min on. Brain concentrations were also higher when l-propranolol was injected, as compared to the concentrations obtained with the same dose of d-propranolol (see table 2). Because of the high lipid solubility of the drug, the brain to serum ratio increased rapidly with time until a ratio of 48 ± 3 is obtained.

It is interesting to note that 30 min following i.v. injection of 16 mg/kg of d,l-propranolol, only 8% of the total amount administered was accounted for in the tissues studied. The distribution of this 8% was as follows: liver (44%), brain (30%), lung (14%), blood (8%) and heart (4%). The remainder undoubtedly was taken up by other tissues having high lipid content, which were not analyzed, and a fraction was also metabolized. The data point out the large relative accumulation of propranolol in the liver and brain. In terms of concentration (ng/g), however, the lung exhibits the highest value (fig. 3A), as reported previously for other species (Hayes and Cooper, 1971).

We tried to correlate the protective effect of propranolol on HPO-induced seizures and brain concentrations. According to the data presented in table 2, protection can occur only with l-propranolol, and only after brain concentrations are higher than 4.8 μg/g in the Swiss-Webster mice. However, one can also obtain protection with lower doses of l-propranolol, in the presence of d-propranolol. This is not an additive effect, since a dose of 16 mg/kg of d-propranolol, which gave a brain concentration of 5,800 ng/g, failed to show any protection. This might be interpreted to imply that since only a small fraction of the tissue contains β-adrenoreceptor sites, the bulk of the tissue l-propranolol is taken up by non-specific sites in the brain. If one saturates these non-specific sites with the d-isomer, then more of the l-isomer is available to interact with the specific β-receptor sites. Therefore, in the presence of d-propranolol, the effective dose of l-propranolol could be lowered. This synergistic action may have important consequences for the application of propranolol as a blocker of β-receptors.

Acknowledgement

References

Brownstein, M., J.M. Saavedra and J. Axelrod, 1973, Control of pineal N-acetylserotonin by a beta adrenergic receptor, Mol. Pharmacol. 9, 605.

Evans, G.H., A.S. Nies and D.G. Shand, 1973, The disposition of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat, J. Pharmacol. Exptl. Therap. 186, 114.

George, C.F., T. Fenyvesi, M.E. Conolly and C.T. Dollery, 1972, Pharmacokinetics of dextro-laevo- and racemic propranolol in man, European J. Clin. Pharmacol. 4, 74.

Hayes, A. and R.G. Cooper, 1971, Studies on the absorption, distribution and excretion of propranolol in rat, dog, and monkey, J. Pharmacol. Exptl. Therap. 176, 302.

Kawashima, K., A. Levy and S. Spector, 1976, Stereospecific radioimmunoassay for propranolol isomers, J. Pharmacol. Exptl. Therap. 196, 517.

Mayers, M.G., P.J. Lewis, J.L. Reid and C.T. Dollery, 1974, Brain concentration of propranolol in relation to hypertensive effect in the rabbit with observations on brain propranolol levels in man, J. Pharmacol. Exptl. Therap. 192, 327.

Ngai, S.H., A.D. Finck, A. Levy, J.C. Yang and S. Spector, 1976, Hyperbaric oxygen induced seizures: Effects of available light, CNS norepinephrine and β-adrenergic blockade, Fed. Proceedings, 60th Meeting, 237.

Nies, A.S., G.H. Evans and D.G. Shand, 1973, The hemodynamic effects of beta adrenergic blockade on the flow-dependent hepatic clearance of propranolol, J. Pharmacol. Exptl. Therap. 181, 716.

Shand, D.G., E.M. Nuckolls and J.A. Oates, 1970, Plasma propranolol levels in adults with observations in four children, Clin. Pharmacol. Therap. 11, 112.

Shand, D.G., G.H. Evans and A.S. Nies, 1971, The almost complete hepatic extraction of propranolol during intravenous administration in the dog, Life Sci. 10, 1417.

Shand, D.G. and R.E. Rangno, 1972a, The disposition of propranolol. I. Elimination during oral absorption in man, Pharmacology 7, 159.

Shand, D.G., R.E. Rangno and G.H. Evans, 1972b, The disposition of propranolol. II. Hepatic elimination in the rat, Pharmacology 8, 344.

Walle, T. and T.E. Gaffney, 1972, Propranolol metabolism in man and dog: mass spectrometric identification of six new metabolites, J. Pharmacol. Exptl. Therap. 182, 83.

Wilkinson, G.R. and D.G. Shand, 1975, A physiological approach to hepatic drug clearance, Clin. Pharmacol. Therap. 18, 377.