Transport of Propranolol and Lidocaine through the Rat Blood-Brain Barrier

PRIMARY ROLE OF GLOBULIN-BOUND DRUG

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ABSTRACT Basic lipophilic drugs such as propranolol and lidocaine are strongly bound by α_1-acid glycoprotein, also called orosomucoid. Although the liver is known to rapidly clear plasma protein-bound propranolol or lidocaine, it is generally regarded that peripheral tissues, such as brain or heart, are only exposed to the small fraction of drug that is free or dialyzable in vitro. The "free drug" hypothesis is subjected to direct empiric testing in the present studies using human sera and an in vivo rat brain paradigm.

Serum from 27 human subjects (normal individuals, newborns, or patients with either metastatic cancer or rheumatoid arthritis) were found to have up to a sevenfold variation in orosomucoid concentrations. The free propranolol or lidocaine as determined in vitro by equilibrium dialysis at 37°C varied inversely with the orosomucoid concentration. Similarly the rate of transport of propranolol or lidocaine through the blood-brain barrier (BBB) was inversely related to the existing serum concentration of orosomucoid. However, the inhibition of rat brain extraction of drug by orosomucoid in vivo was only about one-fifth of that predicted by free drug measurements in vitro. This large discrepancy suggested orosomucoid-bound drug was readily available for transport into brain in vivo. Studies using purified human orosomucoid in the rat brain extraction assay also showed that orosomucoid-bound propranolol or lidocaine is readily transported through the BBB. Conversely, albumin-bound propranolol or lidocaine was not transported through the BBB. The studies using albumin provide evidence that the in vivo rat brain paradigm used in the present investigations is capable of confirming, when possible, predictions made by the "free drug" hypothesis.

These data suggest that the amount of circulating propranolol or lidocaine that is available for transport into a peripheral tissue such as brain is not restricted to the free (dialyzable) moiety but includes the much larger globulin-bound fraction. Therefore, existing pharmacokinetic models should be expanded to account for the transport of protein-bound drugs into peripheral tissues similar to what is known to occur in liver.

INTRODUCTION

Basic lipophilic drugs such as propranolol or lidocaine are widely used in clinical practice (1, 2). The pharmacologic effect of these agents is proportional to the plasma total drug concentration (3, 4). The clinical interpretation of plasma drug levels is complicated by the fact that many basic lipophilic drugs are bound by a plasma globulin, orosomucoid, i.e., α_1-acid glycoprotein (5–7). Orosomucoid is an acute-phase reactant and serum levels of this protein rise severalfold in a variety of inflammatory illnesses (8). For example, the binding of propranolol (and chlorpromazine, another basic lipophilic drug) by orosomucoid is increased in rheumatoid arthritis, Crohn's disease, chronic renal failure, and hyperthyroidism (5, 9). Orosomucoid binding of lidocaine increases after myocardial infarction, due to increased orosomucoid levels (10). Plasma binding of quinidine, another basic lipophilic drug, increases precipitously in the postoperative period in parallel with increases in plasma orosomucoid concentrations (11). Conversely, high estrogen states, e.g., oral contraceptives, pregnancy, cirrhosis, and the fetal state, are associated with low orosomucoid con-

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centrations and with decreased plasma protein binding of propranolol (12, 13).

Protein-bound propranolol is known to be readily available for entry into liver (14) and this accounts for the large first pass effect by liver on the systemic bioavailability of drug (15). Although it is widely recognized that protein-bound drug is transported into liver (14), it is still generally regarded that only free, nonprotein bound drug is available for entry into peripheral tissues such as the heart and brain (16, 17). This latter view is supported by the observation that the cardiac effect of propranolol correlates well with the free drug in serum (18). However, a demonstration of the correlation between two parameters does not prove a causal relationship and does not provide information regarding the pathway by which circulating propranolol reaches receptor sites in peripheral tissues.

A critical examination of the free drug hypothesis for peripheral tissues has yet to be undertaken, owing to the lack of a suitable methodology to empirically test the hypothesis (19). In these studies, we test the free drug hypothesis with studies of propranolol and lidocaine transport into brain, by a method used previously by us to test the free hormone hypothesis (20–22). The method measures the effects of plasma protein on the transport of hormones and drugs through the rat brain endothelial wall, i.e., the blood-brain barrier (BBB).[1] Since plasma proteins do not cross the BBB, the inhibition of BBB transport of ligand by the plasma protein reflects in vivo binding of ligand within the brain capillary lumen. This in vivo rat brain paradigm provides a means for direct empiric testing of the free drug hypothesis in the in vivo state vis-à-vis conventional in vitro methods such as equilibrium dialysis.

METHODS

Eight samples of cord blood were obtained from three males and five females at term after the cord was clamped. Serum was obtained from six patients with rheumatoid arthritis (all females; 28–65 yr), seven metastatic cancer patients (four females, three males; 27–68 yr), and six healthy subjects (two females, four males; 26–35 yr).

The L-[4-^{3}H]propranolol, 28.7 Ci/mmol; [*carbonyl*-^{14}C] lidocaine 48.3 mCi/mmol; and [*N*-1-^{14}C]butanol, 1.0 mCi/mmol were purchased from New England Nuclear, Boston, MA. The labeled compounds were stored under nitrogen at −20°C in the manufacturer solvent, until use. The radiochemical purity of the labeled drug was >98% as assessed by thin-layer chromatography and radioscanning. The drugs were chromatographed on 250 μm silica gel G plates (Analtech, Inc., Newark, DE) in chloroform/methanol/ammonia (6:4:0.1) (propranolol), and chloroform/methanol (95:5) (lidocaine). Unlabeled propranolol and lidocaine standards were visualized by UV light. Orosomucoid was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA.

The first pass brain or liver extraction of [^{3}H]propranolol relative to [^{14}C]butanol, or [^{14}C]lidocaine relative to [^{3}H]water, was measured with a tissue sampling-single injection technique (23, 24) in barbiturate anesthetized (50 mg/kg sodium pentobarbital i.p.) male Sprague-Dawley rats (200–300 g). The [^{14}C]butanol and [^{3}H]water are differentially labeled highly diffusible internal standards of tissue clearance (23, 25). Since propranolol was commercially available in the ^{3}H-form, [^{14}C]butanol was used as a reference. Similarly, lidocaine was commercially available in the ^{14}C-form, so [^{3}H]water was used as a reference.

In the case of brain transport studies, an ~200 μl bolus of buffered Ringer's solution (pH 7.4; 5 mM Hepes) was rapidly injected (<1 s) into the right common carotid artery via a 27-gauge needle. The injection solution contained 1–10 μCi/ml ^{3}H-compound and 0.25–1.0 μCi/ml ^{14}C-compound and either human serum (80%) or purified plasma proteins. At 15 s after the injection, the rat was decapitated. A sample of the injection solution and the hemisphere ipsilateral to the injection were solubilized in duplicate in 1.5 ml Soluene-350 (Packard Instrument Co., Downers Grove, IL) at 50°C for 2 h before double-isotope liquid scintillation counting.

Drug transport in liver was determined after injection of the same solutions into the portal vein, immediately after ligation of the hepatic artery. At 18 s after injection, the right major lobe was removed. The liver was also solubilized in duplicate in 1.5 ml Soluene-350 before liquid scintillation counting.

Because the rate of injection exceeds the rate of either portal blood flow or carotid blood flow, the injection solution traverses the hepatic and brain microcirculation as a bolus without significant mixing with the circulating rat plasma (20, 21).

Counts per minute were converted to disintegrations per minute by standard quench corrections and the percent brain uptake index (BUI) and liver uptake index (LUI) were calculated as follows:

propranolol BUI or LUI = (^{3}H/^{14}C dpm) in brain or liver/(^{3}H/^{14}C dpm) in injectate × 100; lidocaine BUI or LUI = (^{14}C/^{3}H dpm) in brain or liver/(^{14}C/^{3}H dpm) in injectate × 100.

The BUI or LUI = E_t/E_r, where E_t and E_r represent the extraction of the test compound ([^{3}H]propranolol or [^{14}C]lidocaine) and the reference compound ([^{14}C]butanol or [^{3}H]water), respectively, at 15 s after injection. The E_t or E_r represents the maximal extraction of unidirectional influx into brain minus the back-diffusion of test or reference compound during the period between bolus flow through brain (~2–5 s after injection) and decapitation (at 15 s after injection). With regard to the reference compounds, the maximal extraction ($E_r°$) of [^{14}C]butanol and [^{3}H]water under the experimental conditions is 100 and 62%, respectively (25, 26). The relationship between $E_r°$ and the extraction at 15 s, [E_r (15 s)], is defined as (26),

$$E_r(15\ s) = E_r^{\circ} e^{-kt},$$

where k = the efflux rate constant for the [^{3}H]water, 0.46 min^{-1} (26), or the efflux rate constant for [^{14}C]butanol, 0.67 min^{-1} (27). Substitution of the values for $E_r°$ and k, and using

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[1] *Abbreviations used in this paper*: BBB, blood-brain barrier; BUI, brain uptake index; E_r, extraction of the reference compound; $E_r°$, maximal reference extraction; E_t, extraction of the test compound; K_D (app), apparent dissociation constant; LUI, liver uptake index.

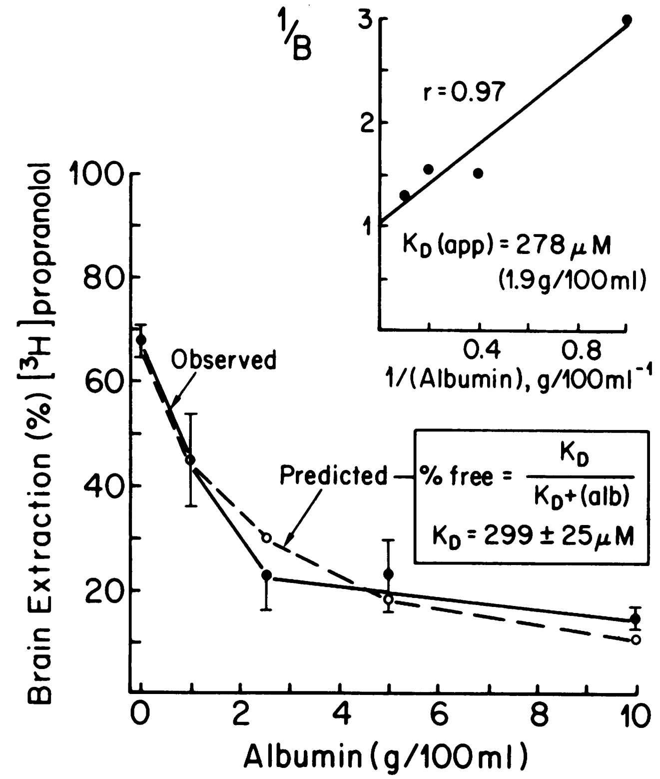

FIGURE 1 The rat brain extraction of $[^3H]$propranolol (mean±SE, $n = 3–6$ rats per point) is plotted against the concentration of bovine albumin in the carotid injection solution (observed line). The predicted line was obtained from the product, $E_t^o \times$ (% free), where E_t^o = the extraction in the presence of 0.1 g/100 ml albumin, i.e., when >95% of drug is free, and (percent free) was determined from the law of mass action (see figure) for each albumin (alb) concentration. K_D was determined by equilibrium dialysis at 37°C. The double-reciprocal plot provides the (app) K_D as determined from the measurements of albumin-bound (B) drug in vivo in the brain capillary, according to the equation, $1/B = 1 + [K_D \text{ (app)}](1/\text{alb})$ (Methods). The equivalence of the K_D (app) in vivo and the absolute K_D in vitro is indicative of the lack of transport of albumin-bound propranolol into brain.

t = 0.17 min (the time between bolus entry into brain and decapitation) into the above equation indicates E_r (15 s) = 58% for $[^3H]$water and E_r (15 s) = 90% for $[^{14}C]$butanol. With regard to the drugs, $[^3H]$propranolol and $[^{14}C]$lidocaine, the E_t (15 s) is essentially identical to E_t^o. Owing to active sequestration of the lipophilic amines by rat brain, similar to processes reported for gonadal steroid hormones (27), the drugs are retained by brain and return to blood slowly ($t_{1/2}$ = 7 min).[2] Therefore, the drug extraction value measured in the present studies represents the maximal extraction of unidirectional influx into brain.

With regard to the calculation of the E_t in liver, the E_r (18 s) has been measured directly for liver, e.g., E_r (18 s) = 84% for $[^{14}C]$butanol and E_r (18 s) = 65% for $[^3H]$water (28). Since drugs such as propranolol and lidocaine are actively sequestered by liver (15), it is assumed that little back-diffusion of drug occurs within the 18-s circulation period. Therefore, the E_t for liver measured in the present studies represents the maximal percent extraction of unidirectional influx into liver.

Since unidirectional influx is the measured parameter, the E_t for each drug is a function only of tissue blood flow, membrane permeability, and plasma protein effects. Factors such as tissue binding or metabolism of drug, which alter the net metabolic clearance of drug, do not influence the unidirectional E_t. Therefore, measurements of unidirectional E_t may not necessarily predict the outcome of systemic drug distribution if the latter is largely influenced by tissue factors. However, measurements of E_t isolate the effects of plasma proteins from the tissue factors (binding and metabolism) and provide direct testing of plasma protein effects in vivo.

Since plasma proteins lower the E_t value due to binding of drug, the fractional binding of drug in vivo (B) is equal to (22),

$$B = 1 - \left\{\frac{E_t(\text{serum})}{E_t(\text{Ringer's})}\right\},$$

where E_t (serum) and E_t (Ringer's) represent the brain or liver extraction of drug after injection in either serum or Ringer's solution, respectively. In previous studies (20, 22), we have shown that the linear equation

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[2] Pardridge, W. M. Unpublished observations.

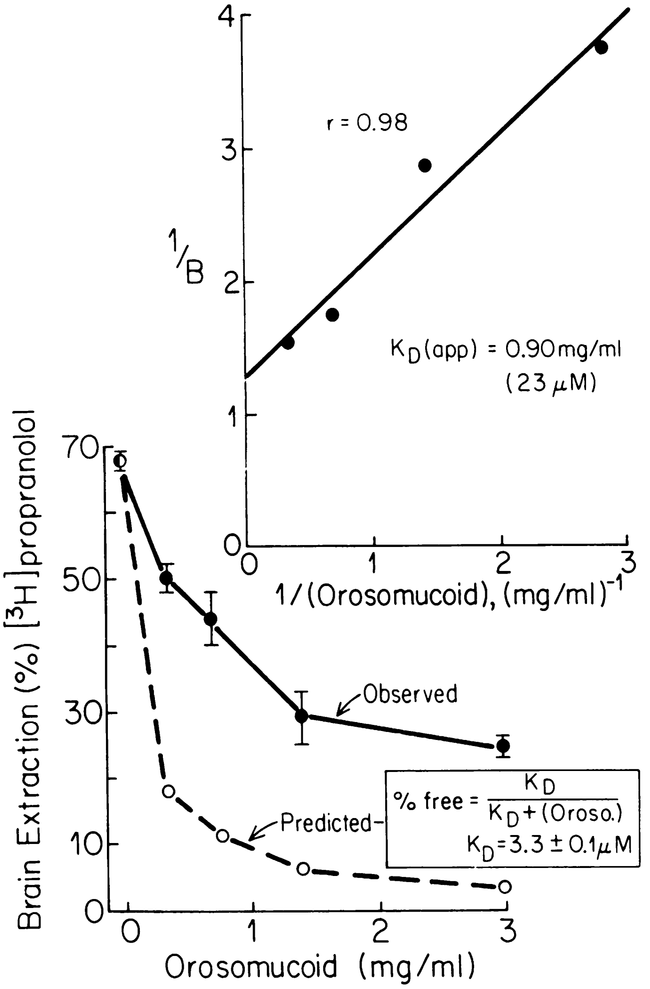

FIGURE 2 The rat brain extraction of [^{3}H]propranolol (mean±SE, n = 3–6 rats per point) is plotted against the concentration of human orosomucoid in the carotid injection solution (observed line). See the legend to Fig. 1 for an explanation of the predicted line. The K_D shown in this figure is the orosomucoid K_D for propranolol binding. The (app) K_D was determined from the double-reciprocal plot and represents the concentration of orosomucoid that inhibits propranolol transport by 50%. See legend to Fig. 1 for explanation of double-reciprocal plot. The sevenfold discrepancy between the K_D (app) in vivo and the K_D in vitro indicates orosomucoid-bound propranolol is readily transported into brain.

$$1/B = 1 + [K_D\,(\text{app})]\cdot\left(\frac{1}{A}\right)$$

relates the in vivo bound fraction (B) to the concentration of plasma protein (A) in the injection solution and the apparent dissociation constant, K_D (app), of protein binding of drug in vivo.

The K_D of albumin or orosomucoid binding of labeled drug in vitro was calculated from the law of mass action, i.e., K_D/n = (% free/% bound) × (protein concentration), where n = the number of drug-binding sites on the plasma protein. The percent free drug in vitro was determined by equilibrium dialysis at 37°C for 16–20 h (20).

Orosomucoid was quantitated by radial immunodiffusion using commercially available plates (Calbiochem-Behring Corp.). Albumin was measured by colorimetric assay (Sigma Chemical Co., St. Louis, MO).

The 1-octanol/Ringer's solution (pH = 7.4) partition coefficients of [^{14}C]lidocaine or [^{3}H]propranolol were determined as previously described (20).

RESULTS

The addition of bovine albumin to the carotid injection solution resulted in a decreased brain extraction of [^{3}H]propranolol (Fig. 1). The concentration of albumin that caused a 50% inhibition of transport, i.e., the apparent (app) K_D of albumin binding of propranolol in vivo, was 1.9 g/100 ml or 278 μM (Fig. 1). The K_D

TABLE I

*Plasma Protein Concentrations in Human Sera**

Group (*n*)	Albumin	Orosomucoid
	g/100 ml	*mg/ml*
Cord (8)	5.1±0.2	0.41±0.05
Normal (6)	5.4±0.4	0.78±0.07
Arthritis (6)	5.1±0.2	1.48±0.10
Metastatic cancer (7)	4.0±0.3	2.93±0.45

* Data are mean±SE.

(app) in vivo was not significantly different from the K_D of albumin binding of propranolol in vitro, 299±25 μM (Fig. 1). The equivalence of the K_D in vitro and the K_D (app) in vivo indicates only the free (dialyzable) portion of propranolol is available for transport in the presence of albumin (20).

The effects of adding human orosomucoid to the injection solution are shown in Fig. 2. Orosomucoid inhibited propranolol transport but to a much lesser extent than that predicted on the basis of the free drug in vitro. The concentration of orosomucoid that resulted in 50% binding in vitro was 3.3±0.1 μM, as opposed to the concentration of protein, 23 μM, which caused a 50% inhibition of propranolol transport in vivo.[3] These studies suggested orosomucoid-bound propranolol was available for transport into brain.

The effects of varying serum concentrations of orosomucoid on the first pass extraction of [^{3}H]propranolol by brain was examined using human sera. As shown in Table I, serum orosomucoid concentrations varied more than sevenfold in comparing cord and metastatic cancer serum. With the exception of the metastatic cancer patients, serum albumin levels did not vary. The free (dialyzable) fraction of propranolol changed inversely with the orosomucoid concentration (Table II), e.g., the free (dialyzable) fraction increased more than fourfold in comparing the metastatic cancer and cord groups. The brain extraction of orosomucoid varied inversely with the serum orosomucoid concentration (Table II). However, the effect of high orosomucoid concentrations on the brain extraction was blunted compared with the change in the dialyzable percentage, e.g., the brain extraction decreased only 24% in comparing cord and metastatic cancer serum (Table II).

TABLE II

*Effects of Human Sera on the Free (Dialyzable) Percentage and on the Rat Brain Extraction of Propranolol and Lidocaine**

Group (*n*)	Propranolol		Lidocaine	
	Dialyzable	Extraction	Dialyzable	Extration
	%		%	
Cord (8)	30.8±1.6	85±3	58.1±4.4	92±7
Normal (6)	18.2±1.2	67±5	43.2±5.9	91±4
Arthritis (6)	11.5±0.9	63±3	ND	87±6
Metastatic cancer (7)	6.9±1.1	50±6	24.4±7.5	77±3

* Data are mean±SE. ND, not determined.

The effects of albumin on the brain extraction of [^{14}C]lidocaine are shown in Fig. 3. Increasing concentrations of albumin resulted in a progressive decrease in the brain extraction. The (app) K_D of albumin binding of lidocaine in the brain capillary was 730 μM (Fig. 3). The K_D of albumin binding of lidocaine was 3,900±600 μM as determined by equilibrium dialysis for 20 h at 37°C.[4]

The effects of orosomucoid on brain extraction of lidocaine are shown in Table III. Large concentrations of orosomocoid, e.g., up to 5 mg/ml (125 μM), had little to no effect on brain extraction of lidocaine but had substantial effects on the free lidocaine in vitro (Table III). The K_D of orosomucoid binding of lidocaine in vitro was 66±5 μM, considerably less than the concentration of orosomucoid needed to inhibit brain extraction of lidocaine (Table III). The effects of human serum on brain extraction of lidocaine was de-

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[3] The ratio of the K_D (app) of orosomucoid binding of propranolol in vivo to the absolute K_D in vitro is 23 μM/3.3 μM or 7.0. We have previously emphasized that the deviation of the in vivo parameter, K_D (app), from the in vitro K_D is a function of BBB permeability (20, 22). It is of interest that the ratio of K_D (app) to K_D is 2,000 μM/261 μM or 7.7 for corticosterone (20, 22), an adrenal steroid hormone. Moreover, the BBB permeability for the two compounds, propranolol and corticosterone, is very similar (20). The approximation of the K_D (app)/K_D ratios for two compounds with similar membrane permeability supports the model that the deviation of in vivo binding parameters from the equilibrium state in vitro is a function of membrane permeability (20, 22).

[4] The fact that the (K_D/n) of albumin binding of lidocaine in vitro (3,900 μM) is not less than the K_D (app) of albumin binding within the brain capillary (730 μM) is evidence that albumin-bound lidocaine is not transported through the BBB; if protein-bound ligand is transported into the brain, then the K_D (app) in vivo > (K_D/n) in vitro (references 20, 22, and Fig. 2). However, in the case of lidocaine binding to albumin, the K_D (app) in vivo < (K_D/n) in vitro (Fig. 3), and the physical basis to this discrepancy is unexplained. Possibly the *n* value, i.e., the number of drug binding sites on albumin, is much greater in vivo than in vitro. The reliability of our estimate of the bovine albumin K_D for lidocaine in vitro is supported by the fact that our lidocaine dialysis data are quantitatively similar to those of Routledge et al. (37) for human albumin binding of lidocaine.

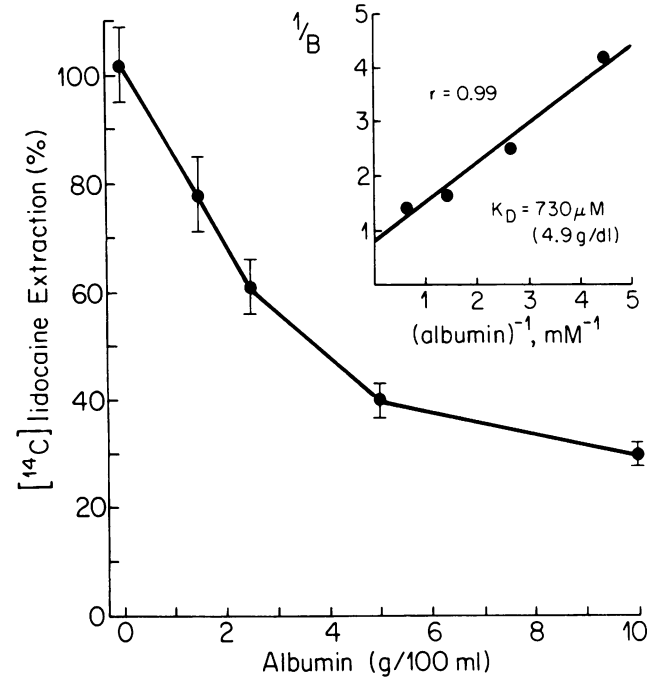

FIGURE 3 The rat brain extraction of [^{14}C]lidocaine (mean±SE, n = 3–4 rats per point) is plotted against the concentration of bovine albumin in the carotid injection solution. See the legend to Fig. 1 for an explanation of the double-reciprocal plot.

creased only 16% in comparing cord and metastatic cancer sera (Table II).

The effects of albumin and human serum on the rat liver extraction of propranolol and lidocaine are shown in Table IV. Drug bound either to albumin or to human serum proteins was freely transported into liver.

The 1-octanol/Ringer's partition coefficients for lidocaine and propranolol were 54±2 and 19±1, respectively.

DISCUSSION

These studies show that albumin-bound drugs, such as propranolol or lidocaine, are not transported through the BBB, but globulin (orosomucoid)-bound drugs are readily available for transport into brain. Since the majority of drug in plasma is bound to orosomucoid (5, 10), the circulating drug that is available for entry into peripheral tissues such as brain is not restricted to the free (dialyzable) moiety but includes the larger protein-bound fraction. Since plasma proteins such as albumin or orosomucoid are not measureably transported across brain capillaries (29) or into liver cells (30) on a single circulatory passage, the transport of protein-bound drugs into tissues represents a process by which the drug is stripped off of the plasma protein by the tissue.[5]

TABLE III

Effects of Human Orosomucoid on the Rat Brain Extraction and the Free (Dialyzable) Percentage of Lidocaine[a]

Orosomucoid	Lidocaine Brain extraction	Lidocaine Dialyzable
mg/ml	%	
1	107±2	73±6
5	93±3	35±3

[a] Data are mean±SE (n = 3–4).

The observation that protein-bound drugs are transported into brain is not predicted by the "free drug" hypothesis, which states that only the fraction of drug that is free in vitro is available for transport into tissues in vivo. Similarly, our previous findings that protein-bound hormone is transported into brain and into liver were not consistent with the "free hormone" hypothesis (20–22). We have proposed the "free intermediate" model to account for the diversity characterizing

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[5] Owing to very large pores and the absence of a basement membrane in liver microvessels, plasma proteins the size of albumin or orosomucoid enjoy instantaneous distribution into the hepatic interstitial space. Therefore, the rate-limiting membrane lining the plasma compartment in liver is the hepatocyte plasma membrane (38).

TABLE IV

Effects of Albumin and Human Serum on the Hepatic Extraction of Propranolol and Lidocaine[*]

Injection solution	Extraction Propranolol	 Lidocaine
	%	
5 g/dl albumin	105±5	86±16
Serum		
Normal human	85±3	83±6
Metastatic cancer	96±10	72±5

[*] Data are mean±SE (n = 4–6).

the transport of plasma protein-bound ligands in tissues in vivo (see Fig. 1 of reference 22). The three primary determinants of the model are (*a*) the capillary transit time, e.g., ~1 s in brain or ~10 s in liver; (*b*) the rate of unidirectional dissociation of ligand from the plasma protein, e.g., milliseconds to seconds; and (*c*) the rate of ligand diffusion through the biological membrane lining the plasma compartment, e.g., the BBB in brain or the hepatocyte cell membrane in liver. This model predicts that given the dual proviso that both ligand dissociation from the plasma protein and ligand diffusion through the membrane are fast relative to the capillary transit time, then protein-bound ligand enters the tissue via a "free intermediate" mechanism. This model is in contradistinction to a receptor-mediated or "collision" model for protein-bound ligand transport.

The present results for brain transport of protein-bound propranolol and lidocaine can be explained within the context of the free intermediate model. Both drugs are highly lipid-soluble (Results) and rapidly traverse the BBB either in the rat (Results) or in man (31). Therefore, the rate of ligand diffusion is fast relative to the brain capillary transit time. However, the rate of propranolol or lidocaine unidirectional dissociation from albumin is probably slow relative to the 1-s brain capillary transit time. The lack of dissociation of drug from albumin within the brain capillary transit time appears to be the most plausible explanation for the absence of albumin-bound transport of drug into brain (Figs. 1 and 3). The albumin data in Fig. 1 are noteworthy for the essentially identical estimates of the K_D of propranolol binding to albumin as determined with either the in vivo carotid injection technique or the in vitro equilibrium dialysis technique. This correlation, and our previously reported correlation between in vivo and in vitro assays of progesterone binding to an antibody (32), indicates the in vivo rat brain paradigm is capable of confirming (or rejecting) predictions made by the "free drug" hypothesis. Although we observe that albumin-bound drug is not transported through the BBB, and thereby confirm the free drug hypothesis in this case, we also observe that orosomucoid-bound propranolol and lidocaine are readily transported into brain (Fig. 2, Tables II and III). These observations are consistent with the hypothesis that the rate of unidirectional dissociation of drug from orosomucoid is fast relative to the brain capillary transit time and may have a half-time of 10^{-1} to 10^{-2} s at 37°C. The dissociation kinetics for drug binding to orosomucoid have apparently not been measured. However, it is known that steroid hormones dissociate from hormone-binding plasma globulins with half-times as short as 12 ms (33).

The proposal that basic lipophilic drugs, such as propranolol and lidocaine, dissociate rapidly from orosomucoid and slowly from albumin is not necessarily at odds with the observation that the K_D of orosomucoid binding of the drugs is about 100-fold lower than the albumin K_D (Results). Since the $K_D = k_{off}/k_{on}$, it may be that the k_{on} is up to 10^3-fold greater for drug binding to orosomucoid as compared with drug binding to albumin. These considerations regarding the kinetics of plasma protein binding are predicted in the process of explaining within the context of the free intermediate model the differential availability for transport of albumin-bound and orosomucoid-bound drug.

The free intermediate model does not provide an explanation for the rapid transport of albumin-bound drug into liver (Table III). Although the liver capillary transit time is 10-fold greater than the brain capillary transit time, it is unlikely that the nearly complete transport of albumin-bound drug by liver could be sustained by a drug-albumin dissociation reaction on the order of 1–10 s. The most plausible explanation for the rapid transport of albumin-bound drug into liver is the operation of a receptor-mediated mechanism for the transport of albumin-bound ligands. Other studies provide support for the hypothesis that free fatty acids (34) and bile salts (35) bound to albumin enter liver via a receptor-mediated mechanism.

The probable receptor-mediated transport of albumin-bound drug into liver notwithstanding, it is unlikely that this pathway accounts for the large hepatic first pass extraction of basic lipophilic drugs. As noted above, the K_D of orosomucoid binding of propranolol is 100-fold greater than the albumin K_D. Since the molar concentration of albumin (~700 μM, Table I) is only ~20-fold greater than the molar concentration of orosomucoid (~40 μM, Table I), the binding index (molar concentration ÷ K_D) is about fivefold greater for orosomucoid than for albumin. Therefore, at least 80% of the protein-bound drug pool in circulating human serum resides with orosomucoid, not with albumin.[6] Since receptors do not exist for native orosomucoid on liver cell membranes (36), it is unlikely that a receptor-mediated mechanism exists for the transport of orosomucoid-bound drug into liver. Therefore, the rapid transport of protein-bound propranolol and lidocaine in human sera into liver (Table III) probably represents transport via the free intermediate mechanism.

Finally, the observation of the present study that globulin-bound drug is transported into a peripheral tissue like brain must be reconciled with the clinical practice of using free plasma drug levels to monitor therapy. One view might be that a kinetic approach such as used in the present studies reveals the pathway of drug movement from the circulation into the tissue. However, the kinetics of the transport process may be so fast that equilibrium between the plasma proteins and the permeability barrier, e.g., the endothelial wall, is established within a fraction of the capillary transit time. Therefore, equilibrium measurements of free drug in vitro may underestimate the exchangeable plasma drug in vivo but equilibrium measurements, assuming tissue factors are constant, will still parallel changes in the exchangeable drug in vivo. However, a dialogue is created by an opposing view that considers it unlikely or, at least, unproven, that a new equilibrium between plasma proteins and the endothelial wall is established within a fraction of the transit time. Therefore, equilibrium measurements in vitro will not necessarily parallel the exchangeable drug in vivo (22). This dialogue and the utility of clinical measurements of free drug levels in plasma will be clarified by future studies, which attempt to reconcile the kinetic and equilibrium descriptions of the exchangeable drug in vivo.

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[6] Our attempts to directly measure the fraction of albumin-bound propranolol in human serum were frustrated by the rapid dissociation of [^{3}H]propranolol from serum proteins in the course of electrophoretic or gel filtration separation of plasma proteins. (Sakiyama, R., and W. M. Pardridge. Unpublished observations).

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