Blood-to-Brain Transfer of Various Oxicams: Effects of Plasma Binding on Their Brain Delivery

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Purpose. The objective of this work was to assess the influence of binding to plasma proteins and to serum on the brain extraction of four antiinflammatory oxicams.

Methods. The brain extraction of isoxicam, tenoxicam, meloxicam and piroxicam was investigated in rats using the carotid injection technique. Blood protein binding parameters were determined by equilibrium dialysis using human serum, human serum albumin (HSA) and alpha-1-acid glycoprotein (AAG) solutions at various concentrations.

Results. All oxicams had low values of brain extraction, between 19% and 39% when dissolved in serum, i.e. under physiological conditions. Brain efflux rate constants calculated from the wash-out curves were the same in the absence or presence of serum. Brain efflux was inversely related to the polarity of the oxicams, such that the higher their H-bonding capacity, the lower their brain efflux. The free dialyzable drug fraction was inversely related to protein concentration. However, rat brain extraction was always higher than expected from *in vitro* measurements of the dialyzable fraction.

Conclusions. Except for piroxicam whose brain extraction was partially decreased in the presence of proteins, the serum unbound and initially bound fractions of oxicams both seem available for transfer into the brain. Modest affinities for AAG rule out any related effect. More surprising is the apparent lack of effect on brain transfer of the high-affinity binding to HSA and serum. The enhanced brain uptake of meloxicam in the presence of AAG could be a result of interactions between this globular protein and the endothelial wall.

KEY WORDS: blood-brain barrier; drug brain transfer; *in vivo* brain extraction; plasma protein binding; oxicams.

INTRODUCTION

Drugs are generally bound in plasma to a significant extent by several transport proteins, both with high and low affinity. It has long been thought that only the free drug concentration was available for diffusion into tissues (1). Indeed, in most situations, plasma binding may impair the distribution of drugs to tissues, with drug distribution then mainly restricted to the distribution compartment of the drug-binding protein. However, several studies (2,3) have shown that a fraction or the total bound drug can dissociate in some capillaries and thus becomes available for transfer. In such cases, the plasma drug-binding is permissive and does not limit drug distribution to tissues. Thus, a given transport protein may have either a permissive or a restrictive effect on drug distribution, depending on the tissue. For example, HSA exerts a permissive effect on propranolol uptake by the liver, but a restrictive effect on brain transfer (3).

Oxicams are non-steroidal antiinflammatory drugs (NSAIDs) which exhibit a specific pharmacokinetic behavior, namely a high percentage of protein binding, a low apparent volume of distribution (always lower than the volume of body-exchangeable water, 0.6 L kg^{-1}), and a long plasmatic half-life (>20 h). Piroxicam and tenoxicam are zwitterions in the pH range 2–5 and anions at pH 6 and above. Meloxicam is a zwitterion in the pH range 1–4 and an anion above pH 5. In contrast, isoxicam is mainly neutral below pH 4 and mainly anionic above. Therefore, all oxicams will be mainly anionic at physiological pH and as such have a modest capacity to partition into octanol (log $D_{oct}^{7.4} = -0.3$ to 0.1) (4,5). Such properties are expected to markedly influence distribution and tissue penetration. NSAIDs seem to cause headaches and dizziness in a relatively small number of recipients. These reactions are commonly observed with indomethacin, whereas few central nervous reactions have been reported for oxicams.

The aim of our study was to compare the brain extraction of various oxicams and to assess the influence of physicochemical factors and plasma protein binding on their brain extraction.

MATERIALS AND METHODS

Radiolabeled Compounds

[^{3}H]Piroxicam (specific activity = 374 GBq M^{-1}) was a gift from Pfizer (France), [^{14}C]isoxicam (specific activity = 281.2 GBq M^{-1}) from Warner Lambert (USA), [^{14}C]meloxicam (specific activity = 178.0 GBq M^{-1}) from Thomae (Germany) and [^{14}C]tenoxicam (specific activity = 499.5 GBq M^{-1}) from Roche (Switzerland). Their chemical structure is shown in Figure 1.

Radiochemical purities were all >97%, as assessed by TCL on silica plates using four different solvent systems: $CHCl_3/CH_3COCH_3/HCOOH$ (70/30/4 v/v) for piroxicam, $CHCl_3/CH_3OH/HCOOH$ (85/10/5 v/v) for isoxicam, $CHCl_3/CH_3OH/NH_4OH$ (80/20/1 v/v) for meloxicam and $CHCl_3/CH_3COCH_3/CH_3OH/C_2H_5OH$ (70/20/10/2 v/v) for tenoxicam. The labeled compounds were stored at −80°C until use. The specific activity of the internal standards, [^{14}C]butanol and tritiated water (NEN®, Du Pont de Nemours) were 37 GBq M^{-1} and 370 GBq M^{-1}, respectively.

Human Plasma Proteins

Human serum albumin (A1887) (HSA) was purchased from Sigma (Saint-Quentin Fallavier, France) and alpha-1-acid glycoprotein from Behring (Marburg, Germany). Protein frac-

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ABBREVIATIONS: AAG, alpha-1-acid glycoprotein; BBB, blood-brain barrier; E(0), total (endo- and extravascular) brain extraction, extrapolated to time 0 from values measured at 5 s; E(0)*, net brain extraction after endothelial transcytosis (extravascular part), extrapolated to time 0 from values measured at 5 s; F, cerebral blood flow (mL s^{-1}); f_u, unbound drug fraction; HSA, human serum albumin; K_A, association binding constant (M^{-1}); K_D, dissociation binding constant (M); N, binding sites concentration (M).

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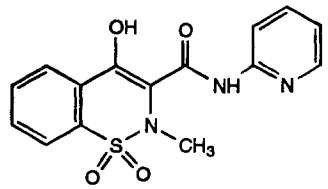
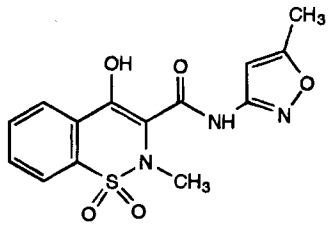


piroxicam



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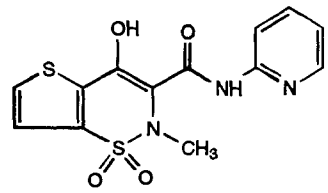
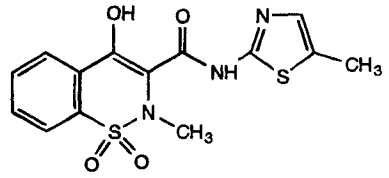


tenoxicam



meloxicam

Fig. 1. Chemical structure of the investigated oxicams.

tions were diluted to the desired concentration with buffered Ringer solution at pH 7.4. Human serum was pooled from healthy adult volunteers.

Brain Uptake Studies

The first-pass brain extraction of oxicams relative to [^{14}C]butanol or tritiated water was measured according to the method of Oldendorf (6) or «brain uptake index» method. Briefly, a 200 μL bolus of buffered Ringer solution was rapidly injected (0.25 s) via a 26-gauge needle (Microlance; 26 G 3/8, 0.45 × 10) into the common carotid artery of anesthetized (50 mg kg^{-1} sodium pentobarbital, i.p.) male Wistar rats (220–250 g). After injection, the needle was left in place to prevent bleeding, and the carotid flow after the puncture site was unimpeded. The injected solution contained 0.29 MBq mL^{-1} of [^{3}H]-labeled drug and 0.037 MBq mL^{-1} of [^{14}C]butanol or 0.037 MBq mL^{-1} of [^{14}C]-labeled drug and 0.29 MBq mL^{-1} of [^{3}H]water, in the absence or presence of isolated human serum protein or serum. At 5 s after injection, rats were decapitated. Samples of the original isotope mixture obtained from the injection syringe added to a control hemisphere and the hemisphere ipsilateral to the injection were dissolved in 1.5 mL of Soluene 350 (Packard Instrument Co.) at 60°C, and left overnight before double isotope liquid scintillation counting (Packard Tri-Carb 460 CD).

Owing to the rapid bolus injection, the injected solution traverses the brain microcirculation as a discrete bolus, mixing only minimally with the circulating rat blood. Previous studies have shown that bolus mixing with rat blood is <5% (6).

Separation of the Net Brain Fraction of the Drug from the Intracapillary Fraction

In these experiments, brain homogenates were depleted of brain microvasculature, so that net cerebral uptake could be measured, as opposed to the fraction of the drug retained by brain capillary endothelium. According to the method previously described by Triguero *et al.* (7), the hemisphere ipsilateral to the injection was quickly removed after decapitation (5 s after carotid injection) and homogenized with a glass homogeniser (8–10 strokes) in 5 mL of physiological glucose buffer (pH 7.4). A 28% Dextran solution (5 mL) was added and homogenized again. After an aliquot of the homogenate was taken, the remainder was centrifuged at 6000 g for 20 min at 4°C in a swinging-bucket rotor (Sorvall RC 28S). The supernatant (brain tissue) and pellet (vascular cells) were carefully separated and prepared, as described earlier, for double-isotope liquid scintillation counting. In this manner, we obtained the intra-endothelial fraction of the drug (f_e) which had remained trapped in the endothelial cells during the blood-to-brain transfer.

Estimation of Drug Brain Uptake

The brain uptake index (*BUI*) was calculated as follows:

$$BUI = \frac{(^{3}H/^{14}C)\ dpm\ in\ tissue}{(^{3}H/^{14}C)\ dmp\ in\ injected\ sample} \quad (1)$$

for piroxicam and

$$BUI = \frac{(^{14}C/^{3}H)\ dpm\ in\ tissue}{(^{14}C/^{3}H)\ dmp\ in\ injected\ sample} \quad (2)$$

for the other oxicams.

The BUI represents the net uptake of the drug normalized by the net uptake of the reference compound. The BUI is, therefore, a direct function of the single-pass extraction of the drug (E_d):

$$E_d = E_r \cdot BUI \quad (3)$$

where E_r is the single-pass extraction of the reference compound.

The brain-to-blood transport of each drug and reference compound were simultaneously determined at 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 300 s after injection. The data were analysed using an iterative non-linear program according to the formula:

$$E_d(t) = E_d(0) \cdot e^{-k \cdot t} \quad (4a)$$

$$E_r(t) = E_r(0) \cdot e^{-k \cdot t} \quad (4b)$$

where $E(t)$ represents the brain extraction at time t, E(0) is the brain extraction extrapolated at time 0 and k is the efflux rate

constant. According to previous results (8), the $E_r(0)$ of butanol is 100% and that of tritiated water 62%.

The uptake index at time *t*, BUI(*t*), is related to the efflux rate constant and initial uptake BUI(*0*) as follows:

$$BUI(t) = BUI(0) \cdot e^{-(k_d - k_r)\cdot t} \quad (5)$$

where the subscripts d and r again refer to the drug and reference compound, respectively. Using Eq. 5 and 3, the brain extraction of the drug at time 0, $E_d(0)$ can be easily calculated from the measured BUI(t).

$E_d(0)$ represents the total brain extraction of the drug, including both brain cells drug fraction and endovascular drug fraction, extrapolated at time 0 from the value $E_d(5)$ measured at 5 s. The net extraction after transfer through the endothelial cell is obtained by the following formula:

$$E_d(0)^* = (1 - f_e) \cdot E_d(0) \quad (6)$$

where f_e is the intra-endothelial brain fraction.

Determination of *In Vitro* and *In Vivo* Drug Binding

According to the law of mass action, we have:

$$f_u = \frac{K_D}{K_D + P_f} \quad (7)$$

where f_u is the unbound fraction of the drug, K_D the dissociation constant of the drug-protein complex and P_f is the concentration of free protein binding sites. In our experiments, we used only a trace concentration of oxicams, therefore the concentration of occupied binding sites (P_b) is negligible relative to the total concentration of protein binding sites (P_t) and $P_f = P_t$. Thus, equation (7) becomes:

$$f_u = \frac{K_D}{K_D + P_t} \quad (8)$$

The *in vitro* drug protein binding was measured by equilibrium dialysis at 37°C during a 3 h period as previously detailed (9). Protein solutions identical to those injected to rats were used, and the K_D values were estimated using Eq. 8. In the case of *in vivo* studies, the exchangeable fraction of drug in brain capillaries, when no binding protein is present, is given by the Crone equation of capillary physiology (10):

$$E = 1 - e^{-PS/F} \quad (9)$$

where P is the permeability on the blood side of the BBB (mL s^{-1} cm^{-2}), S is the brain capillary surface area (cm^2) and F is the rate of cerebral blood flow (mL s^{-1}).

When a protein is present, the free (exchangeable) fraction of drug (f_u) is theoretically diminished because of protein binding, and Eq. 9 becomes:

$$E = 1 - e^{-f_u \cdot PS/F} \quad (10)$$

Substituting Eq. 8 in Eq. 10 yields:

$$E = 1 - e^{-PS/F/(1+(P_t/K_D))} \quad (11)$$

When the drug extraction is measured at various protein concentrations, PS/F and K_D can be estimated by fitting the data to Eq. 11.

Analysis of Data

The binding parameters and PS/F values were estimated by a non-linear regression program using a Gauss-Newton algorithm (Microparm®, INSERM).

All values are presented as means plus standard deviations and sample numbers (n). Means were compared by ANOVA and estimated parameters by their 95% confidence intervals.

Structural Calculations

Molecular Volumes

For all the oxicams, the molecular volumes were expressed by van der Waals volumes (V_w) calculated with the standard software MOLSV (QCPE N° 509) and the atomic radii of Gavezzotti (11). The geometries used to generate van der Waals volumes were optimized with the Tripos force field including an electrostatic term calculated with a dielectric constant ϵ = 1 until the gradient norm was less than 0.001 kcal $Å^{-1}$.

Polarity Parameter Λ

Previous studies (12, 13) have shown that lipophilicity can be factorized into a hydrophobic term and a polarity term designated as Λ:

$$\log P = \text{hydrophobicity} - \Lambda = (a \cdot V_w + c) - \Lambda \quad (12)$$

In this equation, the hydrophobic term encodes all intermolecular forces proportional to the compound size (i.e. mainly hydrophobic forces between the solute and the aqueous phase) whereas the polarity term expresses van der Waals forces and mainly hydrogen bonds between the solute and both phases (13).

For non-polar compounds (where $\Lambda = 0$), the coefficient «a» in Eq. 12 is the slope of the line relating log P and V, and «c» is the intercept. Thus, the hydrophobic term in the octanol/water partition coefficient ($\log P_{oct}$) is easily determined for *n*-alkanes (where $\Lambda = 0$) yielding Eq. 13 (14):

$$\log P_{oct}(n\text{-alkanes}) = 3.087 \cdot 10^{-2}(\pm 0.136 \cdot 10^{-2})V_W + 0.346\ (\pm 0.199)$$

$$n = 14;\ q^2 = 0.995;\ r^2 = 0.997;\ s = 0.145;\ F = 3619 \quad (13)$$

The combination of Eq. 12 and 13 leads to Eq. 14 which allows the polarity parameter of each oxicam to be calculated:

$$\Lambda_{oct} = 3.087 \cdot 10^{-2} V_W + 0.346 - \log P \quad (14)$$

Eq. 14 means that for each oxicam the polarity parameter is the difference between the log P of a virtual alkane of identical volume and the log P of that solute.

Ethics in Animal Investigations

This research adhered to the «Principles of Laboratory Animal Care» (NIH publication #85-23, revised 1985).

RESULTS

Oxicams Uptake and Efflux from the Brain

The washout kinetics of each oxicam was performed in serum, and the efflux rate constants (Table I) were calculated

Table I. Brain-to-Blood and Blood-to-Brain Transfer Parameters and Physicochemical Descriptors of Oxicams

Drug	k (min^{-1})[a]	E(0)* serum[b]	f_e[c]	Λ_{oct}[d]	log P^N	Molecular volume[h]
Tenoxicam	0.54 ± 0.06	0.24 ± 0.02	0.135	7.64	0.80[e,g]	262.1
Meloxicam	0.72 ± 0.08	0.19 ± 0.05	0.228	6.27	2.70[f,g]	279.4
Piroxicam	0.74 ± 0.06	0.39 ± 0.03	0.041	6.99	1.76[e,g]	272.3
Isoxicam	0.90 ± 0.07	0.22 ± 0.03	0.108	5.78	2.83[e]	267.6

[a] k: brain efflux rate constant (min^{-1}).
[b] E(0)* serum: maximal extraction obtained after transcytosis.
[c] f_e: intra-endothelial brain fraction of the drug, measured 5 s after carotid injection.
[d] Λ_{oct}: polarity parameter derived from lipophilicity.
[e] Ref 4.
[f] Ref 5.
[g] Measured at the isoelectric pH.
[h] A^3.

from the washout curves. These values allow the determination of the extrapolated extraction at time 0. We note that, of the four oxicams investigated, isoxicam is the most rapidly cleared from rat brain. The efflux rate constants estimated for butanol (0.75 ± 0.10 min^{-1}) and for tritiated water (0.49 ± 0.07 min^{-1}) are comparable to earlier reported values (8).

All oxicams had low values of brain extraction, between 19% and 39%, when dissolved in serum, i.e. under physiological conditions (Table I). For the four oxicams, we measured low values of f_e (between 0.041 and 0.228), indicating that only a small fraction of the drug was retained in the endothelial cells during their brain transfer.

Correlations were searched between the pharmacokinetic factors in Table I and some physicochemical descriptors. The most interesting trend is that seen between the efflux factor k and the Λ_{oct} parameter (Figure 2; $r^2 = 0.83$) such that the smaller the efflux rate constant of the oxicam, the higher its polarity (H-bonding capacity). In contrast, we did not find any correlation between the extraction value and polarity expressed by Λ_{oct}.

Effect of Protein Binding on Oxicam Brain Transfer

The *in vitro* protein binding parameters (Table II), obtained by dialysis, are similar to those previously reported in our laboratory (15–18). All the four oxicams were highly bound to HSA. Binding parameters were similar for HSA and serum, demonstrating the major contribution of this protein in the serum binding of oxicams. Although significant and saturable, oxicam binding to AAG was <1%. Isoxicam did not bind to AAG.

The effect of protein binding on drug brain transfer is reported in Table III.

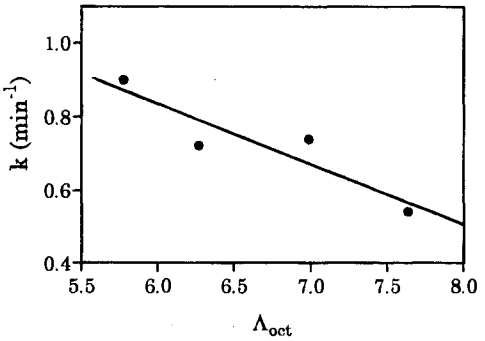


Fig. 2. Correlation between brain efflux and polarity ($r^2 = 0.83$).

Partial Plasma Retention of Piroxicam by Binding to HSA

The brain transfer of piroxicam was significantly reduced by its plasma binding, but to a lesser extent than expected from the initial plasma free fraction as measured *in vitro* (Figures 3A and 3B). The decrease in brain extraction was related to the protein concentration in the injected solution. However, the measured brain extraction *in vivo* was greater than that predicted on the basis of *in vitro* measurements of the dialyzable fraction. The concentration of HSA that caused a 50% reduction in brain extraction, i.e. equal to the *in vivo* dissociation constant K_D, was significantly higher ($p < 0.001$) than that estimated from *in vitro* studies (Figure 3A). The same observation was made for AAG (Figure 3B), but the discrepancy between the two parameters, *in vivo* and *in vitro*, was smaller than for HSA ($p < 0.02$).

The maximal brain extractions in presence of HSA, E(0)HSA = 0.47 and E(0)serum = 0.43, were not significantly different. Surprisingly, despite the low binding to this protein, the maximal brain extraction of piroxicam in the presence of AAG was similar, E(0)AAG = 0.42. Hence HSA and AAG exerted similar restrictive effects on piroxicam brain extraction.

Permissive Effect of Plasma Binding on the Brain Transfer of Tenoxicam and Isoxicam

The entire fractions of tenoxicam and isoxicam bound to HSA, AAG and in serum were readily available for transfer into the brain. Hence, binding of the two oxicams to plasma proteins produced a permissive effect on their brain distribution.

Enhanced Brain Uptake of Meloxicam in the Presence of AAG

Serum and HSA binding of meloxicam allowed the total transfer of the initially bound fractions into the brain. Furthermore, its brain extraction was unexpectedly facilitated by the presence of AAG (20 μM).

Table II. Binding Parameters of Oxicams to HSA, AAG and Serum

	Human serum		HSA (600 μM)		AAG (25 μM)	
	$1 - f_u$ (%)[a]	NK_A[b]	$1 - f_u$ (%)[a]	NK_A[b]	$1 - f_u$ (%)[a]	NK_A[b]
Isoxicam	96.5 ± 0.3	27.9 ± 0.7	96.2 ± 0.9	27.4 ± 1.2	<1	—
Piroxicam	98.3 ± 0.2	55.4 ± 1.5	98.2 ± 0.3	54.8 ± 1.8	<1	2.66 ± 0.37
Tenoxicam	98.4 ± 0.5	87.8 ± 0.9	98.4 ± 0.4	70.8 ± 1.7	<1	0.54 ± 0.05
Meloxicam	99.7 ± 0.05	90.7 ± 15.9	98.5 ± 0.5	60.2 ± 0.5	<1	1.59 ± 0.19

[a] f_u: unbound drug fraction.
[b] NK_A: is the product of the concentration of binding sites N (M) by the association constant $K_A(M^{-1})$.

DISCUSSION

The main results of the present study are: 1) All oxicams have rather low values of brain extraction, about 30% under physiological conditions; 2) The *in vivo* results correlate well with those obtained using a coculture of brain capillary endothelial cells and astrocytes (19); 3) A high H-bonding capacity of the drugs is an obstacle to their brain efflux; 4) Except for piroxicam whose brain extraction was partially decreased in presence of proteins, the entire fraction of oxicam was available for transfer into the brain; 5) Binding to AAG produced three distinct effects on brain extraction.

The oxicam extraction values (E(0)) are similar to those previously obtained for other NSAIDs such as indometacin or diclofenac (19). Pardridge et al. (20) provided a measure of drug and solute transport through *in vitro* and *in vivo* models of the BBB and demonstrated a good correlation for 13 drugs. Adverse central reactions to indometacin are frequent and attributed to salt and water retention and to some chemical similarities of the drug with serotonin. Dizziness and headache are also reported on oxicam therapy in patients treated with doses of 20 mg daily, but with a smaller frequency. The incidence of such adverse effects are comparable for all oxicams, as explained by the similar extraction values of the four studied oxicams.

Lipophilicity is a molecular property expressing hydrophobic and polar contributions and influencing the pharmacokinetics and pharmacodynamic behavior of many classes of drugs. Oldendorf *et al.* (21) showed that the brain transfer of opiate drugs is inversely related to the number of polar functional groups involved in hydrogen bonding. In our work, the experimental log P^N values reported describe the octanol/water lipophilicity of the globally neutral forms, i.e. the mixture of neutral and zwitterionic forms of tenoxicam, meloxicam and piroxicam, and the unionized form of isoxicam. In other words, log P^N is equal to the highest distribution coefficient attainable by these compounds. This parameter did not correlate well with the pharmacokinetic parameters measured here. The same is true for the molecular volume, a measure of hydrophobicity.

In contrast, the polarity factor Λ_{oct} proved interesting. This parameter is calculated as lipophilicity (log P) minus hydrophobicity, and it expresses mostly the H-bonding capacity of a solute. The correlation found between the efflux rate constant k_e and Λ_{oct} (Figure 2) has a negative slope, indicating that a high H-bonding capacity is an obstacle to brain efflux.

It has long been thought that only the free drug in plasma is available for diffusion into tissues and determines its pharmacological and toxic activities. Indeed, Albengres *et al.* (22) showed that the high capacity for binding to plasma proteins associated with a low volume of distribution provides a biological mechanism for limiting dose-dependent cutaneous, gastrointestinal or renal side-effects. Although this «free-drug hypothesis» has been confirmed in many cases, several studies have shown that a fraction of the bound drug in plasma can be dissociated in brain capillaries and thus becomes available for brain transfer (2,23). The most likely explanation for this phenomenon was proposed by Pardridge *et al.* (3) as the «free-intermediate hypothesis». If the transfer of the free fraction of the drug entering the brain capillaries is sufficiently fast, it is conceivable that a new equilibrium is rapidly achieved inside the capillaries, leading to the release of some of the bound drug into a free form that then becomes available for transfer.

The main parameters that govern the blood-to-brain exchange are the capillary transit time (inversely correlated to the cerebral blood flow), the permeability of the cerebral endothelial cells to the drug, and the rate of dissociation of the drug-protein complex. The conventional concept limiting transcapillary transport to the unbound moiety associated with spontaneous dissociation during organ transit does not seem to apply here, considering the respective protein binding associa-

Table III. Effects of Protein Binding on Drug Brain Transfer

	Extrapolated brain extraction at time 0, $E_d(0)$, in the presence of various media			
Drug	Ringer	Serum	HSA	AAG
Piroxicam	0.61 ± 0.05	0.43 ± 0.02***[a]	0.48 ± 0.02***[a]	0.42 ± 0.02***[a]
Tenoxicam	0.25 ± 0.02	0.27 ± 0.02	0.29 ± 0.03	0.24 ± 0.03
Meloxicam	0.27 ± 0.03	0.22 ± 0.03	0.26 ± 0.04	0.43 ± 0.02***[b]
Isoxicam	0.27 ± 0.02	0.26 ± 0.03	0.31 ± 0.04	0.32 ± 0.02

[a] This implies a retention effect of the binding of piroxicam on its brain transfer (***: $p < 0.001$).
[b] This implies an enhanced brain extraction of meloxicam is the presence of AAG (***: $p < 0.001$).

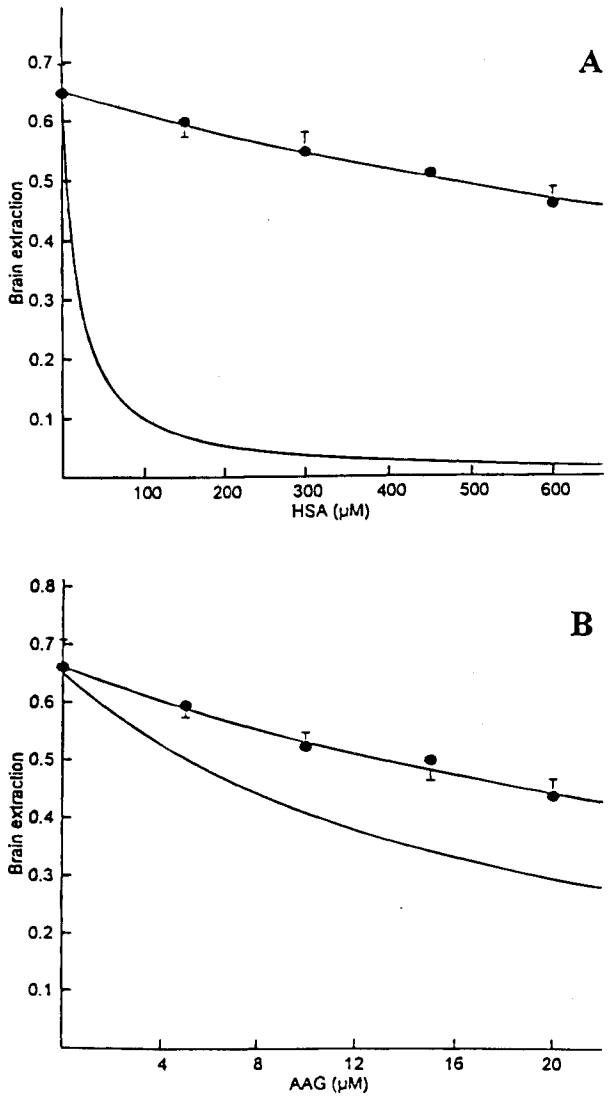


Fig. 3. Effects of protein binding on drug brain transfer. Total (intra and extra-endothelial) brain extraction, E(o), is plotted as a function of the protein (HSA or AAG) concentration of the injected sample. The observed values are the mean ± SD (bars) of groups of five rats. The observed curve was drawn by using PS/F and K_D values estimated from nonlinear regression of the data to equation 11, and the expected curve was simulated from equation 11 by using the previous PS/F estimate and K_D value estimated from in vitro studies: for HSA, PS/F = 1.08; K_D in vivo = 910 ± 105 μM; K_D in vitro = 10.95 ± 0.12 μM; and for AAG, PS/F = 1.05; K_D in vivo = 34.8 ± 3 μM; K_D in vitro = 29.4 ± 0.7 μM. **Fig 3a.** Rat brain extraction of piroxicam plotted as a function of HSA concentration in the carotid injection solution. **Fig. 3b.** Rat brain extraction of piroxicam plotted as a function of AAG concentration in the carotid injection solution.

tion parameters and the brain capillary transit time (only 1 s). Additional factors are probably involved that promote dissociation *in vivo*. Each plasma protein seems to have its own selectivity, both in binding and releasing the drug (2,3). In this study, the HSA-oxicam or AAG-oxicam binding parameters are not inversely related to their brain transfer. Except for piroxicam, whose brain extraction was partly decreased in the presence of proteins, the entire fraction of oxicam seems available for transfer into the brain.

Quite surprising is the lack of effect of HSA, which binds oxicams with high affinity. We note that piroxicam, the only oxicam whose brain extraction is limited by HSA-binding, possesses a lower binding capacity to HSA than meloxicam or tenoxicam. Thus the association constant of the drug-HSA complex is not directly correlated with the plasma retention capacity of protein binding. The same observation was made for the brain transfer of antiprogesterone derivates such as mifepristone, whose AAG-binding was permissive in spite of a very high association constant ($K_A > 10^6\ M^{-1}$) (24). However, in the presence of a binding competitor or a ligand increasing binding affinity, the effect of the protein on brain transfer can be modified. This was observed when associating *in vivo* chlordiazepoxide and valproate, with an increase in the free fraction and brain transfer of chlordiazepoxide (25). Similarly, in the presence of small amounts of free fatty acids, the K_A of the warfarin-HSA complex increased, resulting in a reduction of warfarin transfer into the brain (26). Oie *et al.* (27) showed that albumin enhanced the intrinsic liver uptake of drugs both bound and not bound to albumin, whereas AAG only decreased the elimination of drugs bound to it. Such effects are opposite to those observed by Pardridge (3) where propranolol brain transfer was restricted by HSA and only partially decreased in the presence of AAG.

The three AAG-binding effects on brain extraction demonstrated here were quite unexpected. Since central side-effects of drugs are often dose-related, the effects of AAG on blood-to-brain transfer of oxicams could have clinical consequences, inasmuch as the AAG concentration is increased in inflammatory diseases. The permissive effect observed for tenoxicam and isoxicam can be explained by the low binding capacity of AAG toward these two drugs. Even if piroxicam has the highest affinity parameter for AAG, its partial plasma retention in the presence of this protein, which is similar to those obtained with HSA and serum, is very unusual. As for meloxicam, its enhanced brain uptake in the presence of AAG could result from interactions between this globular protein and the endothelial wall. Indeed, Curry *et al.* (28) showed that plasma AAG modifies the permeability properties of frog mesenteric capillaries, modulating the microvessel wall charge. Furthermore, a receptor-mediated transport could occur after the binding of AAG to cerebral endothelial cell receptors, e.g. lectin-receptor or galactose-receptor. Such a mechanism has been observed in liver capillaries (29). Also, non-specific adsorption of plasma proteins to the endothelial glycocalyx could modify the normal capillary permeability and enhance the dissociation of the protein-drug (30).

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