Morphine and methadone binding to human serum proteins

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Binding to plasma proteins can influence the therapeutic and toxicologic actions of medicinal agents. Most studies have involved acidic compounds and only a few have been concerned with binding of basic drugs or not ionized at plasma pH to human serum (Borga et al 1977; Bickel 1975; Olsen 1975). Evidence is now accumulating that the plasma protein binding of the two groups of drugs is quantitatively different from that of acidic or ionized drugs. We now report the study of the binding to human serum of two narcotic analgesics: morphine and methadone. Recently Judis (1977) reported Scatchard plots with a positive slope for the binding of morphine and methadone and other basic drugs to human serum, and it was implied that such Scatchard plots may be characteristic of alkaloids and bases. The Scatchard relationship (Scatchard 1949) can be applied to a system with one or several classes of binding sites as follows. For an interaction of the following type: Drug + Protein ⇌ Drug-Protein, the concentration of the bound drug may be related to the concentration of unbound drug by the law of mass action:

$$Ka = \frac{(\text{Drug-Protein})}{(\text{Drug})\,(\text{Protein})}$$

When the binding sites n are completely independent and have identical intrinsic affinities the equilibrium may be expressed by:

$\frac{r}{Clf} = Ka\ n - Ka\ r$, known as the Scatchard relationship where Ka is the assocciation constant, r is the concentration of bound drug divided by the concentration of protein, and Clf is the concentration of free drug. For a ligand possessing one or several classes of binding sites, from the relationship:

$$r = \frac{n_1\ Ka_1\ Cl_1\ f}{1 + Ka_1\ Cl_1\ f} + \frac{n_2\ Ka_2\ Cl_2\ f}{1 + Ka_2\ Cl_2\ f} + \ldots + \frac{n_m\ Ka_m\ Cl_m\ f}{1 + Ka_m\ Cl_m\ f}$$

The Scatchard plot can be expressed as $r/Clf = f(r)$.

Materials and methods. [N-Methyl-^{14}C]morphine hydrochloride (56·4 mCi mmol^{-1}) was purchased from the Radiochemical Centre, Amersham (U.K.) and DL-[1-^{14}C]methadone hydrochloride (36 mCi mmol^{-1}) was prepared (Nam et al 1978) at Service des Molécules Marquées, C.E.N. - Saclay, France. Crystalline human serum albumin (Sigma), DL-tryptophan, stearic acid, palmitic acid (Merck), and other chemicals (analytical grade) were used without further purification. Human serum (Centre de transfusion sanguine) contained $9{\cdot}565 \times 10^{-4}$ mol of protein expressed as bovine serum albumin (Lowry et al 1951). Binding of morphine and methadone to protein was measured by equilibrium dialysis according to Weder et al (1971) with a Dianorm apparatus. Dialysis experiments were performed with 2 ml microcells at 37 °C, pH 7·4 (0·074 M phosphate) for 2 h of incubation with constant stirring at 20 rev min^{-1}. Cellulose hydrate membranes (Diackema) of 25 μm thickness and a 5000 mol. wt cut off were used. Morphine and methadone were examined at a range of concentrations (from 10^{-8} to 2×10^{-4} M) prepared by isotopic dilution of a constant amount of ^{14}C-labelled drug with increasing quantities of unlabelled drug. At the end of each experiment, the concentration of free drug in the dialysate was measured by liquid scintillation counting (Intertechnique SL 30. Instagel). Calculations were carried out with a Hewlett Packard (HP 9831 A) programmable calculator by linear regression analysis.

Results. The percentage of binding of morphine to human serum and human albumin varies only to a small extent when the concentration of morphine increases from 2×10^{-6} to 2×10^{-4} M (Table 1). On the contrary, at a constant concentration of morphine the percentage of bound drug increases in proportion to the concentration of albumin present (Fig. 1). Methadone binds much more to human serum than to

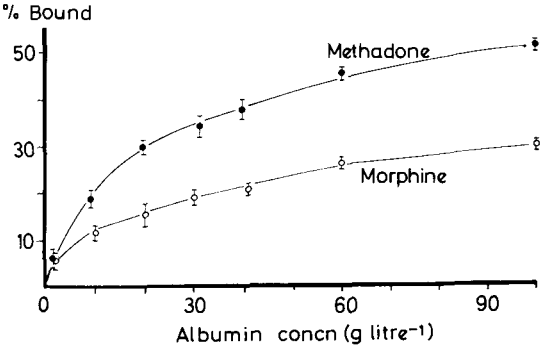

FIG. 1. Percentage of binding of morphine and methadone. The concentration of drugs was held constant (morphine = 0·406, methadone = 0·5 μM) while the albumin concentration (g litre^{-1}) was increasing.

* Correspondence.

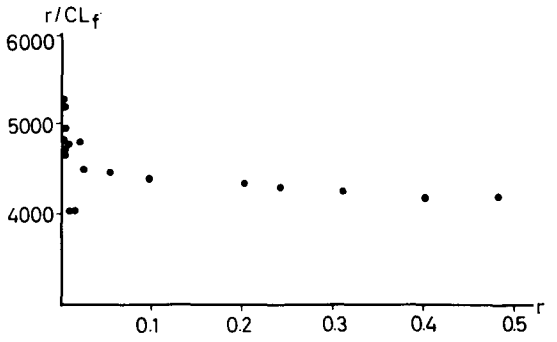

FIG. 2. Scatchard plot of the binding of morphine and methadone to human serum in phosphate buffer pH 7·4, 37 °C. Morphine concentration varied from 0·03 to 266 μM.

albumin. Therefore its binding to other proteins such as globulin is important (Olsen 1973). When the albumin concentration is varied at fixed drug concentration, methadone binds with a small initial binding which increases and stabilizes. As the concentration is increased up to about 2×10^{-6} M, the percentage of methadone bound decreases rapidly. At higher concentrations this decrease becomes smaller until at about 4×10^{-4} M, the binding appears to be relatively independent of drug concentration. When the results were analysed by the method of Scatchard, it was found that for human serum and albumin there is one class of binding sites for morphine (Fig. 2) and at least two classes for methadone (Fig. 3). The product nK for morphine, the number of binding sites in each

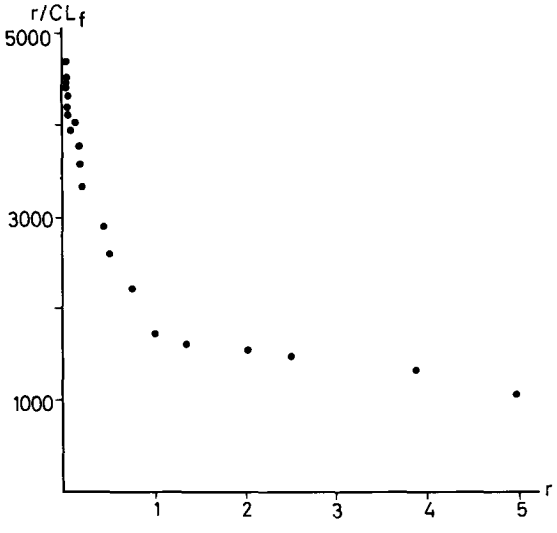

FIG. 3. Methadone concentration varied from 0·11 to 144 μM. Each point is the mean of five experiments.

Table 1. Binding of morphine and methadone to human serum proteins. (each value is the result of five experiments).

Concn (M)	Binding % mean with s.d.		
	Human Serum	Albumin	Albumin free fatty acids
Morphine			
$2{\cdot}73 \times 10^{-7}$	21 s.d. 1·5	19·5 s.d. 0·37	20 s.d. 0·9
$2{\cdot}8 \times 10^{-6}$	18 s.d. 1·2	18 s.d. 1·09	18 s.d. 1·2
$5{\cdot}333 \times 10^{-5}$	17 s.d. 1·15	16·5 s.d. 1·02	17 s.d. 1·05
$2{\cdot}661 \times 10^{-4}$	17 s.d. 0·69	16 s.d. 1·3	16 s.d. 1·4
Methadone			
$2{\cdot}197 \times 10^{-7}$	66 s.d. 0·48	39 s.d. 1·4	40 s.d. 1·1
$6{\cdot}001 \times 10^{-6}$	58 s.d. 0·58	28 s.d. 1·49	28 s.d. 1·2
$5{\cdot}803 \times 10^{-5}$	40 s.d. 1·92	22 s.d. 1·25	21 s.d. 0·5
$1{\cdot}447 \times 10^{-4}$	35 s.d. 0·56	18 s.d. 1·62	18 s.d. 1.3

Table 2. Binding parameters for morphine and methadone calculated from plots r/Clf versus r.

Morphine	Human serum		nk = 4980 M^{-1}		
	Human albumin 4%		nk = 8070 M^{-1}		
		n^1	k^1	n^2	k^2
Methadone	Human serum	0·5	5300 M^{-1}	9·5	190 M^{-1}
	Human albumin 4%	0·1	5350 M^{-1}	1·08	110 M^{-1}
	Human albumin 0·4%	0·3	5300 M^{-1}	3·3	150 M^{-1}

class and the association constant of each class for methadone are shown in Table 2.

There is no difference in the percentage of drug bound for morphine and methadone with normal albumin and albumin free from fatty acids. Tryptophan (100 μM), stearic acid (500 μM) and palmitic acid (500 μM) did not affect the albumin binding.

Discussion. The binding of morphine to human plasma proteins is practically independent of the drug concentration. However, the binding varies proportionally to the concentration of albumin which is the main binding protein for morphine (Olsen 1975). The lack of change of the percentage binding as the drug concentration is varied shows that there is a large number of available binding sites: n is large, K_a is small, therefore it is difficult to saturate all the available sites. These data explain why with this class of drug non-ionized at plasma pH, it is not possible to bring about drug-interference in vivo with a large increase of the free form. We have found that r increases while r/(Clf) slightly decreases. This is consistent with another of our findings that the percentage binding slightly decreases while the drug concentration increases. Judis found that r increases while r/(Clf) greatly increases. This is inconsistent with another of his findings that the percentage binding decreases slightly while the drug concentration increases.

Methadone shows a Scatchard plot with two negative slopes. The first slope corresponds with the

more specific binding: n is small and the sites are rapidly saturated. The second negative slope corresponds with the binding to the second class of sites which are of lower affinity but in greater number; these sites are not saturable. These Scatchard plots are in complete disagreement with those obtained previously by Judis whose positive Scatchard plots indicate that n and/or K_a decrease as the protein concentration increases. One could attribute (Bowmer & Lindup 1978) this phenomenon of positive slopes to the contaminants of the albumin commercial preparation, like fatty acids or tryptophan. We have used simultaneously a normal albumin and an albumin free from fatty acids with no change in the binding percentage. The phenomenon of positive slope can also be attributed to a cooperative binding.

This would be obvious when the protein concentration increases. The Scatchard plot of methadone is the same for a 0·4% albumin or 4% albumin: n is only a little smaller for the 4% albumin, this could be due to the masking of some sites by the folding of the protein molecules.

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On the urinary disposition of phenformin and 4-hydroxy-phenformin and their rapid simultaneous measurement

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Phenformin (β-phenethylbiguanide) is an orally active hypoglycaemic agent used in the treatment of maturity-onset diabetes. It has been found to be metabolized by oxidation to form a single hydroxylated derivative, 4-hydroxy-phenformin, which is excreted, together with unchanged parent drug, in the urine (Beckmann 1967). We have studied the urinary disposition of phenformin and its metabolite in a single subject given phenformin and we describe the method used. A normal male volunteer was given an oral dose of 50 mg phenformin (Dibotin, Winthrop Laboratories). Urine samples were then collected hourly for the first 8 h following this dose and at 10, 13, 24 and 26 h. After recording the volume of each sample an aliquot was stored at −20 °C before analysis as described below. Both parent drug and metabolite could readily be detected in all the urine samples collected and the rates of excretion for the two substances were plotted on a logarithmic scale against time (Fig. 1). Maximum rate of excretion for phenformin and 4-hydroxy-phenformin coincided in the same urine sample, that obtained from 1 to 2 h after dosing, suggesting the existence of a significant first-pass effect. Thereafter the rates of excretion declined exponentially with time enabling estimates of elimination half-life to be estimated which for phenformin was 3·7 h and 4-hydroxy-phenformin 3·8 h. The total recovery of the drug in this subject was 27·6 mg (56·2%) comprising 18·5 mg unchanged phenformin and 9·1 mg metabolite.

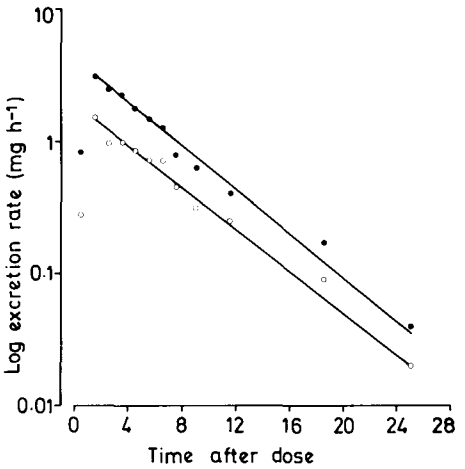

FIG. 1. The rates of excretion of phenformin (●) and 4-hydroxy-phenformin (○) by a single male subject following an oral dose of phenformin (50 mg).

Whereas a number of methods have been described for the estimation of phenformin (Matin et al 1975; Alkalay et al 1976; Hill & Chamberlain 1978), this has not been so for the metabolite. 4-Hydroxyphenformin possesses the strongly basic biguanide group and a weakly acidic phenolic hydroxyl residue, thereby

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