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In vitro binding of ketamine to human serum albumin

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Summary

A study was made of the interaction of ketamine and human serum at 37°C and with human serum albumin at temperatures of 37 and 20°C using an ultrafiltration method. Ketamine concentrations were determined by a gas chromatography technique with a flame ionization detector.

With the aid of an iterative calculation programme, the affinity constants for class 1 and class 2 of binding sites at temperatures of 20 and 37°C were determined. The binding were determined at 20°C and the effect of temperature on binding was established; finally, the thermodynamic parameters $\Delta H°$, $\Delta G°$ and $\Delta S°$ which define binding were determined.

Introduction

Drug–protein binding studies are important for the prediction of drug dynamics in the body (Levy and Shand, 1984). The protein complex serves as the transport system that conveys the metabolite or drug to its site of action. Drug binding can therefore affect the therapeutic, pharmacodynamic and toxicological action of a drug. The binding of drugs by albumin and plasma proteins has been reviewed earlier (Jusko and Gretch, 1976).

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Ketamine, 2-(O-chlorophenyl)-2-(methylamino) cyclohexanone, is an anaesthetic agent derived from phencyclidine and is frequently used in paediatrics (Yoshikawi and Murai, 1971). The study of protein binding by anaesthetics is of great importance in that interaction of these substances with plasma proteins implies a limitation in their access to the CNS and hence in their pharmacological effects.

The data appearing in the specialist literature referring to the binding of ketamine to plasma proteins varies widely; while some workers report that binding is highly significant (Little et al., 1975), others find that percentage bound to human albumin is only 12% (Wieber et al., 1975); on the other hand, binding percentages of as high as 53% have been found for plasma protein in the dog (Kaka and Hayton, 1980).

The aim of the present paper was to determine the percentage of ketamine binding to human serum albumin and to establish the parameters of the interaction between ketamine and human serum albumin.

Experimental

Chemicals

The following reagents were used in the study: ketamine and 2-(O-bromophenyl)-2-(methylamino) cyclohexanone (Parke Davis), and benzene, borax and sodium hydroxide (Merck). Crystalline human serum albumin was obtained commercially (Nutritional Biochemicals Division of INC Life Sciences Group) and the whole human serum was of tissue culture quality (Desiccated TC Human Serum; Difco Laboratories). Water was deionized and double-distilled, with the second distillation performed in an all-glass apparatus. Chemicals in the buffer solutions were of reagent grade.

Measurement of ketamine

The analytical technique used for the determination of ketamine was chromatography using an apparatus fitted with flame ionization (Varian mod. 3700) and a programmable integrator (Varian mod. CDS 111). The details of the technique have been described elsewhere (Pedraz et al., 1983).

Ultrafiltration

The determination of the binding capacity of ketamine to plasma proteins was carried out by ultrafiltration. Filtration was performed using a polycarbonate cell of 15 ml capacity, equipped with a molecular filtration membrane (Millipore), type PTGC 0.13 10, capable of retaining molecules with molecular weights greater than 10^4.

Serum albumin was reconstituted with a 0.003 M phosphate buffer (pH 7.4). The albumin solution at a concentration of 4% in the presence of different amounts of drug were incubated at 37°C for 5 h in order to reach binding equilibrium. 10 ml aliquots of these solutions were stirred at 40 rpm during filtration under nitrogen at 10 p.s.i. taking 0.1-ml samples of ultrafiltrate for their later determination.

Filtration was checked periodically for protein leakage using trichloroacetic acid and later determining the unbound ketamine.

Binding studies for 20°C were carried out under identical conditions with the aim of establishing the thermodynamic binding parameters.

The initial studies for the determination of the binding of ketamine to human serum were also carried out under the same conditions as in the case of serum albumin at 37°C.

All averages were based on a minimum of three replicates.

Data analysis

The equilibrium aspect of such interactions was correlated through the mass law, yielding the familiar equation:

$$r = \frac{nK[A]}{1 + K[A]} \tag{1}$$

where r represents the moles of ligand bound per mole of macromolecule, n is the number of binding sites, K is the binding constant and A is the amount of free ligand. This equation is valid when there is only one class of binding site, so that in general the equation may be written as:

$$r = \sum_{i=1}^{m} \frac{m_i K_i[A]}{1 + K_i[A]} \tag{2}$$

where m represents the number of classes of independent sites such that each class, i, has n_i sites with binding affinity K_i.

The previous treatment is preferred when high values of r are reached, whereas in the opposite case the treatment which establishes the existence of more than one binding site with their corresponding macroscopic constants is of greater advantage. This procedure for the simplest case of one and two centers may be expressed by the following equations:

$$n = 1;\ r = \frac{K_1[A]}{1 + K_1[A]} \tag{3}$$

$$n = 2;\ r = \frac{K_1[A] + 2K_1K_2[A]^2}{1 + K_1[A] + K_1K_2[A]^2} \tag{4}$$

The mathematical treatment of the experimental results and curve-fitting were performed using a non-linear Newton type (Gonzalez and Salvador, 1982) iterative program with the Eqns. 3 and 4 as described elsewhere (Meyer and Guttman, 1968).

Regarding thermodynamic analysis, it was assumed that there is no significant temperature dependence of enthalpy changes within the temperature range in which the interaction was carried out; it is possible to estimate the standard enthalpy

change $\Delta H°$ for the associations of 1 mol of ketamine with 1 mol of the binding site from Eqn. 5.

$$\log \frac{K_{20°C}}{K_{37°C}} = \frac{-\Delta H°}{2.303\ R} \cdot \left(\frac{1}{293} - \frac{1}{310} \right) \tag{5}$$

The value of standard free Gibb's energy for the formation of the complex is given by the equation:

$$\Delta G° = -RT \cdot \ln K \tag{6}$$

Finally, entropy changes, $\Delta S°$, were obtained by substituting the value of $\Delta G°$ in the Gibb's equation:

$$\Delta S° = \frac{\Delta H° - \Delta G°}{T} \tag{7}$$

Results and Discussion

A cross-over study was first carried out to determine the binding capacity of ketamine at different concentrations with human serum and with a solution of 4% human serum albumin, both at 37°C. The concentrations studied ranged between 0.6 and 12.0 μg/ml, the approximate concentration found on arousal from anaesthesia by ketamine. After the ketamine solution had remained in contact with serum and albumin during the incubation period, the binding percentage was determined and it was seen that ketamine is bound in a greater proportion to serum protein than to albumin, as shown in Fig. 1. The binding percentages found by us were different to the values reported by other workers who indicate that binding is less than 12%. Our results resemble more the binding value obtained by Dayton et al. (1983). These authors, studying binding over a similar concentration to our own, found binding percentages as high as 47%, together with a high affinity of ketamine for the α_1-acid glycoprotein, even greater than for human serum albumin. With disease, stress and trauma, α_1-acid glycoprotein may be increased, and the concentration range changes from 0.005 to 0.1%. Thus past variability of the binding of ketamine to human serum is likely to be due to variability in α_1-acid glycoprotein concentration. This phenomenon would therefore justify the greater binding percentages found by us in total serum with respect to the 4% albumin solution.

In order to quantify the binding parameters it was necessary to work with chemically pure materials. Thus, after checking the above results, all later studies were carried out with human serum albumin. The aim in determining the parameters defining the binding of ketamine to serum albumin at 37°C was to obtain the closest approximation to what occurs in physiological conditions after administration of the drug.

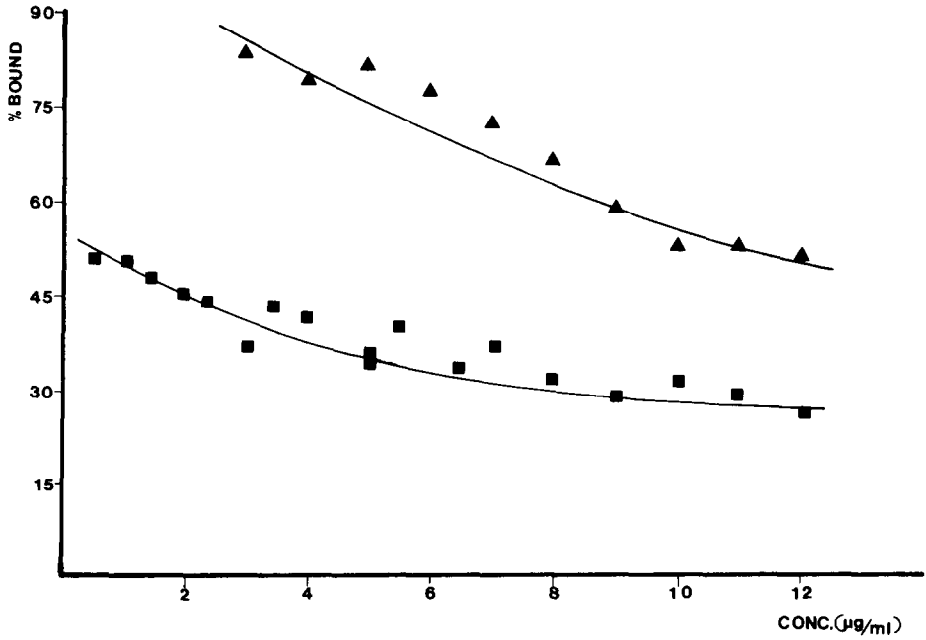

Fig. 1. Binding percentages of ketamine in human plasma and in solutions of human albumin with the same concentration as in serum (4 g%). Serum (▲); human albumin (■).

TABLE 1

EXPERIMENTAL RESULTS OBTAINED FOR THE BINDING OF KETAMINE TO HUMAN SERUM ALBUMIN AT TEMPERATURES OF 37 AND 20°C

37°C			20°C		
µmol/l Free	µmol/l Bound	$r \times 10^{-2}$	µmol/l Free	µmol/l Bound	$r \times 10^{-2}$
1.2	1.2	0.2	0.7	3.5	0.6
2.1	2.1	0.4	1.1	5.1	0.8
2.9	2.8	0.5	1.7	6.7	1.1
4.6	3.7	0.6	2.4	8.1	1.3
5.5	4.2	0.7	2.9	9.7	1.6
7.9	4.5	0.8	4.4	12.3	2.1
8.2	6.2	1.1	6.3	14.7	2.5
9.1	7.1	1.2	7.9	17.2	2.9
13.6	7.5	1.3	10.1	19.3	3.3
13.6	9.1	1.6	12.1	21.1	3.6
18.2	8.9	1.6	18.9	23.1	3.9
18.2	10.9	1.9			
22.7	9.9	1.7			

TABLE 2

BINDING PARAMETERS OF KETAMINE TO HUMAN SERUM ALBUMIN FOR CLASSES 1 AND 2 BINDING SITES AT TEMPERATURES OF 37 AND 20°C, RESPECTIVELY. (S.Q.R. = SUM OF SQUARED RESIDUALS.)

37°C			20°C		
	$K(l \cdot M^{-1})$	S.Q.R.		$K(l \cdot M^{-1})$	S.Q.R.
n = 1	$K_1 = 9.7 \times 10^{-4}$	6.51×10^{-5}	n = 1	$K_1 = 3.5 \times 10^{-3}$	2.0×10^{-4}
n = 2	$K_1 = 9.7 \times 10^{-4}$	6.64×10^{-5}	n = 2	$K_1 = 3.7 \times 10^{-3}$	1.7×10^{-4}
	$K_2 = 2.2 \times 10^{-4}$	6.64×10^{-5}		$K_2 = 2.4 \times 10^{-3}$	1.7×10^{-4}

Table 1 shows the experimental values obtained for a temperature of 37°C. The constants calculated after the fitting for class 1 and class 2 of binding sites are shown in Table 2. In the light of such results, it is not possible to distinguish, under these experimental conditions which are similar to physiological states, the existence of more than one class of binding site. This is a consequence of the low relationship between the moles of bound drug and the total moles of protein in the system (r) which shows the relative usefulness of the classic mathematical treatments of protein binding when, as in our case, it is attempted to reproduce conditions which are similar to those of physiological states, where the value of r is very low.

In order to be able to characterize the thermodynamic parameters that define binding, the whole study was repeated under identical conditions, this time at a

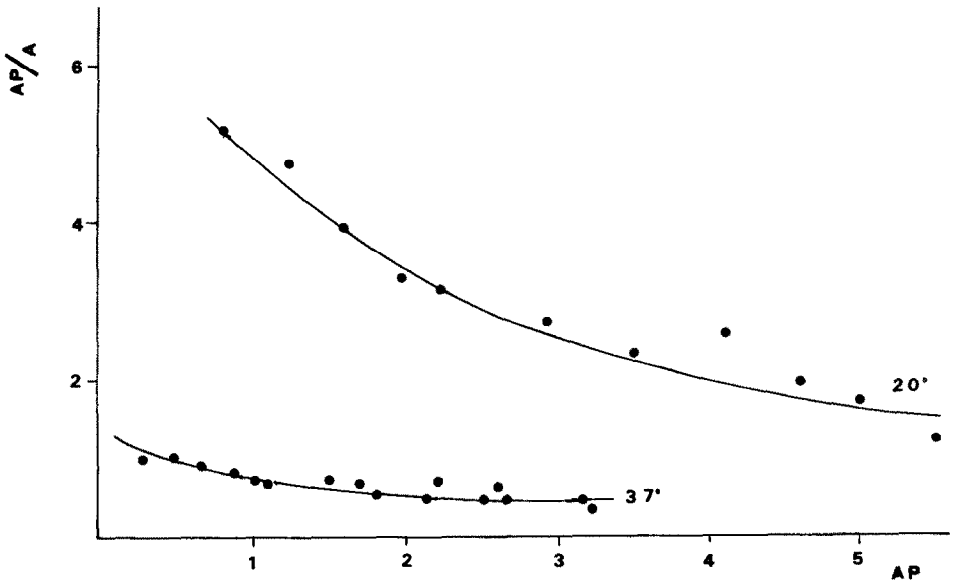

Fig. 2. Effect of temperature on ketamine binding to human albumin by ultrafiltration. A represents free ligand and AP the bound ligand.

TABLE 3

THERMODYNAMIC DATA FOR HUMAN SERUM ALBUMIN/KETAMINE BINDING CONSIDERING ONLY ONE CLASS OF BINDING SITE

T(°K)	K($l \cdot M^{-1}$)	$\Delta H°$ (kcal/mol)	$\Delta G°$ (kcal/mol)	$\Delta S°$ (u.e.)
310	$9.7 \cdot 10^{-4}$	−13.6	4.3	−58
293	$3.5 \cdot 10^{-3}$	−13.6	3.3	−58

temperature of 20°C; it was then possible to check the effect exerted by temperature on the binding of the drug to serum albumin.

The experimental results obtained at this temperature are shown in Table 1 and the value of the constants calculated for class 1 and class 2 of binding sites are shown in Table 2. Similar to what happened at 37°C, it was again impossible to define the existence of more than one class of binding site for the same reasons adduced before.

Fig. 2, representing the relationship between bound and free drug versus the amount of drug bound, gives a general idea of the influence of temperature on the binding capacity of ketamine to human serum albumin; it can be seen how this relationship is greater at a temperature of 20°C for the whole range of combined drug studied.

As a final aim of this study, the thermodynamic parameters defining binding were calculated. Table 3 shows the thermodynamic constants which define the binding of ketamine to human serum albumin, taking into account only one class of binding site for ketamine to the human serum albumin molecule.

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