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EXTENT OF PLASMA PROTEIN BINDING OF AMPHETAMINE IN DIFFERENT SPECIES

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Abstract—The extent of plasma protein binding of amphetamine in different species was determined *in vitro* by the equilibrium dialysis technique using ^{3}H-*d*-amphetamine sulphate and liquid scintillation counting. The per cent of amphetamine bound to plasma proteins was independent of drug concentration within the range of concentrations studied, that is $2{\cdot}5 \times 10^{-7}$ M to $4{\cdot}0 \times 10^{-6}$ M. Protein binding was determined in pony, goat, swine, dog, cat, monkey, rabbit, opossum, rat, mouse, human and chicken plasmas at a drug concentration of $1{\cdot}0 \times 10^{-6}$ M. The extent of binding was less than 45 per cent in all species but varied significantly among species (F-test, $P < 0{\cdot}01$). Protein binding of this base appears to be associated with plasma albumin; there was no binding to 3 per cent equine or procine gamma globulin or to 2 per cent ovalbumin. The extent of protein binding (mean ± S. E.) in intact (23·1% ± 1·7) and nephrectomized uremic (24·3% ± 1·5) dogs was not significantly different. The non-protein bound drug appeared to diffuse freely into cerebrospinal and ocular fluids. The extent of protein binding was independent of drug concentration, similar in normal and uremic plasmas and significantly different among several species. These properties pose the interesting question as to the nature of the binding.

THE EXTENT of plasma protein binding of some acidic drugs has been shown to vary in different species,[1–3] but few comparative studies have been performed on the binding of basic drugs. Borga *et al.*[4] showed that a species difference existed in the plasma protein binding of desipramine. The objective of determining the extent of plasma protein binding of amphetamine in different species was to evaluate the role of protein binding in the pharmacokinetics of this drug.

MATERIALS AND METHODS

The blood donor animals were clinically healthy adults of each of the following species (breed or strain): pony (Shetland-cross), goat (Toggenburg), swine (Yorkshire), dog (mongrel), cat (American short-haired), monkey (Rhesus), rabbit (New Zealand white), opossum, rat (Sprague–Dawley), mouse (Swiss), chicken (Leghorn) and human. Blood from the various species was collected in tubes containing dipotassium EDTA and plasma was separated by centrifugation. The total plasma protein concentrations were determined by refractometry; the plasma was then stored at −10°. The extent of plasma protein binding was determined by the equilibrium dialysis technique using ^{3}H-*d*-amphetamine sulphate (New England Nuclear Corp., Boston, Mass.) and liquid scintillation counting. The specific activity of the amphetamine sulphate was 9·8 c/m-mole (radiochemical purity > 98%).

The dialysis system was based on double equilibrium dialysis cells.* A premoistened cellophane membrane strip was placed between the half-cells and clamped in that position. Plasma (1 ml) was introduced into one half-cell and in the opposing cell 1 ml isotonic dialysis buffer (pH 7·4) containing the labeled amphetamine. The dialysis buffer consisted of 0·16 M KH_2PO_4 + 0·16 M Na_2HPO_4. The dialysis cells were secured on a CRC dialysis cell shaker, placed on a laboratory warming plate with an acrylic plastic hood† and incubated at 37° for 24 hr. Following this incubation period, 100-μl aliquots were removed from each half-cell, added to 0·5 ml Soluene (Packard Instrument Company) and 15 ml Bray's solution,[5] in a liquid scintillation vial. The radioactivity was then assayed in a Packard Tri Carb model 3380 liquid scintillation spectrometer. The per cent of plasma protein binding was calculated according to the formula: (dpm/ml plasma − dpm/ml buffer)/(dpm/ml plasma) × 100. Binding of ^{3}H-*d*-amphetamine to the cellophane membrane was measured by allowing the drug to equilibrate across the membrane; both half-cells contained dialysis buffer. After the incubation period, the membrane was dipped briefly into buffer, placed in a liquid scintillation vial, Soluene (0·5 ml) and Bray's solution (15 ml) were added and the radioactivity was assayed. The effect of varying drug concentration upon the extent of protein binding was established at the following concentrations of amphetamine (2·5, 5, 10, 20, 25, 30 and 40) × 10^{-7} M in dog, cat and goat plasma. Subsequently, the extent of binding was determined in each species under investigation at a drug concentration of 1 × 10^{-6} M.

Experiments performed in vivo. The experimental subjects were seven healthy dogs and eight dogs which were nephrectomized 42–48 hr earlier. The BUN values (mean ± S. E.) in five animals 24 hr after nephrectomy were 88 ± 6·1 mg/100 ml (normal, 10–30 mg/100 ml). A similar dose (0·66 mg/kg, calculated as free base) of *dl*-amphetamine sulphate (Amfetasul, Pitman–Moore, Indianapolis, Ind.) was administered intravenously to each animal. Four hours after drug administration, cerebrospinal fluid from the cisterna magna and venous blood were collected simultaneously. Ocular fluid was also collected from some animals. The concentrations of amphetamine in the biological fluids (plasma, cerebrospinal and ocular fluids) were determined by a sensitive and specific gas chromatographic method.[6] The trichloroacetamide derivative of amphetamine was chromatographed and detected by electron capture.

RESULTS AND DISCUSSION

Binding of labeled amphetamine to the cellophane membrane was low; the number of disintegrations per minute associated with the membrane was one-hundredth that present in buffer in each half-cell. The per cent amphetamine bound to plasma proteins was independent of drug concentration within the range of concentrations studied, that is 2·5 × 10^{-7} M to 4·0 × 10^{-6} M. This concentration range was similar to the plasma amphetamine levels obtained in several species of animals after intravenous injection of 0·66 mg/kg of *dl*-amphetamine sulphate, calculated as free base. Extent of protein binding independent of drug concentration was reported for desmethylimipramine[7] and amphetamine[8] in human plasma. In general, the extent of binding of acidic drugs decreases with increasing drug concentration. The usual Scatchard[9] plot

* Chemical Rubber Company, Cleveland, Ohio.
† Will "Gentle-therm", Will Scientific Inc., Rochester, N.Y.

for the binding of most acidic drugs is a straight line with a negative slope; the X and Y intercepts are respectively the number of binding sites and association constant of drug with albumin. A Scatchard plot of the data obtained in this study indicates that there are a large number of binding sites available for amphetamine within the drug concentration range studied. Preliminary work suggests that the extents of protein binding of chloramphenicol and morphine in dog plasma are independent of drug concentration within the therapeutic range of plasma levels. In contrast, the extent of plasma protein binding of quinidine was concentration dependent.[10]

TABLE 1. TOTAL PLASMA PROTEIN CONCENTRATIONS AND EXTENT OF PROTEIN BINDING OF AMPHETAMINE IN SEVERAL SPECIES OF ANIMALS*

Species (no.)		Bound amphetamine (%)		Plasma protein concn (g/100 ml)	
		Mean	S. E. M.	Mean	S. E. M.
Goat	(12)	40·7	1·7	6·6	0·3
Swine	(6)	39·6	3·0	6·4	0·6
Monkey	(11)	40·2	0·7	7·8	0·2
Rat	(4)	40·5	3·0	6·1	0·8
Rabbit	(4)	31·0	2·9	5·5	0·3
Dog	(17)	27·1	1·5	6·7	0·3
Cat	(12)	26·4	1·2	7·3	0·2
Pony	(9)	25·3	2·6	7·5	0·3
Opossum	(4)	26·0	4·0	6·8	0·4
Mouse	(5)	17·2	3·5		
Human	(7)	16·2	2·3	6·3	0·1
Chicken	(6)	14·5	0·9	3·0	0·3

* Amphetamine concn., 1×10^{-6} M.

The per cent of bound amphetamine (mean $\pm$ S. E.) at a concentration of $1{\cdot}0 \times 10^{-6}$ M and the total plasma protein concentration (Mean $\pm$ S. E.) in each species of animal studied are tabulated (Table 1). The extent of binding was less than 45 per cent in all species but varied significantly among species (F-test, $P < 0{\cdot}01$). At a similar drug concentration, the extent of binding (mean $\pm$ S. E.) in dependent and drug naive human subjects was $23\% \pm 1{\cdot}1$ and $26\% \pm 1{\cdot}0$ respectively.[8] There was no binding of amphetamine to 3 per cent equine or porcine gamma globulin or to 2 per cent ovalbumin, so that binding of this drug appears to be associated with plasma albumin.

Extent of binding in vivo. The extravascular distribution of amphetamine was rapid (less than ten min) in dogs. The rate of attainment of the apparent volume of distribution was considerably greater than the rate of drug elimination. Approximately 30 per cent of the dose administered was excreted unchanged in 24-hr urine of dogs. Eight randomly selected mongrel dogs were bilaterally nephrectomized in an attempt to decrease the rate of amphetamine elimination. The amphetamine concentrations were similar in cerebrospinal and ocular fluid samples (*ca.* 115 ng/ml) collected simultaneously 4 hr after intravenous injection of the drug. These values were lower than the amphetamine concentration in plasma (*ca.* 150 ng/ml) by an amount which corresponded to the extent of protein binding in dog plasma, determined *in vitro*. Since only

non-protein bound drug in plasma was available for diffusion into cerebrospinal fluid, which contains practically no protein, one might conclude that after distribution was complete similar concentrations of amphetamine were present in cerebrospinal and ocular fluids and plasma water. On this basis, the extent of plasma protein binding (*in vivo*) was computed as follows:

$$\text{Per cent bound amphetamine} = \left(1 - \frac{C_{CSF}}{C_{plasma}}\right) \times 100.$$

The extent of binding (mean ± S. E.) in intact (23·1% ± 1·7) and nephrectomized (24·3% ± 1·5) dogs was similar. It thus appears that uremia had no effect upon the extent of protein binding of this drug. Reidenberg *et al.*[11] showed that desmethylimipramine had almost normal binding in plasmas from uremic patients while the extent of binding of diphenylhydantoin was reduced. The extents of protein binding in the dog determined by the equilibrium dialysis technique *in vitro* and by measuring the amphetamine concentrations in plasma and cerebrospinal fluid collected simultaneously were not significantly different (Student's *t*-test, $P > 0{\cdot}05$, Table 2).

TABLE 2. PLASMA PROTEIN BINDING OF AMPHETAMINE IN DOGS*

	Bound amphetamine (%)	
Variable	*In vitro*	*In vivo*†
Amphetamine concn.	135 ng ml^{-1}	100–150 ng m^{-1}
Mean	27·1	23·7
n	17	15
S. E. M.	1·5	1·1

* Student's *t*-test: NS, $P > 0.05$.

† Experimental subjects were seven healthy and eight nephrectomized uremic dogs.

In conclusion, the extent of protein binding was independent of drug concentration, similar in normal and uremic plasmas and significantly different among several species. Since the amphetamine concentrations in CSF and plasma water were similar, protein binding appeared to be the only factor which hindered access, temporarily, of this drug to the central nervous system.

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