Pethidine binding in whole blood: methodology and clinical significance

C. LA ROSA², D. J. MORGAN² & L. E. MATHER¹

¹Department of Anaesthesia & Intensive Care, Flinders Medical Centre, The Flinders University of South Australia, Adelaide, South Australia 5042 and ²Victoria College of Pharmacy Limited, Melbourne, Victoria 3000, Australia

1 A three-compartment equilibrium dialysis method was developed for the simultaneous and direct determination of drug binding in whole blood and in plasma and of the blood to plasma concentration ratio (b/p).

2 The unbound fraction of pethidine in the blood of six healthy volunteers (0.63, s.d. 0.09, $n = 23$ determinations) was significantly different from that in the blood of six patients (0.72, s.d. 0.08, $n = 24$ determinations).

3 There was no significant difference in the b/p of six patients (0.84, s.d. 0.09, $n = 24$ determinations) and six volunteers (0.90, s.d. 0.14, $n = 23$ determinations).

4 The observed unbound fraction of pethidine in blood (0.6 to 0.7) was considerably lower than previously reported.

5 As this value for the unbound fraction (0.6 to 0.7) is similar to the reported estimated hepatic extraction ratio of the drug in man, it is proposed that pethidine elimination should be described as 'capacity limited, binding sensitive' rather than 'flow-limited'.

Keywords pethidine binding whole blood methodology

Introduction

There have been numerous studies on the binding of drugs to plasma proteins. However, relatively little consideration has been given to the binding of drugs by erythrocytes or to the binding in whole blood, even though it has been emphasised that whole blood data are more relevant for interpretation in physiological terms (McArthur *et al.*, 1971; Rowland, 1972). The application of classical plasma protein binding methods to the study of drug binding in whole blood is recognised as being difficult and subject to error (Cruze & Meyer, 1976). Dialysis may cause haemolysis of erythrocytes or denaturation of other blood components, and separation of blood components may arise from ultrafiltration methods. Although there have been binding studies using whole blood (for example, McArthur *et al.*, 1971; Borodny *et al.*, 1973; Bickel, 1975; Ehrnebo & Odar-Cederlof, 1975; Cruze & Meyer, 1976) most investigators have

been concerned with erythrocyte uptake (for example, Abshagen *et al.*, 1971; Bratlid, 1972) or with the correlation of unbound fraction in plasma with another variable such as the blood to plasma concentration ratio (b/p) of the drug (for example Kurata & Wilkinson, 1974; Taylor & Turner, 1981).

Where binding studies have been performed in whole blood, essentially three different variants in methodology have been utilised. First, there are those studies in which whole blood in cellulose sacs was dialysed against 0.9% sodium chloride usually with pH adjustment (McArthur *et al.*, 1971; Gazzard *et al.*, 1973; Borodny *et al.*, 1973). Others have used polymethacrylate dialysis cells to dialyse whole blood against isotonic sodium phosphate buffer at pH 7.35 (Ehrnebo *et al.*, 1974; Ehrnebo & Odar-Cederlof, 1975). A variation of this method was the use of Dianorm® apparatus by Bickel (1975)

who investigated the blood binding of the basic drugs chlorpromazine and imipramine. The third methodological variant was used by Cruze & Meyer (1976) who attempted to adapt the dynamic dialysis technique of Meyer & Guttman (1968) to the quantitation of drug binding to whole blood. It was found that mechanical agitation caused considerable haemolysis and that this haemolysis altered drug binding. Furthermore it was found that the nature of the binding sites in the complex whole blood system appeared to alter during the study. As a consequence the authors concluded that the data obtained by this technique did not lend itself to analysis by classical methods.

In the present study, the classical two-cell equilibrium dialysis system was extended so that both the binding of drug in whole blood and the blood:plasma concentration ratio (b/p) could be measured simultaneously. The technique was applied to investigate the inter-subject variation in pethidine whole blood binding—a subject which, in the past, has caused considerable controversy because of its importance to the physiological interpretation of pethidine total body clearance.

Methods

Sample preparation

Blood and plasma samples freshly collected from patients and volunteers were used throughout the study. Blood was collected via the median cubital vein using a 21 gauge scalp vein cannula and 30 ml plastic syringes. Blood was immediately transferred into commercially available 10 ml plastic tubes containing lithium heparin (Kayline, South Australia) to which had been added (unless otherwise stated) 20 mg sodium fluoride. Part of the sample was centrifuged to collect the plasma, the remainder being used for the binding determination and for the measurement of haematocrit. The procedure outlined above was completed quickly so that not more than 30 min elapsed from collecting the blood sample to the beginning of the binding experiment and no initial pH adjustments were made to either the blood or plasma. Blood samples were obtained from a group of six patients presenting for routine haematological tests in the outpatient clinic. The patients' ages ranged from 20 to 63 years and two were in the third trimester of pregnancy. Volunteers were all healthy and aged between 19 and 35 years. Neither patients nor volunteers were taking medications at the time of the study. Informed consent was obtained from each subject.

Dialysis technique

Chemicals and solutions were the same as described previously (La Rosa *et al.*, 1984) except the apparatus used previously was modified by the addition of a third 2 ml chamber. Two ml of buffer (0.067 M Sorensen's phosphate, pH 7.4) was placed in the central compartment, a 2 ml aliquot of blood was pipetted into one outer chamber and a 2 ml aliquot of plasma from the same blood sample was pipetted into the other outer chamber. [^{14}C]-pethidine HCl (150 μl , 1035 d/min/ μl , 0.023 mg/ml base) was added to the buffer. The initial pH of blood buffer and plasma were recorded as before (La Rosa *et al.*, 1984) but no pH adjustments were made. The dialysis unit was sealed, gently rotated by hand several times and placed in an oscillating water bath (100 oscillations/min at 37°C for 5 h). Preliminary experiments had shown that this time was sufficient for the system to reach equilibrium and that loss of [^{14}C]-pethidine to the apparatus was negligible.

After 5 h, pH was measured in each chamber and duplicate 0.5 ml samples were taken from each chamber. The samples were made alkaline with NaOH (0.1 M; 0.2 ml) and extracted into scintillation grade toluene (2 ml) by vortex mixing (2 min). The organic layer was added to scintillation cocktail (six parts PCS (Amersham): three parts scintillation grade toluene; 8 ml) and counted in a scintillation counter using the preset radionuclide window for [^{14}C]. Quench correction was performed with each experiment using the channels ratio method. Preliminary experiments indicated that plasma, blood or buffer extracts caused no significant quenching. The overall extraction of pethidine of [^{14}C]-pethidine was found to be 85.0 (s.d. 3.1, $n = 8$) percent from plasma and 85.3 (s.d. 3.6, $n = 6$) percent from blood. The difference between these was not significant. The extraction from buffer was 92.0 (s.d. 1.6, $n = 4$) percent and this was greater than from plasma or blood. The extent of binding in plasma and in blood, and the blood to plasma ratio (b/p) were calculated after correcting for extraction efficiency, according to Equations (1–3).

$$\begin{aligned} \text{unbound fraction in plasma} = \\ \frac{\text{mean total d/min in 0.5 ml buffer aliquots}}{\text{mean total d/min in 0.5 ml plasma aliquots}} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{unbound fraction in blood} = \\ \frac{\text{mean total d/min in 0.5 ml buffer aliquots}}{\text{mean total d/min in 0.5 ml blood aliquots}} \end{aligned} \quad (2)$$

$$\begin{aligned} \text{b/p} = \\ \frac{\text{mean total d/min in 0.5 ml blood aliquots}}{\text{mean total d/min in 0.5 ml plasma aliquots}} \end{aligned} \quad (3)$$

Results and discussion

Blood pH change during dialysis

Dialysis without the addition of sodium fluoride to the blood collection tube demonstrated a lack of acid-base equilibrium (Figure 1), presumably due to the accumulation of acid metabolites from the blood cells. Since pethidine plasma protein binding is pH sensitive (La Rosa *et al.*, 1984) control of pH is imperative. The addition of sodium fluoride to the blood collection tube was found to control the pH during dialysis, presumably due to the inhibition of cellular glucose metabolism. No haemolysis was evident after dialysis of the sodium fluoride-treated blood. Figure 1 also shows that the pH equilibrated within the first hour and was maintained at 7.40 ± 0.05 in each compartment thereafter.

It is difficult to ascertain whether the addition of sodium fluoride affected the binding determination. Since the plasma binding data arising from these experiments are similar to those reported previously (La Rosa *et al.*, 1984) in comparable experiments where no fluoride was used, it would be reasonable to assume that no gross distortions have been introduced.

Clinical studies

The blood binding method described was used to investigate the inter-subject variation in the *in vitro* binding of [^{14}C]-pethidine in whole blood from six healthy volunteers (Table 1) and from six patients (Table 2). There were significant differences (Student's *t*-test) between volunteers and

Figure 1 pH change in each chamber (a) during dialysis of whole blood (---) and plasma (---) against buffer (—) and (b) during dialysis of plasma and sodium fluoride-treated whole blood against buffer (mean $\pm$ s.d. of three observations in each case).

Table 1 Whole blood binding of [^{14}C]-pethidine in six healthy volunteers (s.d. in parentheses)

Subject	Sex	Age (years)	Haematocrit	% blood unbound	% plasma unbound	b/p	Final total blood concentration of pethidine ($\mu\text{g/ml}$)
CL	F	24	42	55 (4)	60 (2)	1.1 (0.1)	0.75 (0.04)
DF	F	19	34	65 (15)	55 (3)	0.88 (0.17)	0.54 (0.06)
RU	M	20	45	63 (10)	56 (2)	0.91 (0.13)	0.54 (0.06)
LA	F	35	39	64 (12)	55 (11)	0.91 (0.13)	0.61 (0.06)
GP	F	32	36	66 (3)	54 (2)	0.83 (0.03)	0.53 (0.02)
JP	M	30	39	64 (7)	56 (3)	0.89 (0.12)	0.53 (0.04)
Grand mean		27	39	63	56	0.90	0.56
s.d.		7	4	9	5	0.14	0.09

patients with respect to both the percent unbound in plasma ($P < 0.01$) and the percent unbound in blood ($P < 0.001$). These data indicate that caution should be taken in applying pethidine binding data from healthy volunteers to patient groups. The b/p in both groups showed appreciable inter-subject variation. There was no significant difference between the mean b/p of the two groups ($P > 0.1$) but a small difference may have been obscured by the relatively small sample size and the small range of protein concentrations, especially among the volunteers. The mean value of b/p from the data of both groups was 0.86 s.d. 0.18 , $n = 47$).

In conclusion, the unbound fraction of pethidine in blood is around 0.6 – 0.7 and that in plasma is around 0.5 – 0.6 when pH is controlled to 7.4 . These values are considerably higher than previously reported. The pharmacokinetic consequence of this finding is that the generally held view that pethidine is a drug cleared non-restrictively may, reasonably, be questioned. The present study revealed an unbound fraction of around 0.6 , a value similar to the commonly

accepted hepatic extraction ratio of pethidine in man (Blaschke, 1977). It appears, therefore, that the unbound fraction of pethidine does not exceed, markedly, the hepatic extraction ratio. This is characteristic of a drug that undergoes restrictive hepatic elimination.

Thus, changes in the extent of binding in whole blood would be expected to result in altered total clearance of the drug but with unchanged concentrations of unbound drug at steady-state. Interestingly, Verbeeck *et al.* (1981), using a prolonged blood sampling time after a single dose, have reported that the total systemic blood clearance of pethidine was 500 to 600 ml/min in healthy volunteers, a value similar to that determined directly in post-operative patients receiving prolonged (32 h) infusions (Austin *et al.*, 1981). Both of these values contrast with reports of 900 ml/min as previously reported (see Mather & Meffin, 1978). It is suggested, therefore, that the classification of pethidine pharmacokinetics be changed from the category of 'flow limited' to 'capacity limited, binding sensitive' as defined by Blaschke (1977).

Table 2 Whole blood binding of [^{14}C]-pethidine in six patients (s.d. in parentheses)

Subject	Sex	Age (years)	Haematocrit	% blood unbound	% plasma unbound	b/p	Final total blood concentration of pethidine ($\mu\text{g/ml}$)
MM (pregnant)	F	20	34	79 (2)	67 (5)	0.85 (0.08)	0.47 (0.06)
BS (pregnant)	F	25	38	69 (11)	62 (4)	0.92 (0.15)	0.54 (0.08)
JF	M	63	38	70 (5)	57 (3)	0.81 (0.07)	0.52 (0.04)
CJ	F	23	28	77 (4)	59 (1)	0.76 (0.03)	0.47 (0.02)
SS	F	22	41	66 (3)	59 (1)	0.88 (0.03)	0.51 (0.01)
JL	F	55	37	80 (11)	57 (3)	0.82 (0.09)	0.52 (0.06)
Grand mean		35	36	72	60	0.84	0.51
s.d.		19	5	8	5	0.09	0.04

References

- Abshagen, U., Kerwitz, H. & Rietbrock, N. (1971). Distribution of digoxin, digitoxin and ouabain between plasma and erythrocytes in various species. *Naunyn. Schmiedebergs Arch. Pharmac.*, **270**, 150-161.
- Austin, K. L., Stapleton, J. V. & Mather, L. E. (1981). Pethidine clearance during continuous intravenous infusions in post-operative patients. *Br. J. clin. Pharmac.*, **11**, 25-30.
- Bickel, M. H. (1975). Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components. *J. Pharm. Pharmac.*, **27**, 733-738.
- Blaschke, T. F. (1977). Protein binding and kinetics of drugs in liver diseases. *Clin. Pharmacokin.*, **2**, 32-44.
- Borondy, P., Dill, W. A., Chang, T., Baughman, R. A. & Glazko, A. J. (1973). Effect of protein binding on the distribution of 5,5-diphenylhydantoin between plasma and red cells. *Ann. N. Y. Acad. Sci.*, **226**, 82-87.
- Bratlid, D. (1972). Bilirubin binding by human erythrocytes. *Scand. J. clin. lab. Invest.*, **29**, 91-97.
- Cruze, C. A. & Meyer, M. C. (1976). Binding of salicylate and sulfathiazole by whole blood constituents. *J. pharm. Sci.*, **65**, 33-37.
- Ehrnebo, M., Agurell, S., Boreus, L. O., Gordon, E. & Lonroth, U. (1974). Pentazocine binding to blood cells and plasma proteins. *Clin. Pharmac. Ther.*, **16**, 424-429.
- Ehrnebo, M. & Odar-Cederlof, I. (1975). Binding of amobarbital, pentobarbital and diphenylhydantoin to blood cells and plasma proteins in healthy volunteers and uraemic patients. *Eur. J. clin. Pharmac.*, **8**, 445-453.
- Gazzard, B. G., Ford-Hutchinson, A. W., Smith, M. J. H. & Williams, R. (1973). The binding of paracetamol to plasma proteins of man and pig. *J. Pharm. Pharmac.*, **25**, 964-967.
- Kurata, D. & Wilkins, G. R. (1974). Erythrocyte uptake and plasma binding of diphenylhydantoin. *Clin. Pharmac. Ther.*, **16**, 355-362.
- La Rosa, C., Mather, L. E. & Morgan, D. J. (1983). Pethidine binding in plasma: Effect of methodological variables. *Br. J. clin. Pharmac.*, **17**, 411-415.
- McArthur, J. N., Dawkins, P. D. & Smith, M. J. H. (1971). The binding of indomethacin, salicylate and phenobarbitone to human whole blood *in vitro*. *J. Pharm. Pharmac.*, **23**, 32-36.
- Mather, L. E. & Meffin, P. J. (1978). Clinical pharmacokinetics of pethidine. *Clin. Pharmacokin.*, **3**, 359-365.
- Meyer, M. C. & Guttman, D. E. (1968). Novel method for studying protein binding. *J. pharm. Sci.*, **57**, 1627-1629.
- Nation, R. L. (1981). Meperidine binding in maternal and fetal plasma. *Clin. Pharmac. Ther.*, **29**, 472-479.
- Rowland, M. (1972). Influence of route of administration on drug availability. *J. pharm. Sci.*, **61**, 70-74.
- Taylor, E. A. & Turner, P. (1981). The distribution of propranolol, pindolol and atenolol between human erythrocytes and plasma. *Br. J. clin. Pharmac.*, **12**, 543-548.
- Verbeeck, R. K., Branch, R. A. & Wilkinson, G. R. (1981). Meperidine disposition in man: influence of urinary pH and route of administration. *Clin. Pharmac. Ther.*, **30**, 619-628.

(Received July 19, 1983,
accepted December 1, 1983)