BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **17**: 447–452 (2003)
Published online Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.269

ORIGINAL RESEARCH

HPLC investigation of free and bound propofol in human plasma and cerebrospinal fluid

Andrzej L. Dawidowicz and Rafał Kalityński

Department of Chemical Physics and Physicochemical Separation Methods, Faculty of Chemistry, Maria Curie-Skłodowska University, PL 20-031 Lublin, pl. Marii Curie-Skłodowskiej 3, Poland

Received 20 January 2003; accepted 24 February 2003

ABSTRACT: The paper compares the total propofol concentration in the cerebrospinal fluid (CSF) with the free drug concentration in plasma measured in 35 humans scheduled for elective neurosurgical procedures during propofol anaesthesia. The concentrations of total and free propofol in the blood and CSF samples were measured by means of HPLC using liquid–liquid extraction and ultrafiltration in the sample preparation procedure. The arterial blood and CSF samples (collected from intraventricular drainage) were taken at the same time. According to the obtained results, the usually expected equality between free drug concentration in plasma and its total concentration in CSF is not valid for propofol: the unbound propofol concentration in plasma is not equal to its total concentration in CSF ($p < 0.05$). This difference suggests a substantial contribution of active transport in propofol transfer from blood into CSF. Moreover, the paper shows the presence of bound propofol in CSF, which is a novel finding. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: propofol anaesthesia; liquid–liquid extraction; ultrafiltration; UV and fluorescence detection

INTRODUCTION

In organisms, drugs are transported by blood as complexes with proteins and blood cells (the bound form) as well as solved in plasma (the free form). Since blood is the most easily available tissue of the organism, the drug concentration in blood is frequently correlated with its pharmacological effect. As is known from literature (Mehta, 1989; Ganong, 1999; Mazoit and Samii, 1999), only the free (unbound) form of a drug is its active form. Determining the concentration of an unbound drug in blood requires a complicated and time-consuming procedure of sample preparation (involving dialysis, ultrafiltration, ultracentrifugation, etc.; Mehta, 1989; Pacifici and Viani, 1992; Sebille *et al.*, 1990; Bowers *et al.*, 1984). This is the main reason for limiting the research to the measurement of the total drug concentration only. However, the investigation of some drug concentrations in different body fluids has led to the conclusion that the total concentration of some drugs in saliva and cerebrospinal fluid (CSF) is comparable with the concentration of their free form in plasma (Mehta, 1989).

*Correspondence to: A. L. Dawidowicz, Department of Chemical Physics and Physicochemical Separation Methods, Faculty of Chemistry, Maria Curie-Skłodowska University, PL 20-031 Lublin, pl. Marii Curie-Skłodowskiej 3, Poland.
E-mail: dawid@hermes.umcs.lublin.pl

Abbreviations used: CSF, cerebrospinal fluid; CNS, central nervous system; TCI, target control infusion; TMAH, tetramethylammonium hydroxide.

Propofol is nowadays one of the most frequently applied intravenous anaesthetics. Its clinically relevant properties and applications in anaesthesiology have been extensively reviewed (Kanto, 1988; Langley and Heel, 1988; Morgan *et al.*, 1990; Schütler and Ihmsen 2000). Anaesthetics are drugs which exhibit a strong affinity to the central nervous system (CNS). The specific morphological properties of the choroidal epithelium and the existence of a CSF pathway for drug distribution to different targets in the CNS suggest that the choroid plexus–CSF route is more significant than previously thought for brain drug delivery (Ghersi-Egea and Strazielle, 2001). There is little information about the CSF pharmacokinetics of propofol concentration in humans (Engdahl *et al.*, 1998; Dawidowicz *et al.*, 2002, 2003).

The aim of this paper is to establish whether the unbound propofol concentration in plasma really equals the anaesthetic concentration in CSF. As CSF sampling during anaesthesia is frequently not plausible, the confirmation of this equality would make the CSF propofol concentration known without the necessity for drug determination in this medium, making the propofol concentration in the brain surroundings easier to estimate. This in turn would extend the knowledge about propofol concentration and pharmacokinetics in CNS. In order to answer the above question, free and total propofol concentrations were investigated in blood and CSF samples taken during neurosurgery from patients anaesthetized with propofol.

Table 1. Size, sex composition, demographic data of the study groups and mean time of the intraventricular drainage insertion

Group no.	Patients	Sex		Mean age (years)	Mean body weight (kg)	Mean time of the drainage insertion (min)
		Men	Women			
I	17	10	7	50.41 SD 12.86; SEM 3.12	80.53 SD 11.58; SEM 2.81	105.4 SD 26.1; SEM 4.2
II	18	12	6	47.11 SD 15.51; SEM 3.66	76.61 SD 12.52; SEM 2.95	106.8 SD 18.1; SEM 5.0

MATERIALS AND METHODS

Study group selection and sampling. After obtaining an approval from the University Ethics Committee and consent from the patients, samples were taken from patients without any symptoms of elevated intracranial pressure scheduled for elective procedures of posterior fossa extra-axial tumours removal.

All the patients were premedicated with 10 mg of diazepam administered orally 2 h before anaesthesia. Before induction the patients had a bolus of fentanyl 0.2 mg and were pre-oxygenated with 100% oxygen for 10 min.

Propofol was applied using a Graseby 3500 pump in the form of target control infusion (TCI) with predicted blood induction concentration from 4.5 to 5 $\mu g\,mL^{-1}$ and predicted maintenance blood concentration from 3.5 to 4 $\mu g\,mL^{-1}$ (Coetzee *et al.*, 1995). Tracheal intubation was facilitated by 0.15 $mg\,kg^{-1}$ of *cis*-atracurium. After the intubation the lungs were ventilated to normocapnia with an oxygen–air mixture (fraction of inspired oxygen = 0.33). In addition to the continuous infusion of propofol, anaesthesia was maintained with repeated doses of fentanyl and *cis*-atracurium. The infusion of propofol was stopped immediately after the end of surgery. When necessary, neuromuscular block was antagonized with 2.5 mg of neostigmine bromide preceded by 0.5 mg of atropine. Usual intraoperative fluid administration consisted of crystalloids initial infusion 7 $mL\,kg^{-1}$ followed by 4 $mL\,kg^{-1}\,h^{-1}$ of the same fluids (Prough, 1993).

Before the induction of anaesthesia, an indwelling 17-gauge cannula was inserted in a large forearm vein and used solely for the infusion of propofol. After the induction, two additional cannulas were placed: one (20-gauge) in the radial artery in the contralateral forearm (for blood pressure monitoring and blood sampling), and the other (7 French × 20) in the right subclavian vein (for monitoring central venous pressure). After the surgery area preparation, the External Drainage System (Codman, Johnson & Johnson, UK) was inserted into one of the lateral brain ventricles. The proper position of the drainage system was confirmed by the CSF outflow. The CSF and blood samples were taken just after the drainage insertion. The blood samples (5 mL) were taken from the radial artery into heparinized syringes and CSF samples (2.5 mL) were collected from the drainage into syringes. The presence of red blood cells in any CSF sample from a given patient caused the rejection of all the samples taken from that patient. The intraventricular drainage was maintained for the entire duration of the surgery.

Two groups of patients were investigated: group I—17 patients from whom CSF and blood samples were taken; group II—18 patients from whom only CSF samples were taken. The groups composition, demographic data of the patients and the mean time of drainage insertion are contained in Table 1.

Drugs. The following drugs were used: propofol (2,6-diisopropylphenol) in soybean oil emulsion for infusions (Diprivan; AstraZeneca, Caponago, Italy); diazepam (Relanium; Polfa, Warsaw, Poland); fentanyl (Fentanyl; Polfa, Warsaw, Poland); *cis*-atracurium (Nimbex; Glaxo Wellcome, Dartford, UK); neostigmine bromide (Polstigminum; Pliva, Kraków, Poland); atropine (Atropinum sulphuricum; Polfa, Warsaw, Poland).

Reagents and solutions. All chemicals, except those separately mentioned, were obtained from the Polish Factory of Chemical Reagents-POCh (Gliwice, Poland) and were of analytical grade. A mixture composed of 75% methanol and 25% deionized Milli-Q water was used as mobile phase. Propofol was obtained from AstraZeneca (Caponago, Italy). Stock solutions of thymol and propofol in methanol (1 $mg\,mL^{-1}$) were each prepared and stored at 4°C. Tetramethylammonium hydroxide, TMAH (25% in methanol; Aldrich, Germany), was diluted with 2-propanol (3:37).

Sample preparation and propofol assay. Unbound propofol was isolated by ultrafiltration on Amicon MPS (Millipore, Bedford, MA, USA) units, utilizing the YM-10 membranes (product no. 40424, Millipore, Bedford, MA, USA) of 10 kDa molecular mass cutoff. The ultrafiltration units were centrifuged in a constant rotor angle centrifuge MPW-341 (Mechanika Precyzyjna, Warsaw, Poland). One millilitre of each CSF or plasma sample was put into a sample compartment of the ultrafiltration unit. After the attachment of an ultrafiltrate collection container, the unit was centrifuged at 2500 rpm until 400 µL of ultrafiltrate was obtained.

For propofol assay, to each sample of blood plasma (1 mL) or CSF (400 µL) or CSF ultrafiltrate (400 µL), thymol (internal standard), dihydrogen sodium phosphate (1 mL of 0.1 M NaH_2PO_4) and cyclohexane (5 mL for plasma and 3 mL for CSF and CSF ultrafiltrate) were added. The mixtures were vigorously shaken for 10 min at 200 rpm. After centrifugation (3000 rpm for 5 min), in order to separate the phases, an aliquot of the cyclohexane layer (4 or 2 mL, respectively) was transferred to a clean tube with TMAH solution (20 or 10 µL, respectively). The solvent was evaporated to dryness in a stream of nitrogen. The residue was re-dissolved in mobile phase and injected into the chromatographic column. The propofol detection limit in plasma was 43 $ng\,mL^{-1}$ with

coefficient of variation ($n = 3$) of 2.8% at 150 ng mL^{-1}, 2.3% at 750 ng mL^{-1} and 0.9% at 1500 ng mL^{-1}. The limit of propofol detection in CSF and ultrafiltrates of plasma and CSF was 1.1 ng mL^{-1} with coefficient of variation ($n = 3$) of 11.1% at 5 ng mL^{-1}, 12.1% at 20 ng mL^{-1} and 9.8% at 40 ng mL^{-1}.

Other details of sample preparation and the analytical procedure can be found in the work of Dawidowicz *et al.* (2000, 2001) and Dawidowicz and Fijałkowska (1995).

Chromatographic equipment. The concentrations of propofol were measured by means of high performance liquid chromatography (HPLC) in plasma as well as in CSF. A Gilson liquid chromatograph (Middleton, WI, USA) consisting of a dual high-pressure pump (model 122), integrated with a manometric module and a dynamic mixer, was employed for HPLC analysis. Propofol in plasma (high levels) was detected with an UV/vis variable wavelength detector working at 270 nm (model 155), also from Gilson, whereas propofol in CSF (lower levels) was detected with a fluorescence detector (Jasco FP-920, Japan) set at excitation wavelength 276 nm and at emission wavelength 310 nm. Chromatographic separations were carried out using a 150 × 4.6 mm i.d. C_{18} silica gel column (Kromasil C_{18}, 5 μM). The samples were injected into the column by a model 7125 injection valve from Rheodyne (Cotati, CA, USA).

Statistical analysis. The data are expressed as mean value with 95% confidence interval (CI), standard deviation (SD), and standard error mean (SEM) where applicable. Statistical analysis was performed by Student's *t*-test for dependent samples. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Examples of chromatograms of propofol determination in plasma and CSF are presented in Figs 1(a), (b) and 2(a), (b), respectively. As can be seen, the HPLC system applied has the sufficient resolution for propofol analysis with the sample preparation procedure used. The thymol (T) and propofol (P) peaks are well separated from impurities and sample constituents not removed during sample processing.

As pointed out in the Introduction, the main aim of the present work was to find out whether for propofol its total concentration in CSF equals the concentration of the unbound drug in plasma. The results of the experiments performed in order to answer this question are presented in Fig. 3. The first data point labelled T/PL illustrates the mean total propofol concentration in plasma for group I of the study patients ($n = 17$); the second point labelled F/PL shows the mean free form concentration of the anaesthetic in plasma, and the last point, marked T/CSF, the total propofol concentration in CSF. The comparison of T/CSF and F/PL values indicates that the total propofol concentration in CSF is significantly higher than its unbound form concentration in plasma ($p < 0.05$). As mentioned above, according to Mehta (1989), the unbound form concentration of the drug in plasma is assumed to be equal to its total concentration in CSF. In the light of the presented results, this rule does not apply to propofol. Taking into account the mean values [total plasma propofol concentration 6112.7 (CI 4970.6, 7254.7; SD 2221.19; SEM 538.72) ng mL^{-1}, free propofol concentration in plasma 62.96 (CI 42.19, 83.72; SD 40.39; SEM 9.80) ng mL^{-1}, and total propofol concentration in CSF 95.62 (CI 75.51, 115.73; SD 39.11; SEM 9.49) ng mL^{-1}], the total propofol amount present in plasma in the free form is 1.06%. The relation of total propofol concentration in CSF to total plasma propofol concentration gives a value of 1.80%. The absolute values of the percentages mentioned above are not very different indeed, but their mutual relation shows their substantial difference, confirming the statistical significance of the difference between respective concentrations.

At this point a question arises about the reason of the observed discrepancy. It should be stressed that CSF is not 'pure water' and, apart from molecules inert towards propofol, contains also molecules (e.g. proteins) and cells which have the ability to bind propofol (Pollay, 1996; Hoag, 1992). Their presence and the possiblity

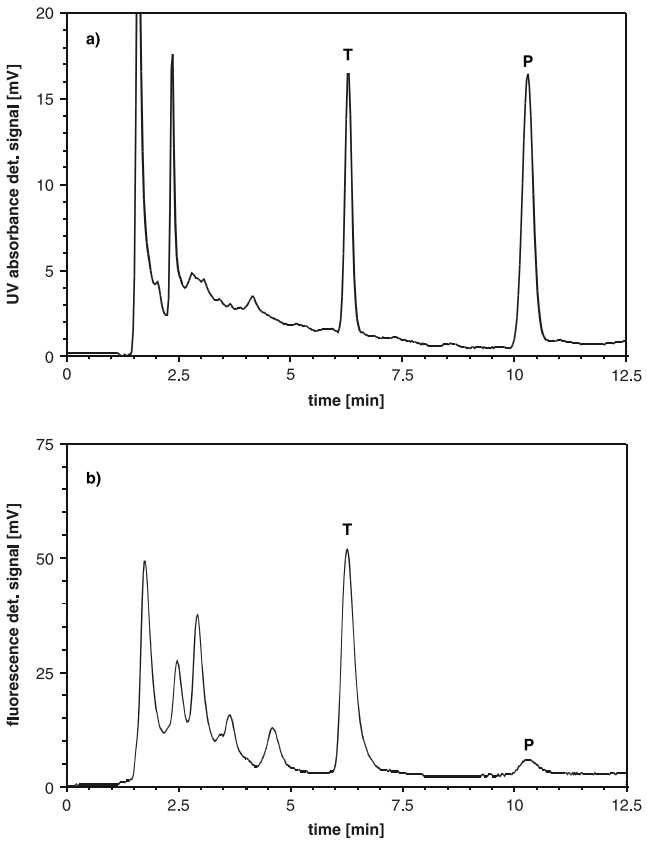

Figure 1. Chromatograms of plasma samples pretreated in order to determine the concentration of: (a) total and (b) unbound propofol. T = thymol (internal standard); P = propofol. Sample preparation procedure and chromatographic conditions—see Experimental section.

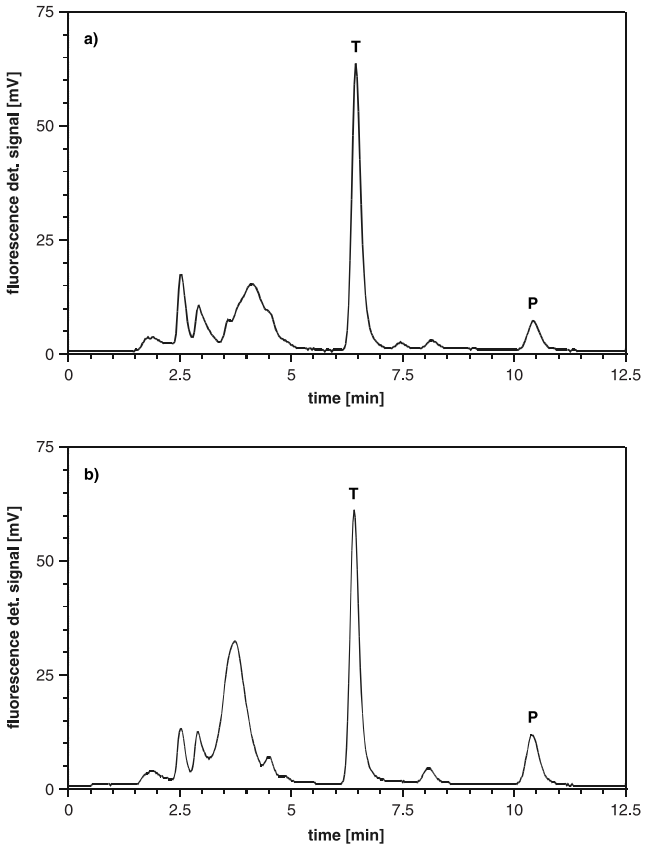

Figure 2. Chromatograms of CSF samples pretreated in order to determine the concentration of: (a) total and (b) unbound propofol. Peaks labelled as in Fig. 1. Sample preparation procedure and chromatographic conditions—see Experimental section.

of propofol complexes formation in CSF may lead to the increase of total propofol concentration in CSF. Moreover, in a paper concerning propofol pharmacokinetics (Dutta *et al.*, 1998), it is claimed that the unexpectedly low effect-site equilibration half-time of propofol can be explained with the provision that not only unbound drug, but also the drug bound to plasma proteins and blood cells can participate in the blood–brain propofol transport.

Both facts above suggest the possibility of bound propofol presence in CSF. In order to verify this hypothesis, another series of investigations was performed, the results of which are depicted in Fig. 4. It presents total propofol concentration in CSF (marked T/CSF) and unbound drug concentration in this medium (F/CSF). According to the outcome of the study carried out with group II, propofol is present in CSF both in the free and in the bound form. As in plasma, the free form concentration of the anaesthetic in CSF is significantly lower than its total concentration ($p < 0.001$). However, the percentage of the free form is not as low as in plasma [its mean value equals 31.14% (CI 25.51, 36.77; SD 11.32; SEM 2.67) of the total CSF propofol concentration]. The much higher content of unbound propofol observed in CSF (in comparison with plasma) is probably the result of lower protein content in CSF (Pollay, 1996). The formation of propofol complexes in CSF can explain why the total propofol concentration in CSF is higher than its free form concentration in plasma.

Concluding, it must be stated that the unbound propofol concentration in plasma is not equal to its total

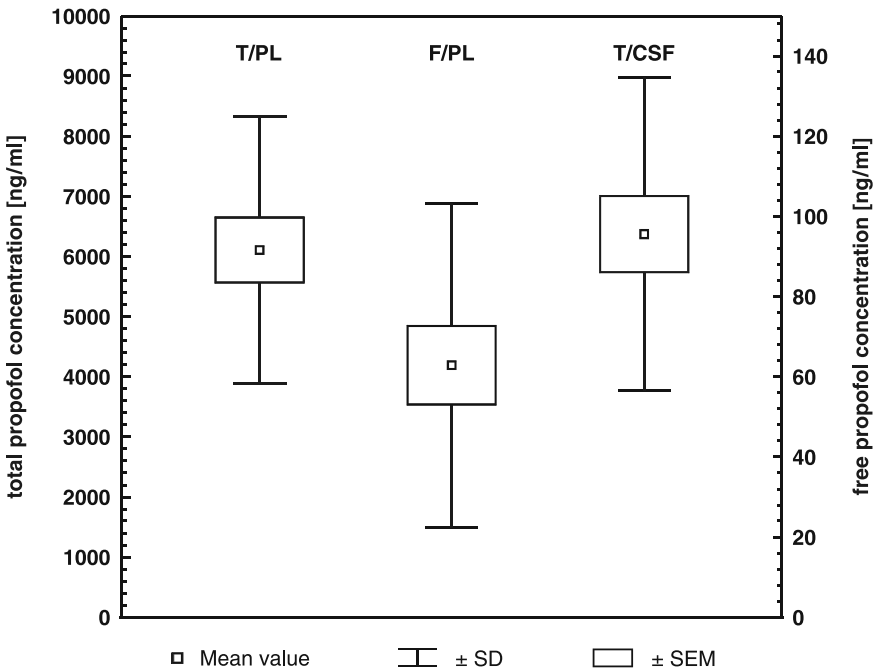

Figure 3. Total (T/PL) and free (F/PL) propofol concentration in plasma, and total propofol concentration in CSF (T/CSF) in patients of group I ($n = 17$). Diagram shows mean values ± SD and SEM.

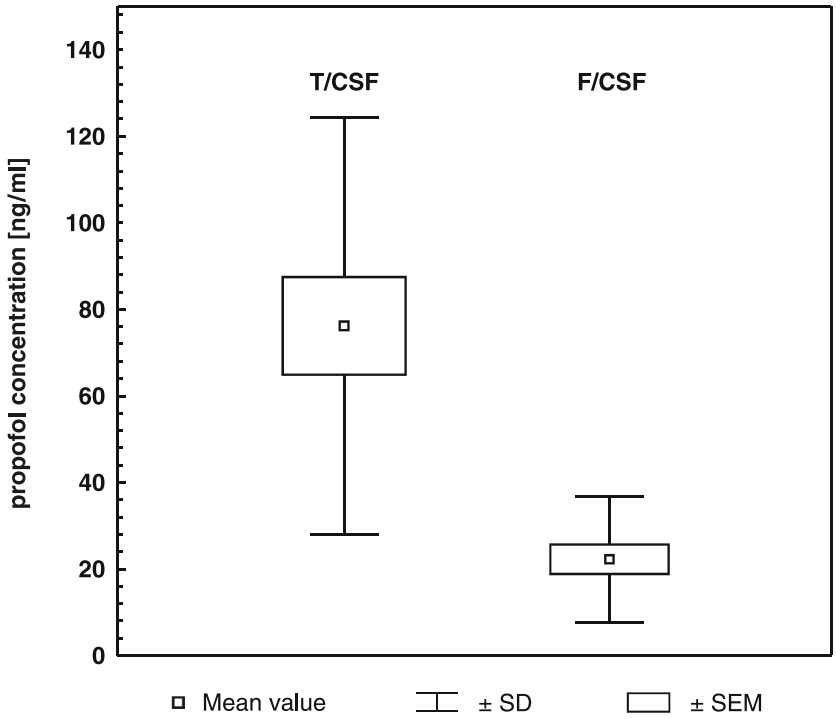

Figure 4. Total (T/CSF) and free (F/CSF) propofol concentration in CSF in patients of group II (n = 18). Diagram shows mean values ± SD and SEM.

concentration in CSF. Thus the unbound drug concentration in plasma cannot be directly related with the drug concentration in the brain surroundings. Propofol exists in CSF not only in the free but also in the bound form. This fact is not described in the generally available scientific literature. The results obtained suggest the substantial contribution of active transport in the propofol transfer from blood into CSF. In consequence, the binding process of the anaesthetic in CSF should be included into the models describing the drug transit from plasma into CNS.

REFERENCES

Bowers WF, Fulton S and Thompson J. Ultrafiltration *vs* equilibrium dialysis for determination of free fraction. *Clinical Pharmacokinetics* 1984; **9** (Suppl.1): 49–60.

Coetzee JF, Glen JB, Winns CA and Boshoff LM. Pharmacokinetic model selection for target controlled infusion of propofol. Assessment of three parameter sets. *Anesthesiology* 1995; **82**: 1328–1345.

Dawidowicz AL and Fijałkowska A. Determination of propofol in blood by HPLC. Comparison of the extraction and precipitation methods. *Journal of Chromatographic Science* 1995; **33**: 377–382.

Dawidowicz AL, Fornal E and Fijałkowska A. Determining the influence of storage time on the level of propofol in blood samples by means of chromatography. *Biomedical Chromatography* 2000; **14**: 249–255.

Dawidowicz AL, Fijałkowska A, Nestorowicz A and Fornal E. The influence of blood sample storage time on the propofol concentration in plasma and solid blood elements. *Biomedical Chromatography* 2001; **15**: 408–412.

Dawidowicz AL, Kalityński R, Nestorowicz A and Fijałkowska A. Changes of propofol concentration in cerebrospinal fluid during continuous infusion. *Anesthesia and Analgesia* 2002; **95**: 1282–1284.

Dawidowicz AL, Fijałkowska A, Nestorowicz A, Kalityński R and Trojanowski T. Cerebrospinal fluid and blood propofol concentration during total intravenous anaesthesia for neurosurgery. *British Journal of Anaesthesiology* 2003; **90**: 84–86.

Dutta S, Matsumoto A, Muramatsu A, Matsumoto M, Fukuoka M and Ebling WF. Steady-state propofol brain:plasma and brain:blood partition coefficients and the effect-site equilibrium paradox. *British Journal of Anaesthesia* 1998; **81**: 422–424.

Engdahl O, Abrahams M, Björnsson A *et al.* Cerebrospinal fluid concentrations of propofol during anaesthesia in humans. *British Journal of Anaesthesia* 1998; **81**: 957–959.

Ganong WF. *Review of Medical Physiology*, 19th edn, Chap. 32. Appleton & Lange: Stamford, CA, 1999.

Ghersi-Egea JF and Strazielle N. Brain drug delivery, drug metabolism, and multidrug resistance at the choroid plexus. *Microscopic Research Technology* 2001; **52**: 83–88.

Hoag G. Urinanalysis, clinical microscopy and fluids. In *Clinical Laboratory Medicine*, Tilton RC, Balows A, Hohnadel DC and Reiss RF (eds). Mosby Yearbook: St Louis, MO, 1992; 402–447.

Kanto JH. Propofol, the newest induction agent of anaesthesia. *International Journal of Clinical Pharmacology Therapy and Toxicology* 1988; **26**: 41–57.

Langley MS and Heel RC. Propofol. A review of its pharmacodynamic and pharmacokinetic properties and use as an intravenous anaesthetic. *Drugs* 1988; **35**: 334–372.

Mazoit JX and Samii K. Binding of propofol to blood components: implications for pharmacokinetics and for pharmacodynamics. *British Journal of Clinical Pharmacology* 1999; **47**: 35–42.

Mehta AC. Therapeutic monitoring of free (unbound) drug levels: analytical aspects. *Trends in Analytical Chemistry* 1989; **8**: 107–112.

Morgan DJ, Campbell GA and Crankshaw DP. Pharmacokinetics of propofol when given by intravenous infusion. *British Journal of Clinical Pharmacology* 1990; **30**: 144–148.

Pacifici GM and Viani A. Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. *Clinical Pharmacokinetics* 1992; **23**: 449–68.

Pollay M. Cerebrospinal fluid. In *The Practice of Neurosurgery*, Tindall GT, Cooper PR and Barrow DL (eds). Williams & Wilkins: Baltimore, MD, 1996; 35–44.

Prough DS. Perioperative fluid management: the use of crystaloid, collloid and hypertonic solutions. In *ASA 1993 Annual Refresher Course Lectures*. ASA: Washington, DC, 1993: 223.

Schütler J and Ihmsen H. Population pharmacokinetics of propofol: a multicenter study. *Anesthesiology* 2000; **92**: 727–738.

Sebille B, Zini R, Madjar CV, Thuaud N and Tillement JP. Separation procedures used to reveal and follow drug—protein binding. *Journal of Chromatography* 1990; **531**: 51–77.

