British Journal of Pharmacology (1999) 128, 83–91
http://www.stockton-press.co.uk/bjp

Electroencephalographic effects of thiopentone and its enantiomers in the rat: correlation with drug tissue distribution

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1 To better understand the pharmacology of the thiopentone enantiomers, we studied their quantitative electroencephalographic effects and their distribution into vital tissues.

2 Adult Wistar rats were infused with *rac*-, R- or S-thiopentone at 4 mg $kg^{-1}min^{-1}$ until death ensued. The EEG signal was acquired continuously; serial arterial plasma and terminal tissue thiopentone concentrations were measured enantiospecifically. Relevant drug tissue : plasma distribution coefficients and plasma concentration-EEG effect relationships were determined.

3 Doses (mg kg^{-1}) (mean ± s.e.mean) for anaesthesia (toe pinch) and lethality (respiratory failure), respectively, decreased in the order R-thiopentone (55.8 ± 2.4 and 176.2 ± 11.2) > *rac*-thiopentone (39.3 ± 2.1 and 97.5 ± 3.9) > S-thiopentone (35.6 ± 1.9 and 74.2 ± 5.2); plasma drug concentrations (μg ml^{-1}) decreased in the order R-thiopentone (66.3 ± 4.5 and 89.8 ± 5.2) > *rac*-thiopentone (56.7 ± 2.0 and 77.8 ± 2.8) > S-thiopentone (55.0 ± 1.9 and 64.1 ± 2.8).

4 Initial EEG activation was similar for all thiopentone forms. Plasma drug concentrations for the same extent of EEG deactivation reflected the potency order.

5 After infusion of *rac*-thiopentone, tissue : plasma distribution coefficients were higher for R- than for S-thiopentone in brain and visceral regions, but not in fat or muscle. After infusion of the separate enantiomers, the relative heart : brain distribution ratio was for S-thiopentone was double that for R-thiopentone.

6 The therapeutic index of R-thiopentone (3.16 ± 0.14) was more advantageous than either *rac*-thiopentone (2.52 ± 0.13) or S-thiopentone (2.10 ± 0.14), possibly due to the relatively greater distribution into CNS tissues than heart. The data suggest that R-thiopentone could make a satisfactory single enantiomer substitute for *rac*-thiopentone.

Keywords: Thiopentone; enantiomers; potency; tissue equilibration; blood-brain barrier

Abbreviations: C_{max}, plasma thiopentone concentrations corresponding to E_{max}; CNS, central nervous system; CSP, chiral stationary phase; EEG, electroencephalogram; E_{max}, maximum activation effect; GABA, gamma-aminobutyric acid; HPLC, high performance liquid chromatography; IC, integrated circuit; IC_0, plasma thiopentone concentrations corresponding to crossing the baseline EEG value; IC_{50}, plasma thiopentone concentrations corresponding to 50% E_{max}; i.m., intramuscular; i.v., intravenous; m, nonlinear regression slope of line of best fit from E_{max} through the remaining data on plasma thiopentone concentration; *rac*-thiopentone, the (clinically used) racemate (±)-(RS)-thiopentone; R-thiopentone, (+)-(R)-thiopentone; s.c., subcutaneous; S-thiopentone, (−)-(S)-thiopentone

Introduction

The countless pharmacological studies of thiopentone performed over the past 60 years have, with few exceptions, ignored the fact that thiopentone is a racemate [(±)-(RS)-thiopentone], i.e. an equimolar mixture of R- and S-thiopentone enantiomers [respectively, (+)-(R)- and (−)-(S)-thiopentone]. These enantiomers differ in potency (Christensen & Lee, 1973; Haley & Gidley, 1976). Similarly, the variety of models proposed to probe the relationships between thiopentone plasma concentrations and its central nervous system (CNS) effects have been based upon thiopentone measured achirally, i.e. as the sum of the enantiomers (Mather & Edwards, 1998). Because the thiopentone enantiomers have also have differences in pharmacokinetics (Cordato *et al*., 1997; Mather *et al*., 1996, 1999b; Nguyen *et al*., 1996) the time course of thiopentone effect involves a changing composition of thiopentone enantiomers. These differences in enantiomer composition and potency would be expected to have pharmacological and/or clinical implications.

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We have recently found similar qualitative CNS effects of *rac*-thiopentone and both of the separate R- and S-enantiomers using the electroencephalogram (EEG), i.e. an initial activation precedes deactivation (Mather *et al*., 1999a). This similarity between the enantiomers was not previously known and is pertinent because, for some barbiturates, one enantiomer may be predominantly CNS depressant and the other excitant (Downes *et al*., 1970). Although our study used a different measurement paradigm (the product of dominant frequency and amplitude) as a quantitative parameter of thiopentone CNS effect, the results were essentially the same as found by others using Fourier transform and aperiodic analysis parameters of the EEG (Ebling *et al*., 1991; Gustafsson *et al*., 1996; Hudson *et al*., 1983).

The purpose of the present study was to perform a quantitative analysis comparing *rac*-thiopentone with its enantiomers, thereby drawing together elements from previous studies (Ebling *et al*.,1991, 1994; Gustafsson *et al*., 1996; Igari *et al*., 1981; Mather *et al*., 1999a) involving behavioural endpoints, quantitative dose-EEG effect relationships and tissue:plasma distribution coefficients to determine whether

there may be an advantage in enantiopure thiopentone over the currently used racemate.

Methods

Overview

The studies were approved by the institutional Animal Care and Ethics Committee. Three groups of animals received a constant rate infusion of *rac*-thiopentone, R- thiopentone or S-thiopentone at 4 mg kg^{-1} min^{-1} until fatal. They were previously prepared with electrodes to record the time course of EEG changes as a function of increasing thiopentone plasma concentrations. After death, the animals were dissected to determine the distribution of the thiopentone enantiomers between various tissues and plasma.

Animals and their preparation

Young adult male Wistar rats (350–400 g) were housed in groups of four, maintained on a constant 12 h light/dark cycle at 23°C, and allowed free access to food and water. After surgery rats were housed individually, and post-operative body weights and fluid intake were monitored.

EEG recordings EEG electrodes were previously implanted under halothane in oxygen anaesthesia with the animals mounted in a stereotaxic frame (Kopf model 900). A midline incision was made to expose the skull, and four holes were made with a 2 mm dental drill, approximately 2–3 mm on each side of bregma and lambda. A fifth screw was inserted to act as an anchor, 3–4 mm lateral to the midline, midway between bregma and lambda. Heat shrink tubing, approximately 4–5 mm in height, was placed around the perimeter of the screws and filled with acrylic dental cement. The electrode ends were subsequently soldered to an 8-pin IC socket, the exposed electrical wire and IC socket were then embedded in acrylic dental cement. The wound was closed by sutures placed on either side of the electrode block. During surgery, body temperature was maintained with a heating pad and monitored with a rectal probe. Rats received amoxycillin (85 mg kg^{-1}, i.m.) and buprenorphine (0.2 mg kg^{-1}, s.c.) post-operatively, and a subsequent dose of amoxycillin (85 mg kg^{-1}, i.m.) was administered the following morning. Body weight was allowed to return to baseline before vascular cannulation was performed.

EEG recordings were taken from a single pair of electrodes positioned contralaterally across the frontal and occipital lobes. The signal was collected with an amplifier module (Biopac Systems, Inc, Santa Barbara, CA, U.S.A.: Model EEG100; gain 5000, 1–30 Hz band pass filter) connected to an analogue to digital converter (Biopac Systems, Inc: Model MP100), and acquired by a personal computer (Pentium 120 MHz) using Acqknowledge III software (Biopac Systems, Inc). Recording electrodes were made from 0-08 × 3/32 stainless steel screws soldered to 1.5 cm lengths of IDC computer cable and connected to the EEG100 amplifier module by a recording cable (2 m length, 7 core shielded electrical cable).

Vascular cannulation Chronic indwelling cannulae were implanted into the jugular vein and carotid artery to allow simultaneous venous infusion and arterial sampling. For this surgery, the animals were anaesthetized by pentobarbitone (30 mg kg^{-1}, i.p. in 1 ml 0.9% saline) followed 5 min later by ketamine (45 mg kg^{-1}, i.p. in 1 ml 0.9% saline). Body temperature was maintained with a heating pad and monitored with a rectal probe. A 1 cm thoracic incision was made just lateral to the midline, and the jugular vein and carotid artery were exposed. The jugular vein was cannulated with Silastic laboratory tubing (Dow Corning, 0.025″ ID × 0.047″ OD) inserted 2.5 cm; the carotid artery was cannulated with Silastic laboratory tubing (Dow Corning, 0.020″ ID × 0.037″ OD) inserted 2.0 cm. The cannulae were tunnelled under the skin and exteriorized above the neck anterior to the scapulae. Each line was filled with a solution of 6 g polyvinylpyrrolidone (MW 40,000; Sigma Chemical Co) in sodium heparin (5 ml, 1000 U ml^{-1}) to maintain line patency. At the completion of surgery rats were administered amoxycillin (85 mg kg^{-1}, i.m.), buprenorphine (0.15 mg kg^{-1}, s.c.), and given 0.9% saline (10 ml, s.c.) for fluid replacement. A subsequent dose of amoxycillin (85 mg kg^{-1}, i.m.) was administered the following morning. Experimental procedures were performed 2 days later.

Drugs *rac*-Sodium thiopentone (Pentothal®, Abbott Australasia Pty Ltd.) was dissolved in deionized water to a final concentration of 20 mg ml^{-1} (18.4 mg ml^{-1} as acid). The peak

Table 2 Mean (±s.e.mean) of the ratio of anaesthetic to lethal doses, and of corresponding arterial plasma concentrations for *rac*-, R- and S-thiopentone in adult male Wistar rats

	Lethality/anaesthesia ratio	
	Doses	*Concentrations*
rac-thiopentone	2.52 ± 0.13*	1.32 ± 0.04**
R-thiopentone	3.16 ± 0.14*	1.37 ± 0.06**
S-thiopentone	2.10 ± 0.14*	1.17 ± 0.04**

rac-, R- and S-thiopentone was infused i.v. 4 mg kg^{-1} min^{-1} until fatal (each $n = 7$). One-way ANOVA: *$P = 0.0001$; R-thiopentone > *rac*-thiopentone > S-thiopentone; **$P = 0.03$; R-thiopentone = *rac*-thiopentone > S-thiopentone.

Table 1 Mean (±s.e.mean) of anaesthetic and lethal doses, and corresponding arterial plasma concentrations for *rac*-, R- and S-thiopentone in adult male Wistar rats

	Anaesthesia		*Lethality*	
	Dose (mg kg^{-1})	*Concentration* (μg ml^{-1})	*Dose* (mg kg^{-1})	*Concentration* (μg ml^{-1})
rac-thiopentone	39.3 ± 2.1*	56.7 ± 2.0**	97.5 ± 3.9†	77.8 ± 2.8‡ $^{+}$
R-thiopentone	55.8 ± 2.4*	66.3 ± 4.5**	176.2 ± 11.2†	89.8 ± 5.2‡
S-thiopentone	35.6 ± 1.9*	55.0 ± 1.9**	74.2 ± 5.2†	64.1 ± 2.8‡

rac-, R- and S-thiopentone was infused i.v. 4 mg kg^{-1} min^{-1} until fatal (each $n = 7$). One-way ANOVA: *$P < 0.0001$; R-thiopentone = *rac*-thiopentone < S-thiopentone; **$P = 0.04$; R-thiopentone > *rac*-thiopentone = S-thiopentone; †$P = 0.0001$; R-thiopentone > *rac*-thiopentone > S-thiopentone; ‡$P = 0.0006$; R-thiopentone > *rac*-thiopentone > S-thiopentone. $^{+}$Student's *t*-test for paired data: R-thiopentone = 37.3 ± 1.1 < S-thiopentone = 40.4 ± 1.8 μg ml^{-1}).

areas of the R- and S-enantiomers were found to be approximately equal upon chiral stationary phase high performance liquid chromatography (CSP-HPLC, see below). R- and S-thiopentone were synthesized chirally in-house, from enantiopure R- and S-citronellic acid as starting materials. The structures were verified by chemical ionization mass spectrometry and nuclear magnetic resonance spectroscopy. Purity was verified by CSP-HPLC and by comparison of melting points and optical rotations to previous literature data (Huang *et al*., 1996). The S-thiopentone was found to be >99% enantiopure. The R-thiopentone was 98.9% enantiopure; S-thiopentone accounted for the remaining 1.1% and was presumed to have arisen from S-enantiomer which was also found to be present in the starting material (Huang *et al*., 1996). Each of the pure enantiomers were dissolved in a minimum volume of 0.1 M NaOH, then diluted to a final concentration of 18.8 mg ml containing 0.06% Na_2CO_3 (w v^{-1}). All solutions contained 2 U ml^{-1} heparin.

Experimental

On the day of the study rats were placed in the recording chamber and allowed to acclimatise for 1 h. After a 75 cm infusion line and a 45 cm sampling line were attached to the chronic indwelling venous and arterial lines, and the recording cable was attached, rats were allowed a further 30 min to settle before commencing the study. An infusion of *rac*-thiopentone, R-thiopentone, or S-thiopentone was delivered at 4 mg kg^{-1} min^{-1} until lethal. A rectal probe to monitor body temperature was inserted as soon as possible, and body temperature was maintained with a heating lamp. The occurrence of a consistent absence of reflex withdrawal upon toe pinch was defined as the onset of anaesthesia. The time of death was taken at the cessation of respiration, following an agonal gasp, accompanied by a pre-existing flat EEG of longer than 1 min duration.

EEG data analysis The product of the rectified amplitude (μV) and rate of signal crossing through 0 μV (Hz) derived from the 1–30 Hz filtered EEG signal was used as a surrogate measure of CNS activity, i.e. activation and depression. This is effectively a null variable derived from the inverse relationship existing between the dominant frequency and amplitude within a given EEG sample (Edmonds & Wauquier, 1986). A data acquisition integral function was used to determine the area under the curve (AUC) of the product of amplitude and

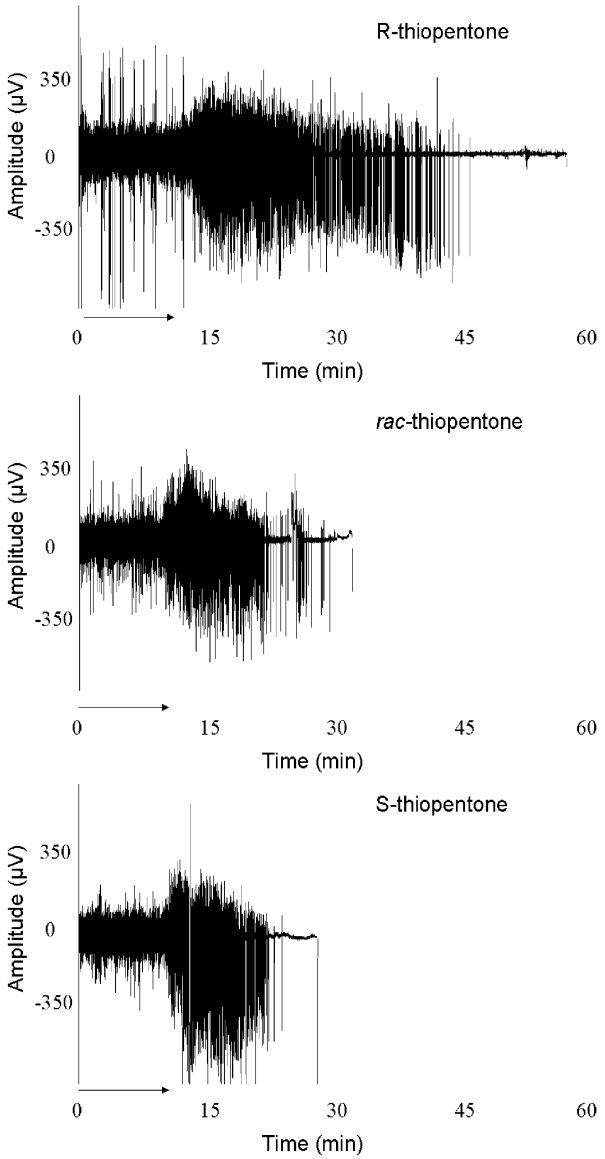

Figure 1 Raw EEG signals recorded in individual adult male Wistar rats having the value of lethal dose closest to the respective group mean values. After an initial baseline recording for 10 min (shown by arrow), *rac*-thiopentone and the individual enantiomers were infused intravenously at a constant rate of 4 mg kg^{-1} min^{-1} until fatal.

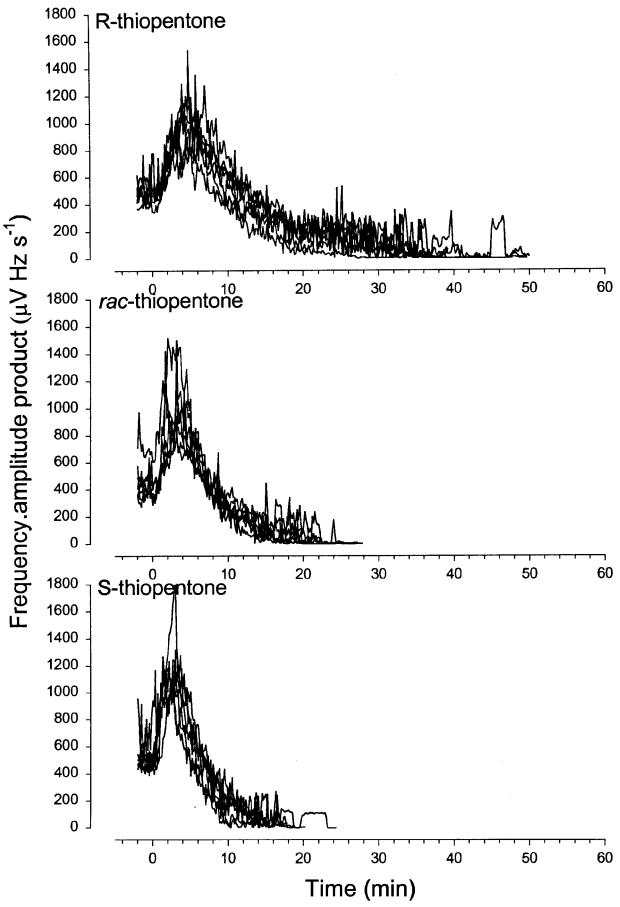

Figure 2 Quantitative processed EEG effect (μV Hz s^{-1}) recorded in individual adult male Wistar rats. After an initial baseline recording for 10 min, *rac*-thiopentone and the individual enantiomers (each $n=7$) were infused intravenously at a constant rate of 4 mg kg^{-1} min^{-1} until fatal.

(dominant) frequency for 10 s epochs over the duration of the recording; from this the mean value of μV Hz s^{-1} for each epoch was determined.

Thiopentone enantiomer assays The plasma concentrations of R- and S-thiopentone were determined by micro-modifications of our previously reported CSP-HPLC procedure (Huang *et al*., 1996). A chiral-AGP column (ChromTech) was used with a Waters 600 MS system and spectrophotometric detection at 287 nm with a Waters 991 Photodiode Array Detector. The non-therapeutic barbiturate, 5-ethyl-5-hexyl barbituric acid, was used as an internal standard. Plasma aliquots (50 μl) in Eppendorf tubes (1.5 ml) were extracted with ethyl acetate in hexane (1.1 ml, 5% v v^{-1}) after the addition of internal standard (50 μl, 50 mg l^{-1}) and H_3PO_4 (10 μl, 2 M). The samples were shaken vigorously (1 min), centrifuged (7000 r.p.m., 2 min), and frozen on dry ice (15 min) before the organic layer was decanted and evaporated to dryness in a rotary vacuum bench evaporator at 40°C. The residue was reconstituted in Na_2HPO_4 (200 μl, 10 mM) containing isopropanol (30% v v^{-1}); 10 μl was injected onto the column. Tissue samples were homogenized (50–100 mg ml^{-1}) into Na_2HPO_4 (0.2 M). An aliquot (200 μl) was taken, internal standard (100 μl) was added, and the sample extracted with hexane (1.0 ml) by shaking vigorously (1 min). After centrifugation (2 min, 7000 r.p.m.) the organic layer was decanted and discarded, the sample was resuspended by sonication and vortexing, acidified with H_3PO_4 (20 μl, 2 M), and extracted with ethyl acetate in hexane (5% v v^{-1}) as before. The mobile phase consisted of isopropanol (3.4% v v^{-1}) in phosphate buffer (100 mM, pH 6.2).

Data analysis

Tissue : plasma distribution coefficients, uncorrected for drug elimination and residual tissue blood volume, were determined from the ratio of relevant tissue concentration to the respective final plasma concentration.

The medians of triplicate values for processed EEG effect, i.e. frequency.amplitude product (μV Hz s^{-1}), over the 30 s period surrounding the sampling interval expressed as a percentage of the mean value for μV Hz s^{-1} found during the 20 min baseline recording, were used for quantitative analysis.

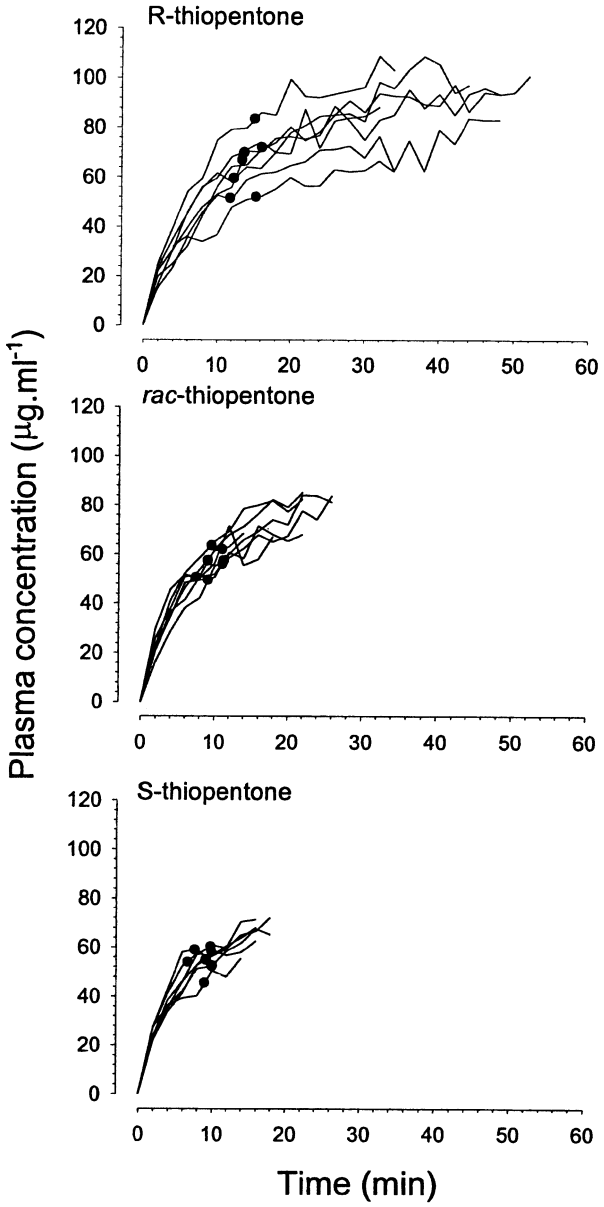

Figure 3 Arterial plasma concentrations of R-thiopentone (top), *rac*-thiopentone (middle) and S-thiopentone (bottom) (each $n=7$) in individual adult male Wistar rats from intravenous infusion at a constant rate of 4 mg kg^{-1} min^{-1} until fatal. The symbols indicate the point of induction of anaesthesia.

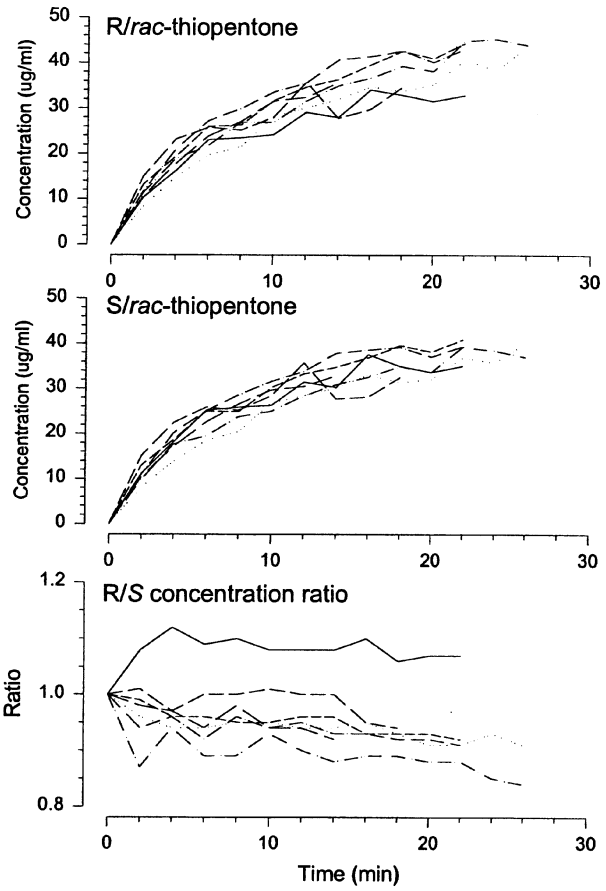

Figure 4 Plasma concentrations of the individual enantiomers from infusion of *rac*-thiopentone in individual adult male Wistar rats ($n=7$). *rac*-Thiopentone was infused intravenously at a constant rate of 4 mg kg^{-1} min^{-1} until fatal. Each line type indicates an individual animal in all three panels.

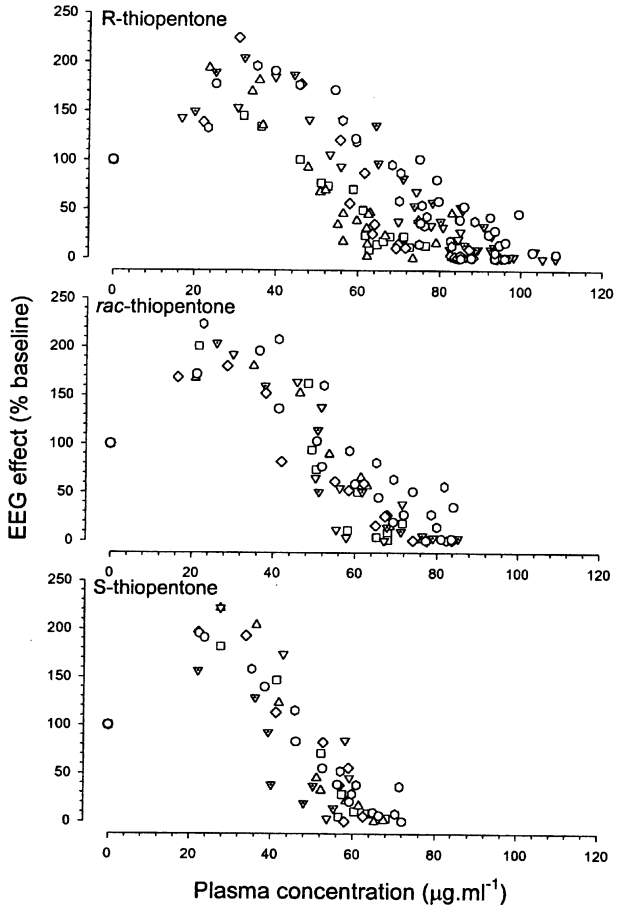

Figure 5 Concentration-EEG effect data in rats infused with R-, *rac*- or S-thiopentone (each $n=7$). The median of triplicate values for μV Hz s^{-1}, over the 30 s period on either side of the interval when blood samples were taken, was expressed as a percentage of the respective mean value over the 10 min baseline recording period. Symbol sets apply to individual animals in each drug treatment group.

Table 3 Mean ($\pm$s.e.mean) values for the descriptors of the biphasic sigmoidal plasma concentration-EEG effect relationship from E_{max} through the remaining data in adult male Wistar rats

	Thiopentone infusion		
Descriptor	rac-*thiopentone*	*R-thiopentone*	*S-thiopentone*
E_{max} (% activation)	210 $\pm$ 11	196 $\pm$ 10	198 $\pm$ 10
C_{max} (μg ml^{-1})*	30.5 $\pm$ 1.9	33.3 $\pm$ 2.1	24.7 $\pm$ 1.0
IC_0 (μg ml^{-1})**	51.3 $\pm$ 0.7	58.3 $\pm$ 1.4	45.1 $\pm$ 1.2
IC_{50} (μg ml^{-1})**	50.8 $\pm$ 1.9	58.5 $\pm$ 3.6	45.2 $\pm$ 0.6
m†	-8.6 ± 1.6	-7.2 ± 0.7	-8.2 ± 0.8

rac-, R- and S-thiopentone (each $n=7$) were infused i.v. 4 mg kg^{-1} min^{-1} until fatal. E_{max} = maximal activation (percentage of baseline); IC_{50} = respective plasma thiopentone concentrations corresponding to 50% E_{max}; M = slope for the line of best fit of the 3 parameter Hill equation; C_{max} and IC_0 = respective plasma thiopentone concentrations corresponding to E_{max} and to crossing the baseline value of EEG signal determined by inspection of the data. One-way ANOVA followed by comparison of mean values by Least Significant Differences method: *$P=0.05$ (*rac vs* S-); $P=0.005$) (R- *vs* S-); **$P=0.005$ (R- *vs* S-); †$P=0.05$ (*rac vs* R-); $P=0.002$ (R- *vs* S-).

The 3-parameter sigmoidal Hill equation was fitted to each set of EEG effect-plasma drug concentration data using a nonlinear least squares regression algorithm (Sigmaplot 4.0, SPSS Inc, Chicago, IL, U.S.A.) on a personal computer. The parameters estimated from this analysis were E_{max} (maximum activation as a percentage of baseline), m (the slope of the line of best fit from E_{max} through the remaining data), and IC_{50} (plasma thiopentone concentrations corresponding to 50% E_{max}). In addition, Cmax (plasma thiopentone concentrations corresponding to E_{max}), and IC_0 (plasma thiopentone concentrations corresponding to crossing the baseline EEG value) were determined by inspection of the data.

Comparisons between infusions of *rac*-thiopentone and the separate thiopentone enantiomers were made by one-way analysis of variance; if significant, subsequent comparisons of individual mean values were performed by the method of least significant differences. Between enantiomer comparisons were made with Student's *t*-test for paired data when *rac*-thiopentone was infused, and with Student's two sample *t*-test when the separate enantiomers were infused. Two-tailed probabilities from significance testing are reported and summary data are reported as mean $\pm$ s.e.mean unless specified otherwise.

Results

The doses and corresponding arterial plasma drug concentrations required for the behavioural endpoints are given in Table 1. One-way anova indicated that the respective mean plasma concentrations and the doses for anaesthesia and for lethality differed between the three thiopentone forms; the values for R-thiopentone were greater than the corresponding values for *rac*- and S-thiopentone.

The lethality/anaesthesia ratios when derived from doses (= 'therapeutic index') (Table 2) decreased in the order R-thiopentone > *rac*-thiopentone > S-thiopentone ($P=0.0001$) and in the order R-thiopentone = *rac*-thiopentone > S-thiopentone ($P=0.04$) when derived from plasma concentrations. The plasma drug concentration for anaesthesia, normalized for dose infused to that point, increased in the order R-thiopentone (ratio = 1.20 ± 0.08) < *rac*-thiopentone ($1.46 \pm 0.0.07$) = S-thiopentone (1.56 ± 0.07). The final plasma concentration measured (at lethality), normalized for dose infused, increased in the order R-thiopentone (ratio = 0.55 ± 0.04) < *rac*-thiopentone (0.89 ± 0.05) < S-thiopentone (1.06 ± 0.04). These bear an inverse relationship to the respective total body clearances.

Examples of the raw EEG, for individual cases corresponding closest to the relevant data group mean values, are shown in Figure 1. These clearly demonstrate activation, followed by depression with time courses reflecting lethal potencies of the three thiopentone preparations. The time courses of the changes to the EEG frequency.amplitude product (uV Hz s^{-1}) and arterial plasma drug concentrations during infusion are shown in Figures 2–4. The EEG changes were qualitatively similar, but quantitatively different, for the three thiopentone forms. Whilst similar activation occurs for all three infusions, deactivation (anaesthesia and ultimately death) occurred at lower plasma concentrations of S-thiopentone than of *rac*-thiopentone or R-thiopentone (Figure 5, Tables 2 and 3). The similarity between the respective values of IC_{50} and IC_0 arises because $E_{max} \cong 200\%$.

Some regions showed enantioselective distribution after administration of *rac*-thiopentone although the magnitude of the difference was small (Table 4). The differences in tissue

Table 4 Mean (±s.e.mean) tissue concentrations in adult male Wistar rats after infusion with *rac*-thiopentone

Tissue	*Concentration* ($\mu g\ g^{-1}$) *R-thiopentone*	*S-thiopentone*	*Thiopentone*	*Probability** *R-* vs *S-*
CNS				
Cortex	67.2±3.3	65.9±3.5	133.1±6.7	n.s.
Striatum	66.6±4.2	65.2±4.2	131.8±8.4	$P=0.034$
Hippocampus	60.1±3.5	59.2±3.3	119.3±6.8	n.s.
Cerebellum	64.5±2.8	64.8±2.8	129.3±5.6	n.s.
Brachial spinal cord	81.1±6.4	79.2±6.6	160.2±13.1	$P=0.009$
Sacral spinal cord	77.7±7.1	77.0±7.8	154.6±14.8	n.s.
Peripheral				
Kidney	101.6±8.9	93.5±8.4	195.1±17.2	$P<0.0001$
Liver	103.2±4.5	102.1±4.8	205.3±9.2	n.s.
Heart	89.5±8.4	88.2±8.0	177.8±16.4	$P=0.034$
Lung	79.7±7.7	79.3±8.0	159.0±15.7	n.s.
Gut	58.9±5.9	57.4±5.4	116.3±11.2	n.s.
Fat	109.4±18.6	114.0±19.0	223.4±37.5	n.s.
Muscle	19.9±1.3	19.8±1.3	39.7±2.5	n.s.

rac-thiopentone was infused iv 4 mg kg^{-1} min^{-1} until fatal ($n=7$; except cerebellum: $n=6$). Thiopentone = sum of enantiomers. **P* values pertain to R- *vs* S- (Student's *t*-test for paired data).

Table 5 Mean (±s.e.mean) tissue concentrations in adult male Wistar rats after infusion with the individual enantiomers of thiopentone

Tissue	*Concentration* ($\mu g\ g^{-1}$) *R-thiopentone*	*S-thiopentone*	*Probability** *R-* vs *S-*
CNS			
Cortex	198.2±8.0	83.5±4.9	$P<0.0001$
Striatum	203.1±14.2	93.1±3.0	$P=0.0002$
Hippocampus	191.4±8.2	88.4±5.0	$P<0.0001$
Cerebellum	189.7±11.4	91.9±5.7	$P<0.0001$
Brachial spinal cord	250.5±15.9	112.8±5.2	$P=0.0001$
Sacral spinal cord	252.9±24.3	116.9±7.5	$P=0.001$
Peripheral			
Kidney	217.6±14.9	135.1±11.4	$P=0.0009$
Liver	259.3±17.7	181.8±11.5	$P=0.0032$
Heart	245.2±22.9	200.8±17.5	ns
Lung	273.2±27.5	111.9±18.5	$P=0.0004$
Gut	189.6±16.5	92.0±7.9	$P=0.0005$
Fat	475.2±49.3	236.2±34.0	$P=0.0018$
Muscle	65.5±6.0	37.5±3.7	$P=0.0019$

Mean doses: 176 and 76 mg, respectively, R-thiopentone and S-thiopentone were each infused i.v. at 4 mg kg^{-1} min^{-1} until fatal (each $n=7$). **P* values pertain to R- *vs* S- (Student's *t*-test).

concentrations found after administration of the racemate and separate enantiomers of thiopentone should be viewed in comparison to the total doses tolerated and the plasma drug concentrations (Table 1). The much greater tissue concentrations of R- than of S-thiopentone (Table 5) was consistent with the much larger dose tolerated of R- than of S-thiopentone; despite this, a significant concentration difference between enantiomers did not occur in the heart.

Tissue:plasma distribution coefficients measured with respect to total (i.e. unbound + bound) plasma drug concentrations are shown in Tables 6 and 7. Table 6 indicates there to be ~10% greater uptake of R-thiopentone than of S-thiopentone into most tissues from *rac*-thiopentone. However, after administration of the separate enantiomers, the distribution coefficients into CNS tissues of R-thiopentone were ~50% greater than those of S-thiopentone. When the relative distribution to the heart and the brain was examined, the values increased in the order R-thiopentone (1.24±0.09) < *rac*-thiopentone (1.39±0.10) < S-thiopentone (2.29±0.25). Thus it is likely that the lesser lethality of R-thiopentone compared to *rac*- and S-thiopentone relates to its relatively lower uptake into the heart than the brain.

Discussion

Many studies of chiral barbiturates in a variety of models have shown that their enantiomers can, and usually do, have quantitative differences in pharmacological potency and/or pharmacokinetics (e.g. Huang & Barker, 1980; Chandler *et al*., 1988; Steen & Michenfelder, 1978; Wahlstrom & Nordberg, 1984). Like many barbiturates, thiopentone contains one chiral centre, its enantiomeric forms resulting from asymmetry of the alpha-carbon of the 5-(2-pentyl) side chain (Andrews & Mark, 1982). With all racemic drugs, the time-course of pharmacodynamic effects derives from the weighted time-course of the sum of the individual enantiomers, being subject to any differences in the pharmacokinetics of the enantiomers. With thiopentone, quantitative differences between enantiomers have been found in potency and pharmacokinetics. Hence, quantitative pharmacological models for thiopentone that ignore its chirality are flawed to some extent.

The pharmacokinetic findings of the present study clearly show that thiopentone undergoes enantioselective distribution and clearance. Formalized fitting of pharmacokinetic models to the data was not entertained since the commonly applied models imply a condition of stationarity (Lassen & Perl, 1979). Stationarity was not a valid assumption in this paradigm (of drug infusion to fatality) and relevant data to support other models, e.g. including thiopentone-induced changes in blood flow (Wada *et al*., 1996), were not intended with the experimental design used.

Enantioselective distribution is shown in the relative tissue drug concentrations. After administration of *rac*-thiopentone, several tissues showed greater concentrations of R- than S-thiopentone but, as plasma drug concentrations also differed, concentrations alone are not a true marker of enantioselectivity of distribution. Tissue : plasma distribution coefficients, in comparison, were greater for R- than for S-thiopentone in most tissues. The difference was magnified when the

Table 6 Mean (±s.e.mean) distribution coefficients for thiopentone and its separate enantiomers in CNS and peripheral tissue in adult male Wisatar rats after infusion with *rac*-thiopentone

Tissue	*Concentration* (μg g^{-1}) *R-thiopentone*	*S-thiopentone*	*Thiopentone*	*Probability** *R-* vs *S-*
CNS				
Cortex	1.81±0.10	1.66±0.14	1.73±0.12	$P=0.05$
Striatum	1.80±0.12	1.64±0.15	1.71±0.13	$P=0.021$
Hippocampus	1.61±0.09	1.48±0.10	1.54±0.09	$P=0.017$
Cerebellum ($n=6$)	1.75±0.06	1.64±0.10	1.69±0.07	n.s.
Brachial spinal cord	2.19±0.19	1.98±0.18	2.07±0.18	$P=0.0092$
Sacral spinal cord	2.09±0.20	1.93±0.21	2.00±0.20	$P=0.021$
Peripheral				
Kidney	2.72±0.21	2.31±0.18	2.51±0.19	$P=0.001$
Liver	2.78±0.14	2.56±0.19	2.66±0.16	$P=0.034$
Heart	2.40±0.21	2.18±0.16	2.28±0.18	$P=0.019$
Lung	2.15±0.23	1.98±0.23	2.06±0.23	$P=0.031$
Gut	1.59±0.17	1.44±0.15	1.51±0.16	$P=0.0086$
Fat	2.89±0.44	2.77±0.39	2.82±0.41	n.s.
Muscle	0.54±0.04	0.49±0.03	0.51±0.03	n.s.

rac-thiopentone was infused iv 4 mg kg^{-1} min^{-1} until fatal ($n=7$; except cerebellum: $n=6$). Thiopentone = sum of enantiomers. **P* values pertain to R- *vs* S- (Student's *t*-test for paired data).

Table 7 Mean (±s.e.mean) distribution coefficients for R- and S-thiopentone in CNS and peripheral tissue of rats after infusion with the individual enantiomers of thiopentone

Tissue	*Distribution coefficient* *R-thiopentone*	*S-thiopentone*	*Probability** *R-* vs *S-*
CNS			
Cortex	2.25±0.17	1.30±0.05	$P=0.0008$
Striatum	2.26±0.10	1.47±0.07	$P<0.0001$
Hippocampus	2.17±0.14	1.38±0.06	$P=0.0008$
Cerebellum	2.13±0.10	1.43±0.06	$P=0.0001$
Brachial spinal cord	2.83±0.21	1.77±0.08	$P=0.0007$
Sacral spinal cord	2.83±0.27	1.82±0.08	$P=0.003$
Peripheral			
Kidney	2.44±0.13	2.11±0.14	n.s.
Liver	2.92±0.19	2.86±0.19	n.s.
Heart	2.71±0.17	3.19±0.33	n.s.
Lung	3.06±0.29	1.72±0.23	$P=0.0032$
Gut	2.11±0.13	1.46±0.16	$P=0.0088$
Fat	5.34±0.52	3.81±0.70	n.s.
Muscle	0.72±0.04	0.59±0.06	n.s.

Mean doses: 176 and 76 mg, respectively, R-thiopentone and S-thiopentone were each infused iv at 4 mg kg^{-1} min^{-1} until fatal (each $n=7$). **P* values pertain to R- *vs* S- (Student's *t*-test).

enantiomers were administered separately, suggesting that enantiomer-enantiomer interactions may occur. Enantioselective clearance is shown by the lower plasma concentrations of R-thiopentone compared to S-thiopentone when administered as *rac*-thiopentone, as well as by the lower plasma concentrations per unit dose when administered separately, although this is also affected to some extent by differences in the duration of the infusions.

The tissue:plasma distribution coefficients of thiopentone (summed enantiomers) from this paradigm of continuous infusion to fatality were generally comparable with values reported by others who have used different drug administration paradigms. Igari *et al*. (1982) determined non-steady state elimination-corrected tissue : blood distribution coefficients of thiopentone 30 min after an intravenous bolus dose. Ebling *et al*. (1994) reported steady state residual blood volume-corrected tissue:blood distribution coefficients after 8 h continuous infusion. Most researchers measure tissue : plasma distribution coefficients and then adjust the results to tissue:blood values by the drug's blood : plasma distribution ratio. This approach can compound errors should the blood : plasma distribution coefficient be sensitive to modifying factors, such as acid-base changes during blood sample storage or to concentration dependence. A blood : plasma distribution coefficient of ~0.8 can be calculated from the data of Igari *et al*. (1982). Our previous experiments have found that the blood : plasma distribution coefficient is enantioselective. Mean values for R- and S-thiopentone 1.02 and 0.92, respectively, were found (Mather *et al*., 1999b).

Most significantly, the enantioselectivity of distribution between heart (unfavourable) and CNS (favourable) decreases in the order S-thiopentone > *rac*-thiopentone > R-thiopentone. Thus the greater tolerance of R-thiopentone than of *rac*-thiopentone or S-thiopentone may derive, at least in part, from a relatively lower myocardial burden of this enantiomer after its administration compared to either *rac*-thiopentone or S-thiopentone.

Despite the different experimental paradigms, the quantitative EEG response–plasma thiopentone concentration relationships found in this study are remarkably similar to those reported by Gustafsson *et al*. (1996) who used aperiodic analysis (number of waves s^{-1}) as their quantitative EEG variable with pseudo-steady state infusions of *rac*-thiopentone. Data reported by Gustafsson *et al*. (1996) indicated that maximal EEG activation occurred at around 20–25 μg ml^{-1} and total deactivation occurred around 70–80 μg ml^{-1}. Our data with separate enantiomers indicate that quantitative, rather than qualitative, enantiomeric differences occur in both activation and deactivation potency.

From the EEG effects in relation to drug plasma concentrations, it is clear that S-thiopentone is more potent than *rac*-thiopentone and R-thiopentone. Christensen & Lee (1973) examined the anaesthetic and lethal doses of *rac*-thiopentone, R-thiopentone and S-thiopentone in mice after intravenous and intraperitoneal injection. They reported that after intravenous injection both the anaesthetic and lethal potencies increased in the order R-thiopentone < *rac*-thiopentone < S-thiopentone; however, the 'therapeutic index' calculated from the ratio of lethal to anaesthetic doses increased in the order *rac*-thiopentone (2.44) < R-thiopentone (3.05) < S-thiopentone (3.21). After intraperitoneal injection

the anaesthetic potencies increased in the order *rac*-thiopentone < R-thiopentone < S-thiopentone but the lethal potencies increased in the order R-thiopentone < *rac*-thiopentone < S-thiopentone; thus the 'therapeutic index' calculated from the ratio of lethal to anaesthetic doses increased in the order S-thiopentone (3.44) < *rac*-thiopentone (3.63) < R-thiopentone (4.47). As drugs injected intraperitoneally are subject to first pass hepatosplanchnic extraction, lesser potency than from intravenous injection is expected; thus, it is likely that a greater hepatic clearance of R-thiopentone would diminish its relative apparent potency more for intraperitoneal than for intravenous injection. However, the effects are complicated by hepatic clearance producing, in part, the metabolite pentobarbitone, itself having similar actions to thiopentone, albeit with lesser potency (Christensen & Lee, 1973).

The data raise the question as to whether enantiopure R-thiopentone for *rac*-thiopentone would be beneficial from a point of margin of safety. This was previously considered as a way of shortening the time to awakening from thiopentone anaesthesia. Mark *et al*. (1977), in preliminary observations made from the incremental intravenous administration of 300 mg of the separate enantiomers of thiopentone to three human volunteer subjects, found that the duration of sleep was shorter from R- than from S-thiopentone. This is in accord with previous systematic studies in mice (Christensen & Lee, 1973; Haley & Gidley, 1976) that reported S-thiopentone to be more potent in producing CNS depression. They also observed that although the plasma half-life of R-thiopentone was shorter than that of S-thiopentone in each subject, R-thiopentone would not offer a clinical advantage because the half-life would not play 'an important part in the process of rapid termination of sedative effect'. It must be added that the plasma half-life, as a sole pharmacokinetic measure, is crude by contemporary standards. Mark *et al*. (1977) instead opted to consider the further development of S-thiohexitone, a more rapidly metabolised barbiturate. Moreover the pharmacodynamic data for the chiral barbiturates, taken together, suggested a receptor selectivity for the S-configuration (Andrews & Mark, 1982; Christensen & Lee, 1973), a view that has been now confirmed by direct measurement of facilitation of $GABA_A$ receptor response (Cordato *et al*., 1999).

Although *rac*-thiopentone has a long history of successful clinical use it also has some recognized drawbacks. It causes myocardial and respiratory depression, and its repeated use leads to prolonged time to awakening (Crankshaw, 1987; Hirshman *et al*., 1975; Huang *et al*., 1997; Upton *et al*., 1997). We believe that there is now sufficient evidence to suggest that substitution of enantiopure R-thiopentone for the clinically used *rac*-thiopentone may have merit. This claim is based upon the following principal observations.

(i) The 'therapeutic index' defined by the ratio of lethal to anaesthetic doses favours R-thiopentone (Table 2). This is consistent with previous data found from studies in the mouse (Christensen & Lee, 1973; Haley & Gidley, 1976). The greater separation of anaesthetic and lethal doses (Figures 1–3), combined with the less steep relationship between effect (EEG) and plasma drug concentration (Table 3), suggests R-thiopentone to have a greater margin of safety than either *rac*-thiopentone or S-thiopentone. This would be of advantage in patients with compromised clinical status.

(ii) Higher distribution coefficients into CNS tissues of R- than of *rac*- or S-thiopentone indicate a larger fraction of the dose distributes into required tissues. The lowest heart:brain distribution ratio for R-thiopentone when administered alone suggests that this is a reason for its greater tolerability. Thus, R-thiopentone has a relatively more favourable distribution into appropriate tissues (CNS) compared to inappropriate tissues (heart) than either *rac*-thiopentone or S-thiopentone. This may confer a greater selectivity of effects.

(iii) Pharmacokinetic studies continually indicate a higher clearance of R- than S-thiopentone. The difference, although small, is favourable towards faster termination of effects and has been documented in every species (rat (Mather *et al*., 1999b), sheep (Mather *et al*., 1996), human (Cordato *et al*., 1997; Nguyen *et al*., 1996)) so far examined. Patient groups having thiopentone for induction of anaesthesia or neuroprotection could benefit from faster recovery and was implied by previous EEG studies showing faster recovery with R-thiopentone than with *rac*-thiopentone or S-thiopentone (Mather *et al*., 1999a).

In summary, this study supports the suggestion that enantiopure substitution of R-thiopentone for *rac*-thiopentone would have pharmacological advantages. Whether these are clinically significant remains to be determined.

The helpful advice of Dr C. Minto, Dr D.R. Stanski and Ms E. Osaki and the support of the National Health and Medical Research Council of Australia are acknowledged with pleasure.

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(Received April 12, 1999
Accepted June 1, 1999)