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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINING THIOPENTAL CONCENTRATIONS IN TWELVE RAT TISSUES: APPLICATION TO PHYSIOLOGIC MODELING OF DISPOSITION OF BARBITURATE

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SUMMARY

A sensitive, selective and reproducible high-performance liquid chromatographic assay of thiopental concentrations in twelve rat tissues was developed using thiamylal as the internal standard. Samples were homogenized in phosphate buffer, extracted into pentane and chromatographed on a microparticulate octadecyl reversed-phase column using ultraviolet detection at 290 nm. A simple digestive step with collagenase prior to homogenization facilitated analysis of thiobarbiturate in skin. Thiopental extraction recovery from fat exceeded 90%. Assay sensitivity was greater than 1 μg/ml for tissue and plasma samples as small as 50 μl. This assay has been applied to physiologic pharmacokinetic studies. The paper also presents typical concentration–time profiles of thiopental in four tissues taken from 74 rats given 20 mg/kg thiopental.

INTRODUCTION

Thiopental is used extensively to induce anesthesia in animals and humans. The intensity and duration of the effect of this barbiturate depend on its redistribution from brain tissue into moderately to poorly perfused high-capacity tissues (fat, muscle and skin) [1–3]. Variations in body composition and cardiovascular physiology greatly influence the time course and duration of thiopental effect. Clinically, the dose required to induce anesthesia adequately while maintaining hemodynamic stability varies widely and appears to be related to

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cardiovascular performance. Dose requirement is affected by age [4], state of hydration and blood volume [5] and the adequacy of cardiac function [6,7].

To examine the complex interaction of cardiovascular physiology on the time course of thiopental anesthetic effects, we developed a rat model in which physiologic and disease state perturbations can be easily performed. Part of this investigation involves examination of the pharmacokinetics of thiopental in relevant tissues. Because redistribution of thiopental from brain to other tissues terminates the anesthetic effect, examining only the plasma concentrations does not accurately quantitate the redistribution process. Examination of the interaction between physiologic and pharmacologic events involved in redistribution of thiopental requires measurement of tissue concentrations of drug over time. Clearly, this can be done only in an animal model allowing sampling of large numbers of tissues in a controlled manner.

Many assays reported in the literature describe the estimation of the concentration of thiopental in plasma. These assays have employed spectrophotometric or fluorometric techniques and liquid–liquid extraction [8–10], radioimmunoassay [11], gas chromatography [12] and high-performance liquid chromatography (HPLC) [13–26]. Several of these techniques quantitate thiopental in tissues but do not use chromatography to ensure specificity [8–10]. Some use inappropriate internal standards [16,19]. Others are not sufficiently sensitive for extensive pharmacokinetic studies in small species [19]. The effective use of the rat for high-resolution pharmacokinetic investigations of the distribution phase of thiopental requires high assay sensitivity to accommodate the small sample volume demanded by this species. Additionally, the assay must be able to quantitate thiopental concentrations reliably in a wide range of tissues having different physical and chemical attributes. This report describes the assay we now use with our pharmacokinetic–pharmacodynamic model of thiopental anesthesia in the rat. This assay permits the estimation of thiopental concentrations of less than 1 μg/ml in samples of rat plasma as small as 50 μl.

EXPERIMENTAL

Chromatographic system and materials

The HPLC system consisted of a continuous-flow constant-volume Model 5000 solvent delivery system, a Model UV-100 absorbance detector and a Model 9176 dual-pen recorder (Varian, Walnut Creek, CA, U.S.A.). Samples were injected onto the system with a Model 710B WISP automatic sample injector (Waters, Milford, MA, U.S.A.). The UV absorbance of thiopental and thiamylal was measured at 290 nm. A C_{18} reversed-phase column (Alltech, Deerfield, IL, U.S.A.; 25 cm $\times$ 4.6 mm I.D., 10 μm particle size) was used to separate the compounds. The HPLC-grade methanol, acetonitrile and pentane used in the extraction procedure and mobile phase were purchased from J.T. Baker

(Phillipsburg, NJ, U.S.A.). The monobasic potassium phosphate and phosphoric acid used in the mobile phase were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.), as was the sodium phosphate and calcium chloride used in the extraction and homogenization buffers. The Trizma hydrochloride and Type I collagenase were obtained from Sigma (St. Louis, MO, U.S.A.). Thiopental acid was donated by Abbott Labs. (North Chicago, IL, U.S.A.), thiamylal by Parke Davis Labs. (Detroit, MI, U.S.A.).

Standard preparation

Standard stock solutions of thiopental in 50% methanol were prepared in the range 5–100 μg/ml; 50 μl of these solutions were added to blank plasma or tissue homogenate to produce standard solutions of thiopental from 0.5 to 100 μg/ml.

Tissue homogenization

Tissue (0.5–1.5 g) was added to 3 ml of Sorensen's phosphate buffer (pH 7.4, 0.07 *M*) and homogenized with 10-s bursts of a Brinkman Polytron homogenizer (Model PT 10/35 with Model PTA 10TS generator). Tubes containing tissue–buffer mixture were chilled in ice during homogenization.

Skin digestion–homogenization

Finely divided skin (0.2 g, subdermal fat removed) was added to 1 ml of Tris buffer (50 m*M* Tris, 5 m*M* calcium chloride, pH 7.2) and digested at 37 °C with 2 mg of collagenase for 90 min. Then, 3 mg of fresh collagenase were added, and the mixture was reincubated for another 90 min. At the end of incubation, 1.5 ml of Tris buffer were added to the digest and homogenized with the Brinkman Polytron homogenizer. The chemical stability of thiopental during this digestive step was evaluated.

Extraction procedure

Plasma sample or tissue homogenate (200 μl to 1 ml) was added to 1 ml of Sorensen's phosphate buffer (pH 6.3, 0.07 *M*) in glass extraction tubes having PTFE-lined screw caps (100 mm × 16 mm). The internal standard, thiamylal (1 μg in 50 μl of 50% methanol) was added. A 5-ml aliquot of pentane then was added, and the tubes were subjected to extraction for 10 min (Labquake, 40 cycles per minute). The tubes were centrifuged to separate phases, and the aqueous phase was frozen in an acetone–dry ice slurry. The pentane phase was decanted to a 75 mm × 13 mm glass culture tube. The pentane was later evaporated to dryness at 40 °C under a nitrogen gas stream.

Chromatography

The residue was reconstituted with approximately 200 μl of acetonitrile–water (25:75, v/v) for injection. The mobile phase consisted of acetonitrile–4

m*M* potassium phosphate buffer (50:50, v/v) (pH 4.0), and the flow-rate was 1.2 ml/min.

Barbiturate recovery

The assay recovery of thiopental and thiamylal from each tissue homogenate was assessed for drug concentrations of 1 and 20 μg/ml in the following manner. Ten plasma or homogenate samples (200 μl) containing each barbiturate were extracted and reconstituted with a fixed volume of acetonitrile–water (25:75, v/v). A fixed 50% aliquot of the resulting solution was injected onto the chromatograph. The same amount of barbiturate (e.g. 0.1 and 2 μg) in acetonitrile–water (25:75, v/v) was injected directly on the chromatograph ten times. The peak heights of the barbiturates in both sets of samples were measured. The assay recovery of each barbiturate was computed using the following equation:

$$\text{percentage recovery} = \frac{\text{peak height, extracted drug}}{\text{mean peak height, direct injection}} \times 100$$

The assay recovery calculated in this manner indicates the amount of aggregate barbiturate loss occurring during extraction and sample handling.

Precision and accuracy

The within-day and day-to-day variability of the assay was assessed at thiopental concentrations of 1 and 20 μg/ml of plasma or tissue homogenate. Ten replicate samples were assayed for within-day variability. Ten samples were assayed on separate days to assess day-to-day variability.

Barbiturate stability in tissue during storage

Thiopental (45 mg/kg) was infused intravenously into five male Wistar rats over 1 min. After 15 min the animals were killed by decapitation. Tissues and organs were dissected and divided in half; the pieces were wrapped in aluminum foil and placed on dry ice until frozen. On the day of the study, tissues were thawed, homogenized and assayed for thiopental. Thirty days later, the other half of the paired tissue samples was assayed for thiopental. The stability of thiopental during storage was assessed by determining the percentage change in thiopental concentration between the paired tissue samples assayed on these two separate occasions.

Barbiturate stability in liver and liver homogenate

Whole liver. The stability of thiopental was assessed in rat liver to estimate drug loss due to metabolism during sample handling. The stability was first determined in whole, excised liver to assess the necessity of rapidly freezing liver after dissecting it out. Thiopental was infused into two male Wistar rats (350 g) at 20 $\mu g\,min^{-1}\,kg^{-1}$ for 0.75 min. The animals were killed 15 min later

by decapitation. The livers were rapidly removed and coarsely divided. A portion of the liver was immediately frozen on dry ice for later assay. The remaining portion of liver was further subdivided and kept at room temperature or 37°C. After 2, 5, 10, 15 and 30 min, 0.5-g portions were homogenized in 15 ml of methylene chloride with 1.0 ml of distilled water to stop any enzymatic degradation of the barbiturate. The phases were separated and the methylene chloride was evaporated to dryness. The internal standard was added and the extract was reconstituted with mobile phase and injected on the chromatograph to determine the concentration of thiopental. Stability of thiopental in whole liver was evaluated by determining the percentage change in concentration from the mean of all concentrations.

Homogenate. The concentration of thiopental in liver homogenate over time was examined to see whether the extraction procedure had to be performed at low temperature to ensure stability of thiopental. After fresh liver was homogenized as described above, 10 ml of homogenate were placed in three tubes. One tube each was kept at 37, 25 and 0°C. Thiopental was added to each 10-ml volume of homogenate, yielding an initial concentration of 15 μg/ml. At 2.5, 5, 9, 13, 18 and 25 min after addition of barbiturate, 1-ml samples of homogenate were extracted into methylene chloride (described above) to stop enzymatic degradation. The methylene chloride extract was assayed for thiopental as above. Stability of thiopental in homogenate was evaluated by determining the percentage change from the expected concentration.

Animal pharmacokinetic studies

Thiopental, 20 mg/kg, was infused through a central venous catheter into 74 male Wistar rats over 0.75 min. Arterial blood samples (200 μl) were collected through a catheter inserted in a caudal tail artery at 0.5, 1, 1.5, 2.5, 4, 6, 9, 13, 20, 30, 45, 75, 110, 150, 180 and 240 min after the start of barbiturate infusion. The catheters were implanted 24–48 h before administration of thiopental. The animals were divided into thirteen groups of five or six animals per group. Each group was assigned a time for decapitation: 1, 2.5, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 and 240 min after the start of the thiopental infusion. Each animal went through the above plasma sampling protocol until its assigned time of sacrifice. Immediately after decapitation, the animal was dissected; organs and tissues were weighed, frozen rapidly on dry ice and stored at −20°C pending assay. The following tissues were collected and stored within 20 min of death: plasma, brain, heart, lung, liver, intestine, spleen, pancreas, kidney, testes, abdominal muscle, perirenal fat and abdominal skin.

RESULTS

Fig. 1, panel 1 shows the chromatogram resulting from HPLC analysis of 50 μl of plasma taken from a rat 60 min after injection of 25 mg/kg thiopental.

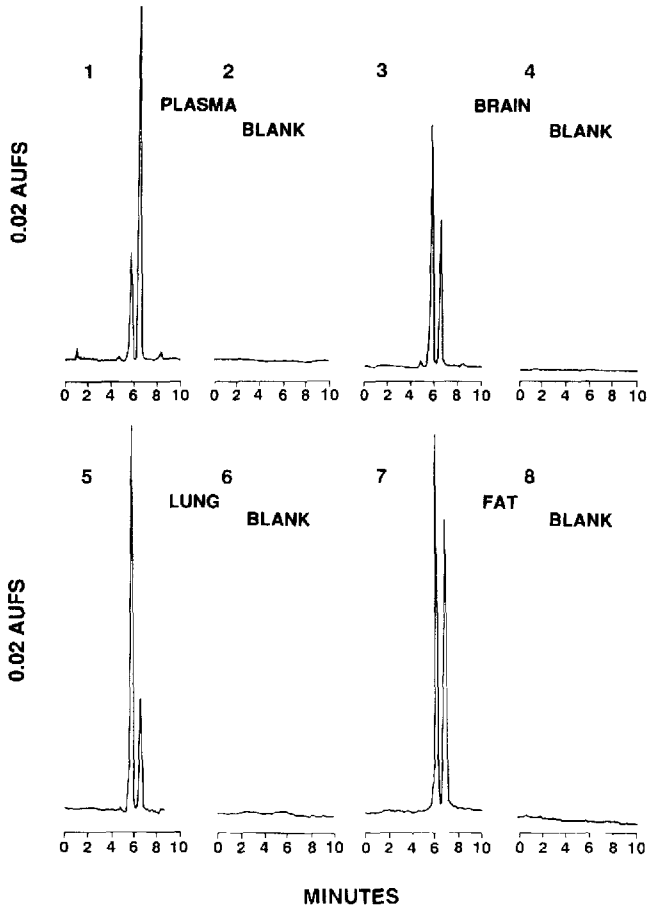

Fig. 1. Representative chromatograms for plasma and tissue extracts after injection of thiopental in rats and for rat plasma and tissue samples containing no thiopental (blanks). (1) Extract of rat plasma containing ca. 10 μg/ml thiopental. Elution of thiopental occurs first, at 5.5 min, followed by thiamylal at 6.5 min. The small peaks before thiopental and after thiamylal may be metabolites of thiopental. (2) Plasma blank. (3) Extract of brain tissue containing ca. 45 μg/g thiopental. (4) Brain tissue blank. (5) Extract of lung tissue containing ca 75 μg/g thiopental. (6) Lung tissue blank. (7) Extract of perirenal fat containing ca. 35 μg/g thiopental. (8) Fat tissue blank.

The large peak at 5.5 min represents elution of thiopental; elution of thiamylal occurred at 6.5 min. The plasma concentration of thiopental was ca. 10 μg/ml. Each barbiturate eluted with sharp peaks and distinct separation at baseline. Although small peaks appear at 5.0 and 8.1 min, they do not interfere with the barbiturates of interest. The early peak may be a metabolite of thiopental. The peak at 8.1 min is an unidentified contaminant in the internal standard. Fig.

1, panel 2 shows the chromatogram resulting from HPLC analysis of blank plasma obtained before administration of thiopental in the same rat.

Fig. 1, panels 3, 5 and 7 provide the chromatograms resulting from HPLC analysis of concentrations of thiopental in brain, lung and perirenal fat, respectively, after injection of thiopental in rats (panels 4, 6 and 8 are chromatograms for the respective tissue blanks). Again, each barbiturate is defined clearly and has no interfering peaks.

The apparent minimum limit of quantitation for thiopental in 50-μl samples of plasma is 50 ng/ml. The minimum detection limit for this barbiturate is ca. 10 ng/ml. This last concentration cannot be quantified; it represents the lowest barbiturate concentration producing a response at least 2.5 times the baseline detector noise.

Table I provides the extraction recovery of thiopental at two concentrations of thiopental in twelve tissues. The mean assay recovery of thiopental for all tissues is 79% after direct injection of 1 μg/ml thiopental and 87% for 20 μg/ml thiopental. Thiamylal assay recovery is also high varying from 75 to 90% in the twelve tissues. The assay recovery for thiopental and thiamylal increases with increasing concentration, however, for the purpose of this assay, this phenomenon appears to be insignificant. Calibration curves of peak-height ratio versus thiopental concentration are linear over the range 1–200 μg/ml. No concentration dependency in normalized peak-height ratio (ratio of thiopental to thiamylal peak heights normalized to thiopental concentration) could be

TABLE I

ASSAY RECOVERY OF THIOPENTAL FROM BUFFER AND RAT PLASMA AND TISSUES

Ten samples from each tissue. Values in parentheses are coefficients of variation (%).

Tissue	Mean recovery (%)	
	1 μg/ml	20 μg/ml
Brain	73 (12.6)	84 (4.6)
Sorensen's phosphate buffer (pH 7.4)	76 (4.9)	83 (3.9)
Fat	88 (21.9)	99 (26.8)
Heart	79 (6.5)	89 (7.5)
Intestine	78 (7.8)	96 (9.7)
Kidney	75 (5 8)	89 (5.8)
Liver	75 (11.3)	84 (5.1)
Lung	89 (4.5)	88 (13.8)
Muscle	67 (5.9)	81 (6.7)
Pancreas	73 (9.0)	83 (5.6)
Plasma	73 (3.2)	82 (4.1)
Skin	70 (6.2)	87 (4.5)
Spleen	96 (14.7)	85 (5.8)
Testes	92 (9.2)	91 (6.5)

demonstrated (Table II). The reason for the apparent concentration dependency in barbiturate assay recovery is not known.

Table III shows the within-day and day-to-day variability of the assay in twelve tissues after injection of low and high concentrations of thiopental. Most coefficients are below 5%; no tissue has coefficients of variation greater than 10%.

The assay was examined for selectivity by injecting other barbiturates into the chromatographic system and comparing their retention times with that of thiopental (Table IV). The assay method clearly separates this series of closely related compounds. Many other conjugated or oxidized metabolites of these barbiturates probably do not warrant concern, as the initial organic extraction step excludes lipid-insoluble biotransformation products from the HPLC column.

Fig. 2 shows the stability of thiopental in metabolizing tissues and in tissue homogenates over a time period consistent with sample handling before and during the assay. The concentration of thiopental in fresh liver, in rapidly frozen liver thawing to room temperature and in liver homogenates did not decrease progressively with time, indicating that significant metabolism did not occur in excised liver. Concentration of thiopental was stable in liver homogenates at three temperatures.

TABLE II

NORMALIZED PEAK-HEIGHT RATIOS FROM BUFFER AND RAT PLASMA AND TISSUES

Ten samples from each tissue. Values in parentheses are coefficients of variation (%)

Tissue	(Thiopental/thiamyl peak-height ratio)/thiopental concentration	
	1 μg/ml	20 μg/ml
Brain	0.208 (3.6)	0.195 (4.8)
Sorensen's phosphate buffer (pH 7.4)	0.206 (0.9)	0.212 (1.2)
Fat	0.218 (2.0)	0.259 (1.9)
Heart	0.206 (5.9)	0.208 (4.2)
Intestine	0.199 (6.8)	0.201 (2.8)
Kidney	0.215 (3.7)	0.202 (4.3)
Liver	0.207 (6.9)	0.194 (3.2)
Lung	0.211 (5.1)	0.196 (6.5)
Muscle	0.190 (4.0)	0.202 (9.3)
Pancreas	0.205 (1.8)	0.200 (2.0)
Plasma	0.204 (1.3)	0.199 (1.1)
Skin	0.207 (3.3)	0.194 (1.9)
Spleen	0.188 (3.6)	0.197 (4.8)
Testes	0.204 (1.9)	0.200 (1.9)

TABLE III

WITHIN-DAY AND DAY-TO-DAY VARIABILITY OF THIOPENTAL ASSAY

Tissue	Coefficient of variation (%)			
	Within-day ($n=10$)		Day-to-day ($n=10$)	
	1 μg/ml	20 μg/ml	1 μg/ml	20 μg/ml
Brain	3.6	4.8	5.5	5.2
Fat	2.0	1.9	2.3	3.4
Heart	5.8	4.2	4 3	4.7
Intestine	9.8	2.8	9.0	4.2
Kidney	3.7	4.3	5.6	4.1
Liver	6.9	3.2	6.5	5.2
Lung	5.1	9.3	3.7	9.7
Muscle	4.0	6.5	4.0	9.7
Pancreas	1.8	2.0	2.7	8.4
Plasma	1.0	2.1	2.9	2.3
Skin	3.6	4.8	8.2	4.3
Spleen	3.3	1.9	4.2	5.4
Testes	1.9	1.9	3.4	1.7

TABLE IV

RETENTION TIMES OF BARBITURATES RELATIVE TO THAT OF THIOPENTAL (4.09 min)

Barbiturate	Relative retention time
Phenobarbital	0.26
Heptabarbital	0.49
Amobarbital	0.50
Pentobarbital	0.50
Hexobarbital	0.59
Secobarbital	0.62
Thiopental	1.00
Methohexital	1.16
Thiamylal	1.26

Thiopental appears stable in frozen tissue over thirty days, as the relative change in thiopental concentrations was less than, or equivalent to, the within-day value of the assay (Table V). The short-team stability of thiopental is consistent with reports from Premel-Cabic et al. [18]. These authors reported thiopental stability in human plasma frozen for eight weeks.

Fig. 3 provides concentration–time profiles for thiopental in four tissues. The profile for thiopental in plasma is characterized by a rapid distribution phase, a longer elimination phase and a half-life of 90 min. The dashed line

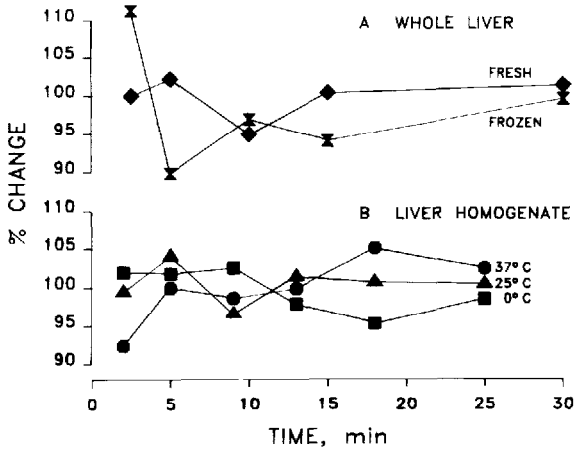

Fig. 2 Stability of thiopental in whole liver or liver homogenates. (A) Percentage change in thiopental concentration in liver at room temperature (♦) or in frozen liver thawing to room temperature (⧗). (B) percentage change in thiopental concentration in spiked fresh liver homogenates kept at 0°C (■), 25°C (▲), and 37°C (●).

TABLE V

STABILITY OF THIOPENTAL IN RAT PLASMA AND TISSUE AFTER THIRTY DAYS IN STORAGE

Five samples for plasma and each tissue.

Tissue	Amount of original quantity remaining (%)	Coefficient of variation (%)
Brain	97.5	5.6
Fat	95.4	7.9
Heart	97.1	3.0
Intestine	99.9	9 6
Kidney	102.0	2.2
Liver	100.7	6.5
Lung	104.5	6.9
Muscle	93.6	10.4
Pancreas	100.3	14.3
Plasma	100.3	1.7
Skin	108.8	13.6
Spleen	97 5	5.6
Testes	104.6	3.9

represents the best triexponential fit to the plasma data. Most of the data were collected before 50 min because of the extensive sacrifice schedule. The time course of the concentration of thiopental in brain tissue parallels that in plasma. Peak concentrations occurred at the 1-min sampling period and attest to the

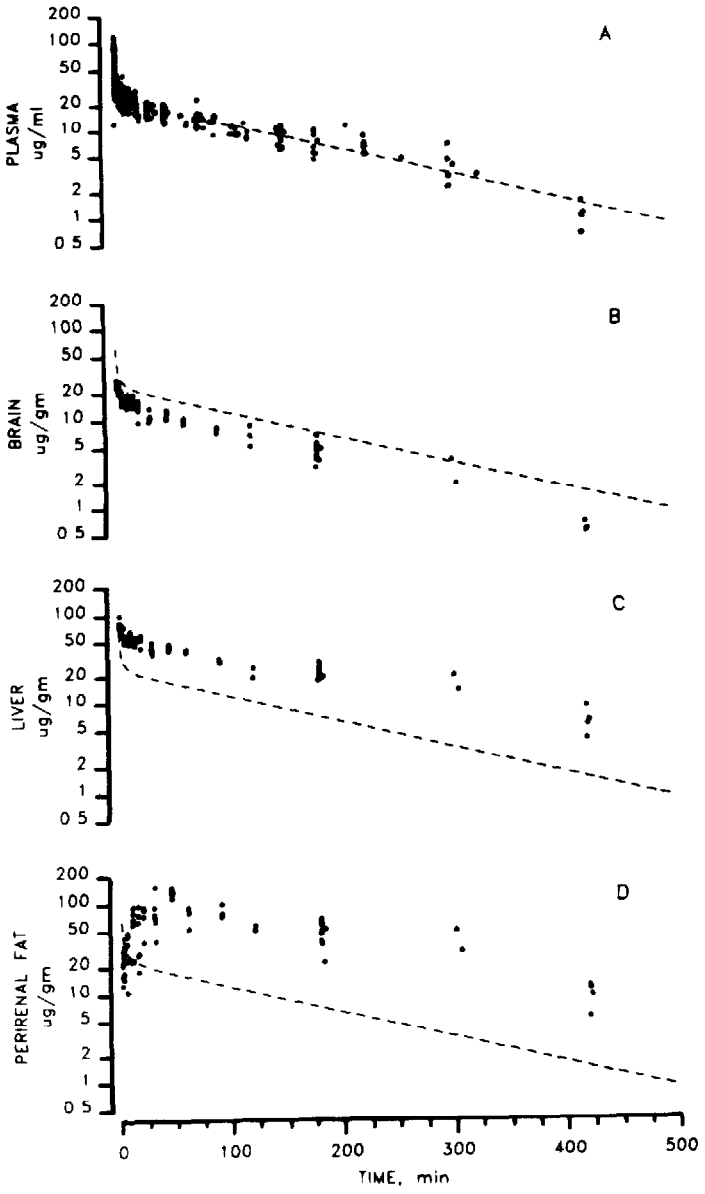

Fig. 3. Profiles of thiopental concentration versus time in plasma (A), brain tissue (B), liver tissue (C) and perirenal fat (D) of 74 Wistar rats after 20 μg/kg injections of thiopental. The dashed line in each panel is the polyexponential best fit describing the plasma decay curve. The large amount of plasma data results from multiple sampling in each rat before decapitation.

rapid plasma-to-brain equilibration of this barbiturate. Rapid plasma-to-brain equilibration is consistent with the fact that blood flow to the brain is high. Although distribution into brain tissue is rapid, it is not extensive: throughout the study, concentrations of thiopental were 40% lower in brain tissue than in plasma. In contrast, distribution into liver tissue is both rapid and extensive. Again, peak concentrations occurred at the 1-min measurement time and then decreased in parallel with concentrations in plasma. This result is consistent with high blood flow to the liver. Liver concentrations remained two to three times higher than plasma concentrations throughout the study. Uptake of thiopental into perirenal fat is slow but extensive. Peak concentrations occurred in the fat more than 60 min after administration of thiopental. During the terminal elimination phase, concentrations of thiopental in fat declined in parallel with, but still remained eight times higher than, concentrations in plasma.

DISCUSSION

The measurement of thiopental by this method is sufficiently efficient, precise, sensitive and selective for high-resolution pharmacokinetic studies in small species. This method permits the drawing of as many as fifteen 100-μl plasma samples from a single 350- to 400-g rat. This amount of sampling does not significantly affect hematocrit or cardiovascular variables. Under our chromatographic conditions, pentobarbital has a sufficiently short retention time to prevent interference from this potential metabolite. The more polar hydroxy, carboxylic acid and conjugated metabolites of thiopental do not warrant concern because they would be excluded from the column by the pentane extraction or would elute before the barbiturates of interest. To date, over 3500 samples have been analysed by this method in studies of the pharmacokinetics and pharmacodynamics of thiopental in the rat.

Thiopental is sufficiently stable in serum and tissue to permit the analysis of this barbiturate from frozen tissue for at least up to one month following sample collection. Some plasma and tissue homogenate samples assayed repeatedly at time intervals over thirty days have yielded identical results. The stability of thiopental in frozen tissues for time periods greater than this period may be a concern. In our laboratory we have examined the stability of thiopental in frozen human plasma for longer periods. In these studies we have found less than 10% loss during the first eight weeks of storage. This is consistent with the short-term stability of thiopental reported by Premel-Cabic et al. [18]. However, we have found that thiopental degradation in human plasma appears to be bi-phasic; after eight weeks of continual storage at $-20\,^{\circ}C$ degradation accelerates. By six months less than 60% of the original barbiturate is detected. The short-term stability of thiopental in rat muscle may be a concern: we found 7% degradation over thirty days for this tissue. The reason for the apparent

instability in muscle is uncertain but may be related to the physical desiccation of the tissue during prolonged freezing. A chemical process may also be operative. We recommend that rat plasma and tissue samples be assayed as soon as possible. If we extrapolate from our human data it appears that thiopental stability in frozen samples cannot be guaranteed for extended periods. Thiopental is intrinsically unstable; the chemical stability of this and other barbiturates has been discussed by Garrett et al. [27].

The tissues are easily homogenized with a Brinkman Polytron homogenizer; extraction of thiopental from tissue homogenate is efficient. Although homogenization of skin required predigestion with collagenase, thiopental was found to be stable during this digestion step. For complete digestion, additional collagenase must be added and the homogenate re-incubated for a second 90-min period.

As with thiopental, the assay recovery of thiamylal from tissue homogenate is 75–90%. The use of the internal standard reduces the variability of the assay, because the two barbiturates have similar extraction characteristics. The low coefficients of variation for the within-day variability studies (Table II) relative to the coefficients of variation for the extraction studies (Table I) attest to the usefulness of thiamylal as an internal standard. Previously reported HPLC methods for thiopental in tissues may have been confounded by use of an inappropriate internal standard. One report used pentobarbital [16], a potential metabolite of thiopental, or phenolphthalein [19], a non-barbiturate with different physical–chemical characteristics. Kelner and Bailey [21] have used thiamylal as an internal standard in an HPLC assay for thiopental in human plasma.

Uptake of thiopental into the highly perfused visceral tissues is rapid. Peak concentrations in brain, lung, heart, liver, intestine, spleen and kidney occurred at the 1-min sacrifice time. Peak concentrations in muscle, skin, fat and testes occurred later. During the terminal elimination phase, concentrations of thiopental were higher in liver, kidney and fat than in plasma; concentrations were lower in muscle, brain and heart tissues than in plasma. This result is consistent with the data on rats by Igari et al. [28]. Our concentration–time profile is also consistent with data on dogs by Brodie et al. [1] after their data are corrected for protein binding. Both of these studies [1,28] used a spectrofluorometric method. The high partitioning of thiopental into liver is consistent with the high lipid content of rat liver [29]; however, Brodie et al. [1] suggested that the high partitioning of thiopental into liver is probably associated with binding to tissue proteins.

We are currently using this assay to obtain tissue concentration data for a comprehensive, physiologically based model of thiopental disposition. Combining these data with regional blood flow data obtained in the anesthetized animal will permit estimation of the transfer rates of thiopental between body tissues. We are establishing the tissue concentration and regional blood flow

data base from which physiologically based simulations can be validated. These simulations will be subjected to animal 'scale-up' principles that enable one to predict human pharmacokinetic profiles accurately from animal data [30–32]. We will then be able to examine how changes in regional blood flow affect the time course of the barbiturate in pharmacokinetically and pharmacodynamically relevant tissues in humans.

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