Pharmacokinetic and Pharmacodynamic Consequences of Thiopental in Renal Dysfunction Rats: Evaluation with Electroencephalography

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The purpose of this work was to investigate the disposition characteristics and pharmacodynamics of a barbiturate, thiopental, in renal dysfunction rats. Normal and renal dysfunction rats were infused with 40 and 20 mg/kg of thiopental, respectively. The quantitative electroencephalographic (EEG) method was used as the surrogate measure of pharmacological response. Signals from two electrodes fitted on the skull of rats were continuously measured, recorded and subjected to off-line analysis. Total amplitude (0.5–29.9 Hz) from aperiodic analysis was taken as the EEG parameter. Thiopental concentration in plasma and cerebrospinal fluid (CSF) was assayed by an HPLC method. Steady-state volume of distribution was increased in renal dysfunction rats due to decreased plasma protein binding, while no change in clearance or volume of distribution based on the plasma unbound concentration was observed. Amplitude changes induced by thiopental in both normal and renal dysfunction rats were characterized by the sigmoidal $E_{\max}$ model. The unbound plasma concentration at half maximal effect was lowered by 30% in renal dysfunction rats as compared to the normal rats. In addition to considerable alteration in the pharmacokinetics of thiopental, it was also evident that renal impairment is associated with an increase in apparent pharmacological sensitivity, which is related to affinity.

Key words thiopental; electroencephalogram; aperiodic analysis; renal failure; pharmacokinetics; pharmacodynamics

Establishing pharmacokinetic–pharmacodynamic relationships for a drug and determining the influence of various factors on this relationship is important from the standpoint of developing rational drug therapy. It has been shown that experimental renal dysfunction in rats, induced by chemicals or by surgical procedure, is associated with increased sensitivity of the central nervous system.^{1,2)} These findings are consistent with the clinical observations of reduced anesthetic dose requirements of thiopental in azotemia.³⁾ However, in these animal studies, measurement of the pharmacological effect is limited to a defined quantal response (loss of righting reflex), which does not allow evaluation of a graded response.

Pharmacokinetic alterations of protein binding, distribution and hepatic metabolism have also been shown in renal dysfunction.^{4,5)} For centrally acting drugs, circumstances may be further complicated due to drug transport to the biophase or to tolerance development.⁶⁾ Thus, the entire concentration–effect relationships in renal dysfunction have not been established.

Recently, electroencephalographic (EEG) methods have been used as a continuous, sensitive and objective measure of the central response of such drugs.^{7–13)} Among the various techniques of EEG analysis, aperiodic analysis has been widely used.^{7–10)} The objective of the present study was to explore the disposition characteristics and pharmacokinetic–pharmacodynamic relationship of a highly lipophilic barbiturate, thiopental, in renal dysfunction rats utilizing aperiodic analysis of the quantitative EEG method as the surrogate measure of pharmacological response.

MATERIALS AND METHODS

Materials Thiopental sodium was purchased from Tanabe Seiyaku Co., Ltd. (Osaka, Japan). Uranyl acetate dihydrate was supplied by Wako Pure Chemical Industries, Ltd., (Osaka, Japan). All other chemicals were commercial products of reagent grade.

Animals Adult male Wistar rats purchased from Japan SLC Inc. (Hamamatsu, Japan) and 225–275 g in weight were used for the experiment. Rats were housed in a normal 12-h light–dark cycle and maintained on laboratory chow and water *ad libitum*.

Induction of Renal Dysfunction Experimental renal dysfunction was induced by administration of 6 mg/kg of a 0.5% solution of uranyl acetate in normal saline *via* the tail vein, 5 d before the experiment. Plasma creatinine, urea nitrogen and glutamate pyruvate transaminase (GPT) levels were measured with a Reflotron S system (Boehringer Mannheim, Mannheim, Germany) before uranyl acetate administration and on the day of the experiment. Body weight was also recorded on these 2 d.

EEG Measurement and Drug Disposition One day before the EEG experiment, stainless steel screws (IMS-143, Intermedical Co., Tokyo, Japan) connected with a coated wire were used as EEG electrodes. For the measurement of EEG signals, electrodes were implanted into the skull of the animal under light ether anesthesia. The animal was fixed in a stereotaxic instrument (Narishige Scientific Instrument Lab., Tokyo, Japan) and two small holes were drilled with a compact drill (C-150, Minitor Co., Ltd., Tokyo, Japan) into the skull, but not through the dura, at a location 2 mm anterior to bregma and 3 mm posterior to lambda. The whole assembly was insulated and fixed to the skull with a dental acrylic cement. For blood sampling and drug administration, cannulas were placed in the right jugular vein and right femoral vein, respectively. The following day, animals were placed in a metabolic cage and allowed to move freely. After a 30-min period of stabilization, baseline EEG signals from the animal were measured at 1 min intervals for 15 min. In order to minimize the contribution of the diurnal rhythm to the cerebral electrical characteristics, all experiments were started between 9 and 10 a.m. The EEG leads were digitized and recorded on a digital oscilloscope (Model 310C, Nicolet Ins. Co., Madison, U.S.A.) at a rate of 500 points/s through a

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bioelectric amplifier (Polygraph System 360, NEC San-ei Co., Tokyo) for subsequent off-line analysis. The normal and renal dysfunction rats were then intravenously given 40 and 20 mg/kg of thiopental, respectively, over 12 min. During and after thiopental infusion, EEG was sampled at 1 min intervals until it returned to the control level value, and about 250 μ l of blood was sampled at fixed time intervals (3, 6, 9, 12, 15, 20, 30, 45, 60, 120, 180, 240, 300, 360 and 420 min), and the plasma separated and stored until analysis.

A group of normal rats were also infused with thiopental at 20 mg/kg, for pharmacokinetic comparison with the renal dysfunction rats.

Plasma-Cerebrospinal Fluid (CSF) Distribution In a separate experiment, groups of normal and renal dysfunction rats were given thiopental at 40 and 20 mg/kg for 12 min, respectively. CSF by cisternal puncture, and blood samples from the jugular vein, were obtained at 240, 300 and 360 min after the infusion. The concentration of thiopental in CSF and plasma, and the plasma protein binding were determined at each time point to assess the difference in distribution kinetics in the two groups. The plasma protein binding of thiopental was determined using a YMT ultrafiltration membrane in the MPS-L micropartition system (Amicon Inc.; Beverly, MA). About 0.5 to 1 ml of plasma was filtered at 3000 rpm for 15 min at 25 °C to afford about 100 to 150 μ l of filtrate which was then analyzed for unbound drug concentration.

Drug Analysis Thiopental concentrations in plasma, CSF and ultrafiltrate from plasma were determined by HPLC. 0.05 ml of *p*-hydroxybenzoic acid *n*-butyl ester (100 μ g/ml) as internal standard, 0.5 ml of 100 mM phosphate buffer (pH 7.4) and diethyl ether (7 ml) were added to a 0.1 ml sample. After shaking for 10 min in a mechanical shaker and centrifugation at 3000 rpm for 10 min, 5 ml of the supernatant was evaporated to dryness. The sample was then reconstituted with 0.5 ml of mobile phase, of which 0.2 ml was injected into the HPLC. The HPLC system (Shimadzu, Kyoto, Japan) consisted of an auto injector (SCL-10AL), a liquid chromatogram (LC-10AD), a system controller (SCL-10A), a variable wavelength UV detector (SPD-10A), a column oven controller (CTO-10A) and a model recorder (Chromatopac, C-R6A). A C18 column (Nucleosil, Macherey-Nagel, Germany, 15 cm $\times$ 4.5 mm i.d.; 5 μ m particle size) was used to separate the compound. Thiopental was detected at 290 nm. Mobile phase was a mixture of acetonitrile and 0.1% phosphate buffer solution, pH 3.5 (4:6). Flow rate was 1 ml/min and oven temperature was maintained at 40 °C. The detection limit was 0.02 μ g/ml and the coefficients of variation of analyses ($n=5$) were 3.4, 5.8 and 6.0% at 1, 10 and 50 μ g/ml, respectively.

Data Analysis-Pharmacokinetics When a drug is given by rapid intravenous injection, the plasma concentration-time data of the drug is described by a simple exponential model as follows:

$$C = \sum_{i=1}^n C_i \cdot e^{-\lambda_i t} \quad (1)$$

For a drug with a short intravenous infusion over 12 min, as in the present work, convolution of an exponential disposition function with a zero order input function yields the corresponding equation, which describes the plasma concentra-

tion-time course of the drug:

$$C = \frac{\text{Dose}}{T} \sum_{i=1}^n \left(\frac{1 - e^{-\lambda_i t'}}{-\lambda_i} \right) C_i' \cdot e^{-\lambda_i t} \quad (2)$$

Where T is the infusion duration, $C_i' = C_i / \text{Dose}$ and $t' = t$ for $t \leq T$, $t' = T$ for $t > T$. The parameters were obtained by fitting the concentration data of thiopental to Eq. 2 with the weighted iterative nonlinear least squares regression program, MULTI.¹⁴⁾ The reciprocal of the square of observed data was adopted as the weight.

The plasma clearance (CL) and the volume of distribution at steady state (V_{ss}) of the total drug were obtained model-independently:

$$CL = \frac{\text{Dose}}{\sum_{i=1}^n \frac{C_i}{\lambda_i}} \quad (3)$$

$$V_{ss} = \text{Dose} \cdot \frac{\sum_{i=1}^n \frac{C_i}{\lambda_i^2}}{\left(\sum_{i=1}^n \frac{C_i}{\lambda_i} \right)^2} \quad (4)$$

The volume of distribution (V_u) and the clearance (CL_u) based on the unbound drug concentration in the plasma are given in Eqs. 5 and 6, respectively:

$$CL_u = CL / f_u \quad (5)$$

$$V_u = V_{ss} / f_u \quad (6)$$

where f_u is the fraction unbound in the plasma.

Data Analysis-Pharmacodynamics The recorded EEG signals of 4.096-s time intervals were analyzed off-line by aperiodic analysis. Programs were written in FORTRAN 77 and executed with a digital computer (PC-9801 RA, NEC, Tokyo, Japan). The algorithm proposed by Gregory and Pettus was used for aperiodic analysis.¹⁵⁾ This algorithm determines the amplitude and period of each EEG on a wave by wave basis, and defines a wave as a fluctuation in voltage that occurs between local minima in voltage. The amplitude of the wave was defined as the average of the differences in voltage between a local maximum peak and the previous local minimum and those between the local maximum peak and the next local minimum. The frequency of the wave was defined as the inverse of the difference in time between the occurrence of local minima. For the simultaneous occurrence of slow waves (from 0.5 to 7.9 Hz) in the presence of fast waves (from 8 to 29.9 Hz), both waves were separated within the algorithm, and only the wave with zero volt crossing was assigned as the slow wave. Time points containing artifacts with amplitude greater than 0.8 mV were excluded from further calculations. Finally, the mean amplitude per second (AMP) from 0.5 to 29.9 Hz was used as the measure of the effect of thiopental on brain.

When the temporal delay between the plasma concentrations and EEG effect is recognized, a hypothetical effect compartment linked to the plasma compartment by a first-order process is postulated:

$$\frac{dC_e}{dt} = k_{eo}(C - C_e) \quad (7)$$

where C_e is the effect compartment concentration and k_{eo} is a first-order rate constant, describing the rate of equilibration

between the plasma and effect site. The k_{eo} was estimated by collapsing the hysteresis curve of EEG effect vs. effect compartment concentration, using the technique described by Fuseau and Sheiner.¹⁶⁾

AMP responded in a biphasic manner to increasing concentrations of thiopental. Under relatively low concentrations, however, AMP showed a monophasic increasing response. For the monophasic response, the relationship between thiopental concentration and AMP was analyzed by fitting the data of individual rats separately with the sigmoidal $E_{\max}$ model as follows:

$$E = E_0 + \frac{E_{\max} \cdot C^N}{EC_{50}^N + C^N} \quad (8)$$

where E_0 is the baseline EEG effect; $E_{\max}$ is the maximum amplitude stimulated by thiopental; C is the concentration of thiopental at the effect site; EC_{50} is the concentration of thiopental at half maximal effect and N is the Hill coefficient. AMP was also analyzed with the log concentration-response model as follows:

$$E = E_0 + m(\log C - r) \quad (9)$$

where m is the slope of the E versus $\log C$ plot, and r is a constant.

Statistical Analysis The statistical significance of difference was calculated using ANOVA, and then Scheffe's multiple comparison methods.

RESULTS

Table 1 summarizes the effect of uranyl acetate administration on plasma creatinine, urea nitrogen concentration, GPT activity and body weight of rats used in the EEG experiment. Plasma urea nitrogen concentration was found to be significantly higher on the 5th day. Although we could not detect the exact level of creatinine in normal rats before uranyl acetate administration, since the sensitivity of the instrument was limited to concentrations above 0.5 mg/dl, the levels were significantly higher on the 5th day. Plasma GPT levels were not different from normal.

Pharmacokinetics Normal and renal dysfunction rats were intravenously given 40 or 20 mg/kg of thiopental over 12 min. The typical plasma concentration profiles are shown in Fig. 1. The plasma concentrations in all experiments decayed biexponentially. The calculated values using Eq. 2 are depicted as solid lines in the figure. At the dose of 20 mg/kg, plasma concentrations were higher in normal rats than in the renal dysfunction rats. In normal rats, the elimination half-life of thiopental had a tendency to increase with increasing dose.

In another set of experiments, unbound drug concentration in plasma and CSF was investigated at different time points and the results are summarized in Table 2. The upper part of the table shows the biochemical parameters of the rats. Normal and renal dysfunction rats were treated with 40 and 20 mg/kg of thiopental, respectively. A significant increase in the unbound plasma and CSF to total plasma concentration ratio was observed, while CSF to unbound plasma concentration ratio was not altered in renal dysfunction rats. The mean values of f_u in normal and renal dysfunction rats were 0.23 ± 0.06 and 0.45 ± 0.08 , respectively.

Table 1. Characteristics of the Renal Dysfunction Rats Used in the EEG Study of Thiopental

Parameters	0 day ^{a)}	5 day ^{b)}
Body weight (g)	249 ± 5	237 ± 7
Plasma creatinine concentration (mg/dl)	<0.5	4.74 ± 0.53
Plasma urea nitrogen concentration (mg/dl)	17.1 ± 0.4	167 ± 12*
GPT (U/l)	48.9 ± 4.4	44.0 ± 5.9

Results are expressed as mean ± S.E.M. of five experiments. * Significantly different from control value, $p < 0.05$. a) Shows the value prior to uranyl acetate administration. b) EEG experiment was carried out on the fifth day of uranyl acetate administration.

Fig. 1. The Concentration-Time Profiles of Plasma for Thiopental in Normal and Renal Dysfunction Rats Following i.v. Administration over 12 min

Each symbol and line represent the observed data and the calculated value from Eq. 2, respectively. Doses are ○, 40 mg/kg in a normal rat; □, 20 mg/kg in a normal rat; ●, 20 mg/kg in a renal dysfunction rat.

In Table 3, the pharmacokinetic parameters obtained for thiopental, 20 or 40 mg/kg infused over 12 min in normal and renal dysfunction rats are shown. V_{ss} was significantly higher in the renal dysfunction rats than in the normal rats, while no change in V_u was observed. CL in the renal dysfunction case also increased though not significantly, whereas CL_u did not differ. Moreover, in normal rats, both CL_u and V_u were not significantly different at doses of 20 and 40 mg/kg.

Pharmacodynamics Figure 2 shows the typical AMP-time profiles induced by thiopental. Thiopental in normal and renal dysfunction rats was given at 40 and 20 mg/kg over 12 min, respectively. Taking into account the difference in drug sensitivity, the dose of thiopental was reduced to half in the renal dysfunction rats to avoid an unusually prolonged effect. The AMP in both groups increased rapidly after the infusion of thiopental was started. Though a temporary decrease of AMP was observed during a short period around 12 min when the infusion was stopped, the high AMP levels remained constant for a while, then slowly returned to the control level. In spite of the difference in dose of thiopental, the recovery periods were not significantly different between normal and renal dysfunction rats.

The plasma concentration-AMP curve induced by thiopental in a normal rat is shown in Fig. 3. AMP with increasing concentrations of thiopental had biphasic characteristics, that is, an increase followed by a decrease. For the decreasing part at high concentration ranges, however, only a few data

Table 2. Kinetics of Thiopental in CSF and Plasma of Normal and Renal Dysfunction Rats

Parameters	240 min		300 min		360 min	
	Normal ^{a)}	Renal dysfunction ^{b)}	Normal ^{a)}	Renal dysfunction ^{c)}	Normal ^{c)}	Renal dysfunction ^{c)}
Body weight (g)	239 ± 9	212 ± 9	242 ± 2	231 ± 11	244 ± 11	234 ± 5
Plasma creatinine concentration (mg/dl)	<0.5	4.93 ± 0.48	<0.5	4.62 ± 0.52	<0.5	4.60 ± 0.83
Plasma urea nitrogen concentration (mg/dl)	18.2 ± 0.4	166 ± 17	17.8 ± 0.5	171 ± 29	16.8 ± 1.1	104 ± 13
GPT (U/l)	56.0 ± 0.9	53.5 ± 2.8	53.7 ± 2.5	45.6 ± 2.7	40.3 ± 1.5	47.3 ± 4.4
Free : plasma	0.23 ± 0.02	0.45 ± 0.03 ^{d)}	0.24 ± 0.03	0.47 ± 0.02 ^{d)}	0.27 ± 0.03	0.43 ± 0.06 ^{d)}
CSF : plasma	0.22 ± 0.02	0.43 ± 0.03 ^{e)}	0.22 ± 0.03	0.46 ± 0.03 ^{d)}	0.26 ± 0.01	0.46 ± 0.09 ^{d)}
CSF : free	0.98 ± 0.02	0.96 ± 0.03	0.91 ± 0.04	0.99 ± 0.03	0.98 ± 0.04	1.04 ± 0.07

Results are expressed as mean ± S.E.M. a) Four experiments. b) Six experiments. c) Five experiments. There is no significant difference in any concentration ratio among the sampling time within each treatment group. d) and e) Significantly different from the normal rat group (240, 300 and 360 min), $p < 0.05$ and $p < 0.01$, respectively.

Table 3. Pharmacokinetic Parameters for Thiopental in Normal and Renal Dysfunction Rats

	Dose (mg/kg)	C_1 (μg/ml)	λ_1 (min ⁻¹)	C_2 (μg/ml)	λ_2 (min ⁻¹)	CL (ml/min/kg)	V_{ss} (l/kg)	$CL_u^{a)}$ (ml/min/kg)	$V_u^{a)}$ (l/kg)
Normal (n=5)	40	40.2 ± 11.9	0.192 ± 0.047	29.5 ± 3.30	0.0032 ± 0.0004	4.51 ± 0.86	1.35 ± 0.13	19.6 ± 3.75	5.88 ± 0.59
Normal (n=5)	20	14.7 ± 2.50	0.279 ± 0.149	17.5 ± 3.80	0.0051 ± 0.0008	5.76 ± 0.46	1.13 ± 0.19	25.0 ± 2.00	4.89 ± 0.81
Renal dysfunction (n=5)	20	12.2 ± 1.60	0.195 ± 0.032	8.30 ± 0.93	0.0042 ± 0.0011	9.25 ± 1.71 ^{b)}	2.39 ± 0.27 ^{c)}	20.6 ± 3.80	5.32 ± 0.50

Results are expressed as mean ± S.E.M. a) f_u values were 0.23 and 0.45 for normal and renal rats, respectively. b) Significantly different from normal rat 40 mg/kg ($p < 0.05$). c) Significantly different from normal rat 40 mg/kg ($p < 0.05$) and 20 mg/kg ($p < 0.01$).

Fig. 2. Typical EEG Effect-Time Profiles in Normal and Renal Dysfunction Rats after Thiopental i.v. Administration over 12 min

A) 40 mg/kg dose in a normal rat (rat number 1 in Table 4); B) 20 mg/kg dose in a renal dysfunction rat (rat number 3 in Table 4).

points could be obtained.

Although hysteresis between plasma concentration and AMP was observed in nine data sets, the obtained rate constant for equilibration between the plasma and effect site, calculated using Eq. 7, had an extremely large value ($5.95 \pm 2.42 \text{ min}^{-1}$). Accordingly, the plasma compartment and the effect site were considered to be kinetically indistinguishable. The AMP data from maximal stimulation to awakening, after stopping the drug infusion, together with the control EEG, were fitted using Eqs. 8 and 9 with respect to the plasma con-

centrations. The values of Akaike's information criterion (AIC), calculated using Eq. 8, were smaller than those using Eq. 9 for most experiments (Table 4). These results indicated that the sigmoidal $E_{\max}$ model is a better model of the relationship between EEG amplitude and thiopental plasma concentration. Figure 4 shows the observed amplitude changes induced by thiopental in normal and renal dysfunction rats, as well as the calculated values. The pharmacodynamic parameters with the sigmoidal $E_{\max}$ model are also listed in Table 4. There was a statistically significant difference between the

EC_{50} values of normal ($9.61 \pm 0.79 \mu\text{g/ml}$) and renal dysfunction rats ($3.48 \pm 0.24 \mu\text{g/ml}$) based on the total plasma concentration, with this value about 64% lower in uranyl acetate pre-treated rats. The corresponding EC_{50} values based on the unbound plasma concentration ($2.21 \pm 0.03 \mu\text{g/ml}$ and $1.57 \pm$

$0.06 \mu\text{g/ml}$) by the use of f_u (Table 2), were also significantly different ($p < 0.05$). On the other hand, no difference in the baseline EEG value ($E_0 = 0.34$ and 0.37), $E_{\max}$ value (1.28 and 0.93) and steepness ($N = 3.4$ and 2.44) of curves was observed.

DISCUSSION

Thiopental is an ultrashort-acting barbiturate used to induce anesthesia in the central nervous system. Thiopental binds to plasma protein moderately¹⁷ and is mainly metabolized in the liver after intravenous administration and less than 1% of a dose is excreted in the urine as intact drug.¹⁸ Though many drugs acting on the central nervous system are eliminated primarily by hepatic metabolism, the effect of renal failure on the distribution and disposition characteristics of drugs eliminated *via* hepatic metabolism has not been well documented. An increase in the sensitivity of the brain towards barbiturates has been reported in renal dysfunction. Hence, investigation of the kinetic consequences of renal failure seems to be important. Furthermore, a quantitative relationship between pharmacokinetics and pharmacodynamics in renal disease needs to be established.

Fig. 3. Thiopental Plasma Concentration and EEG Effect Relationship in a Normal Rat

The observed amplitude data of rat number 2 in Table 4 was plotted against the corresponding plasma concentration, calculated using Eq. 2. The time course is expressed as arrows.

Table 4. Pharmacodynamic Parameters for Thiopental in Normal and Renal Dysfunction Rats

Rat No.	AIC ^(a)	AIC ^(b)	E_0 (mV/sec)	$E_{\max}$ (mV/sec)	EC_{50} ($\mu\text{g/ml}$)	N
Normal rats						
1	1153	995	0.24 (fixed)	1.30 (0.03)	11.36 (0.24)	2.86 (0.18)
2	702	673	0.41 (0.05)	1.29 (0.09)	10.96 (0.43)	3.57 (0.42)
3	1487	1477	0.29 (0.05)	0.94 (0.07)	7.12 (0.48)	2.11 (0.31)
4	2415	2077	0.38 (0.05)	0.85 (0.05)	10.06 (0.21)	7.13 (0.69)
5	2473	2480	0.38 (0.11)	2.00 (0.24)	8.56 (0.11)	1.33 (0.21)
Mean $\pm$ S.E.M.			0.34 ± 0.03	1.28 ± 0.20	9.61 ± 0.79	3.40 ± 1.00
Renal dysfunction rats						
1	414	278	0.18 (0.03)	0.97 (0.06)	3.08 (0.15)	2.17 (0.27)
2	1220	1213	0.39 (0.03)	1.15 (0.12)	3.16 (0.33)	1.60 (0.19)
3	872	847	0.41 (0.03)	0.91 (0.11)	3.46 (0.56)	1.23 (0.17)
4	1234	1151	0.17 (0.02)	0.68 (0.03)	3.09 (0.08)	3.34 (0.23)
5	1024	999	0.61 (0.04)	1.00 (0.06)	4.39 (0.13)	3.80 (0.41)
Mean $\pm$ S.E.M.			0.37 ± 0.09	0.93 ± 0.07	$3.48 \pm 0.24^*$	2.44 ± 0.49

* Significantly different from control value, $p < 0.05$. Numbers in parentheses show standard deviation of the estimated parameters a) Log-linear model (Eq. 9). b) Sigmoidal $E_{\max}$ model (Eq. 8).

Fig. 4. The EEG Effect-Plasma Concentration Profiles for a Typical Rat after i.v. Administration of Thiopental over 12 min

The solid and dotted line represent observed and calculated values using Eq. 8, respectively. Doses are A, 40 mg/kg in a normal rat (rat number 1 in Table 4); B, 20 mg/kg in a renal dysfunction rat (rat number 1 in Table 4).

Thiopental has a low extraction ratio in the liver. For a drug with a low extraction ratio, the hepatic plasma clearance depends on the protein binding and the unbound clearance corresponds to the intrinsic hepatic clearance. The constant CL_u in normal and renal dysfunction rats (Table 3) suggests that the hepatocellular metabolic activity in renal dysfunction induced chemically by uranyl acetate does not change. The saturable metabolism of thiopental has been reported in man and rats.^{19,20} The present experiment, using 40 and 20 mg/kg doses in normal rats, however, could not recognize the differences in plasma clearance; this might be due to the narrow dose range in this study.

The unbound fraction of thiopental in plasma (f_u) increased almost 2 fold, from an average of 0.23 to 0.45 in the uranyl acetate treated rats. The decrease of protein binding in the plasma was associated with an increase in V_{ss} (Table 3). V_u , however, was not influenced. Thiopental is a highly lipophilic barbiturate and is extensively distributed in whole tissues. Thus, the distribution of unbound drug did not change, irrespective of the change in f_u . Furthermore, the unchanged V_u value suggests that the tissue binding of thiopental is not altered. Only the unbound drug can pass through most biological membranes. Accordingly, this value is more closely related to tissue distribution than the total drug. It appears that both distribution and elimination kinetics of thiopental are unchanged in renal dysfunction rats.

The ratio of CSF to unbound plasma concentration was also similar, being almost equal to 1 in the normal and renal dysfunction rats (Table 2). CSF has been considered to be in an equilibrium with the site of action of barbiturates.^{1,2} Although we did not examine thiopental concentration in the brain, its site of action, the rate of entry into the brain is known to be related to its lipid solubility.¹⁹ In our analysis also using the link model (Eq. 7), it was shown that thiopental at the effect site reached 90% of equilibrium with that in the plasma in less than 30 ($=\ln 10/k_{eo}$) seconds. The distribution of thiopental in the brain does not appear to differ in both groups of rats. Thus, it can be expected that the unbound concentration of thiopental in the plasma is equivalent to that at the site of action at the steady state.

Special attention should also be given to the role of active metabolites in the contribution to the effect of a drug. Pentobarbital is considered to be the main metabolite of thiopental. In this study, the concentration of pentobarbital was below 100 ng/ml in plasma samples obtained from both renal dysfunction and normal rats. Pentobarbital has been shown to produce loss of righting reflex at an estimated plasma concentration of 12.5 $\mu\text{g/ml}$.²¹ Based on these facts, it can be concluded that active metabolites had a negligible role in the contribution of the EEG effect.

Measurement of the continuous response for centrally acting drugs was a problem for a long time. Electroencephalographic methods have recently been recognized as a continuous, objective and sensitive technique for measuring the pharmacological effect of centrally acting drugs. These features provide the opportunity to characterize the pharmacokinetic and pharmacodynamic relationships for these drugs in individuals. Two methods, fast Fourier transform and aperiodic analysis have been commonly applied for the evaluation of EEG. Numerous reports have described the application of aperiodic analysis since it has recently gained popularity for

the evaluation of central effects of single drugs as well as for combined drug effects.⁷⁻¹¹

For barbiturates, the pharmacological relevance of the EEG with respect to depth of anesthesia has been established to a greater extent. The biphasic effect site concentration-EEG characteristics for barbiturates have been documented, and evaluated by aperiodic EEG analysis.^{7,8} We adopted aperiodic analysis to evaluate the effect of thiopental on the brain. The characteristic EEG pattern of thiopental was reproduced and the biphasic plasma concentration-AMP relationship was observed (Fig. 3). A pharmacodynamic model, to quantify the plasma concentration of thiopental and its effect, however, was limited to the ranges from the awakening to the stage associated with loss of a movement response to the pinching tail. We were unable to model the concentration-effect relationship for moderate deep anesthesia, that is, stage 2, because a relatively small dose was used and there were not enough data points for accurate pharmacodynamic modeling. However, the present study could determine whether acute tolerance develops to the effects of thiopental on the brain. The equilibrium between the plasma compartment and the effect-site compartment was very rapid. Hence, the equivalent effect at the ascending and descending plasma concentration of thiopental (Fig. 3) suggests that acute tolerance to thiopental does not develop under both normal and renal dysfunction conditions.

EEG properties before the administration of thiopental did not differ in renal dysfunction and normal rats. Thus, the baseline pharmacological parameter (E_0) was not changed. The changes in the maximal effect and Hill coefficient were also not seen in renal dysfunction rats. However, the EC_{50} value based on the unbound drug in plasma, decreased significantly as compared to the normal rats. The decrease of about 30% in renal dysfunction rats suggested increased sensitivity of the central nervous system to thiopental. Previous experiments have demonstrated decreases in CSF concentration of phenobarbital and heptobarbital at onset or offset of loss of righting reflex in renal dysfunction rats.^{1,2} The decrease of 30% in CSF concentration at half maximal effect for thiopental in this study is also comparable to that of other barbiturates with respect to the onset or offset of righting reflex. Our experiment further represents evidence that this change in apparent sensitivity is related to a change in potency, rather than intrinsic efficacy or slope factor, which was not distinguishable in previous studies employing quantal drug effect. In this context, the parallel shift of the concentration-effect curve along the concentration axis from normal rats to renal dysfunction rats is operative and thus might be applicable to different assay systems.

It is well known that certain endogenous compounds are accumulated during renal impairment. There are reports that show that accumulated endogenous compounds may play a role in increased sensitivity of phenobarbital during renal insufficiency.²² On the other hand, no sensitivity change was observed for heptobarbital in nephrotic syndrome.²³ We did not recognize any change in the concentration-amplitude relationship of oxazepam, a benzodiazepine, in renal dysfunction rats, indicating that accumulated benzodiazepine-like compounds do not have a direct action. However, in radioligand binding studies with rat brain membranes, the dialysate of serum from renal dysfunction rats induced by uranyl ac-

etate, enhanced the potency of thiopental in competing for *tert*-butylbicycloorthobenzoate (TBOB) binding to the γ -aminobutyric acid (GABA) receptor/chloride channel complex (data not shown). Although it is unclear whether the same compounds are responsible for *in vivo* and *in vitro* effects, they might, at least in part, play a role in enhancing the potency of thiopental. It is thus clear that further investigation is required to clarify the role of these endogenous compounds.

It follows from the above that in renal dysfunction rats, a change in disposition of thiopental occurs (associated with a change in protein binding), together with increased sensitivity, that can be attributed directly to intrinsic pharmacodynamic factors.

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