Anesthesiology
1996; 84:415–27

Quantitation of Depth of Thiopental Anesthesia in the Rat

Lars L. Gustafsson, M.D., Ph.D., William F. Ebling, Ph.D.,† Eileen Osaki,‡ Donald R. Stanski, M.D.§*

Background: **In contrast to that of inhalational anesthetics, quantitation of anesthetic depth for intravenous agents has not been well defined. In this study, using rodents, the relationship between the constant plasma thiopental concentrations and the clinical response to multiple nociceptive stimuli were investigated characterizing the anesthetic state from light sedation to deep anesthesia and correlated to the degree of electroencephalogram (EEG) drug effect.**

Methods: **Thirty rats were instrumented with chronically implanted EEG electrodes, arterial and venous catheters. A computer-driven infusion pump was used to rapidly attain and then maintain constant, target plasma thiopental concentrations ranging from 7 to 100 μg/ml. Three different target plasma thiopental concentrations were achieved in each rat. Electroencephalographic effects were monitored with aperiodic waveform analysis. The following nociceptive stimuli were applied: (1) unprovoked righting reflex, (2) provoked righting reflex, (3) noise stimulus, (4) tail clamping with an alligator clip, (5) constant tail pressure with an analgesiameter, (6) corneal reflex, and (7) tracheal intubation. For tail clamping, tail pressure, and intubation, either purposeful extremity movement or abdominal muscle contraction response was noted to be present or absent. The clinical responses (present or absent) were modeled using logistic regression to estimate the Cp_{50}, the plasma thiopental concentration with a 50% probability of no response.**

Results: **The following mean Cp_{50} values (95% confidence interval) were obtained: unprovoked righting reflex, 15.9 (15.1–16.6) μg/ml; provoked righting reflex, 21.4 (20.2–22.7) μg/ml; noise stimuli, 31.3 (29.7–33.0) μg/ml; tail clamp and limb movement, 38.3 (36.1–40.4) μg/ml, tail pressure and limb movement, 39.2 (37.1–41.3) μg/ml; tail pressure and abdominal muscle contraction, 52.5 (50.0–55) μg/ml; tail clamping and abdominal muscle contraction, 56.1 (50.0–56.2) μg/ml; corneal reflex, 60.0 (56.6–63.4) μg/ml; and limb movement or muscle abdominal contraction response to intubation, 67.7 (59.2–76.1) μg/ml. At an EEG-effect of 9.1 and 2.2 waves/s, there was a 50% chance of limb movement response to tail clamping and tracheal intubation, respectively. There was a poor relationship between the plasma thiopental concentration and the percent increase of either heart rate or mean arterial blood pressure after applying either tail pressure or tail clamp stimuli.**

Conclusions: **A range of nociceptive stimuli and their observed clinical responses can be used to quantitate thiopental anesthetic depth, ranging from light sedation to deep anesthesia (isoelectric EEG and unresponsive to intubation) in the rodent. Clinical response can be mapped to surrogate EEG measures. (Key words: Anesthetics, intravenous: thiopental. Infusions, computer-controlled; intravenous. Intravenous agents: pharmacodynamics; pharmacokinetics.)**

THE quantitation and assessment of anesthetic depth is an essential feature of the clinical practice of anesthesia. Unfortunately, although this topic has significant clinical and scientific importance, there is poor agreement about the definition and quantitation of anesthetic depth, especially among different classes of anesthetic drugs.[1] In an editorial comment, Prys-Roberts discusses the conceptual approach to defining anesthetic depth. He states that "depth of anesthesia is difficult to define because anesthesiologists have approached the issue in terms of the drugs available for anesthesia rather than the patient's needs during surgery." He suggests that anesthesia should be defined "in terms of the drugs producing unconsciousness and subsequent modification of physiological responses to noxious stimuli."[2]

* Associate Professor in Clinical Pharmacology, Division of Clinical Pharmacology, Huddinge University Hospital.

† Assistant Professor, Department of Pharmaceutics, School of Pharmacy, State University of New York, Buffalo.

‡ Research Assistant, Department of Anesthesia, Stanford University School of Medicine.

§ Professor and Chairman, Department of Anesthesia, Stanford University School of Medicine.

Received from the Division of Clinical Pharmacology, Huddinge University Hospital, Huddinge, Sweden; the Department of Pharmaceutics, School of Pharmacy, State University of New York, Buffalo, Buffalo, New York; and the Department of Anesthesia, Stanford University School of Medicine, Stanford, California. Submitted for publication June 21, 1995. Accepted for publication October 29, 1995. Supported in part by the National Institute of Aging grant R01-04594; the National Institute of Health Fogarty International Research Fellowship grant 4199; the Anesthesia/Pharmacology Research Foundation of Palo Alto, California; the Swedish Cancer Fund (2480); the Swedish Medical Research Council (8951 and 3902); and The Erik and Edit Fernstrom Foundation.

Address reprint requests to Dr. Gustafsson: Division of Clinical Pharmacology, Department of Medical Laboratory Sciences and Technology, Karolinska Institute at Huddinge University Hospital, S-141 86 Huddinge, Sweden.

In an editorial, Kissin[3] expanded this concept, which emphasizes that the spectrum of effects that constitutes the state of general anesthesia should not be regarded as several components of anesthesia resulting from one anesthetic action but represents separate pharmacologic actions, even if produced by only one drug.

Anesthetic depth assessment has been best established in humans and animals for the inhalational anesthetics using the minimum alveolar concentration (MAC) concept.[4–7] This has occurred because of the relative ease of obtaining constant alveolar, end-tidal, plasma, and biophase inhalational anesthetic concentrations, the standard nature of the most common noxious stimuli (skin incision in humans, tail clamping in animals), and the ease of observation of the purposeful movement response. Other stimuli have been used to define MAC_{awake}, $MAC_{intubation}$, and MAC_{bar}, which quantitate the depth of an inhalational anesthetic either greater or less than that defined by skin incision/tail clamping.[8–10] The MAC concept has successfully allowed the definition of pathologic or physiologic changes in humans or animals on inhalational anesthetic pharmacodynamics.[5]

For intravenously administered anesthetic drugs (hypnotics, opioids), assessment of anesthetic depth has only recently become a topic of investigation and increasing knowledge.[11–14] The inability to rapidly attain and then maintain constant anesthetic plasma concentration has confounded efforts to quantify the concentration *versus* effect relationship of intravenous anesthetics. Such studies require that constant plasma (and biophase) drug concentrations be maintained whenever defined stimuli are applied and the clinical response to such stimuli observed. The advent of computer-controlled infusion pumps (CCIP) has eliminated this technical limitation.[14–16] Recently, we described the application of CCIP technology to rodents to obtain constant or pseudosteady-state plasma thiopental concentrations.[17]

While the movement response to skin incision/tail clamping has been the most commonly used noxious stimulus/clinical response measure, for intravenously administered drugs, a wider range of noxious stimuli intensities are necessary to explore the entire range of anesthetic depth. For the opioid alfentanil in humans, Ausems *et al.*[11] used intubation, skin incision, intraoperative dissection, skin closure, and ventilation as defined noxious stimuli. They examined a series of clinical responses including hemodynamics, movement, and autonomic responses categorized into a single quantal measure of adequate or inadequate anesthesia. In 1978, Becker[12,13] first attempted to quantitate thiopental anesthetic depth using defined noxious stimuli (eyelid/corneal reflex, trapezius muscle squeeze) and the purposeful movement response during a constant thiopental infusion. More recently, Hung *et al.*,[14] using a CCIP, applied five defined stimuli (verbal, electrical tetanus, trapezius muscle squeeze, laryngoscopy, and intubation) while observing purposeful movement response during induction of anesthesia with thiopental.

The purpose of the current study was to extend the concepts of anesthetic depth assessment for intravenously administered drugs by applying defined noxious stimuli when constant or pseudosteady-state plasma concentrations were obtained in a chronically instrumented rodent animal model allowing electroencephalogram (EEG) recording as described previously.[18] This was done for the intravenous anesthetic thiopental, thus allowing comparison of these methods and concepts from humans[14] to rodents. Additionally, the relationship of clinical depth of anesthesia in the rodent was compared to the EEG changes that were measured concurrently.

Methods

The Stanford University Animal Use and Care Committee approved all studies involving live animals. Rats were instrumented with chronically implanted vascular catheters and EEG electrodes as previously described.[18] Animals were randomly assigned to receive different target pseudosteady-state plasma concentrations of thiopental.[17]

Animal Care

Chronic Instrumentation of the Rat. Male Wistar rats (Sprague-Dawley, Indianapolis, Indiana) weighing between 300 and 400 g and aged 3–4 months were used. EEG electrodes, and arterial and venous cannulas were implanted as described previously by our laboratory.[18] The venous catheter was used to infuse thiopental and to compensate for fluid losses. The arterial catheter was used for hemodynamic recording, to monitor blood gases, and to obtain blood samples for plasma thiopental concentration determination.

Behavioral Adaptation. Animals were conditioned to the laboratory environment and experimental con-

ditions during at least three training sessions, each occurring on separate days. Each training session lasted 30 min, during which the EEG recording cable was attached to the rat and the animal was allowed to move freely within its cage. Training sessions promoted consistent baseline hemodynamic and EEG measurements and increased the ease of handling the awake instrumented animal.

Clinical Care and Physiologic Assessment. During each study, arterial blood gases were measured periodically. Arterial blood gases were determined from 40-μl blood samples in split capillary mode with a Corning model 170 blood gas analyzer (Corning Medical and Scientific, Medfield, MA). Animal lungs were mechanically ventilated with a positive-pressure, small animal ventilator (Harvard Instruments, model 50-1908, South Natick, MA) whenever thiopental plasma concentrations were targeted to 40 μl/ml or greater. Ventilation was performed using a custom-designed face mask. This technique can maintain adequate ventilation while obviating the need for tracheal intubation. The animals received 5% dextrose in water at a rate of 2.75 $ml \cdot h^{-1} \cdot kg^{-1}$ body weight to compensate for urinary and insensible water losses. This infusion rate, determined from previous studies, maintains anesthetized rats at a constant weight. A brief infusion of saline equal to 2.5 times the sample volume of blood was administered after any blood sample to further account for volume losses. Hematocrit values remained above 36% in all experiments. Body temperature was maintained between 37°C and 38°C throughout the experiments using a water-controlled heating pad (K-Module K-20, Baxter Inc., Valencia, CA), blankets, and, if necessary, rheostat-controlled warming lamps.

Blood pressure waveform was recorded from the arterial catheter using a model MS20 transducer (Electromedics, Inc., Englewood, CA), which was amplified and recorded using a Grass P7D polygraph (Grass Instruments, Quincy, MA). The mean arterial blood pressure, heart rate, and EEG were recorded as described previously.[18] We used EEG electrode pairs with an impedance of less than 5000 Ω. The EEG was recorded on an Accutrace EEG machine (Beckman Instruments, Palo Alto, CA) with a 50-Hz low pass and a 0.5-Hz high pass filter. The EEG was stored on a FM tape recorder (Vetter Co, Phillipsburg, PA) for off-line analysis with a LifeScan aperiodic EEG analyzer (Neurometrics Inc, San Diego, CA).[18,19]

Applied Methods

Computer-controlled Infusion Pump. A CCIP was used to rapidly attain and then maintain constant arterial plasma thiopental concentrations. The thiopental pharmacokinetic parameters used to drive the CCIP were reported previously.[17]

Nociceptive Stimuli. Pilot experiments were performed to find useful nociceptive stimuli with easily observed responses that covered the full range of anesthetic depth: light sedation to deep unconsciousness. Most methods are standard but some have been modified by us. The various nociceptive stimuli, devices needed to implement the stimuli, and the observed clinical responses are detailed in table 1. Unprovoked and provoked righting reflex have been used frequently in experimental pharmacologic studies.[20] The unprovoked righting reflex required the rat to spontaneously right itself within 15 s. The provoked righting reflex, using a hemostat stimulus on the tail, was performed whenever the unprovoked righting reflex was not present. We used noise stimulus as a measure of the startle reflex.[21] Hand clapping was used to test for this reflex.

An alligator clip (10 AMP, Type 85, length $2\frac{1}{8}''$ with jaws that maximally open to $\frac{5}{16}''$, Newark Electronics, Dublin, CA) was used for tail clamping. We monitored two responses by visual inspection: (1) movement of extremities or the head, and (2) abdominal muscle contraction. The abdominal muscle contraction response was present for quite some time after disappearance of purposeful head/extremity movement response and appeared to be a unique measure of drug effect. Movement response of the extremities or head to tail clamping is well established, originally introduced by Eger and coworkers for their MAC studies of anesthetic agents.[4] A separate noxious stimuli tail pressure was applied with an analgesiameter (Analgesymeter, Ugo Basile, Varese, Italy).[22] It has been described in the Randall-Selitto test in which the pressure response of the paw is tested for analgesic drugs. We modified this method and applied a constant pressure to the tail at the maximum possible pressure of the device: 2 kg/cm^2. This was achieved by using five lead plates (total weight of 445 g), which replaced the factory-provided weights. The rat's tail was placed on a platform of polytetrafluorethylene and a 0.7-mm pressure plate was applied. The clinical responses observed for the tail pressure stimulus were identical to those for the tail clamping stimulus. Corneal stimulus has been used in animals to test anesthetic depth.[23] Corneal reflex was tested by stroking the cornea lightly with a

Table 1. Applied Nociceptive Stimuli and Recorded Responses

Procedure	Stimulus	Response
Unprovoked righting reflex[20]	Rat placed on its side in the cage	Posturing on all 4 feet within 15 s
Provoked righting reflex[20]	Rat placed on its side Gentle pressure applied closing a hemostat to its first rachet	Posturing on all 4 feet within 15 s
Noise[21]	Clapping of open hands manually	Forceful contraction of the spine muscles
Tail clamp[4]	Alligator clip applied on the middle third of the rat tail for a maximum of 60 s	Forceful movement of the extremities or the head
Tail pressure[22]	A pressure plate 0.7 mm in diameter placed on the middle third of the rat tail Pressure of 2 kg/cm^2 applied until response, max 60 s	Forceful movement of the extremities or the head
Tail pressure[22]	Pressure plate (0.7 mm in diameter) placed on the middle third of the tail Pressure of 2 kg/cm^2 applied for a maximum of 60 s	Abdominal muscle contraction
Tail clamp[4]	Alligator clip applied on the middle third of the tail for a maximum of 60 s	Abdominal muscle contraction
Corneal reflex[23]	Gauze applied in the lateral corneal edge	Blinking
Tracheal intubation[24]	16-G intubation catheter placed into the trachea	Movement of extremities, head, or contraction of abdominal muscles

few fibers of a cotton swab and observing the presence or absence of a blink reflex. To ensure that the eyes did not dry we regularly applied 0.9% saline solution.

Tracheal intubation[24] was performed at the end of each experiment when we had confirmed that the arterial blood gases were normal. We used a modified nasal speculum as a rodent laryngoscope. A 12-V grain of wheat lamp (Wathers model 800-743-102, Milwaukee, WI) was secured to the inner surface of the left blade 8 mm from the tip. This permitted illumination of the pharynx, epiglottis, and trachea during laryngoscopy. The endotracheal cannula was made by cutting a 14-G polytetrafluorethylene intravenous catheter to a length of 6.4 cm. A blunt wire was used as a stylet to keep the cannula rigid during insertion into the trachea. The rat was placed on its back and held by an assistant. The response was considered positive if the rat showed movement of extremities, head, or abdominal muscles. Correct tube placement was checked by inspecting that bilateral movement of the thorax occurred with manual ventilation after end of the experiment. The intubation was completed within 20 s. The success rate was 24 out of 26 rats.

Analytic Chemistry. The high-performance liquid chromatographic method was developed in our laboratory to assay plasma thiopental concentrations in the rat.[25] This assay requires 50 μl plasma at thiopental plasma concentrations of 1–10 μg/ml and 20 ml for concentrations greater than 10 μg/ml. Within-day and between-day variability is less than 5% at plasma thiopental concentrations greater than 1 μg/ml. All samples were analyzed within 6 weeks of collection to ensure that no degradation occurred because of the storage process.[25] Measured plasma concentrations represented total (protein bound and unbound) thiopental. Whereas the free or unbound thiopental is the pharmacologically active moiety, in the homogeneous rodent population we studied, the degree of variation in protein binding is minimal and unlikely to change interpretation of the data.

Study Design

Achieving Pseudosteady-state Plasma Thiopental Concentrations. Each rat was randomly assigned to one of four schedules of target plasma thiopental concentrations (7, 20, and 60 μg/ml (A); 10, 40, and 60 μg/ml (B); 14, 40, and 100 μg/ml (C); or 24, 50, and 60 μg/ml (D); figs. 1 and 2 and table 2). Each target concentration was maintained for a minimum of 40 min. Whenever animal lungs were mechanically ventilated, arterial blood samples (40 μl) were drawn to determine arterial blood gases. If gases were abnor-

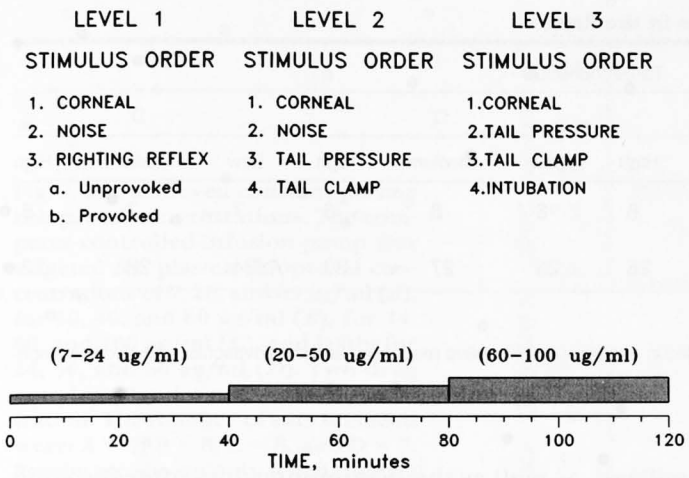

Fig. 1. An overview of the study design. The computer-controlled infusion pump maintained each target concentration for a minimum of 40 min. Target levels were 7, 20, and 60 μg/ml (A); 10, 40, and 60 μg/ml (B); 14, 40, and 100 μg/ml (C); or 24, 50, and 60 μg/ml (D). At each occasion, the nociceptive stimuli were repeated three times with 1–3-min intervals. Intubation was performed only once.

mal, ventilation was adjusted, and gases were reconfirmed. Two blood samples (250-μl each) were obtained for the determination of plasma thiopental concentrations. At higher target concentrations, these samples were drawn only when arterial blood gases were confirmed to be normal. Blood samples for thiopental determination were taken close to the application of nociceptive stimuli. Because the equilibration half-time between blood concentration and EEG drug effect is 1.3 min in the rodent, 6–7 min are required to establish a constant concentration-effect relationship after pseudosteady-state plasma thiopental concentrations have been achieved.[18]

Application of Nociceptive Stimuli. The sequence for applying noxious stimuli is shown in figure 1. Stimuli were repeated three times in a row except for intubation, which was performed only once. The interval between each stimulus was determined by how rapidly behavior and cardiovascular parameters returned to baseline, prestimulus values. In our study, rats required at least 1 min to return to baseline for righting reflex, noise, and corneal reflex, thus we chose 2 min as the interval before repeating stimuli for these measures. For tail clamping and tail pressure tests, the animals returned to baseline within 2 min, thus for these measures, we waited at least 3 min between application of stimuli. No stimuli was applied unless EEG effect intensity remained relatively constant.

In general, the following clinical behavior was found relative to targeted thiopental plasma concentrations. At target plasma thiopental concentrations of 7–14 μg/ml, the rats were sedated, reacted to external stimuli, and were able to ambulate. At target plasma thiopental concentrations of 20–24 μg/ml, they were sedated and did not move until nociceptive stimuli were applied. At target concentrations of 40 μg/ml or greater, the rats appeared anesthetized and could not maintain adequate ventilation, therefore, they were mechanically ventilated without intubation to maintain normal blood gases.

Data Analysis and Statistical Methods

Statistical Methods. We used mean and 95% confidence intervals, linear regression analysis, and chi-square analysis when appropriate (SYSTAT).‖ Logistic regression analysis was performed to model concentration-effect relationships. At each of the nine target plasma thiopental concentrations (7, 10, 14, 20, 24, 40, 50, 60, and 100 μg/ml), the median performance error was calculated as described previously.[17]

Methods for Pooling of Data (Stimulus-Response Pairs). It is not possible to obtain a sufficient number of effect measurements in each rat to characterize the plasma-thiopental concentration *versus* response in each individual animal. Each experimental session had to be limited in time, 3 h, to ensure that stable physiologic conditions were maintained, a prerequisite for stable thiopental concentration-effect data. We pooled data from all animals when performing concentration-effect modeling. We excluded any effect measurements from the analysis if the animal was hypoxic, or showed abnormal blood *p*H or P_{CO_2} values. The number of observations used in our analysis of each concentration-effect relationship are summarized in table 3. Each experimental session was limited to 3 h. During this period, we were generally able to maintain stable physiologic conditions (body temperature, fluid balance, normal blood gases). Censoring data based on abnormal physiologic state ensured the recovery of reliable concentration-effect relationships, unperturbed with physiologic artifact.

Logistic Regression Analysis. Each quantal (present or absent) response to nociceptive stimulus and the measured plasma thiopental concentration were pooled for all animals. Logistic regression analysis was

‖ Wilkinson L: SYSTAT: The System for Statistics. Evanston, SYSTAT, 1988.

Table 2. Predictive Performance of the Computer-driven Infusions in the Rats

	Target Schedule											
	A			B			C			D		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Rats (n)*	7	7	5	8	6	6	8	8	6	7	7	6
Pump performance (MDPE, %)	19	28	27	29	30	26	25	27	30	24	28	26

MDPE = median prediction error.

* Number of rats corresponds to those with normal blood gases and therefore included in analysis of nociceptive responses and cardiovascular and electroencephalographic effects.

performed using the NONLIN module of SYSTAT‖ to estimate a function describing the probability of response for each stimulus. Regression analysis was performed using a maximum likelihood estimator. The steady-state thiopental concentration corresponding to a 50% probability of response, Cp_{50}, and the 95% confidence interval around this concentration, were calculated during the logistic regression analysis. The number of responses included for each stimulus-response variable are the maximum possible that could be observed and are shown in table 3.

Blood Pressure and Heart Rate Responses to Nociceptive Stimuli. Mean arterial blood pressure and heart rate were recorded whenever tail clamp or tail pressure stimuli were applied. The mean hemodynamic values during 2 min preceding the stimulus were calculated, as well as the maximum values after the stimulus was applied. Linear regression analysis was used to correlate pseudosteady-state thiopental concentrations *versus* change (%) of mean arterial blood pressure and heart rate, respectively, for each of the two stimuli using a pooled-data analysis. Data were included when no movement responses were seen.

Electroencephalogram Measurements. The Life Scan EEG monitor employs an EEG analysis technique called aperiodic analysis. This technique has been used extensively in our laboratory and others to establish barbiturate and benzodiazepine concentration *versus* EEG effect relationships in the rat.[18,19,26,27] The total number of EEG waves/s from 0.5 to 30 Hz was used as a measure of thiopental EEG drug effect on the central nervous system. Using this measure of EEG effect

Table 3. Calculated Plasma Thiopental Concentrations for 50% Probability to Respond to Nociceptive Stimuli (Cp_{50} Values) and Corresponding Electroencephalographic Effects

Event	Rats (n)	Valid Observations (n)	C_{p50} Value (μg/ml)	95% Confidence Interval (μg/ml)	Electroencephalographic Effect (waves/s)
Baseline electroencephalogram	30	56*	—	—	11.2
Unprovoked righting reflex	30	110†	15.9	15.1–16.6	15.2
Provoked righting reflex	30	120†	21.4	20.2–22.7	18.1
Noise	30	180†	31.3	29.7–33.0	12.0
Limb movement response to tail clamping	30	168‡	38.3	36.1–40.4	9.5
Limp movement response to tail pressure	30	171‡	39.2	37.1–41.3	9.1
Abdominal muscle contraction response to tail pressure	30	171‡	52.5	50.0–55.0	5.5
Abdominal muscle contraction response to tail clamping	30	168‡	56.1	50.0–62.2	5.2
Corneal blinking response	30	245‡	60.0	56.6–63.4	4.0
Extremity, head, or abdominal muscle contraction response to inbutation	24	24§	67.7	59.2–76.1	2.2

*†‡§ maximum number of observations (60, 120, 180, and 30, respectively) if all tests had been carried out and with valid results.

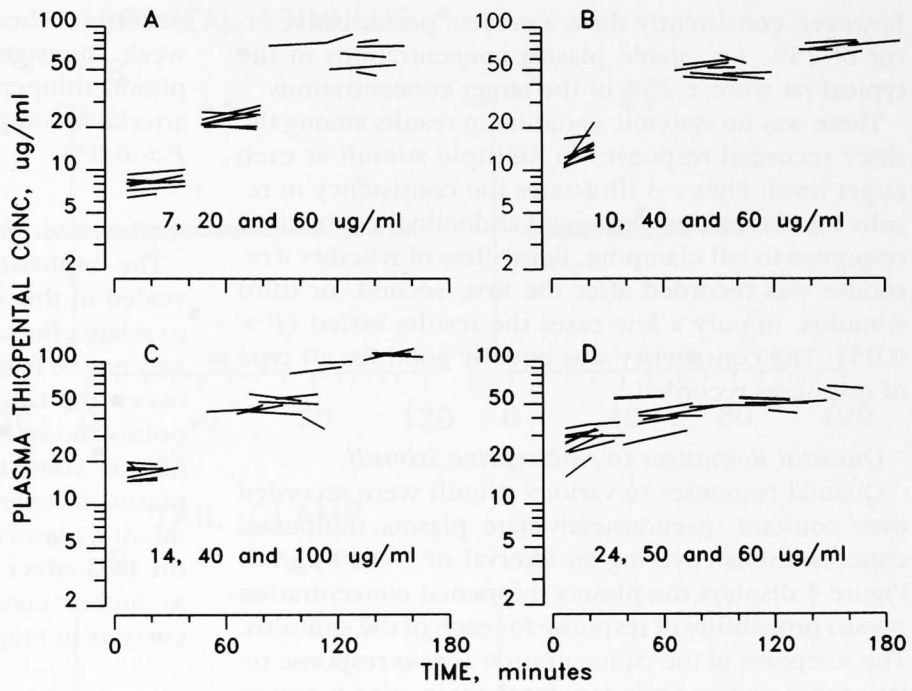

Fig. 2. The achieved constant plasma thiopental concentrations. The computer-controlled infusion pump was targeted for plasma thiopental concentrations of 7, 20, and 60 μg/ml (*A*), for 10, 40, and 60 μg/ml (*B*), for 14, 40, and 100 μg/ml (*C*), and lastly for 24, 50, and 60 μg/ml (*D*). Two drug samples were drawn at each concentration. The number of rats included were: A = 7, B = 8, C = 8, and D = 7. Results are shown only when the rats had normal arterial blood gases.

we have previously quantified k_{e0}, the rate constant characterizing the time delays between arterial thiopental concentrations and EEG effect, as well as the biphasic thiopental concentration EEG effect relationship in rodents.[18]

Two baseline EEG effect values (mean results for 5 min each) were calculated in the individual rat. For each measured plasma thiopental concentration, we calculated the corresponding EEG-effect for the period within 2 min of when drug samples were collected. We usually had eight pairs of concentration-effect data for each rat. We used results only when stable physiologic conditions (blood gases as well as hemodynamics) were present. The data were pooled to characterize the relationship between plasma thiopental drug concentration and EEG effect. For each nociceptive response, we calculated the mean number of EEG waves/s at its corresponding Cp_{50} value. We included EEG results (based on observations from 6–10 rats) from the plasma thiopental concentration interval defining the Cp_{50} value and its 95% confidence interval.

Repeated Stimuli Assessment

It can be argued that the rat will show adaptive changes after experiencing a series of repeated noxious stimuli. To test this hypothesis, we examined the pairs of (A) the first *versus* second, (B) second *versus* third, and (C) the first *versus* third response to each stimuli. A positive response was assigned 0, and no response 1. We then subtracted the A, B, and C-pairs. We analyzed the relationship between plasma thiopental concentration and the difference values for each pair by stimulus to examine the consistency of our data relative to the repeated application of stimuli. Statistical differences were evaluated using the chi-square test.

Results

A total of 30 rats were studied. Data from each of these animals were used, if normal blood gases and stable ventilation were maintained. At the high target concentrations (60 and 100 μg/ml), some of the data from approximately every fifth animal had to be excluded (table 3) because ventilation, blood gases, and oxygenation were inadequate during a major portion of the time that the target concentration was maintained.

Overall Performance of Applied Methods

Figure 2 shows that the CCIP maintained stable pseudosteady-state plasma thiopental concentrations for the study period of 120–160 min, the time required to complete all stimuli/response measurements. We did not have systemic bias in pump performance at low, medium, or high target levels (table 2). The pump did,

however, consistently show a median performance error of 25%, *i.e.,* stable plasma concentrations in the typical rat were ± 25% of the target concentrations.

There was no systemic variation in results among the three recorded responses to multiple stimuli at each target level. Figure 3 illustrates the consistency in results for corneal responses and abdominal contraction responses to tail clamping. Regardless of whether a response was recorded after the first, second, or third stimulus, in only a few cases the results varied ($P > 0.05$). The consistency was equally good for all type of responses recorded.

Quantal Responses to Nociceptive Stimuli

Quantal responses to various stimuli were recorded over constant, pseudosteady-state plasma thiopental concentrations covering an interval of 7–120 μg/ml. Figure 4 displays the plasma thiopental concentration *versus* probability of response for each of the stimulus. The steepness of the concentration *versus* response relationship creates a window for the transition between response and no response for each stimulus. This window is narrow for responses to unprovoked and provoked righting reflex but wider for other effects, in particular, the responses to the corneal stimuli and intubation. The calculated Cp_{50} values have confidence intervals that are no more than 10% of the estimated Cp_{50} value, except for abdominal contraction response to tail clamping and intubation (fig. 4 and table 3). In the latter two cases, the corresponding figures are approximately 25% for each of the two effects (fig. 4 and table 3). Figure 5 presents the probability function curves in order of increasing degree of noxious stimuli. It is clear that the responses to unprovoked and provoked righting reflexes as well as noise effects statistically separate from more intense, noxious stimuli. Limb movement responses showed overlapping 95% confidence intervals for tail clamping and tail pressure stimuli. These two response curves separate from noise and provoked/unprovoked and from the curves characterizing deep levels of anesthesia (corneal reflex, intubation). The Cp_{50} values for tail clamping/abdominal contraction, corneal reflex, and intubation overlap as evaluated using the 95% confidence intervals.

Cardiovascular Responses to Nociceptive Stimuli

Figure 6 shows plasma thiopental concentration *versus* percentage change in mean arterial blood pressure and heart rate after tail clamping and tail pressure in rats in which no movement response to these stimuli occurred. There is no clear relationship except for a weak, nonsignificant negative correlation between plasma thiopental concentration and increase of mean arterial blood pressure after tail clamping ($r = -0.26$, $P > 0.05$).

Electroencephalogram Effects

The biphasic concentration-effect relationship revealed in this study and others,[18,28] confounds efforts to relate clinical effect measures to the EEG (fig. 7). It may not be feasible to define a unique relationship between the Cp_{50} values of the lighter anesthetic endpoints and an EEG effect measure such as waves/s. The EEG is consistently isoelectric (*i.e.,* 0 waves/s.) at plasma thiopental concentrations greater than 80 μg/ml. At a plasma thiopental concentration of 40 μg/ml, the EEG-effect has returned to baseline 11.2 waves/s. At higher concentrations, the thiopental-EEG effect curve is monophasic and continuously decreasing.

Discussion

This study demonstrates that a full range of thiopental depth of anesthesia, as defined from light sedation to pronounced ventilatory depression requiring assisted ventilation, can be characterized by a series of defined noxious stimuli and observed responses. The unprovoked and provoked righting reflexes yield surprisingly consistent results as demonstrated in the narrow confidence intervals. Our results agree with the long tradition of using these tests as measures of central nervous system effects of hypnotic depressants including barbiturates.[20]

We used the presence or absence of abdominal muscle contraction as a response measure to tail clamping and tail pressure. Abdominal muscle contraction response appears to be a measure of a deeper level of anesthesia because it has a higher Cp_{50} than extremity movement response to the same stimuli (tail clamping or pressure). Extremity movement response is similar to the measured effect that Eger and coworkers used to define the MAC concept.[6–8] Consequently, it should be possible to compare the confidence intervals of the various MAC values obtained for different inhalational anesthetics with the Cp_{50} values we obtained for thiopental. Such comparisons will help to elucidate possible differences in the interindividual variability in response between inhalational anesthetics and intravenously administered hypnotic agents. The confidence

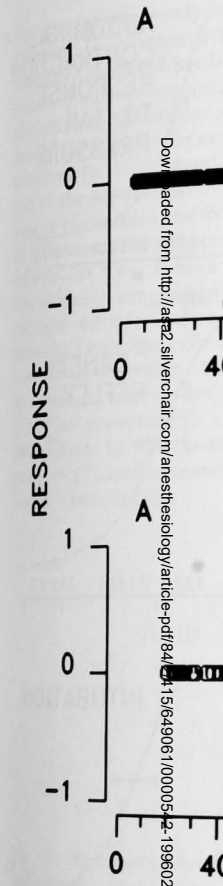

Fig. 3. Response consisten
positive response corresp
and *C*) are calculated. The
thiopental concentration
shows the relationship be
responses from 27 rats).

interval of the Cp_{50} va
tracheal intubation wa
plained by a smaller n
only one intubation w
the intrinsic difficultie
bation in the rat in a st
variability. We found
(heart rate and mean ar
useful for monitoring th
of thiopental.

The major purpose of
full range of anesthetic
isoelectric EEG by apply
uli and recording the n

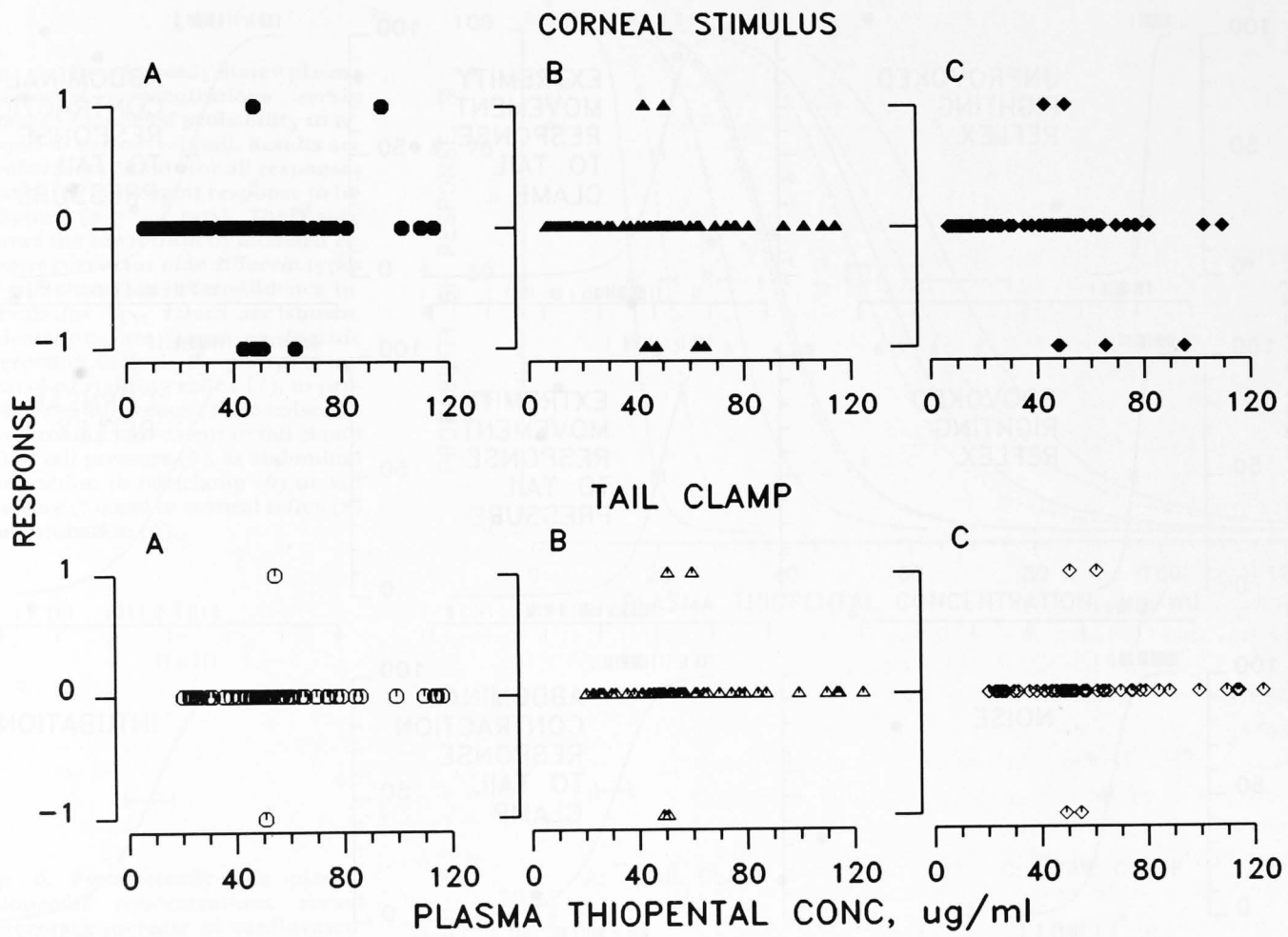

Fig. 3. Response consistency to corneal stimulus and tail clamping. Each rat had three stimuli applied in a row (*A*, *B*, and *C*). A positive response corresponds to 1, and a negative response to 0. The subtracted values between (*A* and *B*), (*A* and *C*), and (*B* and *C*) are calculated. The pooled results are shown in (*A*), (*B*), and (*C*), respectively. The *top* shows pseudosteady-state plasma thiopental concentration *versus* response consistency for the corneal stimulus (triplete responses from 28 rats). The *bottom* shows the relationship between plasma thiopental concentration and abdominal contraction response to tail clamping (triplete responses from 27 rats).

interval of the Cp_{50} value for movement responses to tracheal intubation was relatively wide. This is explained by a smaller number of observations because only one intubation was performed per rodent. Also, the intrinsic difficulties in performing tracheal intubation in the rat in a strictly consistent way could add variability. We found that cardiovascular parameters (heart rate and mean arterial blood pressure) were not useful for monitoring the intensity of anesthetic depth of thiopental.

The major purpose of this study was to describe the full range of anesthetic depth from light sedation to an isoelectric EEG by applying multiple nociceptive stimuli and recording the multiple different responses. It is not feasible to get sufficient number of pairs of stimulus-response for modeling of the concentration-effect relationship in a single rodent. It was necessary to use a pooled-data analysis because we used a random design and had a relatively large number of animals. Because we could not demonstrate signs of behavioral adaptation in response between the series of repeated stimuli, this confounding factor can be excluded.

Because of biphasic nature of the EEG response, it was not possible to use a pharmacokinetic/pharmacodynamic model to relate the EEG-effects to plasma concentrations. Therefore, it is not feasible to define a unique relationship between Cp_{50} values and the number of waves derived from the raw EEG recordings. The

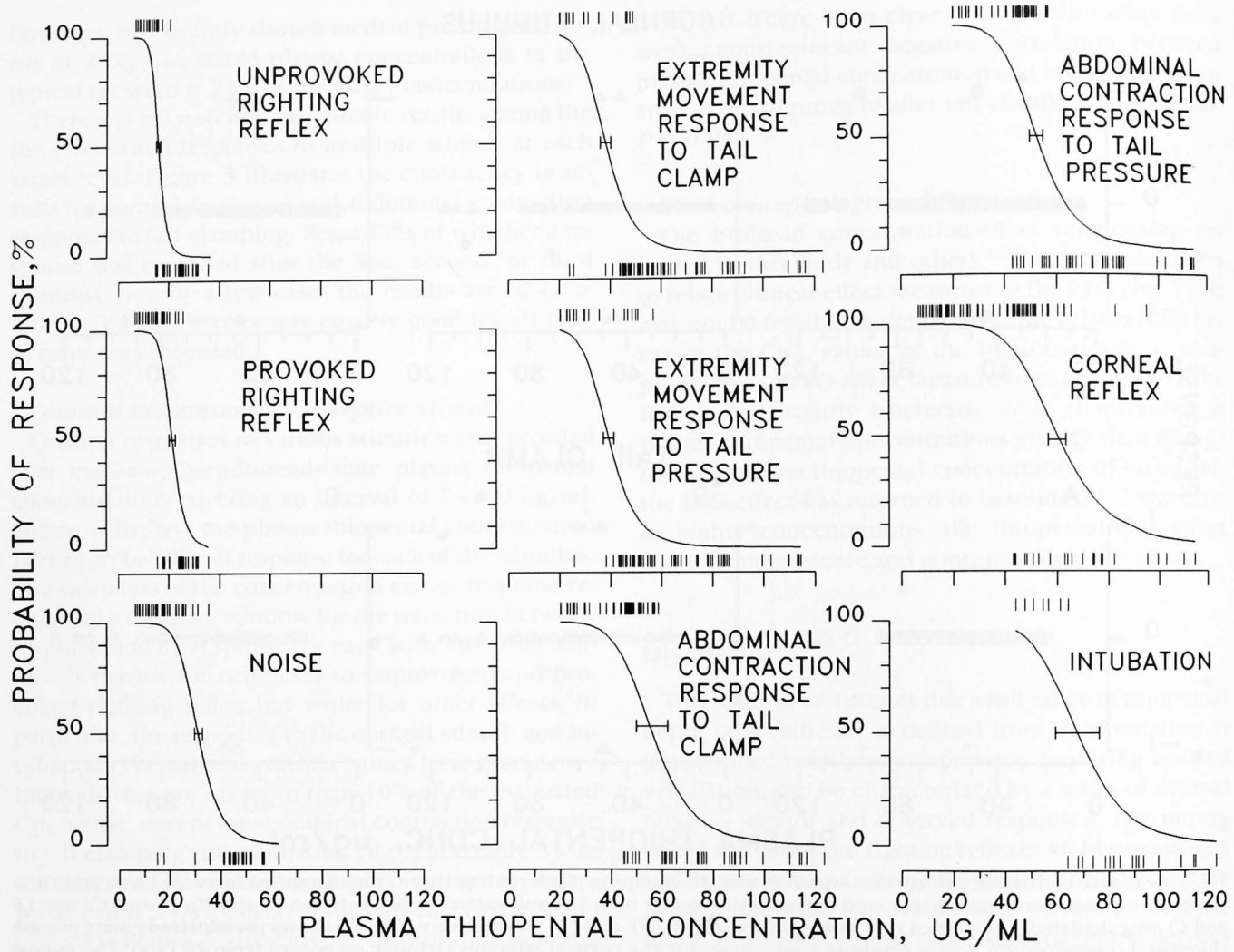

Fig. 4. Pseudosteady-state plasma thiopental concentrations *versus* probability of responding to nociceptive stimuli. Individual responses from 30 rats are shown as vertical bars located just below the 0% and above the 100% probability mark, respectively. The curves are modeled responses from all individual rats. The Cp_{50} values (plasma concentration for 50% chance to respond) are derived with logistic regression analysis. The 95% confidence interval is given.

difficulties with biphasic pharmacodynamic modeling with this has been discussed extensively.[14,18] However, starting at a plasma thiopental concentration of 40 μg/ml, the number of waves/s returns to an awake baseline value of 11.2. At higher concentration, there is a direct monophasic relationship that can be used to relate the number of waves/s and the probability to respond to extremity movement responses to tail clamping, to abdominal movement response induced by either tail pressure or tail clamping. The same is true for the response to the corneal reflex and the movement response to intubation (fig. 6 and table 3). The biphasic nature of the EEG-response to increasing thiopental concentrations was noticed in rabbits by Soltero and coworkers in the 1950s.[28] They showed the feasibility of using EEG as a clinical measure for the administration of ether anesthesia in patients.

Our results are in close agreement with experimental studies of thiopental anesthesia in humans using a similar approach by Hung *et al.* in our laboratory.[12] In these human studies, verbal responses to repeated questions showed a Cp_{50} value of 15.6 μg/ml, which approximates the Cp_{50} value for the unprovoked righting reflex in the rat. Humans demonstrate a Cp_{50} value of 39.8 μg/ml for movement response to trapezius muscle squeeze, which is similar to our results for ex-

Fig. 5. Pseudosteady-s
thiopental concentrati
chance of modeled prob
spond to various stimuli
pooled from 30 rats for a
except for movement re
tubation (n = 24 rats)
shows the separation of
sponse curves for nine di
of stimulus. The 95% co
tervals for Cp_{50} values
Calculations are based
regression analysis. Resp
provoked righting reflex
voked righting reflex (2)
as extremity movement
(4) or tail pressure (5), a
contraction to tail clam
pressure (7), and to corn
and intubation (9).

Fig. 6. Pseudosteady-st
thiopental concentrati
percentage increase of
lar parameters after tail c
tail pressure, respectively
from rats showing no m
sponses with these stimu
centage increase of mo
pressure and heart rate ar
from a 2-min baseline va
maximum increase in
Pooled results are shown.
n = 25 rats and 55 observ
n = 26 and 62 observati
tively, show thiopental
tion *versus* percent increa
arterial blood pressure
clamping and tail pressu
tively. (*C*) and (*D*) n = 25
observations, and n = 26
servations, respectively,
crease of heart rate after
ing and tail pressure, re
The straight lines show r
linear regression analysis
Inadequate oxygenation o
blood gases explain wh
highest target concentra
times had to be excluded.
rats had frequently prese
responses could be record

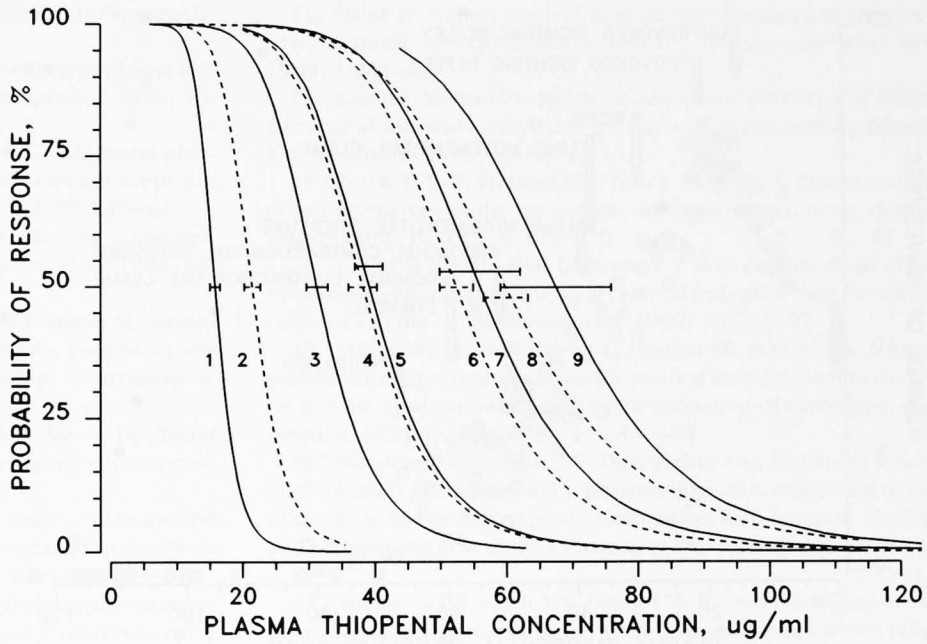

Fig. 5. Pseudosteady-state plasma thiopental concentrations *versus* chance of modeled probability to respond to various stimuli. Results are pooled from 30 rats for all responses except for movement response to intubation (n = 24 rats). The figure shows the separation of modeled response curves for nine different types of stimulus. The 95% confidence intervals for Cp_{50} values are shown. Calculations are based on logistic regression analysis. Responses to unprovoked righting reflex (1), to provoked righting reflex (2), to noise (3), as extremity movement to tail clamp (4) or tail pressure (5), as abdominal contraction to tail clamp (6) or tail pressure (7), and to corneal reflex (8) and intubation (9).

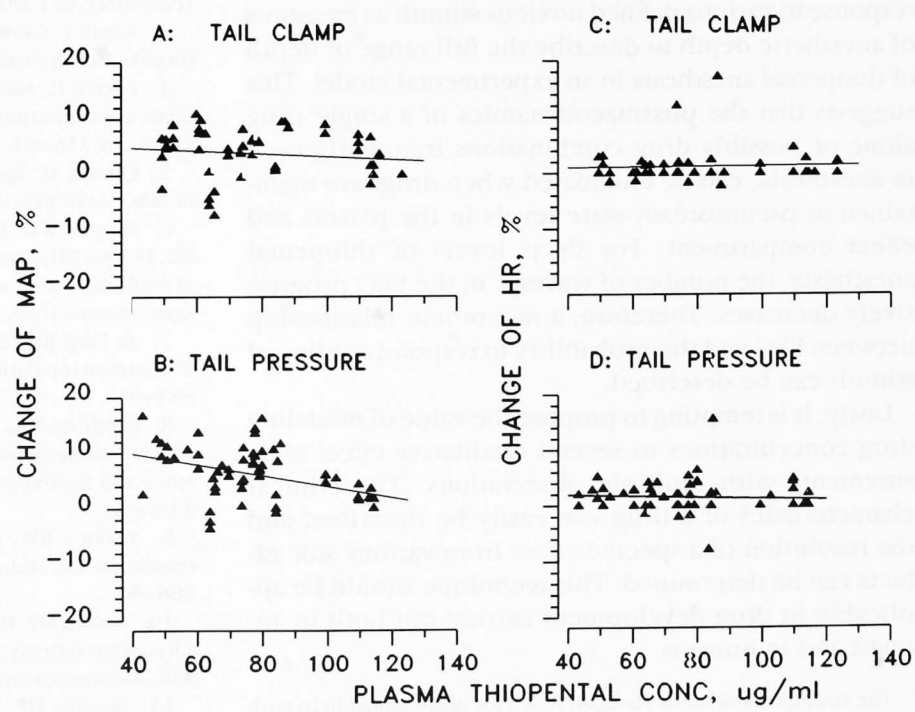

Fig. 6. Pseudosteady-state plasma thiopental concentrations *versus* percentage increase of cardiovascular parameters after tail clamping and tail pressure, respectively. Results are from rats showing no movement responses with these stimuli. The percentage increase of mean arterial pressure and heart rate are calculated from a 2-min baseline value and the maximum increase in each rat. Pooled results are shown. (*A*) and (*B*), n = 25 rats and 55 observations, and n = 26 and 62 observations, respectively, show thiopental concentration *versus* percent increase of mean arterial blood pressure after tail clamping and tail pressure, respectively. (*C*) and (*D*) n = 25 rats and 55 observations, and n = 26 and 62 observations, respectively, show % increase of heart rate after tail clamping and tail pressure, respectively. The straight lines show results with linear regression analysis. Comment: Inadequate oxygenation or abnormal blood gases explain why data at highest target concentration sometimes had to be excluded. Thus, data from 25–26 rats only were used. At lower target concentrations of such excluded rats, the rats had frequently presented movement and responses and thereby no meaningful heart rate or mean arterial blood pressure responses could be recorded.

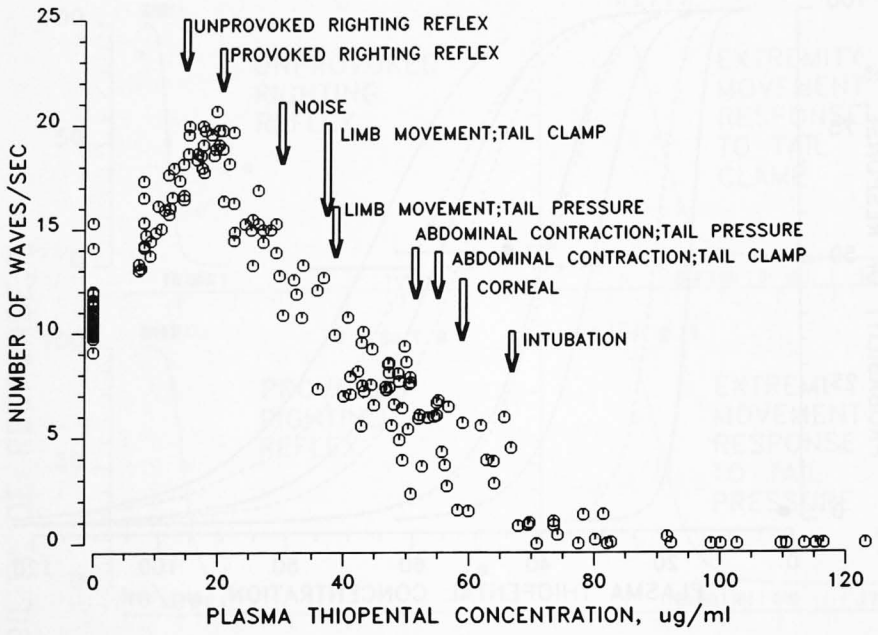

Fig. 7. EEG effect *versus* pseudosteady-state plasma thiopental concentration in a pooled analysis of results from 30 rats. In each rat, two baseline EEG-values and a maximum of two results from each of the three steady-state levels are included (149 of maximum 180 EEG results). The modeled Cp_{50} values for the different stimuli are shown.

tremity movements to either tail pressure or tail clamping. Finally, in the human study, change of either heart rate or blood pressure was a poor marker for depth of thiopental anesthesia (unpublished observations).

In summary, we have used presence or absence of response to various defined noxious stimuli as measures of anesthetic depth to describe the full range of depth of thiopental anesthesia in an experimental model. This suggests that the pharmacodynamics of a single drug alone or possibly drug combinations frequently used in anesthesia, can be elucidated when drugs are maintained at pseudosteady-state levels in the plasma and effect compartment. For deep levels of thiopental anesthesia, the number of waves/s in the EEG progressively decreases. Therefore, a monotonic relationship between EEG and the probability to respond to clinical stimuli can be described.

Lastly, it is tempting to propose the value of modeling drug concentrations to several qualitative effect measurements with multiple observations. The clinical characteristics of a drug can easily be described and the resolution of a specific effect from various side effects can be determined. This technique should be applicable in drug development carried out both in animals and in humans.

The authors thank Sandy Harapat, B.Sc., for professional help with the analysis of thiopental plasma concentrations, and Georgette Bozovich and Margareta Fogelstrom, for secretarial and editorial assistance.

References

1. Stanski DR: Monitoring depth of anesthesia, Anesthesia. 3rd edition. Edited by Miller RD. New York, Churchill-Livingston, 1989, pp 1001–29

2. Prys-Roberts C: Anaesthesia: A practical or impossible construct? (editorial). Br J Anaesth 1987; 59:1341–5

3. Kissin I: General anesthetic action: An obsolete notion? (editorial). Anesth Analg 1993; 76:215–8

4. Eger E II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: a standard of anesthetic potency. ANESTHESIOLOGY 1965; 26:756–63

5. Quasha AL, Eger E II, Tinker JH: Determination and applications of MAC. ANESTHESIOLOGY 1980; 53:315–34

6. Stevens WD, Dolan WM, Gibbons RT, White A, Eger EI, Miller RD, DeJong RH, Elashoff RM: Minimum alveolar concentrations (MAC) of isoflurane with and without nitrous oxide in patients of various ages. ANESTHESIOLOGY 1975; 42:197–200

7. de Jong RH, Eger EI II: MAC expanded: AD_{50} and AD_{95} values of common inhalation anesthetics in man. ANESTHESIOLOGY 1975; 42: 384–9

8. Stoelting RK, Longnecker DE, Eger EI II: Minimum alveolar concentrations in man on awakening from methoxyflurane, halothane, ether and fluroxene anesthesia: Mac awake. ANESTHESIOLOGY 1970; 33:5–9

9. Yakaitis RW, Blitt CD, Angiulo JP: End-tidal halothane concentration for endotracheal intubation. ANESTHESIOLOGY 1977; 47: 386–8

10. Roizen MF, Horrigan RW, Frazer BM: Anesthetic doses blocking adrenergic (stress) and cardiovascular responses to incision—MAC BAR. ANESTHESIOLOGY 1981; 54:390–8

11. Ausems ME, Hug CC Jr, Stanski DR, Burm AGL: Plasma concentrations of alfentanil required to supplement nitrous oxide anesthesia for general surgery. ANESTHESIOLOGY 1986; 65:362–73

12. Becker KE: Plasma levels of thiopental necessary for anesthesia. ANESTHESIOLOGY 1978; 49:192–6

13. Becker KE, Tonnesen AS: Cardiovascular effects of plasma levels of thiopental necessary for anesthesia. ANESTHESIOLOGY 1978; 49: 197–200

14. Hung RH, Varvel JR, Shafer SL, Stanski DR: Thiopental pharmacodynamics: 2. Quantitation of clinical and electroencephalographic depth of anesthesia. ANESTHESIOLOGY 1992; 77:237–44

15. Shafer SL, Siegel LC, Cooke JE, Scott JC: Testing computer-controlled infusion pumps by simulation. ANESTHESIOLOGY 1988; 68: 261–6

16. Buhrer M, Maitre PO, Hung OR, Ebling WF, Shafer SL, Stanski DR: Thiopental pharmacodynamics: 1. Defining the pseudo steady-state serum concentration-EEG effect relationship. ANESTHESIOLOGY 1992; 77:226–36

17. Gustafsson LL, Ebling WF, Harapat S, Osaki E, Stanski DR, Shafer SL: Plasma concentration clamping in the rat using a computer-controlled infusion pump. Pharm Res 1992; 9:800–7

18. Ebling WF, Danhof M, and Stanski DR: Pharmacokinetic and pharmacodynamic modeling of the electroencephalographic effects of thiopental in rats. J Pharmacokin Biopharm 1991; 19:123–44

19. Gregory TK, Pettus DC: An electroencephalographic processing algorithm specifically intended for analysis of cerebral electrical activity. J Clin Monit 1986; 2:190–7

20. Danhof M, Levy G: Kinetics of drug action in disease states. I. Effect of infusion rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J Pharmacol Exp Ther 1984; 229:1–7

21. Davis M: Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. Neurosci Biobehav Rev 1980; 4:241–63

22. Kissin I, Morgan PL, Smith LR: Anesthetic potencies of isoflurane, halothane, and diethylether for various end points of anesthesia. ANESTHESIOLOGY 1983; 58:88–92

23. Tervo T, Jo F, Huikuri KT, Toth I, Palkama A: Fine structure of sensory nerves in the rat cornea: An experimental nerve degeneration study. Pain 1979; 6:57–70

24. Cole DJ, Shapiro HM: Different 1.2 MAC combinations of nitrous oxide-enflurane cause unique cerebral and spinal cord metabolic responses in the rat. ANESTHESIOLOGY 1989; 70:787–92

25. Ebling WF, Mills-Williams L, Harapat SR, Stanski DR: A high-performance liquid chromatographic method for thiopental in twelve rat tissues: Application to physiologic modeling of barbiturate disposition. J Chromatogr 1989; 490:339–53

26. Mandema JW, Sansom LN, Dios-Vieitez MC, Hollander-Jansen M, Danhof M: pharmacokinetic-pharmacodynamic modelling of the EEG effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. J Pharmacol Exp Ther 1991; 257:472–8

27. Mandema JW, Kuch MT, Danhof M: In vivo modelling of the pharmacodynamic interaction between benzodiazepines which differ in their intrinsic efficacy. J Pharmacol Exp Ther 1992; 261:715–28

28. Soltero DE, Faulconer A Jr, Bickford RG: The clinical application of automatic anesthesia. ANESTHESIOLOGY 1951; 12:574–82