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 analogimeter, (6) corneal reflex, and (7) tracheal intubation. For tail clamping, tail pressure, and intubation, either purposefully extreme 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 C95, the plasma thiopental concentration with a 95% probability of no response.

Results: The following mean C95 values (95% confidence interval) were obtained: unprovoked righting reflex, 15.9 (15.2–16.6) 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 (37.4–41.3) μg/mL; tail pressure and abdominal muscle contraction, 52.5 (50.0–55.3) 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. 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.”

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In an editorial, Kissin \(^1\) 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 well established in humans and animals for the inhalational anesthetics using the minimum alveolar concentration (MAC) concept.\(^1\)\(^-\)\(^7\) 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\(_{\text{awake}}\), MAC\(_{\text{infusion}}\), and MAC\(_{\text{bar}}\), 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.\(^9\)

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 anesthetic concentrations.\(^15\)

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 \textit{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 \textit{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.\(^14\) 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 conditions during at least 2 h per day, for 5 days, during which time they were left unrestrained freely within its cage. During the 12 h of recording, the rats were housed inside a small cage and were fed and watered ad libitum.

#### Clinical Care and Monitoring

During each study, animals were housed individually and monitored periodically. Animals were fed a commercial diet and water ad libitum. Animals were placed in a temperature-controlled environment (Corning Medical Analysis, Corning, NY). The animals were placed in an isoflurane anesthetic chamber. The chamber was maintained at a constant temperature and humidity. The chamber was equipped with a heating pad (Fisher Scientific, Pittsburgh, PA), and a cooling unit for temperature control.

Blood pressure was continuously recorded with the LabMaster system (Honeywell, Melville, NY). The arterial catheter was used for blood pressure measurement. The EEG was recorded with a Medelec Sigma System (Medelec Systems Ltd., Stoke-on-Trent, UK). The EEG was recorded continuously during the study period and was analyzed using the Medelec Advanced Analysis System (Medelec Systems Ltd., Stoke-on-Trent, UK).

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dations 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·h⁻¹·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. 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).

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 previously reported.

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 righteous reflex required the drug to spontaneously right itself within 15 s. The provoked righteous reflex, using a hemostat stimulus on the tail, was performed whenever the unprovoked righteous reflex was not present. We used noise stimulus as a measure of the startle reflex. Hand clapping was used to test for this reflex.

An alligator clip (10 AMP, Type 85, length 2 1/4” with jaws that maximally open to 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 was well established, originally introduced by Egger and coworkers for their MAC studies of anesthetic agents. A separate noxious stimuli tail pressure was applied with an analgesiometer (Analgesimeter, Ugo Basile, Varese, Italy). 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/cm². 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 polytetrafluoroethylene 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. Corneal reflex was tested by stroking the cornea lightly with a...
Table 1. Applied Nociceptive Stimuli and Recorded Responses

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Stimulus</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprovoked righting reflex</td>
<td>Rat placed on its side in the cage</td>
<td>Posturing on all 4 feet within 15 s</td>
</tr>
<tr>
<td>Provoked righting reflex</td>
<td>Rat placed on its side</td>
<td>Posturing on all 4 feet within 15 s</td>
</tr>
<tr>
<td>Noise</td>
<td>Clapping of open hands manually</td>
<td>Forceful contraction of the spine muscles</td>
</tr>
<tr>
<td>Tail clamp</td>
<td>Alligator clip applied on the middle third of the tail</td>
<td>Forceful movement of the extremities or the head</td>
</tr>
<tr>
<td>Tail pressure</td>
<td>A pressure plate 0.7 mm in diameter placed on the middle third of the rat tail</td>
<td>Forceful movement of the extremities or the head</td>
</tr>
<tr>
<td>Tail pressure</td>
<td>Pressure plate (0.7 mm in diameter) placed on the middle third of the tail</td>
<td>Abdominal muscle contraction</td>
</tr>
<tr>
<td>Tail clamp</td>
<td>Alligator clip applied on the middle third of the tail</td>
<td>Abdominal muscle contraction</td>
</tr>
<tr>
<td>Corneal reflex</td>
<td>Gauze applied in the lateral corneal edge</td>
<td>Blinking</td>
</tr>
<tr>
<td>Tracheal intubation</td>
<td>16-G intubation catheter placed into the trachea</td>
<td>Movement of extremities, head, or contraction of abdominal muscles</td>
</tr>
</tbody>
</table>

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 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 lamp of wheat was placed 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 polytetrafluoroethylene 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 assess plasma thiopental concentrations in the rat. 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. Measured plasma concentrations represented total (protein bound and unbound) thiopental. Whereas the free or unbound thiopental is the pharmacologically active moiety, in the homogenous 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-
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<table>
<thead>
<tr>
<th>LEVEL 1</th>
<th>LEVEL 2</th>
<th>LEVEL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>STIMULUS ORDER</td>
<td>STIMULUS ORDER</td>
<td>STIMULUS ORDER</td>
</tr>
<tr>
<td>1. CORNEAL</td>
<td>1. CORNEAL</td>
<td>1. CORNEAL</td>
</tr>
<tr>
<td>2. NOISE</td>
<td>2. NOISE</td>
<td>2. TAIL PRESSURE</td>
</tr>
<tr>
<td>3. TAIL PRESSURE</td>
<td>3. TAIL CLAMP</td>
<td>4. INTUBATION</td>
</tr>
<tr>
<td>a. Unprovoked</td>
<td>b. Provoked</td>
<td></td>
</tr>
</tbody>
</table>

(7–24 ug/ml) (20–50 ug/ml) (60–100 ug/ml)

0 20 40 60 80 100 120

TIME, minutes

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 ug/ml (A); 10, 40, and 60 ug/ml (B); 14, 40, and 100 ug/ml (C); or 24, 50, and 60 ug/ml (D). At each occasion, the nociceptive stimuli were repeated three times with 1–3-min intervals. Intubation was performed only once.

normal, ventilation was adjusted, and gases were recon- stured. 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.

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 mea-

sures, 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 ug/ml, the rats were sedated, reacted to external stimuli, and were able to ambulate. At target plasma thiopental concentrations of 20–24 ug/ml, they were sedated and did not move until nociceptive stimuli were applied. At target concentrations of 40 ug/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 ug/ml), the median performance error was calculated as described previously.

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 pH or PCO₂ 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

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

<table>
<thead>
<tr>
<th>Rats (n)</th>
<th>Pump performance (MDPE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Low</td>
<td>Medium</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>B Low</td>
<td>Medium</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>C Low</td>
<td>Medium</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>D Low</td>
<td>Medium</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

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 thioental concentration corresponding to a 50% probability of response, $C_{p50}$, 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 clamps or tail pressure stimuli were applied. The mean hemodynamic values during 5 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 thioental 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. The total number of EEG waves/s from 0.5 to 30 Hz was used as a measure of thioental EEG drug effect on the central nervous system. Using this measure of EEG effect

Table 3. Calculated Plasma Thioental Concentrations for 50% Probability to Respond to Nociceptive Stimuli ($C_{p50}$ Values) and Corresponding Electroencephalographic Effects

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

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

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Fig. 2. The achieved constant thioental concentrations for plasma thioental concentrations of 7, 20, and 70 µg/ml were drawn at 10, 40, and 60 µg/ml and 180 µg/ml (C). A: 7, 20, and 70 µg/ml and B: 40, 60, and 60 µg/ml (E). Results are shown only when there were no movements after intubation. The number of rats were A = 7, B = 10, C = 8, D = 4.
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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 $k_{ae}$, the rate constant characterizing the time delays between arterial thiopental concentrations and EEG effect, as well as the biphase thiopental concentration EEG effect relationship in rodents. 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 Noxious Stimuli

Quantal responses to various stimuli were recorded over constant, pseudosteady-state plasma thiopental concentrations covering an interval of 7–120 $\mu$g/ml. Figure 4 displays the plasma thiopental concentration versus probability of response for each of the stimuli. 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 $C_{50}$ values have confidence intervals that are no more than 10% of the estimated $C_{50}$ 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 $C_{50}$ values for tail clamping/abdominal contraction, corneal reflex, and intubation overlap as evaluated using the 95% confidence intervals.

Cardiovascular Responses to Noxious 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, confounds efforts to relate clinical effect measures to the EEG (fig. 7). It may not be feasible to define a unique relationship between the $C_{50}$ 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 $\mu$g/ml. At a plasma thiopental concentration of 40 $\mu$g/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 responses 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.

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 $C_{50}$ 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. Consequently, it should be possible to compare the confidence intervals of the various $C_{50}$ values obtained for different inhalational anesthetics with the $C_{50}$ 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 interval of the $C_{50}$ value for tracheal intubation was explained by a smaller number of rats; only one rat would be possible under the intrinsic difficulties of this measure in the rat in a small cage. We found that heart rate and mean arterial pressure are useful for monitoring the depth of thiopental.

The major purpose of this full range of anesthetic effect isoelectric EEG by applying it and recording the results.
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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 Cp₅₀ 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 Cp₅₀ values and the number of waves derived from the raw EEG recordings. The
difficulties with biphasic pharmacodynamic modeling with this has been discussed extensively.\textsuperscript{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.\textsuperscript{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.\textsuperscript{12} In these human studies, verbal responses to repeated questions showed a $C_{p_{90}}$ value of 15.6 μg/ml, which approximates the $C_{p_{90}}$ value for the unprovoked righting reflex in the rat. Humans demonstrate a $C_{p_{90}}$ value of 39.8 μg/ml for movement response to trapezius muscle squeeze, which is similar to our results for ext...
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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 Cₚ₀ 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.

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tremor 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.

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