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Plasma, Brain, and Spinal Cord Concentrations of Thiopental Associated with Hyperalgesia in the Rat

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Background: **Although low doses of barbiturates are widely believed to increase sensitivity to pain, studies of the electrophysiologic effects of these drugs on the neurons involved in nociception in the spinal cord have detected only depressant effects. The goal of the studies reported here was to quantify the hyperalgesia resulting from low-dose thiopental infusions and to measure the associated concentrations of thiopental in the plasma, brain, and spinal cord.**

Methods: **Nociception was measured using the threshold for motor response to pressure stimulation of the tail (nociceptive threshold) and tail flick latency in the rat. Thiopental was administered by intravenous infusions designed to produce plasma concentrations that either slowly increased or remained at a steady state. Plasma and tissue thiopental concentrations were measured by high-performance liquid chromatography.**

Results: **We observed a reduction in nociceptive threshold that was correlated with the plasma thiopental concentration over the range 2–20 $\mu g \cdot ml^{-1}$ (7.6–76 μM). The relationship was nonlinear. Nociceptive threshold reached a nadir (36% less than control values) at a mean plasma thiopental concentration of 13.7 $\mu g \cdot ml^{-1}$ (51.9 μM). The steady-state study showed a similar reduction in nociceptive threshold, with an equilibrium plasma thiopental concentration of 7.6 ± 1.3 $\mu g \cdot ml^{-1}$ (28.8 ± 4.9 μM). Concentrations of thiopental in brain and spinal cord samples were 1.7 ± 0.03 and 3.5 ± 1.7 $\mu g \cdot g^{-1}$, respectively.**

Conclusions: **These studies confirm previous reports of hyperalgesia in association with small doses of thiopental. Reductions in nociceptive threshold and tail flick latency were observed in association with spinal cord concentrations of thiopental in a range reported by others to depress the electrophysiologic activity of neurons involved in nociception. (Key words: Anesthetics, intravenous: thiopental. Pain: antianalgesia. Spinal cord: nociception.)**

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FOR more than 30 yr researchers have presented conflicting evidence concerning the influence of barbiturates on nociception in experimental animals and on pain perception in humans. A recent study *in vitro* by Jewett *et al.*[1] has emphasized the controversy. Although several studies of nociception that evaluated behavior or perception in the intact organism have supported a hyperalgesic effect of these drugs,[2–4] electrophysiologic studies have shown barbiturates to depress nociceptive neurons of the spinal cord.[1,5] In an accompanying editorial, Kitahata and Saberski[6] suggested a need for further study of low-dose infusions of barbiturates in a model in which hyperalgesia can be measured.

The purpose of this study was to examine the influence of low-dose infusions of thiopental on the nociceptive threshold (NT) for tail pressure as well as tail flick latency (TFL) in the rat and to characterize the range of doses and the plasma, brain, and spinal cord concentrations of thiopental that were associated with any observed changes in NT.

Materials and Methods

After the protocol had been approved by the University of Calgary Animal Care Committee, 40 male Sprague-Dawley rats weighing 250–325 g were studied.

Animal Model

Experiments involving thiopental infusions were performed under conditions of partial restraint similar to those used for radioautographic studies in the rat.[7] After each animal was weighed, femoral arterial and venous catheters for blood sampling and drug administration, respectively, were inserted during general anesthesia with 2% halothane in oxygen. The animal then was partially restrained in a plaster cast that extended from ankles to midthorax and was taped to a wooden block. The animal was placed under a warming

lamp and were allowed to eliminate the halothane for 2 h before study.

The general health of each animal was assessed by visual inspection, by comparison of body weight with those of its litter mates, and by measurement of physiologic variables. Arterial blood gas tensions, hematocrit, and rectal temperature were measured before testing to confirm that each animal was physiologically within the usual limits for our laboratory (arterial oxygen tension > 65 mmHg, arterial carbon dioxide tension 30–45 mmHg, *p*H 7.30–7.45, hematocrit 35—55 vol%, and rectal temperature 36—38° C). Animals with blood test values outside of these ranges were excluded from the study. Isolated deviations in temperature were corrected by warming or cooling the animal before proceeding. Rectal temperature was maintained at 37–38° C with a warming lamp throughout the experiment. Rectal and tail temperatures were measured with a thermistor probe thermometer (model 43 TD, Yellow Springs Instruments, Yellow Springs, OH). Blood withdrawn for thiopental determinations was replaced with an equal volume of saline.

Measurements of Nociception

Tail Pressure Threshold. This was quantified by measuring the threshold of the motor response to pressure applied to the tail by an Analgesy-Meter (Ugo Basile, Milan, Italy).[8,9] Using this device, the distal part of the tail was supported by a plinth while linearly increasing pressure (32 $g \cdot s^{-1}$, cutoff 500 g) was applied with a cone-shaped pusher. The distal 4 cm of the tail were marked, and, with each trial, the pressure point was positioned 0.25 cm proximal to the previous site. The end point was defined as the first motor response of the animal to the pressure on the tail. The measurement scale on the device was hidden from the observer by a screen; an assistant recorded each end point. The NT for each trial was calculated as the mean of three consecutive determinations. Preliminary studies of NT were performed on 12 normal animals to determine the effect of repeated measurements and the effects of the insertion of the vascular catheters and the partial restraint.

After response to the Analgesy-Meter was lost, the animals were evaluated using tail clamp of the distal 2 cm of the tail. A specific hemostatic clamp closed to the first ratchet was used in this test. The animals were declared to be anesthetized at the first failure of the tail clamp to elicit any motor response, purposeful or not.

Tail Flick Latency. TFL was determined by placing the distal 2 cm of the tail in a container of water maintained at 54° C and measuring with a stopwatch the time from immersion to the first flick movement. Each TFL was calculated as the average of three consecutive measurements.

Measurements of Thiopental Concentrations

Thiopental concentrations in plasma and in nervous tissue were measured by high-pressure liquid chromatography.[10] For plasma samples, 50 μl deionized water/acetonitrile (1:4) was added to 20 μl plasma. The mixture was vortexed and centrifuged at 10,000 rpm for 5 min. Twenty microliters of the supernatant was injected onto the high-pressure liquid chromatography column.

The right frontal lobe of brain or spinal cord samples were homogenized in 250 and 100 μl of a 1:1 deionized water/acetonitrile mixture, respectively. The homogenates were centrifuged at 10,000 rpm for 5 min, and 20 μl of the supernatant was injected onto the high-pressure liquid chromatography column.

Chromatographic equipment consisted of a Perkin-Elmer series 10 pump equipped with an LC-85 spectrophotometric detector (set at 290 nm) and a sigma 1B integrator (all from Perkin-Elmer, Norwalk, CT). A 5-μm, 10-cm × 4.6-mm Whatman column was used for the analyses. The mobile phase was 1/1 deionized water/acetonitrile at a flow rate of 1.5 $ml \cdot min^{-1}$.

Minor variations in detector response were accounted for by running a standard curve on each day of analysis. Standard curves were obtained by adding thiopental standard to blank plasma or brain.

The limit of detection for sodium thiopental was 0.3 $\mu g \cdot ml^{-1}$ (1 μM). The coefficient of variation (for repeated analyses, 1 standard deviation/mean value × 100, determined for the highest value on the standard curve, 42 $\mu g \cdot ml^{-1}$) (159 μM) was ±2.2%. The accuracy (determined from the standard curves) was ±4.1%.

Study Outline

Preliminary investigations were performed (1) to establish the mean values and variability for NT in 12 unrestrained animals and (2) to determine whether the anesthetic or the insertion of vascular catheters and partial restraint altered the NT. Seven animals were tested on 3 consecutive days and before and after general anesthesia, placement of vascular catheters, and partial restraint as outlined above.

After these preliminary investigations had been completed, three separate studies, outlined below, were performed consecutively. Animals were randomly assigned to the treatment groups within each of the study protocols (block randomization[11]), and the observer was blinded to the assignment.

Study 1: Non–Steady-state Thiopental Infusion. To determine the plasma thiopental concentrations at which reductions in NT might be observed, NT was measured repeatedly during a low-dose continuous intravenous infusion of thiopental designed to produce a gradual increase in plasma thiopental concentration.

According to a previous report,[4] we assumed that a reduction in NT would be observed before the thiopental rendered the rats unresponsive to stimuli. The infusion strategy was derived from a preliminary investigation of seven animals in which the level of consciousness was tested by stimulating the rat's vibrissae and moving the head at each sample time. Consciousness was classified as follows: alert = brisk response of head; drowsy = diminished movement; unresponsive = complete absence of response to head stimulation; and anesthetized = failure to respond to tail clamp.

Nine animals received a thiopental infusion (0.15 $ml \cdot min^{-1}$, 0.67 $mg \cdot kg^{-1} \cdot min^{-1}$, 2.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Seven animals received a saline infusion (0.15 $ml \cdot min^{-1}$) and served as controls. NT was measured before and at 2, 4, 6, 8, 12, 16, and 20 min after the start of the infusion. An arterial blood sample (250 μl) was withdrawn to coincide with each of the NT measurements. After 20 min of the infusion, many animals were unresponsive to tail pressure (NT) but remained responsive to tail clamping. The infusion was continued with intermittent tail clamping and blood sampling every 5 min until the first lack of response to tail clamping, at which time final blood samples were drawn and the brain and upper cervical spinal cord samples removed by decapitation.

The effect of the thiopental infusion and blood sampling on physiologic variables was evaluated by comparing initial measurements with values obtained when the animals were unresponsive to tail clamping or, in the case of the control animals, after 30 min of infusion.

In eight animals receiving a thiopental infusion at the rate described above, thiopental concentrations were measured in plasma, brain, and spinal cord samples obtained after 8 min of the infusion (table 4).

Study 2: Comparison of Nociceptive Measurements. The goal of this study was to determine whether a decrease in the TFL (a second measure of nociception) occurred at the same time as the nadir of the NT observed in study 1.

In six animals receiving a thiopental infusion at the same rate as in study 1, concurrent measurements of NT, TFL, and plasma thiopental concentration were made before and after 8 min of the infusion. Four animals receiving saline infusions (0.15 $ml \cdot min^{-1}$) served as controls. The observer was blinded to both the composition of the infusion and the NT values.

Study 3: Steady-state Thiopental Infusion. The goal of this study was to measure NT and tissue and plasma thiopental concentrations during a steady-state thiopental infusion designed to achieve the central nervous system tissue concentrations observed at the nadir of the NT observed in study 1.

In six animals, NT was measured during a bolus-and-infusion strategy designed to produce a steady-state plasma thiopental concentration of 8–10 $\mu g \cdot ml^{-1}$ (30–38 μM). The parameters for the bolus and the infusions were derived from a preliminary study of the clearance of a thiopental bolus (3 $mg \cdot kg^{-1}$, 11.4 $\mu mol \cdot kg^{-1}$) during the first 30 min after injection. Using the method described by Bailey,[12] combined with trial and error, the derived strategy consisted of a bolus of 1.6 $mg \cdot kg^{-1}$ (6.1 $\mu mol \cdot kg^{-1}$) followed by infusions of 0.25 $mg \cdot kg^{-1} \cdot min^{-1}$ (0.95 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) for the first 8 min and 0.125 $mg \cdot kg^{-1} \cdot min^{-1}$ (0.47 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) for the next 8 min. Blood was sampled for thiopental concentrations before the infusion and at 0.5, 4, 8, and 12 min, after which brain and spinal cord samples were obtained as above.

Statistical Analysis

The values of the physiologic variables at the onset and the termination of the experiments (table 1) were compared by two-way analysis of variance (ANOVA) combined with the Student–Neuman–Keuls (SNK) test to identify the individual intergroup differences. The body weights of the animals failed the equal variance test and were analyzed with the Mann-Whitney rank sum test.

Study 1. To identify time-related changes in NT, linear regression analysis was performed, with the mean values of NT for each of treatment group as the dependent variable and the duration of the infusion as the independent variable. Individual differences among the sample times were isolated using two-way repeated-

Table 1. Physiologic Variables

Variable	Saline Group		Thiopental Group	
	Initial	Final	Initial	Final
Pa_{CO_2} (mmHg)	35 ± 1	29 ± 2	33 ± 1	33 ± 1
Pa_{O_2}	73 ± 2	74 ± 3	74 ± 2	77 ± 3
*p*H	7.41 ± 0.01	7.42 ± 0.02	7.41 ± 0.01	7.36 ± 0.01
Hematocrit (vol%)	46 ± 1	44 ± 4	45 ± 1	43 ± 1
MAP (mmHg)	144 ± 4	147 ± 11	147 ± 3	141 ± 7
Rectal temperature (° C)	3.71 ± 0.2	37.1 ± 0.6	37.4 ± 0.4	36.8 ± 0.3

Values are means ± standard deviations. Data are from 16 animals, with 9 in the thiopental group.

Pa_{CO_2} and Pa_{O_2} = the arterial partial pressures of oxygen and carbon dioxide, respectively.

No significant differences detected by two-way repeated-measures analysis of variance.

measures ANOVA combined with the SNK test for multiple comparisons. The influence of plasma thiopental concentration on NT was characterized by polynomial regression analysis.

Study 2. The values for NT and TFL at the two measurement times were analyzed separately by two-way repeated-measures ANOVA followed by the SNK test.

Study 3. The NT values at each of the four measurement times were compared using one-way repeated-measures ANOVA combined with the SNK test.

Results

Table 1 shows the physiologic variables at the onset and termination of the experimental protocols. No significant differences in the physiologic variables data were identified, either between the treatment groups or between the initial and final determinations. As a group, the saline-treated animals were heavier (median body weight 312.5 g, 25th–75th percentiles 300–330 g) than the thiopental-treated group (median body weight 300 g, 25th–75th percentiles 285–330 g) ($P < 0.001$). During the infusion, the plasma thiopental concentrations associated with the behavioral classifications described above were as follows: drowsy = 13 ± 3 $\mu g \cdot ml^{-1}$ (49 ± 11 μM); unresponsive = 20 ± 2 $\mu g \cdot ml^{-1}$ (76 ± 7.6 μM); and anesthetized = 33 ± 3 $\mu g \cdot ml^{-1}$ (125 ± 11 μM)—values significantly different from each other by repeated-measures ANOVA and SNK ($F = 133$, $P < 0.001$).

The results of the preliminary investigations are summarized in table 2. Seventy-five measurements of NT in 12 normal, unrestrained animals had a mean value of 175 g, with a standard deviation of ± 39 g. Seven animals were subjected to daily NT measurements for 3 days as well as determinations of NT before and after halothane anesthesia, insertion of vascular catheters, and partial restraint on the 4th day. No significant differences were found among the different measurement times (table 2). With this design, the power to detect a 30-g change in NT was 0.908.

Study 1

The influence of the saline or thiopental infusions on NT is shown in figure 1. The initial NT values did not differ between the two groups. The NT increased with time during the saline infusion at a rate of 2.9 g/min (2.9 g = 1.1% of the baseline NT) ($P < 0.001$). During the thiopental infusion the NT decreased in a linear fashion ($P = 0.003$) by 6.65 g/min (6.65 g = 3% of the baseline value of 216 ± 36 g) until a nadir (159 ± 48 g) was reached after 8 min infusion. When cor-

Table 2. Variability within Repeated Measures of Nociceptive Threshold

Measurement Time (day)	Nociceptive Threshold (g)
1	204 ± 26
2	180 ± 29
3	178 ± 42
4, before preparation	184 ± 37
4, 2 h after preparation	219 ± 24

Values are the mean ± SD for seven animals.

"Preparation" refers to the insertion of vascular catheters and partial restraint during general anesthesia with halothane.

One-way repeated measures analysis of variance did not reveal any significant differences in nociceptive threshold among the measurement times ($F = 2.135$, $P = 0.101$).

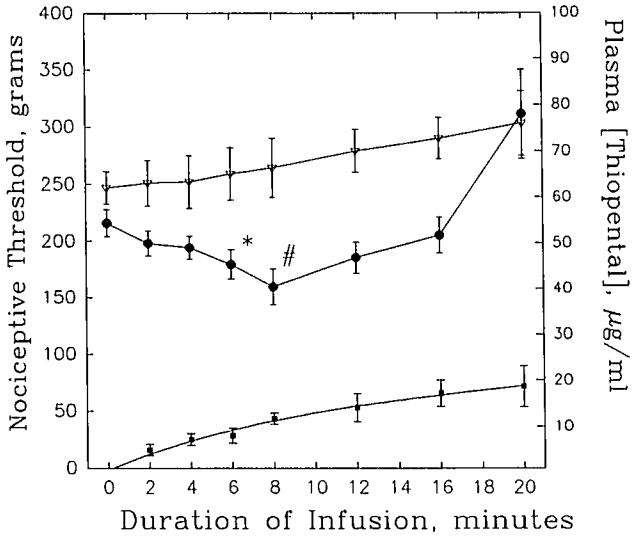

Fig. 1. The influence of plasma thiopental concentration (squares) on nociceptive threshold during the constant infusion of saline (triangles, seven animals) or thiopental (circles, nine animals). All symbols represent mean values ± standard error of the mean. The 6-min (*P = 0.016) and 8-min (#P = 0.01) values of NT in the thiopental-treated animals differ significantly from the corresponding values in the saline group.

rected for the time-related increase in NT observed in the control group, this represents a 35.6% decrease in NT. The NTs after 6 and 8 min of infusion were less than the initial value for the thiopental group and also less than the corresponding values in the animals infused with saline (F = 11.9, P = 0.004). Thereafter, NT increased progressively in the thiopental-treated animals.

Failure to respond to tail clamping occurred after a median infusion duration of 42 min (range 30–92 min). The mean rate of change of the plasma thiopental concentration for the 5 min preceding the first failure to respond was 0.8 ± 0.8 $\mu g \cdot ml^{-1} \cdot min^{-1}$ (3 ± 3 μM).

Figure 2 shows the relationship between NT and the plasma thiopental concentration during the infusion. For this analysis, the NT values were corrected for the increases with time observed in the saline-treated animals before the data were fitted to a third-order polynomial. A significant (F = 8.56, P = 0.043) fit was found with the following equation:

$$NT = 2.14\ e^{2} + 2\ e^{[thiopental]} - 1.86\ e^{[thiopental]2} + 2.4\ e^{[thiopental]3}$$

Study 2

Concurrent determinations both of NT and of TFL showed a significant decrease after 8 min of the thiopental infusion (table 3). NT decreased by 32% of the control value. In these animals, the plasma thiopental concentration associated with this decrease was 13.7 ± 1.93 $\mu g \cdot ml^{-1}$ (51.8 ± 7.3 μM).

Study 3

The effects of the steady-state thiopental infusion on NT are shown in figure 3. The NT was decreased below control at the first measurement time (4 min) and remained significantly less than control until the end of the experiment (F = 4.847, P = 0.02). The NT values at 4, 8, and 12 min were not significantly different from each other.

The thiopental concentrations in brain, spinal cord, and plasma with and without steady-state plasma conditions are summarized in table 4.

Discussion

The results of these experiments confirm the conclusions of earlier studies[2–4] of the hyperalgesic effects of

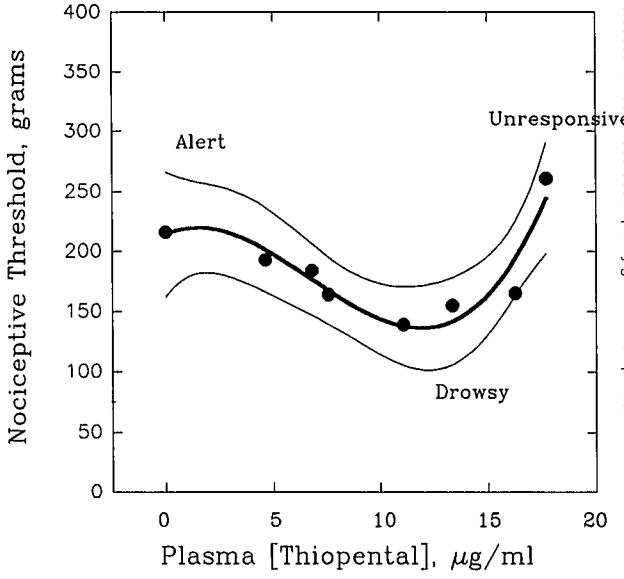

Fig. 2. The relationship between plasma thiopental concentration and nociceptive threshold. Circles = mean values for nociceptive threshold; thick line = third-order polynomial (see text for equation) that fits the data (P = 0.04); thin lines = 95% confidence limits for the relationship. Note that this curve is derived from a non-steady-state experiment. The steady-state relationship between these variables is likely to be shifted approximately 5 μg/ml to the left.

Table 3. Comparison of Nociceptive Threshold With Tail-flick Latency during a Thiopental Infusion

Measurement	Treatment	Initial Value	8-min Value
Nociceptive threshold (g)	Saline	211 ± 80	249 ± 60
	Thiopental	196 ± 41	133 ± 36*
Tail-flick latency (s)	Saline	1.78 ± 0.28	1.71 ± 0.48
	Thiopental	1.43 ± 0.32	1.03 ± 0.37†

Values are mean ± standard deviation.

Nociceptive threshold and tail-flick latency were determined concurrently during infusion of saline (four animals) and thiopental (0.67 mg · kg^{-1} · min^{-1}, six animals).

Two-way repeated-measures analysis of variance indicate that both measurements are decreased after 8 min of thiopental infusion:

* $F = 21.9$, $P = 0.002$.

† $F = 7.004$, $P = 0.029$.

thiopental and, in addition, extend the results to include measurements of NT during conditions of stable plasma thiopental concentrations.

In human subjects, Dundee[4] observed that an intravenous bolus of thiopental (effective dose range 0.6–2.5 mg/kg) was associated with an increase in the sensitivity to a painful stimulus. Similar results were seen with boluses of pentobarbital (50–100 mg).[4] In mice, thiopental, methohexital, pentobarbital, and phenobarbital all increased the tail's sensitivity to electrical stimulation, an effect not seen with other hypnotic agents such as chloral hydrate, paraldehyde, or glutethimide.[2]

Our findings are subject to the limitations common to studies of nociception in animals—namely, uncertainty as to the perception of the stimulus by the animal, considerable variability in the behavioral response, and the difficulties of relating the results to mechanisms of human pain perception. In addition, it is difficult with studies of this design to determine the region(s) or mechanism(s) in the nervous system responsible for hyperalgesia.

Although pressure analgesimetry has been evaluated both in animals[8] and in humans,[13,14] we do not know the specific sensation to which the animal is responding with the first motor response to tail pressure or whether the perception of the stimulus is consistent over time. The stimulus is probably a complex one comprising several components, including pressure, pain, and physical restraint of the tail. For many of these stimuli, the rate of change of the stimulus may play an important role in eliciting the response. In human studies using pressure analgesimetry, Dundee[4] identified two different end points: "threshold," the pressure when pain was first noted, and "response," the pressure at which the pain became unbearable. It was not possible to identify these two end points in our animal model.

The results of study 2 confirm the effects of thiopental on two separate measures of nociception. In addition, the reduction in NT (32% of baseline) and the plasma concentration of thiopental (13.7 ± 1.9 $\mu g \cdot ml^{-1}$) (51.8 ± 7.3 μM) in study 2 serve to confirm the findings after 8 min of infusion in study 1 (36% reduction in NT, plasma thiopental concentration 13.6 ± 2.2 $\mu g \cdot ml^{-1}$) (51.5 ± 8.3 μM).

A methodological limitation of these studies is the use of non–steady-state conditions in study 1, which casts doubt on the relationship between NT and thiopental concentration as shown in figure 2. We would expect that at any particular level of reduction of the NT, the plasma thiopental concentration would be greater than at equilibrium, reflecting continuing flux of drug from plasma to effect site. The magnitude of this error can be estimated from the rate of increase in the plasma thiopental concentration (1.3 $\mu g \cdot ml^{-1} \cdot min^{-1}$ after 6–12 min of infusion) and the plasma concentration–response equilibration time. The equilibration of EEG effects occurs 2–3 min after a change in plasma thiopental concentration (half-time less than 80 s).[15] Thus, we estimate that the error in study 1 would be that for any value of NT during the

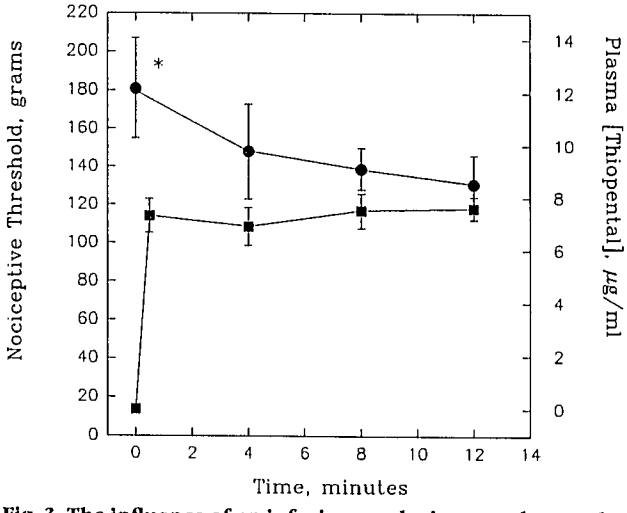

Fig. 3. The influence of an infusion producing pseudo–steady-state thiopental concentrations (squares) on nociceptive threshold (circles). Symbols represent mean values ± standard errors. *The control value for nociceptive threshold differed from the values at the other three measurement times (P = 0.02). After initiation of the infusion strategy, plasma thiopental measurements did not differ.

Table 4. Thiopental Concentrations in Plasma, Brain, and Spinal Cord: Non–Steady State Compared to Steady State

Group	Thiopental Concentration			
	Plasma ($\mu g \cdot ml^{-1}$)	Brain ($\mu g \cdot g^{-1}$)	Spinal Cord ($\mu g \cdot g^{-1}$)	Brain/Plasma
Non–steady state, NT reduced (n = 8)	13.6 ± 2.2*	1.6 ± 0.3	5.1 ± 1.1	0.12 ± 0.02†
Steady state, NT reduced (n = 6)	7.6 ± 1.3*	1.7 ± 0.3	3.5 ± 1.7	0.23 ± 0.07
Steady state, anesthetized (n = 10)	30.7 ± 7.4*	6.9 ± 2.5‡	23.5 ± 6.8‡	0.23 ± 0.09

Values are mean ± standard deviation; n = number of animals in each group.

NT = nociceptive threshold; "non–steady state" refers to the state produced by the infusion in study 1; "anesthetized" refers to the steady-state measurements performed at the first failure to respond to tail clamp in study 1.

Analysis by Kruskal-Wallis one-way analysis of variance on ranks:

* Each of the three values is different from the other two ($P < 0.001$).

† The "non–steady state, NT reduced" value differs from both of the "steady state" values ($P < 0.007$).

‡ The "anesthetized" value differs from both of the "NT reduced" values ($P < 0.001$).

infusion, the measured plasma thiopental concentrations are approximately 5 $\mu g \cdot ml^{-1}$ (18.9 μM) greater than the value that would be observed during steady-state conditions. We could predict that, were study 1 performed during steady-state conditions, the nadir of the NT seen in figure 1 would be associated with a plasma thiopental concentration of approximately 8 $\mu g \cdot ml^{-1}$ (30.3 μM), rather than the 13 $\mu g \cdot ml^{-1}$ (49.2 μM) shown in figure 2.

These estimates are supported by the results in table 4. In the NT-reduced groups, both the steady-state and the non–steady-state infusions produced brain concentrations of 1.6–1.7 $\mu g \cdot g^{-1}$ (6.1–6.4 $nmol \cdot g^{-1}$). However, the associated plasma concentration in the steady-state infusion was 7.6 ± 1.3 $\mu g \cdot ml^{-1}$ (28.8 ± 4.9 μM) compared to 13.6 $\mu g \cdot ml^{-1}$ (51.5 μM) in the non–steady-state infused animals. The optimal design to evaluate the plasma thiopental–NT relationship would use sequential plasma concentration clamping[16] to obtain steady-state thiopental concentrations, provided that sufficient precision could be achieved within the narrow range of concentrations that appear to be associated with hyperalgesia.

To estimate the effect site thiopental concentrations, we measured tissue concentrations of thiopental to supplement the information obtained from the plasma thiopental values. The tissue thiopental concentrations can reflect the concentration at the effect site only indirectly because effect site location is unknown. We would predict that for effect sites with high blood flow, plasma concentrations would provide a good estimate (see below), whereas whole tissue concentrations provide some information about the average thiopental concentrations throughout the sample. The results in table 4 are similar to those reported by others in whole brain tissue samples.[17–19] The concentrations were less in the brain tissue than in the plasma because brain regions with low flow were included within the sample. The measurements therefore represent volume-weighted averages of regional drug concentrations in an organ with very considerable variations in regional blood flow.

For example, in the conscious restrained rat, regional cerebral blood flow in the parietal cortex was 184 ± 15 $ml \cdot min^{-1}$, whereas in the globus pallidus, regional cerebral blood flow was only 70 ± 4 $ml \cdot min^{-1}$.[20] Thiopental equilibrates so rapidly with brain tissue that regional cerebral blood flow is the main determinant of regional tissue concentrations.[21] In the current study, the thiopental concentrations in samples of parietal cortex (a high-flow region) were 11.6 ± 3.5 and 5.7 ± 2.5 $\mu g \cdot g^{-1}$ in the non–steady-state (study 1) and steady-state animals (study 3) animals, respectively. These tissue values approach the plasma concentrations for these animals shown in table 4 and would be even greater than reported if precautions had been taken to prevent diffusion of thiopental within the brain after decapitation. These results confirm that in high-flow regions, the tissue thiopental concentrations can be estimated from the plasma values. The differences in the tissue thiopental concentrations between the brain and the spinal cord likely reflect differences in the mean blood flow to these tissues.

In an experimental model similar to that used in the current study, subhypnotic doses of barbiturates were shown to reduce or abolish the increase in NT that, in untreated animals, was observed to follow an acute stress.[22] That result raises two issues with regard to the

findings presented here. First, did the vascular catheterization and partial restraint have the effect of an acute stress, raising the NT? Second, did the halothane anesthesia have residual effects that might have had an antianalgesic effect?

We do not believe that the general preparation and partial restraint of the animal constituted an acute stress, at least as far as the NT was concerned. The model of vascular cannulation and restraint used in this study is commonly used for radioautographic studies and has been shown not to be associated with increases in cerebral blood flow and metabolism that accompany stimulation of the central nervous system.[7] After restraint the animals were calm and not struggling. Although NT testing produced minor agitation, the animal returned to a calm resting state between tests. We see little evidence for stress-induced analgesia in the preinfusion NT values. The NT values in the instrumented, restrained animals in our studies were 219 ± 24 g (preliminary study, table 2); 216 ± 36 and 247 ± 42 g (study 1); 196 ± 41 and 211 ± 80 g (study 2); and 181 ± 58 g (study 3). These values are not significantly different from the values in unrestrained animals in which the NT was determined once (table 2). These baseline values in restrained animals in the current study are less than the values reported by others in both unrestrained animals (329 ± 33 g, n = 6) and in animals with stress-induced analgesia (486 ± 62 g, n = 6),[22] suggesting that in our model, any effects of stress-induced analgesia that may have been present were small.

The increase in NT observed in the saline-infused group of study 1 may be due to stress-induced analgesia, to habituation of the animal to the stimulus, or to desensitization of the tail from the damage of the repeated stimulation.

We also believe than any residual effects on the NT of the halothane anesthesia used for insertion of the vascular catheters at the start of the experiments were small, because there was no significant difference between the baseline NT values before and after the catheter insertion (table 2). The average duration of the anesthesia for catheter insertion was 15 min, and no animal received more than 30 min of anesthesia. The animals usually were alert within 15 min of being restrained.

In summary, our findings suggest that in this animal model, the maximal reduction in NT due to thiopental (30–40% of baseline values) occurred with plasma thiopental concentrations of 8 $\mu g \cdot ml^{-1}$ (30 μM), brain concentrations of 1.7 ± 0.03 $\mu g \cdot g^{-1}$, and spinal cord concentrations of 7.6 ± 1.3 $\mu g \cdot g^{-1}$. These concentrations were within the ranges (1–10 μM) used in electrophysiologic studies[1,5] that reported depressant effects of thiopental on nociceptive neuronal activity. The absence of a biphasic relationship between electrophysiologic activity and plasma thiopental concentration within this concentration range suggests that the neural mechanism(s) for hyperalgesia may lie outside of the spinal cord nociceptive pathways that have been studied to date.

References

1. Jewett BA, Gibbs LM, Tarasiuk A, Kendig JJ: Propofol and barbiturate depression of spinal nociceptive neurotransmission. ANESTHESIOLOGY 77:1148–1154, 1992

2. Neal MJ: The hyperalgesic action of barbiturates in mice. Br J Pharmacol 24:170–177, 1965

3. Clutton-Brock JC: Some pain threshold studies with particular reference to thiopentone. Anaesthesia 15:71–72, 1960

4. Dundee JW: Alterations in response to somatic pain associated with anaesthesia: II. The effect of thiopentone and pentobarbitone. Br J Anaesth 32:407–414, 1960

5. Kitihata LM, Ghaz-Saidi K, Yamashita M, Kosaka Y, Bonikos C, Taub A: The depressant effect of halothane and sodium thiopental on the spontaneous and evoked activity of dorsal horn cells: Lamina specificity, time course and dose dependence. J Pharmacol Exp Ther 195:515–521, 1975

6. Kitahata LM, Saberski L: Are barbiturates hyperalgesic? (editorial). ANESTHESIOLOGY 77:1059–1061, 1992

7. Soncrant TT, Holloway HW, Stipetic M, Rapoport SI: Cerebral glucose utilization is not altered by hind limb restraint or by femoral artery and vein cannulation. J Cereb Blood Flow Metab 8:720–726, 1988

8. Green AF, Young PA: A comparison of heat and pressure analgesiometric methods in rats. Br J Pharmacol 6:572–585, 1951

9. Kissin I, Brown PT, Robinson A, Bradley EL: Acute tolerance in morphine analgesia: Continuous infusion and single injection in rats. ANESTHESIOLOGY 74:166–171, 1991

10. Crankshaw DP, Boyd MD, Bjorksten AR: Plasma drug efflux: A new approach to optimization of drug infusion for constant blood concentration of thiopental and methohexital. ANESTHESIOLOGY 67: 32–41, 1987

11. Spiliker B: Randomization procedures, Guide to Clinical Trials. New York, Raven Press, 1991, pp 70–71

12. Bailey JM: A technique for approximately maintaining constant plasma levels of intravenous drugs. ANESTHESIOLOGY 78:116–123, 1993

13. Thorpe MH: Tibial pressure analgesimetry: The significance of changes in pain threshold with reference to the measurement of analgesia. Br J Anaesth 38:198–206, 1968

14. Dundee JW, Moore J: Alterations in response to somatic pain associated with anaesthesia: I. An evaluation of the method of analgesimetry. Br J Anaesth 32:396–406, 1960

15. Ebling WF, Danhof M, Stanski: Pharmacologic characterization of the electroencephalographic effects of thiopental in rats. J Pharmacokinet Biopharm 19:123–143, 1991

16. Gustafsson LL, Ebling WF, Osaki E, Harapat S, Stanski DR, Shafer SL: Plasma concentration clamping in the rat using a computer-controlled infusion pump. Pharmacol Res 9:800–807, 1992

17. Bolander HG, Wahlström G: Interaction between probenecid and two lipid-soluble barbiturates in the rat. Neuropharmacology 23:977–981, 1984

18. Büch U, Altmayer P, Isenberg J Ch, Büch HP: Increase of thiopental concentration in tissues of the rat due to an anesthesia with halothane. Arzneimittelforschung 41:363–366, 1991

19. Schliamser SE, Broholm K-A, Wahlström G: Interaction between ciprofloxacin and thiopental in the central nervous system of the male rat. Pharmacol Toxicol 71:348–352, 1992

20. Archer DP, Pappius HM: Effects of two dihydropyridine calcium channel blockers on cerebral metabolism and blood flow in traumatized rat brain. Neurochem Path 5:117–130, 1986

21. Mark LC, Burns JJ, Campomanes CI, Ngai SH, Trousof N, Papper EM, Brodie BB: The passage of thiopental into brain. J Pharmacol Exp Ther 119:35–38, 1957

22. Kissin I, Mason JO III, Vinik HR, McDanal J, Bradley EL: Barbiturates inhibit stress-induced analgesia. Can J Anaesth 34:146–151, 1987