Laboratory Investigations

Hyperalgesia during sedation: effects of barbiturates and propofol in the rat

Alastair Ewen MBChB FRCA FRCPC,*
David P. Archer BSc MDCM MSc FRCPC,*
Naaznin Samanani BSc,* Sheldon H. Roth PhD*†

Subhypnotic doses of thiopentone are considered to possess antianalgesic or hyperalgesic properties. In this study, we have tested the hypothesis that the coincidence of sedation and hyperalgesia is a property of both barbiturate and non-barbiturate anaesthetic agents. In a randomized, prospective, blinded study, the effects of slow (20 min) iv infusions of thiopentone, pentobarbitone, methohexitone or propofol on nociceptive threshold were measured in rats by tail pressure analgesimetry and compared with saline-infused control animals. Nociceptive thresholds were correlated with measurements of plasma drug concentrations and behavioural assessments. Comparison of pre-infusion nociceptive threshold with the lowest threshold obtained during drug infusion revealed decreases in all four treatment groups. As a percentage of the pre-infusion values, the decreases were: thiopentone: 42.5% ($P < 0.001$), pentobarbitone: 27.8% ($P = 0.014$), methohexitone: 24.9% ($P = 0.013$), propofol: 21.6% ($P = 0.006$). There were no changes in nociceptive threshold in the control groups. The relationship between nociceptive threshold and plasma drug concentration was usually characterized by an initial decline followed by a rise in nociceptive threshold as the plasma concentration and degree of sedation increased. The results support the hypothesis that hyperalgesia is a property of different anaesthetic agents when administered at sub-hynotic concentrations.

Key words
ANAESTHETICS, INTRAVENOUS: methohexitone, pentobarbitone, propofol, thiopentone;
MEASUREMENT: nociceptive threshold, sedation;
PAIN: hyperalgesia, nociception.

From the Department of Anaesthesia,* and Department of Pharmacology and Therapeutics,† University of Calgary.

Address correspondence to: Dr. Alastair Ewen, Department of Anaesthesia, Foothills Hospial, 1403 29th Street NW, Calgary, Alberta, Canada T2N 2T9.

Accepted for publication 10th February, 1995.

On considère que les doses sous-hypotiques de thiopentone possèdent des propriétés antianalgésiques ou hyperalgésiques. Cette étude vérifie l'hypothèse selon laquelle la coïncidence de la sédation avec l'hyperalgie constitue à la fois une propriété des agents anesthésiques barbituriques et des non barbituriques. Au cours d'une étude randomisée, prospective et à l'aveugle, les répercussions sur le seuil nociceptif d'une perfusion lente (20 min) de thiopentone, pentobarbitone, méthohexitone et de propofol sont mesurées par analgésiométrie de compression sur la queue du rat et comparées avec des animaux de contrôle sous perfusion au soluté physiologique. Les seuils nociceptifs sont corrélés avec les mesures de concentration plasmatique des drogues et par l'évaluation du comportement. La comparaison du seuil nociceptif avant la perfusion avec le seuil le plus bas obtenu pendant la perfusion révèle des diminutions dans tous les groupes de traitement. En pourcentage des valeurs de pré-infusion, les diminutions sont les suivantes: thiopentone: 42,5% ($P < 0.001$), pentobarbitone: 27,8% ($P = 0,014$), méthohexitone: 24.9% ($P = 0,013$), propofol: 21,6% ($P = 0,006$). Il n'y a pas de changement dans les groupes contrôle au regard du seuil nociceptif. La relation entre le seuil nociceptif et la concentration plasmatique des drogues est ordinairement caractérisée par une baisse initiale suivie par une augmentation du seuil nociceptif à mesure que la concentration plasmatique et le niveau de sédation augmentent. Ces résultats supportent l'hypothèse selon laquelle l'hyperalgie serait une propriété de plusieurs anesthésiques quand ils sont administrés à des concentrations sous-hypnotiques.

The belief that sub-hypnotic doses of barbiturate agents enhance pain perception has been a tenet of anaesthetic practice for >30 yr. In 1960, Dundee[1] used tibial pressure analgesimetry to demonstrate a reduction in nociceptive threshold (NT) in humans following low doses of thiopentone or pentobarbitone. Since then, similar actions of barbiturates have been demonstrated in both human[2] and

animal[3] studies. Contradictory evidence, however, has come from other studies which suggest that barbiturates (and other *iv* anaesthetic agents) possess only antinociceptive properties.[4,5]

The methodological differences among the preceding studies are considerable. A recent editorial by Kitahata and Saberski[6] cautioned against extrapolating the conclusions of *in vitro* studies to support observations in the intact animal. Qualitative differences among such things as the method of restraint or the type of nociceptive stimuli militate against comparisons of different studies.[7] Investigations of non-barbiturate anaesthetic agents, notably propofol,[4] have generally failed to demonstrate hyperalgesia. In spite of the recent interest in the subject, the question of barbiturate or non-barbiturate induced hyperalgesia remains conjectural.

Recently, using tail pressure analgesimetry in rats, we demonstrated that sub-hypnotic doses of thiopentone were associated with a reduction in NT which we believe reflected a hyperalgesic action of this drug.[8] We speculated that both barbiturate and non-barbiturate anaesthetic agents would cause hyperalgesia at plasma concentrations which caused sedation. To test the hypothesis, we performed this prospective, blinded study in two parts. In the first part of the investigation, we used the technique of tail pressure analgesimetry in rats to study the effects of slowly increasing plasma concentrations of thiopentone, methohexitone or pentobarbitone on NT, and to correlate these effects with assessments of the animals' behaviour.

In the second part of the study, we used the same measure of nociception, in a similar study design, to compare the effects on NT of sedative concentrations of propofol and thiopentone.

Methods

The study protocols were approved by the University of Calgary Animal Care Committee. A total of 55 male Sprague-Dawley rats, each weighing between 250–325 g, were studied.

Animal model

The investigations were performed with animals restrained in a manner similar to that previously described for autoradiographic studies in the rat.[9] After weighing, each animal was anaesthetized with 2% halothane in oxygen. Femoral arterial and venous catheters were inserted under direct vision. The animal was partially restrained by wrapping it from ankles to midthorax in a plaster cast which prevented escape whilst permitting free movement of the head and forelimbs. It was then taped to a wooden block and placed under a warming lamp for two hours to allow elimination of halothane. Before inclusion in the study, the physiological status of each animal was evaluated. Mean arterial pressure was measured by an electromanometer (model 33-260, Baxter Healthcare Corp., Irvine, CA., U.S.A.). A sample of arterial blood was withdrawn for measurement of arterial oxygen tension, carbon dioxide tension, pH and haematocrit. We sought to confirm, for each animal, that these measurements lay within the normal limits for our laboratory ($PaO_2 > 65$ mmHg, $PaCO_2$ 30–45 mmHg, pH 7.30–7.45 and haematocrit 35–55 vol%). Rectal temperature was maintained between 36°C and 38°C with a warming lamp. This was confirmed, at the beginning and end of the experiments, with a thermistor probe thermometer (model 43 TD, Yellow Springs Instruments, Yellow Springs, OH., U.S.A.). Each sample of blood withdrawn during the experiment was replaced immediately with an equivalent volume of normal saline.

Measurement of nociceptive threshold

The thresholds for responses to noxious mechanical stimuli were measured by tail pressure analgesimetry, a modification of the Randall and Selitto paw pressure test.[10] Nociceptive threshold was defined as the weight (in grams), applied to the tail, which caused a brisk withdrawal response. The stimulus was applied by an Analgesy-Meter (Ugo-Basile, Milan, Italy).[11] Using this device, the tail was supported on a plinth while pressure was exerted by means of one or two weights via a cone-shaped pusher at a rate which increased linearly by 32 $g \cdot sec^{-1}$ from zero to 250 g when one weight was used, or by 64 $g \cdot sec^{-1}$ from zero to 500 g when two weights were used. We stimulated the distal 4 cm of the tail, avoiding, as much as possible, repetitive use of the same pressure point. The measurement scale on the Analgesy-Meter was hidden from the observer by a screen; an assistant recorded each end point. The NT was calculated as the mean of three consecutive measurements. Prior to each infusion, baseline measurements of NT were performed in the manner described above. In both parts of the study, NT measurements were repeated at 4, 8, 12, 16, and 20 min after the start of the infusion. In part 1 of the study, additional measurements were made at two and six minutes.

Arterial blood samples (250 μl) were withdrawn at the corresponding times for analyses of plasma barbiturate or propofol concentrations.

Infusion strategy

The infusion rates for the different barbiturates were chosen with the knowledge of their respective potencies and on the basis of our previous observations of the animals' behaviours during thiopentone infusions. Over the course of a 20 min infusion, we attempted to produce a spectrum

of behaviour proceeding from an awake animal to one that was unresponsive to non-noxious stimuli. After the two-hour recovery period, and following confirmation of acceptable physiological variables, each animal was randomly allocated to receive an intraveous infusion of one of the following solutions:

1 Thiopentone (STP group) 650 $\mu g \cdot kg^{-1} \cdot min^{-1}$
2 Pentobarbitone (PB group) 375 $\mu g \cdot kg^{-1} \cdot min^{-1}$
3 Methohexitone (MTX group) 335 $\mu g \cdot kg^{-1} \cdot min^{-1}$
4 Normal saline (NS group)

The solutions were infused at a rate of 0.15 $ml \cdot min^{-1}$ by a syringe driver (Sage, model 351, Orion Research Inc., Boston, MA., U.S.A.)

Our hypothesis, and evidence from preliminary studies (results not shown), suggested that, if propofol causes hyperalgesia, then this would be likely to be demonstrated in our model at plasma propofol concentrations which cause sedation, i.e., between 0–2 $\mu g \cdot ml^{-1}$. During preliminary studies of continuous infusions, we observed that the pharmacokinetic properties of propofol differed markedly from those of thiopentone. In most animals, plasma propofol concentrations reached a plateau after approximately eight minutes of infusion, whereas thiopentone did not reach a steady-state until after 20 min of infusion. Consequently, we estimated that at least two different infusion rates would be required to span the plasma concentration range needed to reproduce the behavioural effects observed during the thiopentone infusion used in part 1 of this investigation. By trial and error, we determined that appropriate infusion rates for propofol were 340 and 680 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Thus, each animal was randomly allocated to receive an infusion of one of the following solutions:

1 Thiopentone (STP group) 650 $\mu g \cdot kg^{-1} \cdot min^{-1}$
2 Propofol (PROP1 group) 340 $\mu g \cdot kg^{-1} \cdot min^{-1}$
3 Propofol (PROP2 group) 680 $\mu g \cdot kg^{-1} \cdot min^{-1}$
4 Normal saline (NS group)

The infusion rate was again 0.15 $ml \cdot min^{-1}$. The opacity of the propofol emulsion required that the syringe and tubing be shielded from view in order to meet the requirement that the observer be blinded to the group allocation for each animal.

After 20 min, the infusions were stopped. Arterial blood gas tensions, pH, haematocrit, mean arterial pressure and rectal temperature were again measured.

Sedation scores

The level of sedation of each animal was assessed prior to each NT measurement by observation of spontaneous behaviour, and by testing the animal's responses to passive movement of the head and stimulation of the vibrissae. A sedation score was assigned as follows:

0 Awake: actively exploring, brisk head response.
1 Slightly drowsy: not actively exploring, easily stimulated by manipulating head or vibrissae.
2 Drowsy: responds only to vigorous manipulation of head or vibrissae.
3 Unresponsive to head manipulation.

Determination of plasma barbiturate concentrations

Barbiturate concentrations in plasma were measured by high-performance liquid chromatography (HPLC).[12]

The limit of detection was 0.3 $\mu g \cdot ml^{-1}$ for thiopentone and 1 $\mu g \cdot ml^{-1}$ (when 60 μl of plasma was extracted) for pentobarbitone and methohexitone. The coefficient of variation was ±2.2%, ±3.5%, and ±6.5% for thiopentone, pentobarbitone, and methohexitone, respectively (1 SD/100 × mean value), determined for the highest value on the standard curve (30 $\mu g \cdot ml^{-1}$ for thiopentone: 20 $\mu g \cdot ml^{-1}$ for both pentobarbitone and methohexitone). The accuracy of the method, determined from standard curves, was ±2% for thiopentone, ±3% for pentobarbitone and ±6.5% for methohexitone.

Determination of plasma propofol concentrations

Propofol was measured in plasma samples by HPLC.[13] For extraction, 35 μl acetonitrile was added to an equal volume of plasma. The mixture was vortexed and then centrifuged at 15,000 rpm for five minutes. A 50 μl aliquot of the supernatant was injected directly on to a Perkin-Elmer C_{18} (3 μm, 3.3 cm) stainless steel column equipped with a Series 4 pump (Perkin-Elmer Corp., Norwalk, CT., U.S.A.) and an LC-electrochemical detector (Bioanalytical Systems Inc., West Lafayatte, IN., U.S.A.). The flow rate of the mobile phase (60% methanol and 40% 0.05M phosphate buffer), was 1.4 $ml \cdot min^{-1}$. The potential of the electrochemical detector was set at 0.78 V in reference to a Ag/AgCl electrode.

With this assay technique, the limit of detection of propofol was 0.1 $\mu g \cdot ml^{-1}$ with a coefficient of variation of ±0.8% (1 SD/mean value × 100), determined for the highest value on the standard curve, 4.3 $\mu g \cdot ml^{-1}$. The accuracy of the method, determined from the standard curves, was ±3.6%.

Statistical analyses

All analyses were performed using Sigmastat software (Jandel Scientific, San Rafael, CA., USA).

Physiological data that met the criteria of equal variance were compared at the beginning and end of each infusion by unpaired t test. Data that failed the equal variance test were compared using the Mann-Whitney U test.

In both parts of the study, for each NS (control) group,

linear regression analysis was performed with NT as the dependent variable and time as the independent variable.

In both parts of the study, values of NT prior to infusion were compared among the three treatment groups by one way ANOVA.

For each of the treatment groups, we attempted to characterize the relationship between NT and plasma barbiturate or propofol concentration by submitting the data to polynomial regression analysis. Data that either could not be characterized thus, or data that obviously demonstrated a linear relationship, were submitted to linear regression analysis.

In addition, for each agent, we compared, using a paired t test, the mean pre-infusion NT value with the lowest mean NT during the infusion.

Results

A total of 55 animals were studied, 29 in part 1 of the study, and 26 in part 2.

Physiological data – thiopentone, pentobarbitone, and methohexitone

In each of the four groups, mean haematocrit decreased during testing. The mean arterial blood pH showed a small decline in the pentobarbitone and methohexitone groups. In all four groups, the mean values of the physiological data were within the normal limits for our laboratory.

Plasma barbiturate concentrations and nociceptive threshold

The mean plasma concentrations of pentobarbitone, methohexitone and thiopentone increased, in a non-linear fashion, during each infusion (Figure 1). Observation of the data from the methohexitone group suggested that an equilibrium plasma concentration was attained before the end of the infusion.

Table I summarizes the results of the measurement of NT during the infusions in the four groups. Linear regression analysis revealed no relationship between NT and time in the NS group ($r^2 = 0.003$, $P = 0.697$). Among the four groups, mean pre-infusion NT was greater in the methohexitone group than in the thiopentone group ($P = 0.034$, one-way ANOVA).

The relationships between NT and plasma barbiturate concentration are demonstrated for thiopentone (Figure 2) and pentobarbitone (Figure 3). For both of these drugs, polynomial regression analysis of the data showed a significant fit to a third order polynomial equation. For pentobarbitone, the equation had a P value of 0.008; for thiopentone, the equation had a P value of 0.039. In the case of methohexitone, third-order polynomial regression analysis failed to demonstrate a relationship between NT

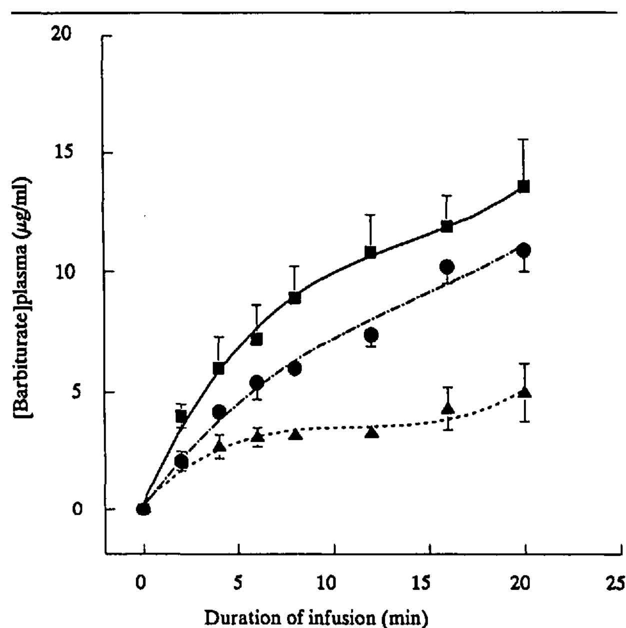

FIGURE 1 Barbiturate pharmacokinetics. Relationship between mean plasma thiopentone (closed squares), pentobarbitone (closed circles) and methohexitone (closed triangles) concentrations and time during the infusions. Values are means ± SD.

TABLE I Nociceptive threshold versus duration of infusion

Group	STP $n = 6$	PB $n = 9$	MTX $n = 7$	NS $n = 7$
Infusion (min)		*Nociceptive threshold (g)*		
0	198 ± 43*	221 ± 43	264 ± 36	230 ± 31
2	185 ± 30	205 ± 44	278 ± 67	211 ± 13
4	183 ± 36	181 ± 68	214 ± 44	209 ± 35
6	149 ± 44	173 ± 60	198 ± 32	209 ± 47
8	127 ± 35	160 ± 51	225 ± 56	207 ± 35
12	131 ± 40	171 ± 45	209 ± 89	206 ± 37
16	156 ± 39	170 ± 45	200 ± 65	230 ± 22
20	161 ± 58	179 ± 32	212 ± 95	220 ± 25

Groups are: thiopentone (STP), pentobarbitone (PB), methohexitone (MTX) and normal saline (NS).
Values are means ± standard deviations.
*Pre-infusion value in STP group significantly less compared with MTX group ($P = 0.034$, one way ANOVA).

and plasma methohexitone concentration. However, linear regression analysis showed an inverse relationship between NT (dependent variable) and plasma methohexitone concentration (independent variable) ($P = 0.046$). The regression line (Figure 4) fitted the equation: $NT = 251.06 - 11.96[MTX]_{plasma}$.

The shaded areas in each of the preceding three figures indicate the range of plasma barbiturate concentrations associated with the onset of drowsy behaviour (sedation score = 1).

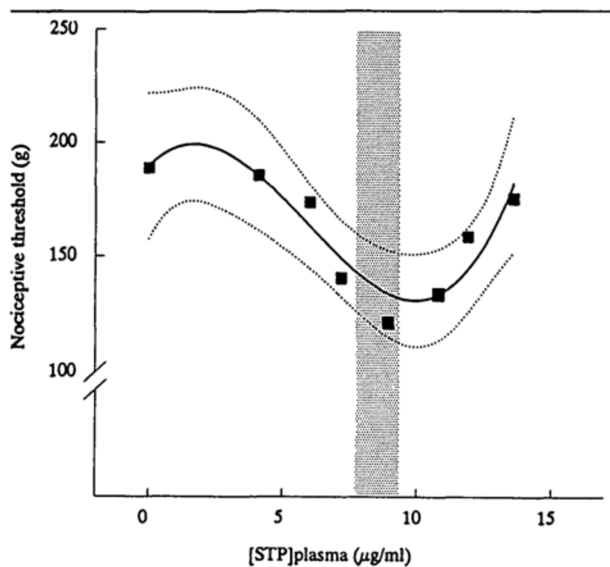

FIGURE 2 Nociceptive threshold versus plasma thiopentone concentration. Solid line shows third order polynomial relationship ($P = 0.039$) between mean values of nociceptive threshold (solid squares) and plasma thiopentone concentrations in six animals. Dashed lines indicate 95% confidence limits for the relationship. Shaded area indicates range of plasma thiopentone concentrations associated with the onset of sedation.

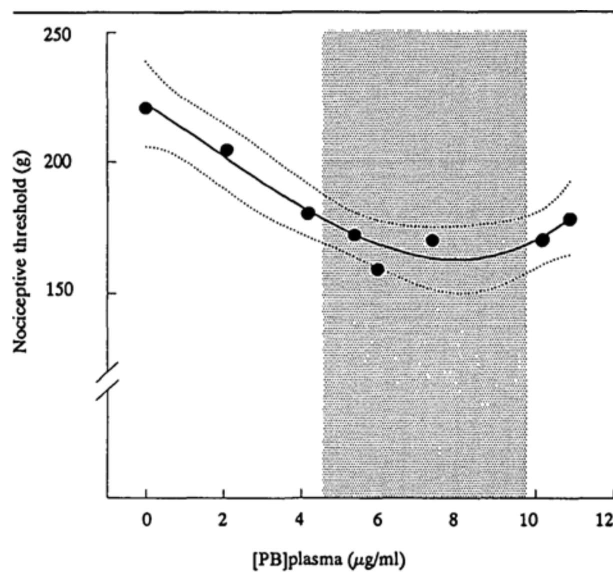

FIGURE 3 Nociceptive threshold versus plasma pentobarbitone concentration. Solid line shows third order polynomial relationship ($P = 0.005$) between mean values of nociceptive threshold (solid squares) and plasma pentobarbitone concentrations in nine animals. Dashed lines indicate 95% confidence limits for the relationship. Shaded area indicates range of plasma pentobarbitone concentrations associated with the onset of sedation.

Physiological data – thiopentone and propofol
Although the mean values for nearly all of the indices fell within the normal range for our laboratory, there were a number of differences from control values noted at the end of the infusions. The mean arterial pressure in all three treatment groups decreased. The magnitude of the decrease was greatest (17.5% of control value) in the PROP2 group.

Plasma thiopentone and propofol concentrations and nociceptive threshold
The mean plasma propofol concentrations in the PROP1 group appeared to reach equilibrium after eight minutes of infusion. In the other two groups, the plasma concentrations of the respective anaesthetic agents gradually increased over the 20 min (Figure 5).

Table II summarizes the nociceptive threshold data in the four groups. Linear regression analysis of NT versus time in the NS group did not reveal any relationship ($r^2 = 0.617$, $P = 0.064$).

The relationships between NT and plasma drug concentrations are shown for thiopentone (Figure 6) and propofol (Figure 7). For the purpose of this analysis, the data from the PROP1 and PROP2 groups were combined. The data in each of the resulting two groups (thiopentone and propofol) were treated with regression analysis to determine the relationship between NT and plasma drug concentration. In the thiopentone group, observation of the data suggested a linear relationship between NT and plasma thiopentone concentration. Linear regression analysis of NT (dependent variable) versus plasma thiopentone concentration (independent variable) demonstrated a decrease in NT as plasma thiopentone concentration increased from zero to 15 $\mu g \cdot ml^{-1}$ ($r^2 = 0.961$, $P < 0.001$). The regression line had the following equation: $NT = 205.84 - 5.70[STP]_{plasma}$.

The relationship between NT and plasma propofol concentration (PROP1 and PROP2 groups combined) could be characterized by a third-order polynomial ($P < 0.001$). In both figures, the shaded areas again illustrate the range of plasma drug concentrations at which the onset of drowsiness occurred (sedation score = 1).

In all groups, the lowest mean NT values were compared with the mean control (pre-infusion) values using paired t tests. The maximum decrease in NT in each of the treatment groups in Part 1 of the study was as follows: thiopentone 35.9% ($P = 0.002$), pentobarbitone 27.8% ($P = 0.014$), methohexitone 24.9% ($P = 0.013$). In the thiopentone group in Part 2, NT decreased by 42.5% ($P < 0.001$) after 20 min. In the combined propofol groups, the nadir occurred at 12 min. Since this value was derived from animals in the PROP1 group only, we decided that the relevant comparison should be with the mean control value from the PROP1 animals

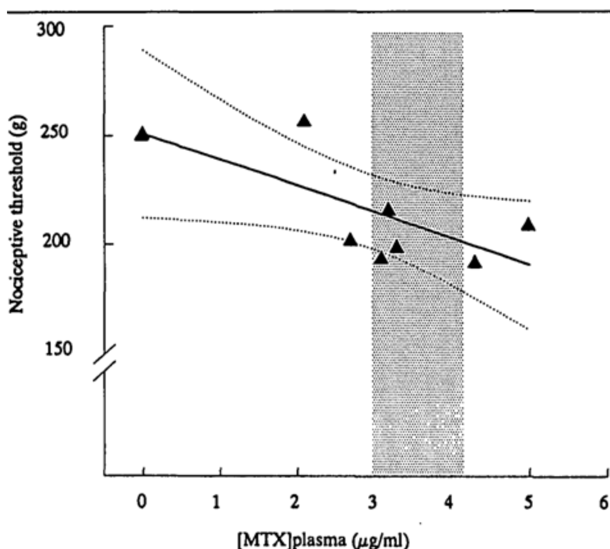

FIGURE 4 Nociceptive threshold versus plasma methohexitone concentration. Solid line indicates linear relationship ($P = 0.046$) between mean values of nociceptive threshold (solid triangles) and plasma methohexitone concentrations in seven animals. Dashed lines indicate 95% confidence limits for the relationship. Shaded area indicates range of plasma methohexitone concentrations associated with the onset of sedation.

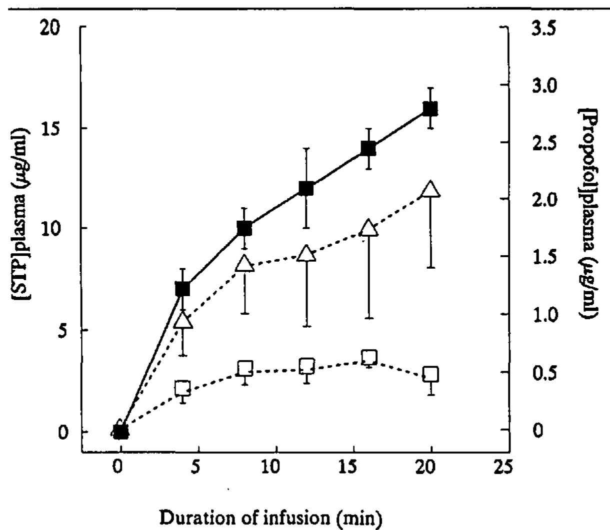

FIGURE 5 Thiopentone and propofol pharmacokinetics. Relationship between mean plasma concentrations of thiopentone (closed squares), propofol - low infusion rate (open squares) and propofol - high infusion rate (open triangles) and time during the infusions. Values are means ± SD.

only. The resultant decrease in NT was 21.6% of control. On this basis, the maximum decrease in NT was greater in the thiopentone group than in the propofol groups (2-way repeated measures ANOVA, $F = 9.465$, dof = 11, $P < 0.001$).

TABLE II Nociceptive threshold versus duration of infusion

Group	*STP* $n = 7$	*PROP1* $n = 6$	*PROP2* $n = 5$	*NS* $n = 8$
Infusion (min)	*Nociceptive threshold (g)*			
0	207 ± 14	213 ± 18	261 ± 27	217 ± 32
4	165 ± 32	190 ± 12	206 ± 20	225 ± 45
8	153 ± 32	185 ± 19	250 ± 74	213 ± 42
12	125 ± 32	167 ± 26	268 ± 33	199 ± 24
16	129 ± 36	178 ± 33	319 ± 61	194 ± 19
20	119 ± 28	178 ± 21	304 ± 13	204 ± 24

Groups are: thiopentone (STP), propofol (PROP1 and PROP2) and normal saline (NS).
Values are means ± standard deviations.

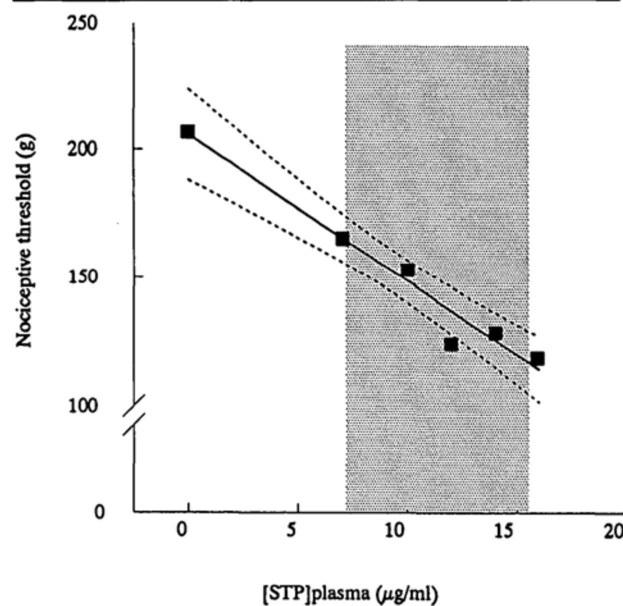

FIGURE 6 Nociceptive threshold versus plasma thiopentone concentration. Solid line indicates linear relationship ($P < 0.001$) between mean values of nociceptive threshold (solid squares) and plasma concentrations in seven animals. Dashed lines indicate 95% confidence limits for the relationship. Shaded area indicates range of plasma thiopentone concentrations associated with the onset of sedation.

Discussion

The results of this study substantiate previous evidence[8] that subanaesthetic plasma concentrations of thiopentone are associated with hyperalgesia, as defined by a decrease in NT. In addition, we have demonstrated that this effect is shared by two other intravenous barbiturate anaesthetic agents, pentobarbitone and methohexitone, when administered in subanaesthetic doses. Given the structural and pharmacodynamic similarities among the three drugs, the

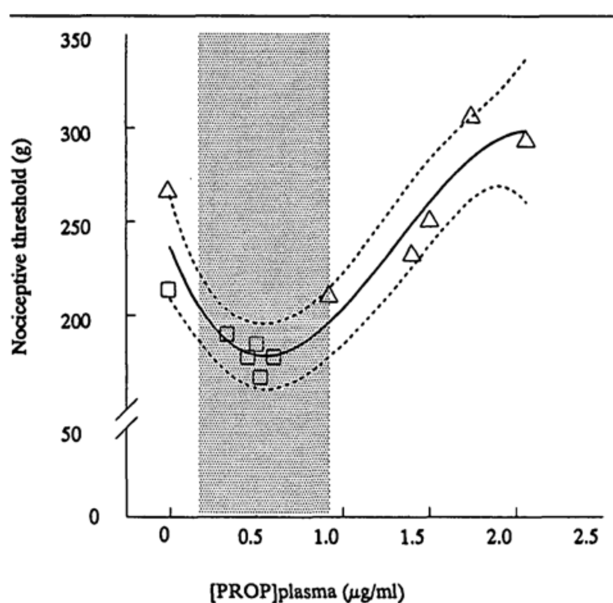

FIGURE 7 Nociceptive threshold versus plasma propofol concentration. Solid line shows third order polynomial relationship ($P < 0.001$) between mean values of nociceptive threshold (open squares and open triangles) and plasma propofol concentrations in 13 animals (PROP1 and PROP2 groups combined). Dashed lines indicate 95% confidence limits for the relationship. Shaded area indicates range of plasma propofol concentrations associated with the onset of sedation.

results are not surprising. Qualitatively similar results were obtained by Neal,[3] using an electrical stimulus in mice, following intraperitoneal administration of the same three barbiturates.

We have also demonstrated that propofol, at subhypnotic plasma concentrations, produces hyperalgesia of a magnitude similar to the barbiturates. Propofol, although structurally quite different from the barbiturates, has been shown to occupy a binding site within the same $GABA_A$ receptor/chloride channel complex as the barbiturates.[14] The results of part 2 of the study demonstrate that hyperalgesia is a feature of both barbiturate and non-barbiturate anaesthetic agents at plasma concentrations which cause sedation.

Part 2 of the study was designed to compare the effects of thiopentone and propofol on NT directly. We observed a difference between the two agents. The maximum decrease in NT during thipentone infusions was nearly twice as great as that observed with propofol (42.5% versus 21.6%, respectively). However, simple interpretation of this result as a measure of relative potencies is hindered by a number of factors. Firstly, under non steady-state conditions, the nadir of NT in the propofol group occurred at 12 min whereas the minimum value for NT in the thiopentone group occurred at the end of the infusion. A linear relationship was demonstrated between plasma thiopentone concentration and NT which may have continued beyond 20 min raising the possibility of a greater decrease in NT which was not detected because measurements were stopped. Secondly, the difficulties of quantifying the animals' behaviour call into question the comparability of the behavioural state of the animals in both groups at the plasma concentrations associated with the maximum decrease in NT. Thirdly, the intermittent nature of the NT measurements allows the possibility that we may have missed observing the nadir of NT in either of the two groups.

This investigation is open to a number of criticisms, some that are common to all behavioural studies of this type, and others that pertain only to this study. We have addressed, in a previous report,[5] several of the limitations applicable to all studies of nociception in animals. These include the inherent difficulty of inferring pain sensation from a reflex measure such as tail withdrawal. Tail pressure is a more complex stimulus than the thermal stimulus used in the tail flick reflex. It produces a correspondingly more complex response which is considered to be mediated at both spinal and supraspinal sites.[15] It results in both noxious and non-noxious stimuli to which the rat can respond, but between which we cannot distinguish in this model. Neither can we confidently relate the magnitude of the response to the intensity of the stimulus. Tail pressure analgesimetry provides, at best, an indirect measure of pain.

Similarly, our ability to quantify the behaviour of the animals was limited by the imprecision of the scoring system that we employed. This problem was accentuated by the fact that, in the treatment groups, we observed animals under non steady-state conditions in which the variable being measured (sedation) continued to change during the measurement process.

Other limitations, pertinent to behavioural models of this type, include the possible influences on NT of stress-induced analgesia[16] or hyperalgesia,[17] or alterations in the response caused by peripheral mechanisms such as injury to the tail.[18] We believe that comparison with the NS (control) groups, in which NT did not change with time, allow us to discount stress as a possible confounding factor. With regard to the latter concern, we attempted to minimize the possibility of tail damage by moving the site of stimulation with each measurement, and by limiting the maximum weight on the tail to 500 g. Again, however, there was no evidence of a peripheral effect on NT in the control animals which were subjected to the same measurements.

Of the criticisms that are specific to this study, perhaps the most important is the potential for observer bias resulting from the difficulty of adequate blinding. Because

one of the measurements being made was an assessment of sedation, the observer soon became aware whether or not an anaesthetic agent was being administered. Shielding the rest of the animal from the observer measuring the tail response was not considered a practical proposition and would have blunted the definition of the end-point. However, inspection of the relationship between NT and the onset of sedation in the treatment groups (Figures 2, 3, 4, 6 and 7) shows that a decrease in NT usually preceded the onset of sedation and, by implication, revelation of allocation to a treatment group.

Another criticism, specific to this study, relates to the handling of the data for the two propofol groups (PROP1 and PROP2) in part 2 of the study. Although the relationship between NT and plasma propofol concentration (Figure 7) mirrors that for thiopentone (Figure 2), it must be remembered that the propofol data are drawn from two groups in which the animals received propofol at different rates and in which the control values for NT were different. Although this method of data handling was imposed by the difficulties of producing the required behavioural changes with a single infusion rate, our conclusions, drawn on the basis of this analysis, require cautious interpretation.

Notwithstanding these limitations, we believe that the hyperalgesic action of these anaesthetic drugs can be consistently demonstrated using this animal model. Although behavioural models of this type do not allow identification of the predominant site(s) within the central nervous system responsible for either the anaesthetic or hyperalgesic actions of drugs, of the available strategies, they provide the closest representation of the human behavioural response to anaesthetic drugs. We believe that we have strengthened the argument that the hyperalgesia demonstrated in human studies[1] is a feature of low concentrations of all drugs which share the property of facilitation of choride flux at the $GABA_A$ receptor/chloride channel complex.

One possible mechanism for GABA-induced hyperalgesia[19] is inhibition, by $GABA_A$ agonist drugs, of medullary serotonergic neurones that normally inhibit interneurones in the superficial layers of the dorsal horn. The resulting loss of descending inhibition could result in an exaggerated response of dorsal horn neurones to input from peripheral nociceptors.[20] Although this is an attractive explanation, further evidence, involving, at the very least, the use of specific GABA antagonists, would be required to confirm or disprove this hypothesis.

It should be noted our results do not necessarily refute the conclusions of other investigators who have failed to demonstrate an electrophysiological basis for barbiturate or non-barbiturate induced hyperalgesia.[5] Rather, a behavioural approach should be regarded as complementary to other experimental designs in an effort to consolidate, from the mass of evidence, a consistent explanation for the contradictory effects of anaesthetic drugs in the intact animal. In a recent review,[21] Tanelian *et al.* speculated that different anaesthetic drugs may have different affinities for $GABA_A$ receptor subtypes within the CNS which can result in agent-specific behavioural effects at low concentrations, but more homogeneous effects at higher concentrations when receptor specificity is lost. The same hypothesis, albeit with scanty evidence of a site of action, could be advanced to explain the perplexing coincidence of sedation and hyperalgesia.

In conclusion, we have demonstrated, in an animal model of previously established utility, that sub-hypnotic plasma concentrations of three different barbiturates (thiopentone, methohexitone and pentobarbitone), as well as a non-barbiturate anaesthetic agent (propofol), produce a behavioural state characterized by an increased response to noxious stimulation. This may represent a hyperalgesic effect of these drugs.

Acknowledgements
The authors wish to thank Ms. Carol Owen, Zeneca Pharmaceuticals Inc., Canada, for providing analytical samples of diisopropylphenol. We also thank Dr. Chris Eagle, Associate Professor and Chair, Department of Anaesthesia, University of Calgary, for his continued support of the Anaesthesia Research Laboratory.

References

1 *Dundee JW.* Alterations in response to somatic pain associated with anaesthesia. II: The effect of thiopentone and pentobarbitone. Br J Anaesth 1960; 32: 407–14.
2 *Briggs LP, Dundee JW, Bahar M, Clarke RSJ.* Comparison of the effect of diisopropyl phenol (ICI 35868) and thiopentone on response to somatic pain. Br J Anaesth 1982; 54: 307–11.
3 *Neal MJ.* The hyperalgesic action of barbiturates in mice. Br J Pharmacol 1965; 24: 170–7.
4 *Anker-Møller E, Spangsberg N, Arendt-Neilsen L, Schultz P, Kristensen MS, Bjerring P.* Subhypnotic doses of thiopentone and propofol cause analgesia to experimentally induced acute pain. Br J Anaesth 1991; 66: 185–8.
5 *Jewett BA, Gibbs LM, Tarasiuk A, Kendig JJ.* Propofol and barbiturate depression of spinal nociceptive neurotransmission. Anesthesiology 1992; 77: 1148–54.
6 *Kitahata LM, Saberski L.* Are barbiturates hyperalgesic? (Editorial). Anesthesiology 1992; 77: 1059–61.
7 *Ramabadran K, Bansinath M, Turndorf H, Puig MM.* Tail immersion test for the evaluation of a nociceptive reaction in mice. Methodological considerations. Journal of Pharmacological Methods 1989; 21: 21–31.
8 *Archer DP, Ewen A, Roth SH, Samanani N.* Plasma,

brain, and spinal cord concentrations of thiopental associated with hyperalgesia in the rat. Anesthesiology 1994; 80: 168–76.

9 *Soncrant TT, Holloway HW, Stipetic M, Rapoport SI.* Cerebral glucose utilization in rats is not altered by hind-limb restraint or by femoral artery and vein cannulation. J Cereb Blood Flow Metab 1988; 8: 720–6.

10 *Randall LO, Selitto JJ.* A method for measurement of analgesic activity on inflamed tissue. Archives Internationales de Pharmacodynamie 1957; 4: 409–19.

11 *Kissin I, Brown PT, Robinson A, Bradley EL Jr.* Acute tolerance in morphine analgesia: continuous infusion and single injection in rats. Anesthesiology 1991; 74: 166–71.

12 *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 1987; 67: 32–41.

13 *Pavan I, Buglione E, Massiccio M, Gregoretti C, Burbi L, Berardino M.* Monitoring propofol serum levels by rapid and sensitive reversed-phase high-performance liquid chromatography during prolonged sedation in ICU patients. J Chromatogr Sci 1992; 30: 164–6.

14 *Peduto VA, Concas A, Santoro G, Biggio G, Gessa GL.* Biochemical and electrophysiologic evidence that propofol enhances GABAergic transmission in the rat brain. Anesthesiology 1991; 75: 1000–9.

15 *Yaksh TL, Aimone LD.* The central pharmacology of pain transmission. *In*: Wall PD, Melzack R (Eds.). Textbook of Pain, 2nd ed. New York: Churchill Livingstone, 1989: 186, 190.

16 *Kissin I, Mason JO III, Vinik HR, McDanal J, Bradley EL Jr.* Barbiturates inhibit stress-induced analgesia. Can J Anaesth 1987; 34: 146–51.

17 *Vidal C, Jacob J.* Hyperalgesia induced by non-noxious stress in the rat. Neurosci Lett 1982; 32: 75–80.

18 *Raja SN, Meyer RA, Campbell JN.* Peripheral mechanisms of somatic pain. Anesthesiology 1988; 68: 571–90.

19 *Moreau J-L, Fields HL.* Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission. Brain Res 1986; 397: 37–46.

20 *Headley PM, Duggan AW, Griersmith BT.* Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. Brain Res 1978; 145: 185–9.

21 *Tanelian DL, Kosek P, Mody I, MacIver MB.* The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. Anesthesiology 1993; 78: 757–76.