Thiopentone induced enhancement of somatic motor responses to noxious stimulation: influence of GABA_A receptor modulation

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Purpose: This study was conducted to determine whether hyperalgesic effects of subanaesthetic concentrations of thiopentone could be attributed to GABA_A receptor effects.

Methods: All studies were performed on 50 rats in a prospective, randomized, blinded fashion using saline-injected animals as controls. Using a modified Randall-Selitto technique, the motor behavior stimulated by noxious stimulation was quantified by determining the lowest tail pressure required to provoke a withdrawal response (somatic motor response threshold, SMRT). In the first protocol (21 rats), we studied the effects of 0.5, 1.5 and 5 mg·kg⁻¹ iv of the GABA_A agonist, muscimol, on SMRT. In the second protocol (20 rats), the effects of administration of saline, muscimol 0.5 mg·kg⁻¹, or the competitive GABA_A antagonist, bicuculline 0.25 mg·kg⁻¹, upon the SMRT-reducing effects of a standardized thiopentone infusion were observed.

Results: No dose of muscimol produced hyperalgesia. The highest dose of muscimol used (5 mg·kg⁻¹) produced pronounced analgesic effects, raising the SMRT above 750 g. No change in SMRT was detected with the smaller doses of muscimol. Given in combination with muscimol (0.5 mg·kg⁻¹), thiopentone produced analgesia, as shown by an increase in SMRT ($P = 0.009$). In the bicuculline treated animals, SMRT decreased linearly with increasing plasma thiopentone concentrations ($P < 0.001$). The slope of the relationship in the

bicuculline group was not significantly different from that observed in the saline-treated group, indicating that bicuculline did not block the hyperalgesic effects of thiopentone.

Conclusion: The results of these studies suggest that hyperalgesia associated with thiopentone is not mediated primarily by GABA_A receptors.

Objectif: Cette étude visait à déterminer si les effets hyperalgiques produits par des concentrations sous-anesthésiques de thiopentone pouvaient être attribués à la médiation des récepteurs GABA_A.

Méthodes: Toutes les expériences ont été réalisées de façon aléatoire, prospective et en double aveugle sur 50 rats avec des contrôles au soluté physiologique. À l'aide d'une technique modifiée de Randall-Selitto, le comportement moteur après stimulation nocive a été quantifié en déterminant la pression la plus basse requise pour provoquer le retrait de la queue (le seuil de réponse motrice somatique, SRMS). Dans un premier protocole (21 rats), les effets sur le SRMS de 0,5, 1,5 et 5 mg·kg⁻¹ iv d'un antagoniste GABA_A, le muscimol ont été étudiés. Dans un deuxième protocole (20 rats), on a observé les effets de l'administration de sol. phys., de muscimol 0,5 mg·kg⁻¹ ou de l'antagoniste compétitif du GABA_A la bicuculline 0,25 mg·kg⁻¹.

Résultats: Aucune des doses de muscimol n'a provoqué d'hyperalgie. La dose la plus forte de muscimol (5 mg·kg⁻¹) a produit un effet analgésique prononcé, élevant le SRMS au-dessus de 750 g. On n'a pas décelé de changement de SRMS avec doses plus faibles de muscimol. Administré en association au muscimol (0,5 mg·kg⁻¹), le thiopentone produit de l'analgésie, comme le montre l'augmentation du SRMS ($P = 0,009$). Chez les animaux traités à la bicuculline, le SRMS a diminué de façon linéaire avec l'augmentation des concentrations plasmatiques de thiopentone ($P < 0,001$). Dans le groupe bicuculline, la pente de la relation n'était pas différente de celle observée dans le groupe traité au sol. phys., démontrant ainsi que la bicuculline ne bloque pas les effets hyperalgiques du thiopentone.

Key words

ANAESTHETICS, INTRAVENOUS: thiopentone;
PAIN: antianalgesia;
RECEPTORS: GABA_A.

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Conclusion: *Les résultats de ces études suggèrent que l'hyperalgie associée au thiopentone ne se produit pas principalement par la médiation des récepteurs GABA_A.*

Management of the responses to noxious stimuli resulting from acute surgical trauma changes abruptly when the patient moves from the operating theatre into the postanesthetic recovery room (PARR). During general anaesthesia, the administration of analgesics is commonly guided by the presence or absence of cardiovascular signs of autonomic nervous system responses to noxious stimulation. For the unconscious patient, the term "pain" has little meaning. In contrast, during recovery from general anaesthesia, the intensity of pain experienced by patients is inferred primarily from pain behaviour, including verbal complaints, paraverbal sounds (e.g., moaning), and motor behaviour such as facial expressions and body posturing. For the patient who is sedated or emerging from general anaesthesia, the relationships between tissue injury and pain behaviour may be different from those when that patient is fully alert. Subanaesthetic concentrations of barbiturates increase sensitivity to noxious stimuli in humans¹ and enhance nociceptive transmission in animal preparations with intact spinal cords.²⁻⁸ Barbiturates have been shown to depress nociceptive transmission in animals with spinal cord section or forebrain ablation or in *in vitro* preparations.⁸⁻¹⁰ These results suggest that the mechanism(s) underlying the enhancement of nociceptive transmission by barbiturates involve supraspinal sites.^{2,11} It has been proposed that enhanced nociceptive transmission is observed when the effects of reduction of activity in descending inhibitory bulbospinal projections exceed the barbiturate-induced depression of impulses within the spinal cord.^{2,11}

Results from whole animal studies of combinations of barbiturates with opioids have been consistent with the basic physiology studies described above. In studies of the somatic motor response to tail clamping, small doses of barbiturates^{12,13} increased the doses of opioid which were required to produce analgesia. This finding may have been due to facilitation of withdrawal responses by barbiturates, because results from our laboratory¹⁴ have shown that both the somatic motor response (SMR) and the tail flick response to heat were enhanced by small doses of thiopentone. Methohexitone, pentobarbitone and propofol each enhanced the somatic motor response in a fashion similar to that seen with thiopentone,¹⁵ as shown by decreases in the threshold for the motor response (SMRT).

These results suggest that increased pain perception due to enhanced nociceptive transmission may be an

undesirable side effect of small doses or low concentrations of some commonly used anaesthetics, particularly during emergence from anaesthesia.¹⁶ It is not known whether these hyperalgesic and antianalgesic properties of barbiturates are mediated by the same mechanisms that produce other behavioural effects such as sedation and anaesthesia. To design drugs or strategies to avoid the hyperalgesic effects, it would be helpful to understand the mechanism(s) involved. Modulation of GABA_Aergic stimulation is a prominent feature of barbiturate action¹⁷ and GABA_Aergic mechanisms are thought to be important in the brainstem centers proposed to mediate descending modulation of nociception.¹⁸ The experiments in this study were designed to evaluate the influence of GABA_A receptor stimulation and blockade on the facilitation of the withdrawal response by small doses of thiopentone. If GABA_A receptors mediate the withdrawal response enhancement, then we expected that small doses of a GABA_A receptor agonist given alone would mimic the effects of thiopentone, and, when given before thiopentone, would have additive effects. Conversely, we expected that the administration of a GABA_A receptor antagonist would abolish or diminish the withdrawal response enhancement caused by thiopentone. Muscimol is a directly-acting GABA_A receptor agonist which enters the brain relatively slowly; functionally relevant concentrations were achieved 20 min after intravenous injection.^{19,20} Bicuculline is a specific competitive GABA_A receptor antagonist which has been shown to be effective in the central nervous system within 10 min of *iv* injection.²¹

In these studies we tested two hypotheses: (1) systemic administration of muscimol enhances withdrawal responses (as shown by decreases in the threshold for the somatic motor response, SMRT) to a degree similar to thiopentone and (2) systemic administration of muscimol or bicuculline, respectively enhance and depress the facilitated withdrawal responses produced by small doses of thiopentone.

Methods

Following approval by the University of Calgary Animal Care Committee, we studied 50 male Sprague Dawley rats weighing 250–500 g. The hypotheses were tested in two sets of experiments. As a first step, we designed an experiment to determine whether systemic administration of a GABA_A receptor agonist, muscimol, would enhance motor withdrawal responses in a fashion similar to that previously observed with the barbiturates. In the first protocol (21 rats), we compared SMRT measurements after intravenous administration of three doses of muscimol to values obtained in saline-injected

control animals. The second set of experiments were designed to evaluate whether the enhancement of withdrawal responses by thiopentone was altered by the administration of muscimol or the GABA_A receptor antagonist bicuculline. In the second protocol (20 rats), SMRT measurements during a standardized thiopentone infusion were measured in animals pretreated with either muscimol or bicuculline and compared with those in saline-injected control animals.

Animal model

To enable the precise administration of study drugs and to permit frequent plasma sampling, we conducted these studies under the conditions of partial restraint commonly used for radioautographic studies in the rat.²² The effects of partial restraint upon the somatic motor response were evaluated by comparing SMRT measurements made in 29 unrestrained animals with values obtained once the animals were instrumented and partially restrained. Twenty of these animals were entered into Protocol 2 while nine were not studied further.

Briefly, after weighing, each animal was anaesthetized with halothane 2% in oxygen. Femoral arterial and venous catheters were inserted under direct vision. A thin film of lidocaine 2% jelly was applied to the wound edges. The animal was partially restrained in a plaster cast extending from the ankles to the lower thorax and taped to a wooden block. In our laboratory these procedures require 10–20 min of anaesthesia. During the two hours that the animals were allowed for emergence from anaesthesia, body temperature was monitored with a rectal thermometer and maintained at 36–38°C with a heating lamp. Previous measurements (unpublished results) have shown that halothane is undetectable in plasma in this rat model after two hours of emergence. Prior to study, we evaluated the general health of each animal by visual inspection, comparison of its weight with the weights of litter mates and with measurements of arterial blood gas tensions, haematocrit, and mean arterial pressure. Animals with values outside the normal limits for our laboratory (arterial oxygen tension >75 mmHg, arterial carbon dioxide tension 30–45 mmHg, pH 7.30–7.45, haematocrit 36–50 vol%, mean arterial pressure 85–120 mmHg) were excluded from further study. The physiological measurements were repeated 20 min after injection of treatment drugs in Protocol 1 and at the termination of the thiopentone infusion in Protocol 2 to determine whether drug treatment had resulted in deviations of the physiological variables from normal.

Somatic motor response threshold to tail pressure

A modification of the Randal-Selitto test²³ was used to

assess the response to noxious stimulation. The principal outcome measure in this study was the threshold for the first motor response to pressure applied to the tail. An Analgesy-Meter (Stoelting Instruments, Chicago, U.S.A.) applied a constantly increasing pressure to randomly selected points within the distal 2 cm of the tail, which was supported on a plinth. The end-point (somatic motor response threshold, SMRT) was defined as the first movement of the tail which could displace it from the plinth. Two observers performed the measurements; the observer who identified the end-point was blinded to the pressure scale on the Analgesy-Meter and also to the treatment or drug administered to the animal under study.

Drug administration

MUSCIMOL

In the first protocol, animals were randomly assigned to receive intravenous injections of saline (1 ml) or muscimol (0.5, 1.5 or 5 mg · kg⁻¹) dissolved in 1 ml of saline. Since we expected the onset of any effects of muscimol on SMRT to occur during the 20 min after injection,^{19,20} we measured SMRT before and 4, 8, 12, 16 and 20 minutes after the administration of muscimol.

In the second protocol, animals were randomly assigned to receive injections of saline, muscimol 0.5 mg · kg⁻¹, or bicuculline, 0.25 mg · kg⁻¹,²¹ *iv*. SMRT was measured before and 20 min after saline or muscimol injection¹⁹ and 10 min after bicuculline injection.²¹ The thiopentone infusion was then started.

THIOPENTONE ADMINISTRATION AND MEASUREMENT

Animals receiving thiopentone were treated with a continuous infusion of the drug (0.15 ml · min⁻¹, 0.67 mg · kg⁻¹ · min⁻¹).^{14,15} Thiopentone concentrations in plasma were measured by high-pressure liquid chromatography with spectrophotometric detection as previously described.^{14,15} Shed blood was replaced with three times the volume of normal saline. The limit of detection of thiopentone was 0.3 µg · ml⁻¹. The coefficient of variation was +5% (1 standard deviation/mean value × 100, determined for 20 µg · ml⁻¹, the highest value on the standard curve). The accuracy (determined from the standard curves) was ±4.3%.

Behavioural assessment

The sedative effects of the drug treatments were assessed prior to each SMRT measurement and quantified with sedation scores: 0 – awake, actively exploring the environment (sniffing, head movement in response to stimuli); 1 – slightly drowsy, responds briskly to mild vibrissal or head stimulation, 2 – very drowsy, responds

slowly to vigorous vibrissal or head stimulation, 3 – unresponsive.

Data analysis

The SMRT values in unrestrained and partially restrained animals were compared with a paired *t* test. The effect of muscimol administration upon SMRT was evaluated by repeated measures ANOVA at each dose. The SMRT values before and after injection of saline, muscimol and bicuculline were compared by two-way repeated measures ANOVA. For each of thiopentone-treated animals, the SMRT value at each measurement time was expressed as a percent of the control SMRT value in that animal measured 20 min after injection of saline or muscimol and 10 min after bicuculline injection. For each treatment group the relationship between the mean % control SMRT value at each measurement time and the corresponding mean plasma thiopentone concentration was evaluated. When, as in previous studies, inspection of the results suggested a simple decrease in SMRT with increasing thiopentone concentrations, a linear regression analysis was performed. Linear relationships were compared by analysis of variance.

More complex relationships between SMRT and plasma thiopentone concentration were evaluated by incremental polynomial regression analysis. Since we do not know of any physical model for a mechanism relating withdrawal responses to plasma thiopentone concentrations we could not intuitively estimate the best number of model parameters. We selected to use the equation with the minimum number of parameters that lead to a statistically significant result in order to provide an estimate of the relationship of the mean SMRT values with the mean thiopentone values and also the 95% confidence intervals of that relationship. Since we did not estimate the number of parameters before the study, this form of analysis is partly post hoc and requires validation by further studies. The sedative effects of thiopentone were characterized by the maximum degree of sedation observed within each of the treatment groups (saline, muscimol, and bicuculline). A χ^2 test was performed upon the data in a contingency table containing the number of individuals achieving maximal sedation scores of 0, 1, 2, or 3 for each treatment group. In addition, the mean plasma thiopentone concentrations associated with sedation scores 1, 2, and 3 were calculated for each of the treatment groups and compared by *t* test.

For all statistical tests, significance was inferred when $P < 0.05$.

Results

Within each protocol there were no differences in mean body weight among the different treatment groups. All

FIGURE 1 Mean somatic motor response threshold, grams $\pm$ SEM, measured before and after injection of saline (filled circles, $n = 7$), muscimol $0.5 \text{ mg} \cdot \text{kg}^{-1}$ (empty triangles, $n = 7$), muscimol $1.5 \text{ mg} \cdot \text{kg}^{-1}$ (filled triangles, $n = 5$), or muscimol $5 \text{ mg} \cdot \text{kg}^{-1}$ (empty square, $n = 3$). *Indicates that the mean SMRT at 5 min after injection of muscimol $5 \text{ mg} \cdot \text{kg}^{-1}$ ($>750 \text{ g}$) was significantly greater than the mean pre-injection value ($P < 0.05$).

values for mean arterial pressure, rectal temperature, haematocrit and blood gas tensions remained within the normal limits for our laboratory. The volume of blood shed for sampling was approximately 2.5 ml per animal.

Influence of partial restraint on SMRT

No difference was found between the mean SMRT values ($\pm$ standard deviation) in unrestrained ($231 \pm 30 \text{ g}$) and partially restrained animals ($243 \pm 39 \text{ g}$) ($t = -1.48$, 28 df, $P = 0.149$). The paired *t* test had a power of 0.83 ($\alpha = 0.05$) to detect an intercondition difference of 17 g (7% of the mean unrestrained SMRT).

Influence of muscimol on SMRT

Results are summarized in Figure 1. Two way repeated measures ANOVA failed to reveal any effects of treatment ($P = 0.212$), time ($P = 0.324$) or the interaction between treatment and time ($P = 0.579$) on SMRT during the 20 min. after injection of saline or muscimol 0.5 or 1.5 $\text{mg} \cdot \text{kg}^{-1}$ (Figure 1). Animals that received 5 $\text{mg} \cdot \text{kg}^{-1}$ muscimol, became drowsy (sedation scores 1 and 2) and failed to respond to tail pressure applied by the Analgesy-Meter (maximum pressure = 750 g) within four minutes of the muscimol injection. The number of animals randomized to this group was therefore limited

TABLE I Maximum sedation scores observed during infusion of thiopentone in animals pretreated with saline muscimol or bicuculline

Treatment	Sedation score			
	0	1	2	3
Saline (<i>n</i> = 7)	1	2	3	1
Muscimol (<i>n</i> = 5)	0	0	0	5
Bicuculline (<i>n</i> = 8)	7	0	1	0

Values are the number of animals observed in each sedation score category. *N* is the total number of animals in each treatment group. The proportion of animals in each sedation category is related to the treatment ($\chi^2 = 22.11$, df, P ($P = 0.001$)).

to three. Corneal reflexes in these animals remained intact. Animals that received saline remained alert (sedation score 0). Animals that received the smaller doses of muscimol either remained alert (4/6 animals with $0.5 \text{ mg} \cdot \text{kg}^{-1}$, 3/6 animals with $1.5 \text{ mg} \cdot \text{kg}^{-1}$) or appeared slightly drowsy (sedation score 1) (Table I).

Influence of bicuculline and muscimol on SMRT during thiopentone infusion

Before drug injection the mean SMRT values ($\pm$ SEM) were similar in the three groups of animals (saline group – 241 ± 17 g, bicuculline group – 270 ± 11 g, muscimol group 226 ± 16 g). Following drug injection, SMRT values increased by 13% of control in the bicuculline-treated animals (two way repeated measures ANOVA, $F = 3.645$, 2 df, $P = 0.048$) but remained similar to the values before injection in the saline and muscimol groups.

Plasma thiopentone concentrations increased with time at a greater rate in the muscimol group (repeated measures ANOVA, $P < 0.001$) (Figure 2).

During the thiopentone infusion (Figure 3), linear regression analysis revealed that the SMRT (% control) values decreased linearly with increasing thiopentone concentrations in both the saline-treated ($r = 0.891$, $P = 0.003$) and the bicuculline-treated animals ($r = 0.963$, $P < 0.001$). The slopes $\pm$ S.E.M. of these regression lines were $-1.23 \pm 0.14\% \cdot \mu\text{g}^{-1} \cdot \text{ml}^{-1}$ in the saline group and $-0.94 \pm 0.20\% \cdot \mu\text{g}^{-1} \cdot \text{ml}^{-1}$ in the bicuculline group ($t = -1.25$, $P = 0.223$). Bicuculline did block the sedative effects of the thiopentone infusion (Table II).

In the animals treated with muscimol, the SMRT increased during thiopentone infusion (Figure 3); incremental order polynomial regression analysis revealed that the best fit of the data was achieved with a second order polynomial ($\text{SMRT} = 282 - 4.72 \cdot [\text{Thiopentone}]_{\text{plasma}} + 0.2 \cdot [\text{Thiopentone}]_{\text{plasma}}^2$, $P = 0.009$). No specific model was used to derive this relationship and the components do not have any physical meaning. A second order polynomial contained the smallest number of parameters required to produce a statistically significant result.

FIGURE 2 Mean plasma thiopentone concentration, $\mu\text{g} \cdot \text{ml}^{-1} \pm$ SEM, during the continuous infusion, in animals pretreated with saline (filled circles, $n = 7$), muscimol $0.5 \text{ mg} \cdot \text{kg}^{-1}$ (empty triangles, $n = 5$), or bicuculline $0.25 \text{ mg} \cdot \text{kg}^{-1}$ (empty squares, $n = 8$). *Indicates that the increase of thiopentone concentrations over time was greater in the muscimol group than in either of the other treatment groups ($P < 0.001$).

FIGURE 3 The regression lines $\pm 95\%$ confidence intervals for relationships between somatic motor response threshold, and plasma thiopentone concentration in animals pretreated with saline (empty squares, solid line, $n = 7$), muscimol $0.5 \text{ mg} \cdot \text{kg}^{-1}$ (filled circles, $n = 5$), or bicuculline $0.25 \text{ mg} \cdot \text{kg}^{-1}$ (filled squares, fine dashed line, $n = 8$). Symbols represent mean values at the specific measurement times.

TABLE II Plasma thiopentone concentrations associated with sedation scores 1, 2, and 3

Treatment	Sedation Score		
	1	2	3
Saline (<i>n</i> = 7)	14 ± 4 (7)	26 ± 6 (5)	28 ± 11 (3)
Muscimol (<i>n</i> = 5)	14 ± 9 (5)	20 ± 2 (5)	30 ± 15 (5)
Bicuculline	29 (<i>n</i> = 1)	44 (1)	

Results are mean values of plasma thiopentone concentration in $\mu\text{g} \cdot \text{ml}^{-1} \pm \text{SEM}$. Brackets () include the number of observations contributing to each mean value. Mean values are provided for the single animal in the bicuculline group which appeared to be sedated.

Discussion

The results of these experiments suggest that, rather than being a mechanism responsible for thiopentone-induced enhancement of nociceptive transmission, GABA_A receptor stimulation opposed this effect. We expected that if low doses of muscimol were to act in a fashion similar to thiopentone, then the withdrawal reflex would be enhanced and the threshold for the response would therefore decrease. In contrast, what we observed was that systemic administration of $5 \text{ mg} \cdot \text{kg}^{-1}$ muscimol produced sedation and analgesia as shown by marked increases in the somatic motor response threshold. In a smaller dose of $0.5 \text{ mg} \cdot \text{kg}^{-1}$, muscimol did not alter the baseline SMRT values, but when thiopentone was infused, SMRT values increased ($P = 0.009$), indicating an analgesic effect. Consistent with results from other studies,^{24,25} GABA_A-receptor block with bicuculline had a mild analgesic effect, shown by an increased SMRT. However, the relationship between SMRT and plasma thiopentone concentration in the bicuculline-treated animals was similar to that seen in the saline injected group.

Pretreatment with bicuculline blocked the sedative actions of thiopentone (Table II) while preserving the SMR enhancing effects (Figure 3). This dissociation suggests that these two effects may not be mediated by the same receptor or mechanism.

The results of muscimol pretreatment suggest that muscimol may antagonize the enhancement of nociceptive transmission produced by thiopentone. This was probably not due to increased sedation after muscimol. Referring to Table II and Figure 3, when the SMRT values at the plasma thiopentone concentration associated with a sedation score of 2 (saline-treated $26 \pm 6 \mu\text{g} \cdot \text{ml}^{-1}$, muscimol-treated $20 \pm 2 \mu\text{g} \cdot \text{ml}^{-1}$) are compared, the mean SMRT was reduced ($-30 \pm 17\%$) in saline-treated animals while it remained unchanged ($-1 \pm 16\%$) in the muscimol-treated animals ($P = 0.011$). Thus, at the same sedation score, there was a

difference in the SMRT values in the muscimol and saline groups.

We did not anticipate the alteration of pharmacokinetics of the thiopentone observed in the muscimol group (Figure 2), and we do not have any explanation for this result. However, for all treatment groups, measurements of SMRT were made throughout similar ranges of thiopentone concentrations, and since we analyzed the relationships between SMRT and thiopentone concentration, we did not feel that the small differences in pharmacokinetics in the muscimol group altered the conclusions. In previous studies,^{14,15} the hyperalgesic effects of thiopentone were detected at subhypnotic concentrations. As the animals became more profoundly sedated, the hyperalgesic effects were progressively reversed and analgesia predominated.¹⁵ These observations were the basis for our choice of the dose of muscimol for the second protocol. Our concern was that if the dose of muscimol was too large, then the combination with thiopentone, even in the low concentrations of thiopentone at the beginning of the infusion, would produce sedation and analgesia, and we would not detect a hyperalgesic state that would be present at lower concentrations. Consequently, $0.5 \text{ mg} \cdot \text{kg}^{-1}$ muscimol was selected because in preliminary studies (data not shown), this was the largest dose which did not produce sedation and increases of SMRT during the initial phase (first two minutes) of the thiopentone infusion.

Our interest in the mechanisms by which subhypnotic doses of thiopentone may influence somatic motor responses was stimulated by the effects that anaesthetics may have on pain perception during emergence from anaesthesia. Our laboratory observations^{14,15} are consistent with the results of human studies.¹ Using tibial pressure analgesimetry to assess pain perception, Dundee¹ reported that thiopentone in single doses of 6 to $10 \text{ mg} \cdot \text{kg}^{-1}$ produced detectable decreases in pain threshold for up to five hours after injection. He felt that the time course of the hyperalgesia following doses of thiopentone and pentobarbital was consistent with the redistribution kinetics of these drugs. Similar findings have recently been reported by Hugo *et al.*,¹⁶ who observed that patients who received $5 \text{ mg} \cdot \text{kg}^{-1}$ of thiopentone for anaesthesia induction reported more moderate and less mild pain compared to patients who received propofol ($2.5 \text{ mg} \cdot \text{kg}^{-1}$).

Although the changes in SMRT in this study were not large, it must be recalled that the noxious stimulus was not very intense. For example, in a previous study,¹⁴ when the thiopentone concentration was sufficient to render the animals unresponsive to head manipulation and a tail pressure of 750 g, there was still a vigorous response to tail clamping. The magnitude of the thiopen-

tone-related reductions in SMRT in the present report was similar to the reduction in vocalization thresholds observed in a rat model of neuropathic pain,²⁶ and therefore may represent an enhancement of nociceptive processing which is of behaviour significance to the animal. Further studies using supraspinal end-points such as vocalization, in freely moving animals will be required to further assess this issue.

The use of partially restrained animals, although a standard technique for autoradiographic studies, was unusual and potentially problematic for a study involving behavioural measurements. One major concern was that restraint stress might induce analgesia.²⁷ It was possible that the administration of thiopentone, either through sedation or by other mechanisms might non-specifically reverse this stress-induced analgesia, enhancing the somatic motor response. The method of partial restraint used in this study allows freedom of movement of the head, forelimbs and thorax. Two results from the present study suggest that restraint did not have a major influence on the control SMRT measurements. First, consistent with our previous findings,¹⁴ the SMRT results in the unrestrained and restrained animals were not different. From the power analysis above, the largest error that could reasonably be expected in these results is a 7 % increase in SMRT values following restraint, whereas the maximal reduction in SMRT with thiopentone infusion in the saline group was 30% (95% confidence intervals – 22–38%). Second, all but one of the bicuculline-treated animals remained alert throughout the thiopentone infusion and still experienced a reduction in SMRT of 44% (95% confidence intervals – 38–50%). Plasma glucose measurements from our laboratory (unpublished data) did not support the hypothesis that thiopentone sedation reduces stress in partially restrained rats. Sedated (steady state plasma thiopentone concentration, 10 $\mu\text{g} \cdot \text{ml}^{-1}$), partially restrained animals had the same mean plasma glucose concentrations as untreated control animals ($7 \pm 1 \text{ mmol} \cdot \text{dl}^{-1}$ in each group of eight animals). Finally, our SMRT values for control values in restrained animals (200–300 g) in the present report are much less than the corresponding values (490 g) reported by others using this technique in unrestrained rats.²⁸

Although systemic administration of GABA_A agonists produces analgesia in the tail flick²⁹ and paw pressure³⁰ tests, the doses of drug used were relatively large and were accompanied by considerable sedation.³¹ The effects of smaller doses of direct-acting GABA_A agonists on nociception are not well established. Also, although GABA_A agonists produce analgesia in most animal tests, the analgesia is not reliably blocked by the GABA_A antagonists bicuculline or picrotoxin,³¹ casting

some doubt on the receptor specificity. Bicuculline has intrinsic analgesic properties³¹ which were confirmed in the present study.

The effects of thiopentone on the somatic motor response are biphasic, with enhancement of SMR (decrease in SMR threshold) at low concentrations and depression (increase in SMR threshold) at high concentrations.¹⁴ Although it was possible that muscimol could also produce similar biphasic effects upon SMR, the results of present study seem to make this hypothesis unlikely. The SMRT was observed during the time period when small doses (0.5 and 1.5 $\text{mg} \cdot \text{kg}^{-1}$) of muscimol would be gradually entering the central nervous system. We would therefore expect that if low concentrations of muscimol do enhance the somatic motor response to the same degree as thiopentone, this phenomenon would have been detected in these studies. Only one dose of muscimol (0.5 $\text{mg} \cdot \text{kg}^{-1}$) was used in Protocol 2. The SMRT was measured over a full range of thiopentone concentrations from 0–40 $\mu\text{g} \cdot \text{ml}^{-1}$, and no decrease in this variable was observed in the 0–10 $\mu\text{g} \cdot \text{ml}^{-1}$ range as would be expected if the muscimol was enhancing the thiopentone-induced hyperresponsiveness. Our results therefore confirm and extend the observations of others:³³ sedative doses of muscimol (5 $\text{mg} \cdot \text{kg}^{-1}$) are analgesic, while smaller doses (1.5 and 0.5 $\text{mg} \cdot \text{kg}^{-1}$) do not alter SMRT.

In summary, the results of the present study do not support the hypothesis that the enhanced somatic motor response induced by thiopentone is primarily due to GABA_A-ergic effects of the drug. In addition, the studies show that the enhanced withdrawal response produced by thiopentone may occur independently from its sedative effects.

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