Barbiturates inhibit stress-induced analgesia

Igor Kissin MD PH D, John O. Mason III BS,
H. Ronald Vinik MD, Judy McDanal MD,
Edwin L. Bradley Jr PH D

The effect of pentobarbitone and thiopentone on stress-induced analgesia was studied in 40 male Sprague-Dawley rats. Antinociception was determined by measuring motor reaction threshold to the noxious pressure on the tail with the use of an "Analgesy-meter." Stress was induced by placement of a clamp on the hind paw. The stress procedure was found to cause an increase in reaction threshold, which was partially suppressed by naloxone 0.5 mg·kg^{-1}. Pentobarbitone in a subanaesthetic dose of 25 mg·kg^{-1}, SC, almost completely abolished the stress-induced increase in the reaction threshold (an increase in reaction threshold from 329 ± 33 g to 486 ± 62 g in control group, and from 250 ± 26 g to 273 ± 35 g in pentobarbitone group, p < 0.02 for the difference in the threshold changes). Thiopentone used in a dose of 25 mg·kg^{-1}, IV, caused a loss of the righting reflex for 37 ± 10 minutes; stress procedure applied ten minutes after regaining the righting reflex did not cause any increase in the reaction threshold (with an increase in the reaction threshold in control group from 355 ± 50 g to 540 ± 26 g, p < 0.001 for the difference between the groups). The results suggest that the barbiturates in subanaesthetic doses inhibit stress-induced analgesia. Thiopentone used in an anaesthetic dose has the potential for inhibition of stress-induced analgesia in the period of recovery from anaesthesia.

From the Department of Anesthesiology, University of Alabama at Birmingham, School of Medicine, Birmingham, Alabama 35294, where correspondence should be addressed to Dr. Kissin.

In 1957, Beecher[1] summarized data demonstrating stress-induced analgesia in man. Since 1976, many investigators have shown that exposure to stress (i.e., electric foot shock, rotation, cold water) can cause analgesia in laboratory animals.[2–5] Evidence now indicates the existence of multiple endogenous analgesia systems that are activated by stress.[5,6] In some cases, stress-induced analgesia appeared opioid-mediated; in others, this was not found.[5] Data also can be found indicating that stress enhances the analgesia effect of opioid drugs.[7,8]

Barbiturates used in subanaesthetic doses are known to antagonize the analgesic action of opioid drugs. A marked antianalgesic action of thiopentone and pentobarbitone had been demonstrated using the pain threshold method in humans.[9,10] The antiopioid effect of barbiturates was reproduced in mice[11] and in rats.[12] The purpose of the present study was to determine whether subanaesthetic doses of barbiturates antagonize stress-induced analgesia.

The most commonly used model of stress-induced analgesia involves foot shock stress (with hind paw electrical stimulation being the most effective)[13] and measurement of analgesia with the tail-flick test (latency of tail withdrawal in response to tail thermal stimulation).[2,5] We did not feel that this model would be appropriate for the study of antianalgesic effect of barbiturates because it was found previously in man that barbiturates demonstrate an antianalgesic effect with pressure-induced pain, but not with thermal pain.[14,15] This fact agrees with new evidence that different modalities of pain sensation (including those induced by thermal and pressure stimuli) are processed differently by the central nervous system, with involvement of different subtypes of opioid receptors.[16–18] As a result, we used pressure-induced noxious stimulation both for measurement of analgesia and for induction of stress.

Methods

The experiments were performed on 40 male Sprague-Dawley rats weighing 275–325 g. Antinociception was determined by measuring motor reaction threshold to the noxious pressure with the use of an "Analgesy-meter" (Ugo Basel, Milan, Italy), a device that provides increasing pressure. The rat's tail was positioned on a platform,

and the pressure plate (0.7 mm edge) attached to the device was placed 1 to 2 cm from the tip of the tail (the rat was held by the researcher's hand). Pressure was increased at the constant rate of $48 \, g \cdot sec^{-1}$ until the animal made an attempt to escape (coordinated struggle) which represents a clear-cut end-point.[19,20] The pressure at that moment was recorded, and the mean of three consecutive measurements was taken as the individual reaction threshold.

The stress was caused by placement of a clamp on the rat's hind paw for two minutes. To do this, with the rat sitting in a transparent chamber, the hind leg of the rat was extended through the slot in the chamber wall, and the clamp was placed on the extended paw. Branches of the clamp were covered with 1.5 mm rubber coats. Pressure of the clamp on the paw was approximately $40 \, g \cdot mm^{-2}$ with the total area under pressure of $0.7 \, cm^2$. Pressure was strong enough to prevent the rat from drawing his paw into the chamber and, at the same time, was below the pressure level that might damage the paw. The reaction threshold was measured two minutes before and 2, 7, 15, 30, and 60 minutes after the stress procedure; each time the animal was taken out of the chamber for the measurement and returned to it following the measurement.

Three series of experiments were performed: pentobarbitone, thiopentone and naloxone. The pentobarbitone series was comprised of a group of six animals given pentobarbitone and a group of six animals given saline (control). Pentobarbitone was administered in a dose of $25 \, mg \cdot kg^{-1}$, SC, 30 minutes before the stress procedure (the maximal dose that did not cause loss of the righting reflex in our preliminary experiments).

The thiopentone series was also comprised of two groups of six animals each, using thiopentone and saline. The administration of thiopentone posed a problem because the procedure of intravenous injection by itself caused a 15- to 20-minute increase in the reaction threshold. Because the regular induction dose of thiopentone provides a marked and prolonged antianalgesic effect in the recovery period,[9,10] we used this agent in an anaesthetic dose, but stress analgesia was induced when animals had already recovered from anaesthesia. Thiopentone was used in a dose of $25 \, mg \cdot kg^{-1}$, (ED_{95} value for the prevention of movement response to the tail clamp reported in our previous study).[21] This dose caused a loss of righting reflex for 37 ± 10 minutes (mean $\pm$ SD, $N = 6$). Ten minutes after the righting reflex recovery, rats were subjected to the stress procedure. In control (saline) experiments, stress procedure was performed with intervals equal to those between injection of the anaesthetic and the stress test.

The third, naloxone series of experiments were performed to find out whether the stress procedure caused

an opioid or a nonopioid form of analgesia. It comprised a group of eight animals given naloxone ($0.5 \, mg \cdot kg^{-1}$, SC, 15 minutes before the stress procedure) and a group of eight animals given saline.

In all experiments each animal received only one injection of an agent, followed by one stress procedure. Pentobarbitone sodium was obtained from Elkins-Simm, thiopentone sodium from Abbott, and naloxone hydrochloride from DuPont. Injection volume ranged from 0.3 to 1.0 ml, and duration of injection from 30 to 60 sec.

The method of simple randomization was used with alternation of the performance of experiments on rats from control and treatment groups. For purposes of analysis, the pre-stress threshold values were subtracted from subsequent post-stress threshold values for each rat. The mean threshold change in the control group was compared to the mean threshold change in the drug group using a two-sample t-test, at each time period following stress induction. For comparisons of control and drug reaction threshold curves as a function of time after stress, a repeated measures analysis of variance was used.[22] Animal care standards in this study were in accordance with federal and institutional policy and with Standards of the American Association for Accreditation of Laboratory Animal Care as specified in the Guide for the Care and Use of Laboratory Animals.[23]

Results

Figure 1 demonstrates the data on the effect of pentobarbitone on stress-induced analgesia. It shows that pentobarbitone in a subanaesthetic dose of $25 \, mg \cdot kg^{-1}$, SC, abolished the stress-induced increase in the reaction threshold (an increase in the reaction threshold from $329 \pm 33 \, g$ to $486 \pm 62 \, g$ in the control group, and from $250 \pm 26 \, g$ to $273 \pm 35 \, g$ in the pentobarbitone group, $p < 0.02$ for the difference in the threshold changes). Pentobarbitone administration also resulted in a tendency for decrease in the baseline reaction threshold.

Figure 2 demonstrates the stress-induced analgesia during recovery from thiopentone anaesthesia. Thiopentone used in a dose of $25 \, mg \cdot kg^{-1}$, IV, caused a loss of righting reflex for 37 ± 10 minutes; stress procedure applied ten minutes after regaining the righting reflex did not cause any increase in the reaction threshold (with an increase in the reaction threshold in the control group from $355 \pm 15 \, g$ to $540 \pm 26 \, g$, $p < 0.001$ for the difference between the groups).

Figure 3 shows the results of the naloxone series of experiments. Naloxone in a dose of $0.5 \, mg \cdot kg^{-1}$, SC, suppressed the increase in reaction threshold. The suppression was not complete, however (an increase in the reaction threshold from $327 \pm 15 \, g$ to $457 \pm 35 \, g$ in the control group, and from $326 \pm 21 \, g$ to $366 \pm 24 \, g$ in the
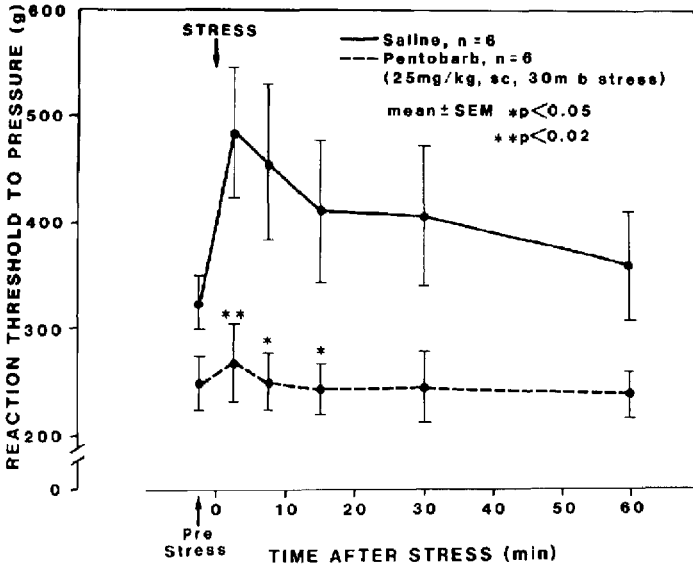
FIGURE 1   Effect of pentobarbitone (25 mg·kg^{-1}, SC) on the stress-induced increase in motor reaction threshold to pressure. P-value represents differences between changes in the threshold in the pentobarbitone and saline groups.
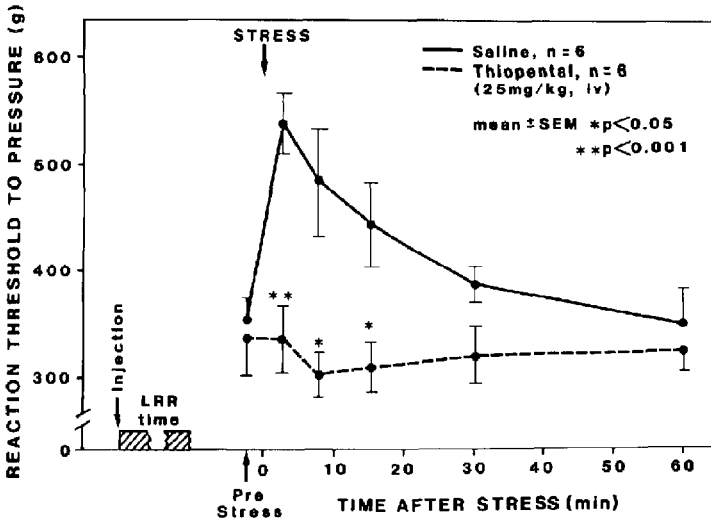

FIGURE 2   Effect of thiopentone (25 mg·kg^{-1}, IV) on stress analgesia induced after recovery of righting reflex. LRR time indicates loss of the righting reflex after injection of thiopentone – 37 ± 10 minutes (mean ± SD). Stressor was applied ten minutes after regaining righting reflex in the thiopentone group and at comparable time intervals (from intravenous injection) in the saline group. P-value reflects the differences between changes in the threshold in thiopentone and saline groups.
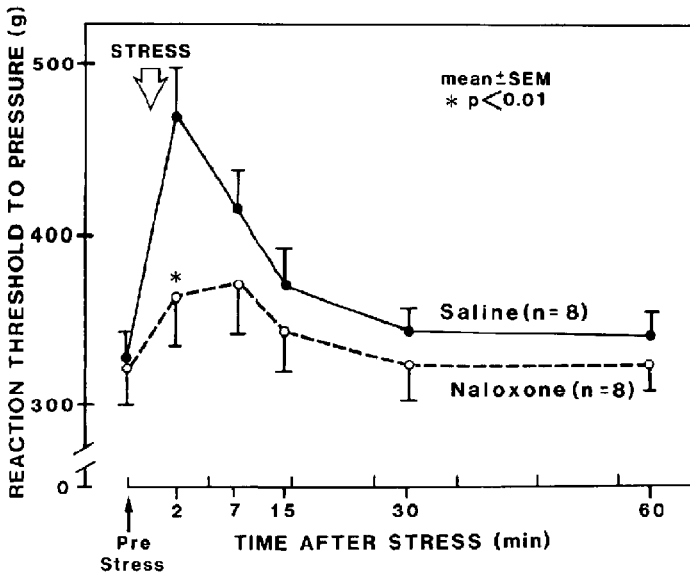
FIGURE 3 Effect of naloxone (0.5 mg·kg^{-1}, SC) on the stress-induced increase in motor reaction threshold to pressure. P-value represents the difference between changes in the threshold in the naloxone and saline groups.

naloxone group, p < 0.01 for the difference in the threshold changes).

Discussion

An endogenous central nervous system pain-modulating network has recently been discovered.[5,6] This system produces analgesia by interfering with afferent transmission of neural messages produced by intense stimuli. The analgesia produced by this system partially depends on the release of endogenous opioid substances.[24] The system is set in motion by clinically significant pain, such as postoperative pain.[25] Barbiturates are known to antagonize the analgesic action of opioid drugs in humans[9,10] and several studies demonstrated that this effect of barbiturates is reproducible in the experimental model in rodents.[11,12,26]

The question arises as to whether subanaesthetic doses of barbiturates also antagonize stress-induced analgesia. The present study shows that pentobarbitone and thiopentone have a potential to antagonize stress-induced analgesia. Both agents abolished the stress-induced increase in the reaction threshold to the noxious pressure in rats.

It was reported that the antiopioid effect of thiopentone injection for the induction of anaesthesia may be present in the period of recovery from anaesthesia.[9,10]

We have found, in the animal model, that the same is true for stress-induced analgesia. After recovery of the righting reflex that was lost following thiopentone injection the animals were unable to increase the reaction threshold in response to the stress.

In the present experiments naloxone attenuated the increase in the reaction threshold, indicating that the stress analgesia induced in our experiments has, at least partially, an underlying opioid mechanism. This fact suggests that antagonism between barbiturates and opioid drugs and barbiturate-induced inhibition of stress analgesia may have some common mechanisms.

It was shown recently that in rats, anaesthetized with halothane, electric foot shock did not cause analgesia.[27] The authors concluded that conscious processes are necessary for the occurrence of stress-induced analgesia. In another study,[5] the effect of pentobarbitone in an anaesthetic dose of 55 mg·kg^{-1} on stress-induced anaesthesia was found to depend on the parameters of stress application: pentobarbitone anaesthesia abolished the analgesic response to intermittent foot shock, but had no effect on analgesia from continuous foot shock in rats. We could find no data in the literature on the effect of subanaesthetic doses of anaesthetics on stress-induced analgesia.

In conclusion, the present study shows that barbitu-

rates, pentobarbitone and thiopentone, antagonize stress-induced analgesia in rats. The clinical implication of this finding might be that barbiturates used perioperatively may inhibit the pain suppression system[25] and thus increase the perception of pain. Because thiopentone has a relatively long half-life (5–12 hours), its antianalgesic effect may last for several hours after the induction of anaesthesia. Our study implies that after recovery from anaesthesia induced by thiopentone, the endogenous pain suppression system may still not be functional. This may have an important implication for management of postoperative pain.

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Résumé

L'effet du pentobarbitone et thiopentone sur l'analgésie induite par le stress est étudié chez 40 rats Sprague-Dawley. L'antinociception fut déterminée par la mesure du seuil de la réaction motrice suite à une pression nocive appliquée sur la queue utilisant un "Analgesy-meter". Le stress était induit en plaçant une pince sur la patte arrière. La manœuvre stressante occasionna une augmentation du seuil de réaction qui était partiellement supprimée par le naloxone 0.5 mg·kg^{-1}. Le pentobarbitone administré à des doses inférieures aux doses anesthésiantes (25 mg·kg^{-1}) en sous cutané a presque complètement aboli l'augmentation du seuil de réaction suite au stress (l'augmentation du seuil de réaction était de 329 ± 33 g à 486 ± 62 g pour le groupe contrôle, et de 250 ± 26 g à 273 ± 35 g pour le groupe pentobarbitone, p < 0.02 quant à la différence des variations du seuil). Le thiopentone utilisé à des doses de 25 mg·kg^{-1} par voie intraveineuse a provoqué une perte du "righting reflex" pour 37 ± 10 minutes. La manœuvre stressante appliquée dix minutes après le regain du "righting reflex" n'a pas provoqué une augmentation du seuil de réaction (l'augmentation du seuil de réaction dans le groupe contrôle était de 355 ± 50 g à 540 ± 26 g, p < 0.001 concernant la différence entre les groupes). Ces résultats suggèrent que les barbituriques à des doses inférieures aux doses anesthésiantes inhibent l'analgésie induite par le stress. Le thiopentone utilisé à des doses anesthésiques a le potentiel d'inhiber l'analgésie induite par le stress en période de réveil de l'anesthésie.