

Halothane Antagonizes Effect of Morphine on the Motor Reaction Threshold in Rats

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The ability of halothane (used in "sub-MAC" concentrations) to modify the effect of morphine on motor response threshold to pressure was studied and compared with pentobarbital in 241 rat experiments. It was found that halothane (0.5-0.7%, insp.) decreased the reaction threshold to pressure, as did pentobarbital. Halothane (0.5%) increased morphine ED₅₀ for the reaction threshold to pressure from 0.21 mg·kg⁻¹ (95% fiducial limits: 0.13-0.29 mg·kg⁻¹) to 0.52 mg·kg⁻¹ (0.28-0.73 mg·kg⁻¹, *P* < 0.0001). Pentobarbital in a dose of 3 mg·kg⁻¹ demonstrated a similar antanalgesic effect. Neither halothane nor pentobarbital antagonized the effect of morphine with motor response to the tail clamp. On the contrary, both agents strengthened this effect. It has been suggested that the effect of morphine on the motor response threshold to pressure results primarily from activation of inhibitory control mechanisms concerned with this response; halothane in a subanesthetic concentration depresses the inhibitory control mechanisms and, therefore, weakens the effect of morphine. (Key words: Analgesics: morphine. Anesthetics, intravenous: pentobarbital. Anesthetics, volatile: halothane. Interactions (drug). Pain: antinociceptive effect.)

IT HAS BEEN SHOWN in our previous experiments¹ that halothane in subanesthetic concentrations profoundly weakened the effect of morphine on cardiac acceleration response to tail clamp in rats. When purposeful movement response to tail clamp was used for the assessment of morphine-halothane interaction, a subanesthetic concentration of halothane enhanced the effect of morphine.¹ Thus, the morphine-halothane interaction with regard to the sympathetic response (heart rate end point) was opposite of that to the somatic response (purposeful movement end point).

The possibility of antagonism between anesthetics and narcotic analgesics first was demonstrated by Clutton-Brock.^{2,3} Using the pain threshold method in man, he has shown that an analgesic-induced increase in the pain threshold to pressure on the anterior surface of the tibia was abolished by thiopental or pentobarbital. The antanalgesic effect of barbiturates was present only when subanesthetic doses were used. Dundee and associates confirmed this observation and reported that analgesic-induced increase in the reaction threshold to pressure was decreased by thiopental in surgical patients.^{4,5} A

number of studies performed in animals with the reaction threshold technique also demonstrated the ability of barbiturates to antagonize the effect of narcotic analgesics.^{6,7} Changes in the threshold in these studies were related to somatic reactions.

Comparing the reported data on morphine-barbiturate antagonism with our results on morphine-halothane interaction with regard to the motor response to tail clamp, it is possible to make two following alternative suggestions. First, halothane, in contrast to barbiturates, does not antagonize the effect of morphine on somatic responses to noxious stimulation. Second, the technique of movement response to tail clamp is not appropriate to demonstrate the antagonism related to the analgesic effect of morphine.

The aim of the present study was to compare the ability of halothane and pentobarbital to modify the effect of morphine using two techniques of assessment of the analgesic action: the reaction threshold to pressure and the motor response to tail clamp.

Methods

Experiments were performed on 241 Sprague-Dawley rats weighing 275-350 g. Motor reaction threshold to pressure was measured with a method suggested by Green and *et al.*⁸ and motor response to tail clamp was studied with a modified Haffner's technique.⁹

Reaction threshold was determined with an "Analgesy-meter" (Ugo Basile, Milan, Italy), a device that provides an increasing pressure via a cone-shaped rod with a pressure point. The rat tail was positioned on a Teflon® platform, and the pressure point of the device was placed in the middle of the tail on its dorsal surface (the rat was held by the experimenter's hand). Pressure was increased at a constant rate of 80 g/s until the animal made an attempt to escape (coordinated struggle). The pressure at that moment was recorded and the mean of three consecutive measurements was determined as the individual reaction threshold. In the series of experiments where analgesic action was assessed with the use of probit analysis, we used the following approach to obtain quantal data. In a group of 20 nontreated rats, the mean reaction threshold to pressure was determined. The effect in the morphine-treated animals was designated as positive if the individual reaction threshold to

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TABLE 1. Series of Experiments

Series	Number of Experiments	Methods	Agents
1	50	Reaction threshold	Halothane
2			
a	25	Reaction threshold	Morphine
b	25	Reaction threshold	Morphine-halothane
3			
a	*	Tail clamp	Morphine
b	*	Tail clamp	Morphine-halothane
4	41	Reaction threshold	Pentobarbital
5			
a	25	Reaction threshold	Morphine
b	25	Reaction threshold	Morphine-pentobarbital
6			
a	25	Tail clamp	Morphine
b	25	Tail clamp	Morphine-pentobarbital

See explanation in the text.

* Data were taken from our previous publication.¹

pressure exceeded the nontreated group mean threshold by two standard deviations of the mean.¹⁰

Noxious stimulation was induced by placement of a hemostat in the middle of the tail (pressure of 0.3 kg/mm) for 60 s. In nontreated rats, this stimulation always caused immediate biting of the hemostat. Only the absence of purposeful movement response to stimulation was considered as a positive effect of a dose.

With each of the two agents under investigation (halothane and pentobarbital), three series of experiments were performed (table 1). First, the effect of various doses of an agent on the reaction threshold was measured—series 1 (halothane) and 4 (pentobarbital). Second, the effect of an agent on the morphine dose–effect curve for the reaction threshold was studied—series 2 (halothane) and 5 (pentobarbital). In addition to this, the effect of an agent on the morphine dose–effect curve for the tail clamp response was tested—series 3 (halothane) and 4 (pentobarbital).

In series 1, 2, and 3 (halothane, or control for halothane), experiments were carried out in a clear chamber where oxygen, or a halothane–oxygen mixture could be delivered (4 l/min). The rat's tail (for stimulation) or hind leg (for morphine injection into the saphenous vein) could be extended from the chamber through a slot. Another airtight opening was provided for the experimenter's hand to hold the rat during reaction threshold measurement. Halothane was vaporized in a Draeger vaporizer, and vapor concentration in the chamber was monitored continuously with a calibrated Beckman® LB-2 infrared analyzer. In experiments

with halothane administration, the animals were exposed to the agent for 30 min prior to testing. In experiments where halothane was used in combination with morphine, the animals were exposed to the halothane for 15 min; then morphine was injected (for the injection, a hind leg was extended through a slot in the chamber) and an additional 15 min of continuing exposure to halothane was followed by a test. In experiments where morphine was used without halothane, the animals were kept in the chamber with oxygen for 15 min; then morphine was injected and the response was tested after an additional 15 min.

In series 4, 5, and 6 (pentobarbital, or control for pentobarbital) were performed without the use of the chamber. Pentobarbital and/or morphine were injected intravenously 15 min before testing.

The effect of halothane on the reaction threshold (series 1) was studied at the following inspired concentrations of halothane: 0.3% (group of five rats); 0.5% (group of 10 rats); 0.7% (group of 10 rats); and 1.0% (group of five rats). The control group in this series of experiments consisted of 20 rats.

The effect of halothane on the morphine dose–effect curve for the reaction threshold was investigated in two subseries of experiments, one (2a) with morphine alone and another (2b) with the morphine–halothane combination. The dose–effect curve for morphine was determined as was described in our previous experiments.¹¹ Briefly, five groups of five rats constituted a morphine dose–effect curve. In one group of animals, the dose of morphine was low enough so that all animals were unaffected, and in another group it was high enough so that all were affected. Dose levels for these two groups were determined in preliminary experiments. In the three remaining groups, the doses of the drug were spaced between the abovementioned marginal doses. As a result, the doses in this subseries (2a) ranged from 0.1 mg·kg⁻¹ to 0.5 mg·kg⁻¹. When morphine was used with halothane (2b), the concentration of halothane was kept constant at 0.5% in all five groups of experiments, and morphine was administered in doses ranging from 0.3 mg·kg⁻¹ to 1.0 mg·kg⁻¹.

The data on the effect of halothane on the morphine dose–effect curve for the purposeful movement response to tail clamp were taken from our previous publication.¹

The effect of pentobarbital on the reaction threshold (series 4) was studied at the following doses: 3 mg·kg⁻¹ (group of five rats); 5 mg·kg⁻¹ (group of six rats); 10 mg·kg⁻¹ (group of five rats); and 20 mg·kg⁻¹ (group of five rats). Control group in this series of experiments consisted of 20 rats.

The effect of pentobarbital on the morphine dose–effect curve for the reaction threshold was investigated in two subseries of experiments, one (5a) with morphine

and another (5b) with morphine-pentobarbital combination. Five groups of five rats constituted a morphine dose-effect curve, with the doses of morphine for the first subseries (5a) ranging from 0.1 mg·kg⁻¹ to 0.2 mg·kg⁻¹. In the second subseries of experiments (5b), the dose of pentobarbital was kept constant at 3 mg·kg⁻¹ in all five groups, while morphine was used in doses ranging from 0.15 mg·kg⁻¹ to 0.7 mg·kg⁻¹.

The effect of pentobarbital on the morphine dose-effect curve for tail clamp response was studied in a similar way in two subseries of experiments, one (6a) for morphine alone and another (6b) for morphine-pentobarbital combination. In the first subseries of experiments (6a), the morphine dose range was from 3 to 10 mg·kg⁻¹. In the second subseries of experiments (6b), the dose range for morphine was from 2 to 6 mg·kg⁻¹ and pentobarbital was kept at a constant level of 3 mg·kg⁻¹.

The drugs used were morphine sulfate (Merck), halothane (Ayerst), and pentobarbital sodium (Butler). Morphine and pentobarbital were injected into the saphenous vein, the duration of injection being 5-10 s, and the volume of the injections was 0.5-1.0 ml. Each animal was given only one dose of the agent (or a combination).

In graded data analysis, to determine the significance of the difference between mean reaction thresholds for various groups of rats, the Duncan multiple-range test was used. In quantal data analysis, the probit method¹² was used. The ED₅₀ values obtained were compared with Student's *t* test. Calculations were performed on an IBM® 4341 computer.

Animal care standards in this study were in accordance with federal and institutional policy and standards of the American Association for Accreditation of Laboratory Animal Care as specified in the Guide for Care and Use of Laboratory Animals.‡

Results

Figure 1 shows the effect of halothane on the reaction threshold to pressure. At inspired concentrations of 0.5% and 0.7%, there was a significant decrease in the threshold from 625 ± 23 g (control group) to 484 ± 30 g and 470 ± 24 g, respectively (*P* < 0.05). With a further increase in the concentration of the agent, the reaction threshold began to increase and at the concentration of 1.0%, in one out of five rats, the threshold

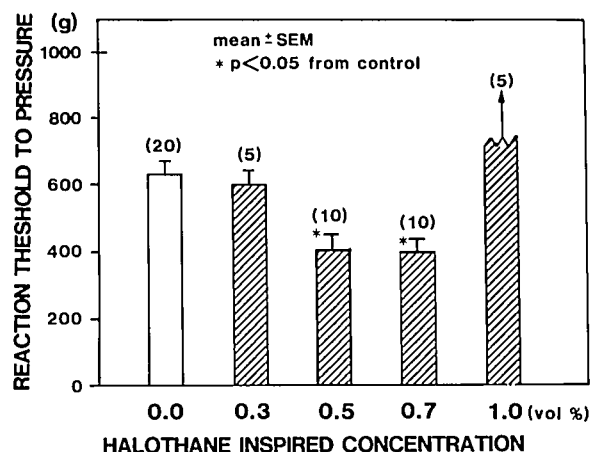


FIG. 1. The effect of halothane on motor reaction threshold to pressure on the tail. The open column represents control animals; each hatched column represents a group of animals at the indicated concentration of halothane. In parentheses is the number of animals in a group. At the concentration of 1.0%, in one out of five rats, the threshold was impossible to measure since the reaction to pressure was absent at the highest force provided by the "Analgesy-meter" (1,250 g).

was impossible to measure because reaction to pressure on the tail was absent at the highest force provided by the "Analgesy-meter" (1,250 g).

The effect of halothane on the morphine dose-effect curve for reaction threshold to pressure is illustrated in figure 2 (left part). The morphine dose-response curve for the reaction threshold to pressure moved to the right along the dose axis when morphine was administered in combination with halothane (0.5%). Morphine ED₅₀ for the reaction threshold was 0.21 mg·kg⁻¹ (95% fiducial limits: 0.13-0.29 mg·kg⁻¹) without halothane and 0.52 mg·kg⁻¹ (0.28-0.73 mg·kg⁻¹) with halothane (*P* < 0.0001). The change in the ED₅₀ demonstrated that halothane weakened the effect of morphine on the reaction threshold.

The right part of figure 2 illustrates, for comparison, previously reported data on the effect of halothane on the morphine dose-effect curve for purposeful movement response to tail clamp.¹ As one can see, halothane moved the morphine dose-effect curve to the left along the dose axis. In other words, halothane produced an effect opposite of that with reaction threshold: it strengthened the effect of morphine on the tail clamp response.

Figure 3 shows that pentobarbital at doses of 5 and 10 mg·kg⁻¹ significantly decreased the reaction threshold to pressure. When the dose was increased up to 20 mg·kg⁻¹, in four out of five animals the reaction threshold to pressure was absent at the pressure level of 1,250 g (highest force on the "Analgesy-meter"). The

‡ Guide for the Care and Use of Laboratory Animals. DHEW Publication No. (NIH)78-23, Washington, D.C., US Government Printing Office, 1978.

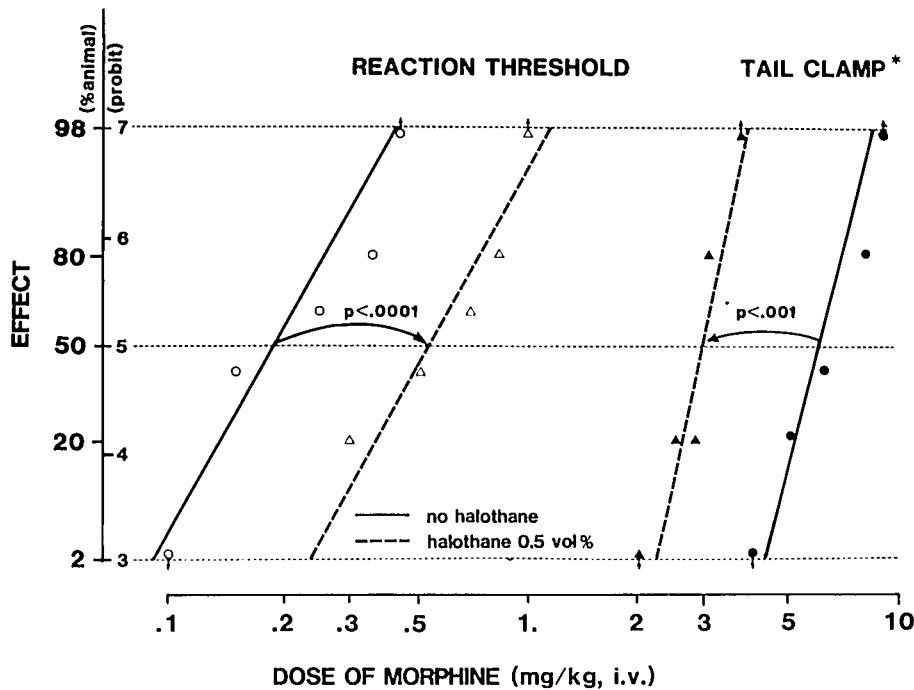


FIG. 2. Halothane-induced changes in morphine dose-effect curves for the reaction threshold to pressure (on the left) and for the tail clamp response (on the right). (Data for the tail clamp curves were taken from our previous publication.¹) Dose of morphine on a log scale. Each dot or triangle represents a group of five animals at the indicated dosage. Solid lines indicate dose-effect curves when morphine was administered alone; interrupted lines indicate when morphine was used in combination with a subanesthetic dose of halothane.

effects of pentobarbital $3 \text{ mg} \cdot \text{kg}^{-1}$ on the morphine dose-effect curve for the reaction threshold and for tail clamp response qualitatively resembled the effects of halothane and are presented in figure 4. The two left curves show that pentobarbital shifted the morphine dose-effect curve for the reaction threshold to the right along the dose axis ($P < 0.0001$), indicating that pentobarbital weakened the effect of morphine on this index. At the same time, pentobarbital $3 \text{ mg} \cdot \text{kg}^{-1}$ caused a shift in the morphine dose-response curve for the tail clamp response to the left along the dose axis

($P < 0.001$), which demonstrates an effect opposite to that with the reaction threshold.

Discussion

The present study demonstrated that halothane 0.5% (inspired) increased morphine ED_{50} for the reaction threshold to pressure from $0.21 \text{ mg} \cdot \text{kg}^{-1}$ to $0.52 \text{ mg} \cdot \text{kg}^{-1}$. By contrast, as was previously reported,¹ halothane decreased morphine ED_{50} for the motor response to tail clamp from $6.0 \text{ mg} \cdot \text{kg}^{-1}$ to $3.1 \text{ mg} \cdot \text{kg}^{-1}$. It was shown by White *et al.*¹³ in rats that the ratio of inspired-to-alveolar halothane (F_i/F_a) progressively decreased, most significantly during the first two hours of anesthesia. After a 30-min exposure to halothane, the F_i/F_a ratio in their experiments was 1.4. Their halothane MAC value was 1.11%. These two values indicate that 0.5% of inspired halothane used in our experiment was probably equal to approximately 1/3 MAC.

While comparing morphine doses obtained in our experiments with those reported by other investigators, two factors should be taken into account. First, morphine potency with intravenous administration is two to four times higher than its potency with subcutaneous or intraperitoneal administration.^{14,15} Second, morphine potency measured with "response to pressure" methods depends on strength of the applied force, and, as a result, the doses of morphine needed to block the response may vary from 2 to $8 \text{ mg} \cdot \text{kg}^{-1}$ (sc administration of the agent).⁸ The maximal reported analgesic doses for morphine were $15 \text{ mg} \cdot \text{kg}^{-1}$ (ED_{50} , Haffner's

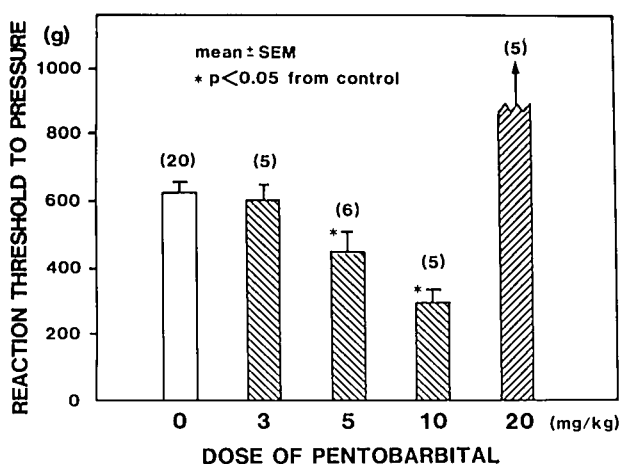


FIG. 3. The effect of pentobarbital on motor reaction threshold to pressure on the tail.

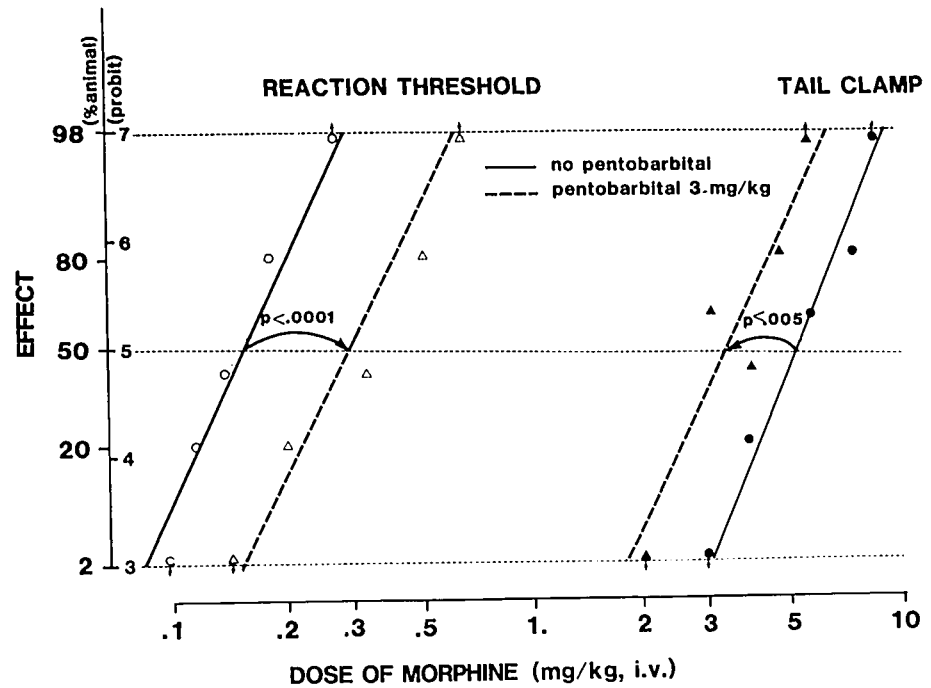


FIG. 4. Pentobarbital-induced changes in morphine dose-effect curves for reaction threshold to pressure (on the left) and for tail clamp response (on the right).

technique; sc administration),¹⁶ and 20 mg · kg⁻¹ (ED₁₀₀, tail flick to hot water, ip administration).¹⁷ The minimal reported dose was 0.16 mg · kg⁻¹ (ED₅₀ phenylquinone-induced writhing, sc administration).¹⁸ The doses of morphine obtained in the present study—0.21 mg · kg⁻¹ iv for the reaction threshold to pressure and 6.0 mg · kg⁻¹, iv for tail clamp response—fell within the range of reported analgesic doses of morphine in rodents.

Changes in the morphine ED₅₀ for the reaction threshold under the influence of the "sub-MAC" dose of halothane were basically the same as with the subanesthetic dose of pentobarbital and indicated an antanalgesic effect of both agents. While this effect is well known for barbiturates, it has not been reported for halothane. However, it should be noted that Dundee and co-authors¹⁹ studied the effect of inhalational anesthetics in subanesthetic concentrations on pain threshold to pressure in unpremedicated volunteers and found that, while nitrous oxide, diethyl ether, and cyclopropane increased pain threshold, halothane had a small tendency to decrease it. It is also possible to see from the Clutton-Brock study on surgical patients that thiopental in a total dose of 50 mg did not decrease the pain threshold when premedication caused no significant increase in it. However, 50 mg thiopental completely abolished the pain threshold increase after premedication.³ This provides the basis for the suggestion that even a small tendency for a decrease in the pain threshold may be indicative of an agent's ability to antagonize the effect of analgesic agents. In the present study, halothane used

alone, like pentobarbital, caused a statistically significant decrease in the reaction threshold to pressure. This effect might be associated with inhibition of some inhibitory control mechanisms in the CNS.

Halothane-morphine antagonism, revealed by the reaction threshold to a pressure test, agrees with halothane-morphine antagonism demonstrated for cardiac acceleration to the tail clamp.¹ When comparing the degree of antagonism for the reaction threshold and for the cardiac acceleration response to the clamp, one can see that antagonism for the sympathetic response is much more pronounced.

It is of interest that neither halothane nor pentobarbital antagonized the effect of morphine with motor response to the tail clamp. On the contrary, both agents strengthened the effect of morphine. It is possible to suggest that halothane-morphine antagonism is related mainly to sympathetic responses to noxious stimulation. With regard to the somatic responses to pain, antagonism is not very pronounced and may be demonstrated only with the reaction threshold method. Since the reaction threshold to pressure may be altered by doses of morphine that are more than an order of magnitude smaller than those changing movement to the tail clamp, the reaction threshold method may be regarded as quite different from the tail clamp method. Motor response to very strong and abrupt noxious stimulation (tail clamp) and response to the weak, gradually increasing pressure (reaction threshold) may involve different mechanisms. Twenty years ago, Dundee and associates

suggested that "the ability of a drug to produce analgesia in subanesthetic concentrations and loss of consciousness in higher concentrations may be two separate entities mediated by different processes."¹⁹ If it is possible to extend this suggestion, one can assume the following: the purposeful movement response to tail clamp, as was suggested by Eger,²⁰ is an index of anesthesia (a hemostat is usually strong enough to cause some tail damage) and the reaction threshold to pressure is an index of analgesia. These two indices may reflect two separate entities mediated by different processes and, as a result, the outcomes of a drug interaction involving these processes are different. Sympathetic responses to tail clamp may be regarded in this case also as an independent entity.

Halothane-morphine antagonism observed with regard to the reaction threshold to pressure is concerned with relatively small doses of morphine. There is evidence that the mechanism of antinociceptive action of morphine, especially with small doses, is associated with activation of the descending inhibitory control systems.²¹⁻²⁴ Halothane used in "sub-MAC" doses may inhibit the inhibitory control systems and, as a result, the effect of morphine diminishes.

We conclude that halothane in "sub-MAC" doses antagonizes the effect of morphine on the reaction threshold to pressure. This effect is similar to the antanalgesic effect of pentobarbital. Although rat experimental data and human clinical experience are rather difficult to correlate, it should be noted that pentobarbital-morphine antagonism regarding somatic movement threshold to noxious stimulation demonstrated in rat^{6,7} agrees with the results reported for humans.²⁻⁴

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