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Pentobarbital-morphine Anesthetic Interactions in Terms of Intensity of Noxious Stimulation Required for Arousal

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Background: **Previous reports suggest that the outcome (synergism, antagonism, summation) of opioid-barbiturate interactions may depend on the depth of anesthesia. One aim of the present study was to determine whether pentobarbital, alone and in combination with morphine, blocks awakening caused by noxious stimulation in a dose-related manner: the more intense the noxious stimulation, the more pentobarbital is required to suppress the response. A second aim of the study was to determine whether the pentobarbital-morphine anesthetic interaction depends on the depth of anesthesia measured in terms of intensity of noxious stimulation required for behavioral arousal (recovery of the righting reflex).**

Methods: **Experiments were performed on rats, with the measure of anesthetic effect being suppression of the righting reflex. The noxious stimulus was pressure on the tail at four levels of intensity: 0.0, 0.25, 2.5, and 3.3 kg, generated with an Analgesy-Meter. Pentobarbital and morphine were injected intravenously *via* chronically implanted catheters. Dose-response curves for pentobarbital given alone and in combination with morphine were determined (by probit analysis) separately for each of the pressure levels.**

Results: **Pentobarbital, alone and in combination with morphine, blocked awakening caused by noxious stimulation of different intensities in a dose-related fashion so that more anesthetic was required to block awakening with more intense stimulation. The pentobarbital ED_{50} values were: 12.0, 19.5, 22.7, and 24.3 mg/kg for 0.0, 0.25, 2.5, and 3.3 kg pressure, respectively. The addition of morphine (1 mg/kg) reduced the pentobarbital ED_{50} values for 0.0, 0.25, and 2.5 kg pressure by 34% ($P < 0.0001$), 39% ($P < 0.0001$), and 21% ($P < 0.005$), respectively. No change was seen in the pentobarbital ED_{50} value at the maximal (3.3 kg) pressure level.**

Conclusions: **The results suggest that the depth of anesthesia can be measured in terms of intensity of noxious stimulation required for arousal and that the outcome of barbiturate-opioid anesthetic interaction depends on the depth of anesthesia. (Key words: Analgesics, opioid: morphine. Depth of anesthesia. Hypnotics: pentobarbital. Interactions, drugs.)**

IT was reported previously[1,2] that the result of opioid-barbiturate interactions depends on the nature of the endpoint of anesthesia. The barbiturate-opioid hypnotic interaction (loss of the righting reflex in rats) was synergistic,[2] whereas the barbiturate-opioid interaction with regard to blockade of the motor response to the tail-clamp resulted in antagonism.[1] This difference might depend not only on the endpoints of anesthesia but also on the depth of anesthesia: anesthetic requirements are much higher for blockade of the tail-clamp response than the righting reflex. Ausems *et al.* determined the alfentanil plasma concentrations necessary to supplement nitrous oxide in suppressing hemodynamic and somatic responses to noxious stimuli of different intensities in surgical patients and found a wide range of alfentanil requirements with different intensities of noxious stimulation.[3] These results could indicate the possibility of assessment of anesthetic depth as a function of intensity of noxious stimulation.

The aim of the present study was to determine whether pentobarbital, alone and in combination with morphine, blocks awakening caused by noxious stimulation of different intensities in a dose-related fashion and how morphine contributes to the pentobarbital-induced anesthesia at its different depths, assessed in terms of intensity of noxious stimulation required for behavioral arousal.

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Materials and Methods

Experiments were performed on male Sprague-Dawley rats weighing 225–275 g. The protocol for this study was approved by the Institutional Panel on Laboratory Animal Care. We performed four series of experiments; in each, pentobarbital requirements for the blockade of the righting reflex were determined with and without the addition of morphine. In the first series, the righting reflex was determined without noxious stimulation. In the other three series, pressure on the tail was used as a noxious stimulation.

To provide pressure, the rat's tail was positioned on a Teflon platform and a plate (0.7-mm edge) transmitting pressure from selected weights was applied on the border between the distal and third quarter of the tail for 60 s with the use of an Analgesy-Meter (Ugo Basil, Milan, Italy). The following three intensities of stimulation were used: 0.25, 2.5, and 3.3 kg. The selection of the levels of stimulation was based on the following approach. We performed a series of experiments with various weights inducing the vocalization (squeak) response in rats sedated with pentobarbital sodium (5 mg/kg, intravenously). This was done to provide a lower anchoring dose for a noxious stimulus. Sedation was used because approximately 20% of rats do not squeak in response to noxious stimulation. When sedated, all animals respond with squeak to weight 250 g and higher. All responses occurred within the 60 s of applied stimulation. Five groups of five animals were used to determine the stimulation-response curve. Animals in each group were stimulated with one of the following weights: 100, 125, 150, 200, or 250 g. The weight was removed at the first vocal response. The weight producing response in 95% of rats has been found to be 0.22 kg (fig. 1). As a result, 0.25 kg was used as a low level of stimulation. The next weight was 10 times greater, 2.5 kg. Pilot experiments revealed that even a small further increase in weight, from 2.5 kg to 3.3 kg, changes the slope of the pentobarbital dose-response curve. Therefore, our maximal stimulation was limited to 3.3 kg. The possibility of damage to the tail with high stimulation intensity was not important because the animals received one episode of noxious stimulation and were not reused.

The righting reflex test was regarded as positive if a rat placed on its back failed to right itself (with all four feet on the table) during the 60 s of stimulation (none of the rats righted itself before the noxious stimulation).

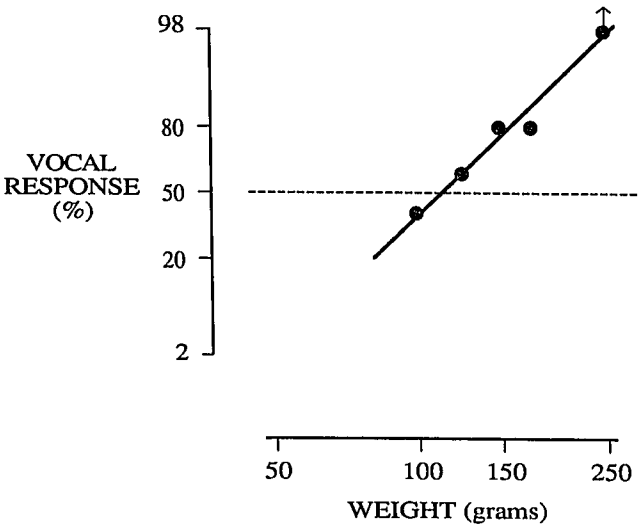

Fig. 1. Pressure-vocal response curve. Along the horizontal axis (on a log scale), pressure on the tail applied through a plate (0.7-mm edge) on the border between the distal and third quarter of the tail for 60 s. Along the vertical axis (on a probit scale), the percentage of animals responding to pressure with a squeak. Each circle represents five animals at the indicated dosage. The arrow superimposed on the upper symbol indicates that its actual location is higher (restriction due to probit scale of the figure).

For the injection of intravenous anesthetics, a catheter was chronically implanted into the jugular vein, and its free end was exteriorized through the skin at the back of the neck. Implantation was performed under ketamine-xylazine (100 mg/kg, intraperitoneally, and 15 mg/kg intraperitoneally, respectively) anesthesia several days before the experiment.

In each of the four series of experiments, two dose-effect curves were obtained (without and with morphine). Four groups of four animals were used for each curve, with the dose range determined in pilot experiments. The levels of the noxious pressure and the doses of pentobarbital and morphine used to determine the dose-effect curves are presented in table 1.

An additional fifth series of experiments was performed in which the possible role of hypercarbia in the outcome of experiments was examined. The rats used in this series of experiments were prepared with a catheter (PE-10) in the aorta placed through the femoral artery (in addition to the intravenous catheter). On the day of experiment, one group of rats (five animals) received 1 mg/kg morphine in combination with 24 mg/kg pentobarbital. Blood samples (0.2 ml) were withdrawn from the catheter before the first injection and 5 min after the second injection (pentobarbital).

Table 1. Doses of Pentobarbital (PTB) and Morphine (MPH) Used to Determine the Dose–Effect Curves

Series (kg, noxious stimulation)	Subseries	Group	N	Dose (mg/kg) PTB	MPH
0.0	PTB	1	4	10	0
		2	4	11	0
		3	4	12	0
		4	4	13	0
	PTB-MPH	1	4	6.5	1
		2	4	7.0	1
		3	4	8.0	1
		4	4	9.0	1
0.25	PTB	1	4	16	0
		2	4	18	0
		3	4	20	0
		4	4	23	0
	PTB-MPH	1	4	8	1
		2	4	10	1
		3	4	12	1
		4	4	14	1
2.5	PTB	1	4	20	0
		2	4	23	0
		3	4	26	0
		4	4	30	0
	PTB-MPH	1	4	16	1
		2	4	18	1
		3	4	20	1
		4	4	24	1
3.3	PTB	1	4	23	0
		2	4	24	0
		3	4	25	0
		4	4	26	0
	PTB-MPH	1	4	23	1
		2	4	24	1
		3	4	25	1
		4	4	26	1

Arterial blood gas tensions were measured using an IL System 1303 Blood Gas Analyzer (Instrumentation Laboratory, Lexington, MA).

In all series of experiments, each animal was given one predetermined dose of an agent or a combination of agents. Time between injections of agents and the tests was based on the time to peak effect for these agents: 15 min for morphine and 5 min for pentobarbital.[4] The peak times were chosen after preliminary experiments. In combined drug experiments, each drug was injected separately to synchronize the occurrence of the peak effects. Morphine sulfate was purchased from Eli Lilly (Indianapolis, IN) and pentobarbital sodium from Abbott (Chicago, IL). The agents or their combinations were injected over 60 s (pentobarbital) and 15 s (morphine). The total volume of injections was not more than 2.5 ml/kg.

Doses of morphine and pentobarbital were expressed in terms of the salts. Dose-response curves and ED_{50} values were determined with probit analysis.[5] Differences between the ED_{50} values were compared by using a *t*-test on the log ED_{50} values. Pairwise comparisons were made between the pentobarbital and pentobarbital-morphine subseries at each pressure level. In addition, within the pentobarbital subseries and within the pentobarbital-morphine subseries, comparisons were made between ED_{50}s for the following pairs of stimulations (kg): 0.0 *versus* 0.25, 0.25 *versus* 2.5, and 2.5 *versus* 3.3. Results were declared significant if $P < 0.05$. A Bonferroni correction to the significance level was used in the case of multiple comparisons.

Results

Figure 1 represents a noxious pressure-vocal response curve that was used to find appropriate pressure levels (see Materials and Methods). The pentobarbital dose-response curves for blockade of the righting reflex (with or without the addition of morphine) are presented in figure 2 and ED_{50} values in table 2. The table demonstrates that behavioral arousal manifested by restoration of the righting reflex occurs at different

Table 2. Pentobarbital (PTB) ED_{50} Values for Blockade of Righting Reflex at Different Intensities of Noxious Stimulation

Stimulation Intensity (kg)	PTB ED_{50} (mg/kg) Without Interacting Agents	Morphine (MPH) (1 mg/kg)	ED_{50} PTB + MPH / ED_{50} PTB
0.0	12.0 (10.7–16.4)	7.9 (7.2–9.6) ($P < 0.0001$)*	0.66 (0.59–0.75)
0.25	19.5 (17.4–23.5)	11.8 (9.6–16.4) ($P < 0.0001$)*	0.61 (0.52–0.70) ($P < 0.02$)†
2.5	22.7 (14.1–27.0)	17.9 (8.8–22.1) ($P < 0.005$)*	0.79 (0.67–0.93) ($P < 0.01$)†
3.3	24.3 (23.0–25.3)	24.0 (23.8–24.2) (NS)	0.99 (0.96–1.02)

Values in parentheses are confidence limits.

* Difference from PTB ED_{50} without MPH.

† Difference from next highest stimulation intensity.

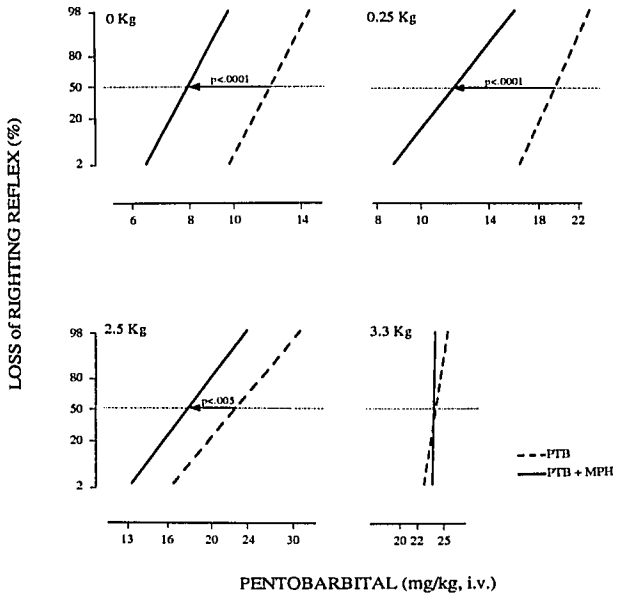

Fig. 2. Shifts in pentobarbital dose-response curves for blockade of the righting reflex induced by addition of morphine. Morphine was added in a fixed dose of 1 mg/kg. Numbers in the upper left corner of each segment indicate the intensity of noxious stimulation in kilograms.

dosages of pentobarbital, depending on the intensity of stimulation. The ED_{50} values for pentobarbital, when used alone, were different for the pressures 0.25 and 2.5 kg with the respective values of 19.5 (17.4–23.5) and 22.7 (14.1–27.0) mg/kg ($P < 0.05$ for the difference). With the pressure of 3.3 kg, the pentobarbital ED_{50} value was 24.3 (23.0–25.3) mg/kg and was not significantly different from the ED_{50} value at 2.5 kg. With the addition of the fixed dose of 1 mg/kg morphine, the difference between pentobarbital ED_{50} values at different noxious pressures became more pronounced: 11.8 (9.6–16.4) mg/kg, 17.9 (8.8–22.1) mg/kg, and 24 (23.8–24.2) mg/kg, respectively ($P < 0.0001$ for any of the differences). These results also are presented in the form of dose-response curves in figure 3. The slopes ± SE of the curves were: 23 ± 9, 16 ± 7, 16 ± 8, and 437 ± 180 for 0.0, 0.25, 2.5, and 3.3 kg pressure, respectively. Only with the pressure of 3.3 kg was the slope of the curve different ($P < 0.02$).

The comparison of pentobarbital ED_{50} values obtained without and with the addition of morphine (table 2) indicates that 1 mg/kg morphine decreased the pentobarbital requirement in all series of experiments except with the highest noxious pressure, 3.3 kg. Without noxious pressure, the addition of morphine decreased the pentobarbital ED_{50} value from 12.0 (10.7–16.4) to 7.9 (7.2–9.6) mg/kg ($P < 0.0001$ for the difference); with the pressure of 0.25 kg, from 19.5 (17.4–23.5) to 11.8 (9.6–16.4) mg/kg ($P < 0.0001$ for the difference); and with the pressure of 2.5 kg, from 22.7 (14.1–27.0) to 17.9 (8.8–22.1) mg/kg ($P < 0.005$ for the difference). In terms of percentile change, 1 mg/kg morphine decreased the pentobarbital ED_{50} values by 34%, 39%, and 21% with 0.0, 0.25, and 2.5 kg, respectively. The addition of morphine did not result in any significant change of the pentobarbital ED_{50} value with 3.3 kg of pressure.

The results with arterial blood gas tensions indicated that the outcome of the righting reflex experiments is unlikely to be influenced by the changes in the Pa_{CO_2} or Pa_{O_2}. The 1 mg/kg morphine and 24 mg/kg pento-

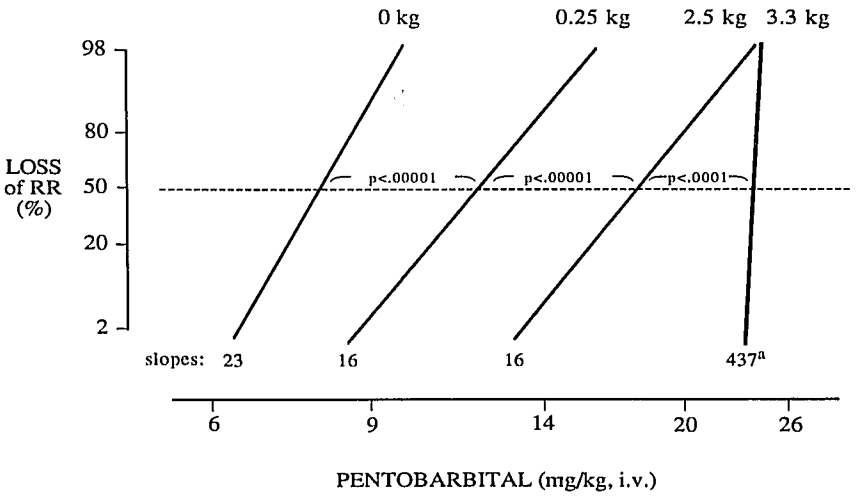

Fig. 3. Pentobarbital dose-response curves for loss of the righting reflex at different intensities (0.0, 0.25, 2.5, and 3.3 kg) of noxious stimulation. Morphine was added in a fixed dose of 1 mg/kg with all curves. Along the vertical axis, the percentage of animals (on a probit scale) with blocked righting reflex. Along the horizontal axis, doses of pentobarbital (on a log scale). At the bottom of each line, the slope of the log dose-probit line (a-p < 0.02 for the differences between the slope of the curve at 3.3 kg and the other slopes).

barbital combination changed the Pa_{CO_2} value from 42 ± 3 to 47 ± 4 mmHg (mean ± SD) and the Pa_{O_2} value from 89 ± 2 to 73 ± 9 mmHg (mean ± SD).

Discussion

Our results indicate that the pentobarbital-opioid combination blocks awakening caused by noxious stimulation of different intensities in a dose-related fashion so that more anesthetic is required to block awakening with more intense stimulation. As a result, the probability of behavioral arousal as a function of intensity of noxious stimulation could be used for measurement of the depth of barbiturate-opioid hypnosis. With this approach, the restriction imposed by the use of the all-or-none phenomenon for the measurement of hypnosis (all-or-none indices like loss of the righting reflex cannot indicate the depth of the process they reflect) is circumvented.

There are two principal approaches to the definition of anesthesia. One is based on the suggestion that anesthesia is composed of several components[6]; the most important of these are unconsciousness, blockade of motor somatic responses to noxious stimulation, and suppression of autonomic responses to noxious stimulations. Another approach relies on the view that only loss of consciousness preventing the patient from perceiving noxious stimulation can be viewed as general anesthesia; all other effects of an anesthetic should be regarded not as components of anesthesia, but as useful additional pharmacologic effects of the drugs.[7]

With the first approach to the definition of anesthesia, a fixed scale of endpoints reflecting different components of anesthesia (where one endpoint comes before or after another) can be used for measurement of anesthetic depth. With the second approach, it seems logical to expect that measurement of anesthetic depth to be directly related to the state of unconsciousness. However, as an all-or-none hypnotic effect, it does not provide an opportunity to measure the depth of the effect. This contradiction can be resolved if the depth of anesthesia is treated as a function of intensity of noxious stimulation necessary for recovery of consciousness, the same way in which it was demonstrated for the recovery of the righting reflex in this study.

Our results demonstrated that, when hypnosis was not counteracted by noxious stimulation, morphine significantly decreased the pentobarbital requirements. This outcome agrees with the result of the previously reported study with isobolographic analysis of fixed-ratio combinations of morphine and thiopental that demonstrated a profound hypnotic synergism.[2] With mild noxious stimulation, the pentobarbital hypnotic requirements also were reduced by morphine. The degree of the morphine-induced effect with the noxious stimulation of 0.25 kg was similar to that without noxious stimulation (39% and 34%, respectively). It was possible to expect here that, with the relatively weak noxious stimulation, the pentobarbital-morphine combination would have provided greater effect than that without noxious stimulation because, in addition to the pentobarbital-morphine hypnotic synergism, the antinociceptive property of morphine would have contributed to the outcome. The antianalgesic property of pentobarbital should be taken into consideration to explain the absence of this outcome. We have reported previously that pentobarbital shifts the morphine dose-response curve for analgesic effect to the right along the dose axis.[8] The morphine-induced decrease in the pentobarbital hypnotic requirement was also present with the noxious stimulation of 2.5 kg. At the same time, with the strongest of the noxious stimulations (3.3 kg), the effect of morphine was not noticeable (see also ED_{50} PTB + MPH/ED_{50} PTB ratios in table 2). With the 3.3-kg stimulation, the slope of the pentobarbital dose-response curve differed from the other slopes (fig. 3). The difference in the slopes of dose-response curves is indicative of the difference in the mechanisms of action.[9] It is possible to suggest that the absence of the effect of morphine on pentobarbital requirements at 3.3 kg of pressure is related to the change in the mechanisms involved in the pentobarbital-morphine interaction with the very strong noxious stimulation. The steep slope at 3.3 kg also may be an indication that, between 2.5 and 3.3 kg, the plateau of the dose-response curve for pentobarbital has been reached.

The absence of the effect of morphine at 3.3 kg coincides with the previously reported relative antagonism when the barbiturate-opioid combinations were studied in relation to the blockade of somatic motor response to strong noxious stimulation with the use of isobolographic analysis of fixed ratio components.[1] Although the nature of somatic motor response with the above two endpoints are different (righting *vs.* any purposeful movement), the strength of noxious stimulation and the dose range of the barbiturate required for suppression of these two responses are relatively close. This comparison indicates that the type of a barbiturate-opioid interaction depends on the strength of

noxious stimulation: The barbiturate-opioid interaction regarding somatic motor response might be synergistic if the strength of noxious stimuli initiating this response is relatively weak.

It is possible to suggest that the morphine-pentobarbital interactions are synergistic with regard to hypnotic effect and antagonistic with regard to the antinociceptive effect. With relatively weak noxious stimuli, the pentobarbital-morphine hypnotic synergism has a predominant effect and, therefore, 1 mg/kg morphine shifts the pentobarbital dose-response curve for awakening to the left along the dose axis. With strong noxious stimuli the antagonistic morphine-pentobarbital antinociceptive interaction plays a predominant role and prevents similar shift of the pentobarbital dose curve for awakening.

The differences between the pentobarbital ED_{50} values with the range of noxious stimulations used is greater with the addition of morphine (table 2). This phenomenon depends on the profound effect of morphine on the pentobarbital requirements with weak noxious stimulation and the absence of any effect with strong noxious stimulation.

In conclusion, pentobarbital, alone and in combination with morphine, blocks awakening caused by noxious stimulation of different intensities in a dose-related fashion so that more anesthetic is required to block awakening with more intense stimulation. In other words, the depth of anesthesia can be measured by intensity of noxious stimulation required for arousal. The morphine-induced decrease in pentobarbital requirements necessary to prevent awakening caused by noxious stimulation depends on the intensity of noxious stimuli. With mild noxious stimulation, the decrease in pentobarbital requirements has a degree similar to that without noxious stimulation. With the strong noxious stimulation, the pentobarbital sparing effect of morphine dramatically declines.

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