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Morphine and Fentanyl Hypnotic Interactions with Thiopental

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The effects of morphine-thiopental and fentanyl-thiopental combinations on the righting reflex were studied in rats. Doses that block the righting reflex for the agents given alone and for their combinations were determined with a probit procedure and compared with an isobolographic analysis. Interaction between morphine or fentanyl and thiopental on blockade of the righting reflex were both found to be synergistic. The degree of synergism was relatively small for the fentanyl-thiopental combination; the maximal deviation from additive interaction (expected/observed ratio) for ED_{50} dose level was 1.15 ($P < 0.01$). The degree of synergism was greater for the morphine-thiopental combination; the maximal deviation from additive interaction was 2.27 ($P < 0.001$). Comparison of the results with the outcomes of opioid-barbiturate interactions in relation to blockade of purposeful movement response to tail clamping, which was antagonistic, shows that the combination of a barbiturate with an opioid gives different outcomes for different end-points of anesthesia. This suggests that the anesthetic effect of an agent is composed of several components, each with a different mechanism of action. (Key words: Analgesics: fentanyl; morphine. Anesthetics, intravenous: thiopental. Interactions (drugs). Potency, anesthetic: ED_{50}.)

BARBITURATES IN SUBANESTHETIC doses are known to antagonize the analgesic effect of opioid drugs.[1–4] Recently, Shingu *et al.* has used the motor response to tail clamping in rats to determine ED_{50} for thiopental and fentanyl in a manner analogous to determining MAC for inhaled anesthetics.[5] When this end point was used to investigate the interactions between thiopental and fentanyl in rats, it was found that both fentanyl and morphine have a less than additive or an antagonistic interaction with thiopental.[6] However, this antagonism was relative, in that it did not increase the requirement for one agent upon the addition of the other agent. Based on the above, it was concluded that interaction between opioid drugs and barbiturates may represent absolute antagonism (increase in opioid requirement to produce analgesia after thiopental) when small doses of agents are used, but relative antagonism (no increase in anesthetic requirement for one component of a combination) when anesthetic doses of barbiturates and opioid drugs were used.

We hypothesized that an anesthetic effect is composed of several components, each with a different mechanism of action. As a result, depending on the specific component in question, the effect of the combination of a barbiturate with an opioid will vary. Although the barbiturate-opioid interaction with regard to the blockade of motor nociceptive reflex was antagonistic, the question arises as to what might be the nature of the opioid-barbiturate interaction for the hypnotic effect. Loss of the righting reflex is a standard measure of hypnotic effect.

The aim of the present study was to define the interaction (synergism, summation, antagonism) between morphine or fentanyl, on one hand, and thiopental, on the other, with regard to loss of the righting reflex.

Methods

Experiments were performed on 198 male Sprague-Dawley rats weighing 275–325 g. The righting reflex test was regarded as positive if the rat failed to right itself (with all four feet on the table) within 15 s after being placed on its side. The experiments were carried out in a clear chamber, 30 × 25 × 40 cm in size, where oxygen was delivered (4 l/min). The rat's hind leg (for injection into the saphenous vein) could be extended to the outside of the chamber through a slot.

Each animal was given one predetermined dose of an agent or a combination of agents. The following agents were used: morphine sulfate (Lilly, Indianapolis, IN), fentanyl citrate (Janssen, Piscataway, NJ), and thiopental sodium (Abbott, Chicago, IL). Doses of morphine and thiopental were expressed in terms of the salt; the doses of fentanyl referred to the free base. The agents or their combinations were injected intravenously, fentanyl and morphine in 15 s, and thiopental in 60 s. Volume of injections was 0.5–1.0 ml. Times between injections of agents and the righting reflex test were based on the times to peak effect for these agents: 15 min for morphine, 5 min for fentanyl, and 2 min for thiopental. The peak times were chosen after preliminary experiments in which the peak effects were determined by measuring the latency of the righting reflex to the pressure on the tail (1 kg/0.25 cm^2). The animals were placed in the chamber with oxygen at least 15 min before a first injection.

Two series of experiments were performed: morphine-thiopental and fentanyl-thiopental. In each series, the interaction between the agents was determined in two steps:[7] First, dose-effect curves were obtained and ED_{50} values calculated; second, isobolographic analysis[8]

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TABLE 1. Morphine-thiopental Series of Experiments

Subseries	Groups	Agents	Doses ($mg \cdot kg^{-1}$)	Weight Ratio of Doses*
A	1	Thiopental	9	—
	2	Thiopental	10	—
	3	Thiopental	11	—
	4	Thiopental	12	—
	5	Thiopental	14	—
B	1	Thiopental	5.5	1:0.7
		Morphine	3.9	
	2	Thiopental	6.0	1:0.7
		Morphine	4.2	
	3	Thiopental	6.5	1:0.7
		Morphine	4.5	
	4	Thiopental	7.0	1:0.7
		Morphine	4.9	
	5	Thiopental	8.0	1:0.7
		Morphine	5.6	
C	1	Thiopental	2.0	1:3.5
		Morphine	6.9	
	2	Thiopental	2.2	1:3.5
		Morphine	7.6	
	3	Thiopental	2.4	1:3.5
		Morphine	8.3	
	4	Thiopental	2.6	1:3.5
		Morphine	9.0	
	5	Thiopental	2.8	1:3.5
		Morphine	9.7	
D	1	Thiopental	0.84	1:17
		Morphine	14.0	
	2	Thiopental	0.96	1:17
		Morphine	16.0	
	3	Thiopental	1.08	1:17
		Morphine	18.0	
	4	Thiopental	1.20	1:17
		Morphine	20.0	
	5	Thiopental	1.32	1:17
		Morphine	22.0	
E	1	Morphine	25	—
	2	Morphine	30	—
	3	Morphine	35	—
	4	Morphine	45	—

* Based on thiopental ED_{50} of 11 $mg \cdot kg^{-1}$ and morphine ED_{50} of 38 $mg \cdot kg^{-1}$.

was used to define the type of drug interaction. Isobolographic analysis is a technique which allows one to readily visualize the nature of an interaction. An isobol is a line on a dose-dose surface denoting all dose combinations which elicit the same response magnitude.

With the first step, five dose-effect curves (five subseries of experiments) were determined in each series of experiments: two with the components given alone (A and E subseries), and three with their various combinations (B, C, and D subseries). Five (or four) groups of four animals were used to determine the dose-effect curve for a drug or a drug combination in each subseries of experiments, with doses equally spread to give a range of doses that block the righting reflex in none or all of the animals in a group. The results obtained in the experiments where agents were given alone were used to determine doses and weight ratios for the components in the combined subseries of experiments. In the combined subseries of experiments, the doses of both components were increased by steps from one group of rats to another with the constant weight ratio between the components. Doses of the agents used in the first and second series of experiments are presented in tables 1 and 2, respectively.

Determination of ED_{50} values from corresponding dose-effect curves was based on the probit procedure.[9] Isobols (lines connecting equi-effective doses) were determined at the ED_{50} level. ED_{50} values from all five subseries of morphine-thiopental (or fentanyl-thiopental) experiments were plotted in a dose field (fig. 1). The isobols connected five points: two points were on the respective single-drug dose coordinates of the isobologram (points A and E), and three (for various com-

TABLE 2. Fentanyl-thiopental Series of Experiments

Subseries	Groups	Agents	Doses ($mg \cdot kg^{-1}$)	Weight Ratio of Doses*
A	1	Thiopental	9	—
	2	Thiopental	10	—
	3	Thiopental	11	—
	4	Thiopental	12	—
	5	Thiopental	14	—
B	1	Thiopental	7.0	1:0.0005
		Fentanyl	0.0035	
	2	Thiopental	7.5	1:0.0005
		Fentanyl	0.0037	
	3	Thiopental	8.9	1:0.0005
		Fentanyl	0.0040	
	4	Thiopental	8.5	1:0.0005
		Fentanyl	0.0042	
	5	Thiopental	9.0	1:0.0005
		Fentanyl	0.0045	
C	1	Thiopental	4.4	1:0.0025
		Fentanyl	0.0112	
	2	Thiopental	4.6	1:0.0025
		Fentanyl	0.0117	
	3	Thiopental	4.8	1:0.0025
		Fentanyl	0.0122	
	4	Thiopental	5.0	1:0.0025
		Fentanyl	0.0127	
	5	Thiopental	5.2	1:0.0025
		Fentanyl	0.0133	
D	1	Thiopental	1.60	1:0.0125
		Fentanyl	0.020	
	2	Thiopental	1.68	1:0.0125
		Fentanyl	0.021	
	3	Thiopental	1.76	1:0.0125
		Fentanyl	0.022	
	4	Thiopental	1.84	1:0.0125
		Fentanyl	0.023	
E	1	Fentanyl	0.020	—
	2	Fentanyl	0.024	—
	3	Fentanyl	0.028	—
	4	Fentanyl	0.032	—
	5	Fentanyl	0.036	—

*Based on thiopental ED_{50} of 11 $mg \cdot kg^{-1}$ and fentanyl ED_{50} of 0.028 $mg \cdot kg^{-1}$.

binations of morphine and thiopental, points B, C, and D) were within the dose field. The deviation of combined ED_{50} points of an isobol from an additive line (joining single-drug ED_{50} points) was measured as the distance between the combined ED_{50} point and a corresponding reference point on the additive line. The reference point on the additive line was determined by the intersection of the additive line with a line connecting the origin and the combined ED_{50} point (fig. 1). This distance was used to determine if a statistically significant difference was present. The standard error of this distance was computed by the method of propagation of error§ and error estimates from a combined ED_{50} point, as well as single-drug ED_{50} points, were used. An approximate t test used to test the assumption of additivity was then obtained as the ratio of the measured distance to its standard error.

In a separate series of experiments, the effects of morphine-thiopental and fentanyl-thiopental combinations on Pa_{CO_2} were studied. The rats used in this series of experiments were prepared with a catheter (PE-10) in the aorta (placed through the femoral artery). The peripheral end of the catheter was tunneled subcutaneously and exteriorized at the back of the neck. Patency of the catheter was maintained with heparinized saline (100 U/ml). On the day of the experiment, the rats received intravenous injections of morphine-thiopental or fentanyl-thiopental in equipotent doses (ED_{50} for loss of the righting reflex) determined in a previous series of experiments. Blood samples (0.2 ml) were withdrawn from the catheter before the first injection (opioids) and 2 min after the second injection (thiopental). Immediately before the investigator took a sample, 0.15 ml of blood was withdrawn from the catheter to prevent 0.1 ml dead space from affecting the Pa_{CO_2} measurement (the 0.15-ml volume was injected back after the sample was taken). Arterial blood gas tensions were measured using an IL System 1303 Blood Gas Analyzer® (Instrumentation Laboratory, Inc.). For comparisons between means of the groups where mor-

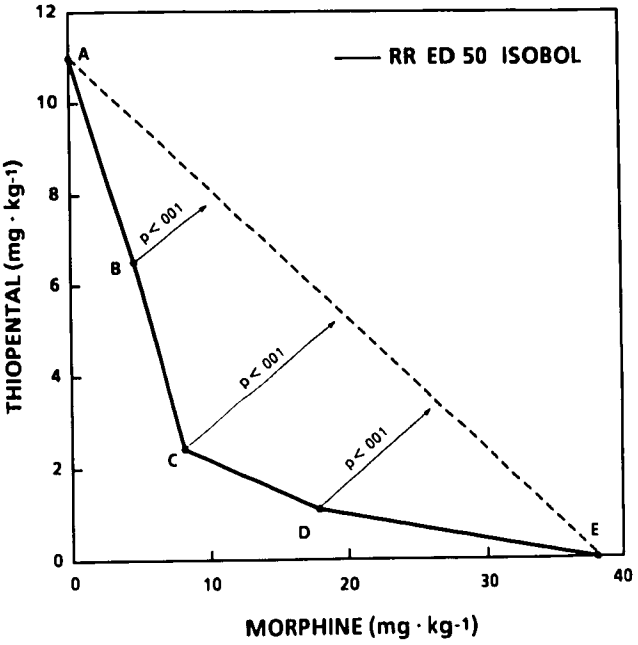

FIG. 1. ED_{50} isobologram for the interaction of morphine and thiopental as characterized by blockade of the righting reflex. ED_{50} values generated by probit analysis indicate the dose level that provides the effect in 50% of animals. A and E are ED_{50} values for thiopental and morphine given alone. B, C, and D are ED_{50} values for thiopental-morphine combinations. The ED_{50} isobol has been generated by connecting adjacent ED_{50} points. The dashed straight line connecting the single-drug ED_{50} points, A and E, is an additive line. P values indicate the level of statistical significance for deviations of the combined ED_{50} points from the additive line.

§ Ku HH: Notes on the use of propagation of error formulas. J Res Natl Bureau Stand 70:263–273, 1966

TABLE 3. Morphine-thiopental Interaction as Characterized by Loss of Righting Reflex

Subseries	Equi-effective Doses (ED_{50}) of Morphine-thiopental Combination				Sum of Fractions	Expected Sum of Doses for Additive Interaction (Fractions)	Deviation from Additive Interaction (Expected/observed Ratio)
	Morphine Component		Thiopental Component				
	Fraction of ED_{50}	Dose in mg · kg^{-1}	Fraction of ED_{50}	Dose in mg · kg^{-1}			
A	0.00	0.0	1.00	11.0 (9.8, 12.5)	1.00	—	—
B	0.12	4.6 (4.1, 5.2)	0.59	6.5 (5.8, 7.4)	0.71	1.0	1.41 $P < 0.001$
C	0.22	8.2 (7.5, 9.0)	0.22	2.4 (2.2, 2.6)	0.44	1.0	2.27 $P < 0.001$
D	0.47	17.9 (15.7, 20.1)	0.10	1.1 (0.9, 1.2)	0.57	1.0	1.75 $P < 0.001$
E	1.00	38.2 (30.3, 52.1)	0.00	0.0	1.00	—	—

Fiducial limits in parentheses.

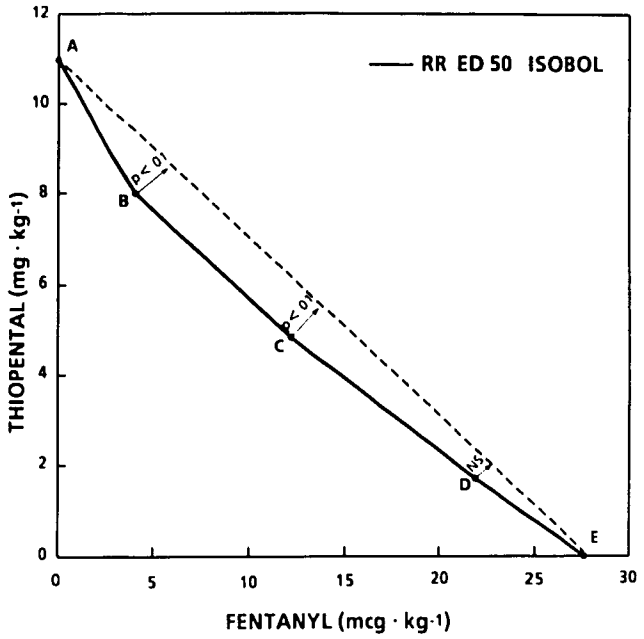

FIG. 2. ED_{50} isobologram for the interaction of fentanyl and thiopental as characterized by blockade of the righting reflex. A and E are ED_{50} values for thiopental and fentanyl alone. B, C, and D are values for their combinations.

phine (or fentanyl) was used in combination with thiopental, a two sample *t* test was used. For changes within a group, a paired *t* test was used.[10]

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

¶ Guide for the Care and Use of Laboratory Animals. Washington, U. S. Government Printing Office, 1985

TABLE 5. Effects of Morphine-thiopental and Fentanyl-thiopental Combinations on Pa_{CO_2}

Series	n	Pa_{CO_2} mmHg (Mean ± SEM) Before	After	Change
Combined morphine-thiopental ED_{50}*	5	33.0 ± 0.5	44.6 ± 2.7	11.6 ± 2.6 $P < 0.02$
Combined fentanyl-thiopental ED_{50}†	5	33.9 ± 1.3	43.9 ± 2.5	9.9 ± 2.4 $P < 0.02$

* Morphine 8 $mg \cdot kg^{-1}$ and thiopental 2.5 $mg \cdot kg^{-1}$.
† Fentanyl 12 $\mu g \cdot kg^{-1}$ and thiopental 4.8 $mg \cdot kg^{-1}$.

Results

The morphine-thiopental ED_{50} isobologram for blockade of the righting reflex is presented in figure 1. The isobol (the line interconnecting adjacent ED_{50} values) deviates to the left of the additive line (joining single-drug ED_{50} doses), indicating synergism. Comparison of the observed ED_{50} doses for morphine-thiopental combinations with the expected doses for an additive interaction is presented in table 3. The expected/observed ratio ranged from 2.27 to 1.41, all of which were greater ($P < 0.001$) than 1.0.

The fentanyl-thiopental ED_{50} isobol is shown in figure 2, and comparison of the observed ED_{50} doses with those expected is shown in table 4. The fentanyl-thiopental interaction was also synergistic, but only to a small degree. The expected/observed ratio in subseries B and C was 1.15 ($P < 0.01$), and in the subseries D the interaction was not different from additive.

In an attempt to explain the marked difference between the expected/observed ratio in the morphine-thiopental and the fentanyl-thiopental series of experi-

TABLE 4. Fentanyl-thiopental Interaction as Characterized by Loss of Righting Reflex

Subseries	Equi-effective Doses (ED_{50}) of Fentanyl-thiopental Combinations: Fentanyl Component Fraction of ED_{50}	Fentanyl Component Dose in $\mu g \cdot kg^{-1}$	Thiopental Component Fraction of ED_{50}	Thiopental Component Dose in $mg \cdot kg^{-1}$	Sum of Fractions	Expected Sum of Doses for Additive Interaction (Fractions)	Deviation from Additive Interaction (Expected/observed Ratio)
A	0.00	0.00	1.00	11.0 (9.8, 12.5)	1.00	—	—
B	0.14	4.0 (3.7, 4.3)	.73	8.0 (7.4, 8.5)	0.87	1.0	1.15 $P < 0.01$
C	0.44	12.2 (11.7, 12.8)	.44	4.8 (4.6, 5.0)	0.88	1.0	1.15 $P < 0.01$
D	0.78	21.7 (20.7, 23.0)	.16	1.7 (1.7, 1.8)	0.94	1.0	1.06 NS
E	1.00	27.7 (23.2, 32.1)	0.00	0.0	1.00	—	—

ments (2.27 *vs.* 1.15) by the possible role of ventilatory depression (hypercarbia) which might be quite different with the two combinations, we performed the Pa_{CO_2} series of experiments. Table 5 presents data on the effect of morphine-thiopental and fentanyl-thiopental combinations at ED_{50} level with opioid-thiopental potency ratio of 1.0:1.0. The Pa_{CO_2} in both groups of animals was increased to a similar degree (11.6 ± 2.6 mmHg with morphine-thiopental *vs.* 9.9 ± 2.4 mmHg with fentanyl-thiopental).

Discussion

The isobolographic analysis used in the present study demonstrated synergistic morphine-thiopental and fentanyl-thiopental interactions in relation to loss of the righting reflex. The degree of synergism was relatively small with the fentanyl-thiopental interaction (maximal expected/observed ratio of 1.15, $P < 0.01$), and very pronounced with the morphine-thiopental combination (2.27, $P < 0.001$).

One explanation for the difference observed is that morphine-thiopental has a greater depressant effect on ventilation than the fentanyl-thiopental combination. However, the Pa_{CO_2} measurements showed: 1) no difference in the degree of increase in Pa_{CO_2} with morphine-thiopental and fentanyl-thiopental combinations, and 2) relatively small increases in Pa_{CO_2}: 11.6 ± 2.6 and 9.9 ± 2.4 mmHg. Increasing Pa_{CO_2} up to 95 mmHg does not affect halothane MAC,[11] and, in the dog, halothane MAC is reduced to zero by Pa_{CO_2} of 245 mmHg.[12] Thus, we feel that the increases in Pa_{CO_2} in our experiments were too small to influence the combined anesthetic effect.

These results showing synergism following morphine-thiopental and fentanyl-thiopental combinations were opposite to those obtained with morphine-thiopental and fentanyl-thiopental interactions when purposeful movement response to tail clamping was the end point. In the later case, isobolographic analysis demonstrated an antagonism.[6]

Combined drug administration may result not only in pharmacodynamic, but also in pharmacokinetic, interactions with appropriate changes in the concentrations of interacting agents at their sites of actions. Measurement of blood, or, even better, brain concentrations of the interacting agents would be the best approach to eliminate the possibility of pharmacokinetic basis for the morphine-thiopental hypnotic synergism. However, with synergism between morphine (or fentanyl) and thiopental, with regard to the hypnotic effect and antagonism between the same agents with regard to the motor response to the tail clamping, pharmacokinetic factors probably cannot be considered as playing a decisive role in morphine-thiopental hypnotic interaction. Because the sites of action of opioids (opioid receptors) are different from the sites of action of barbiturates (independent binding sites on GABA receptor-ionophore complex[13,14]), the opioid-barbiturate interaction probably has a functional nature when two different agents, acting at different sites, affect the same physiologic function.

The results may be explained by the hypothesis that the anesthetic effect of an intravenous anesthetic is composed of several components, each with different mechanism of action. As a result, the combination of a barbiturate with an opioid gives opposite outcomes for different components of anesthesia.

In conclusion, the interaction between morphine or fentanyl, on one hand, and thiopental, on the other, with respect to blockade of the righting reflex in rats, may be defined as synergistic.

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