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Agonist-Antagonistic Interactions of Pentazocine With Morphine Studied in Mice

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SHIMADA, A., H. IIZUKA AND T. YANAGITA. *Agonist-antagonistic interactions of pentazocine with morphine studied in mice.* PHARMACOL BIOCHEM BEHAV **20**(4) 531–535, 1984.—Interactions between the antinociceptive effects of pentazocine and morphine were studied in mice. In the tail-pressure test, the antinociceptive effect of pentazocine, 4.75 to 9.5 mg/kg, SC, was synergistic to that of morphine, 0.69 to 1.38 mg/kg, SC. In the acetic acid writhing test, the effect was also synergistic with pentazocine, 7.13 to 9.5 mg/kg, SC, and morphine, 1.03 to 1.38 mg/kg, SC. In the tail-pinch test, larger doses of morphine than those above were required to suppress the nociceptive response, and simultaneous administration of pentazocine, 2.38 to 19.0 mg/kg, SC, and morphine, 2.75 mg/kg, SC, produced antagonistic effects. Pentazocine, 19.0 mg/kg, completely antagonized the effect of morphine, 2.75 mg/kg, with simultaneous administration at these doses always nearly equipotent to administration of pentazocine alone. These results suggested that when pentazocine and morphine are simultaneously administered, pentazocine synergizes or antagonizes to antinociceptive effects of morphine depending on the dose sizes of morphine and pentazocine, and that the relative saturation levels of morphine and pentazocine at the receptor may be important factors in determining whether the interaction of pentazocine with morphine is antagonistic or synergistic.

Antinociceptive effect Morphine Pentazocine Opioid receptors Mouse

FOLLOWING the discoveries of the agonist-antagonistic property of nallorphine and pentazocine [13] and of the relatively pure antagonist property of naloxone [11,12], a number of studies have been conducted concerning the mode and site of the dual action of agonist-antagonist analgesics. Separation of the antinociceptive property from the antagonistic property of these drugs has led to the concepts of competitive antagonism, competitive dualism [2], and receptor dualism [16]. Furthermore, Martin *et al.* basing upon studies in chronic spinal dog, proposed the concept of multiple opioid receptors which were designated the μ-(morphine type), κ-(ketocyclazocine type), and σ-(SKF-10,047 type) receptors [17]. These opioid receptor subtypes have been confirmed by other investigators *in vivo* tests [5, 10, 23, 27]. Many of the studies on pentazocine are related to its dual agonist-antagonistic property [4]. It has been reported that pentazocine inhibits [9] and/or enhances [24,25] the antinociceptive effect of morphine in animals and man. Blane and Dugdall, by observing the effects of the combination of morphine and pentazocine on bradykinin-induced nociception in rats, found that pentazocine at a low dose (0.35 mg/kg) antagonized the antinociceptive effects of morphine but at intermediate doses (0.36 and 1.25 mg/kg) behaved additively with morphine, while again showing antagonism at higher doses (2.5 and 5.0 mg/kg) [3]. Ankier noticed some unexplainable potentiation of morphine by pentazocine in the hot-plate test in mice [1]. Since these results indicate that pentazocine produces both synergistic and antagonistic effects depending on the dose relationship of the two drugs, the influence of the dose levels on the interaction between the two drugs was studied by three tests that allowed observation of nociceptive responses over a wide range of doses in mice; the tail-pressure, acetic acid writhing, and tail-pinch tests.

METHOD

Drug-naive male Jcl-ICR mice (Nihon Clea Co., Japan) were used in all experiments. The mice were housed for more than a week before the start of the experiment in group cages in a room regulated for temperature, humidity, and light cycle. The animals were fed on a solid diet (CA-1, Nihon Clea Co., Japan) with access to tap water ad lib. The drug solutions were respectively prepared with pentazocine lactate (Winthrop Laboratories, Japan) being dissolved in 0.1 N HCl solution and then diluted to the appropriate concentrations with 0.9% saline, and with morphine hydrochloride (Takeda Pharmaceutical Co., Japan) being dissolved in 0.9% saline. These drugs were separately administered subcutaneously to the nape of the neck and the skin of the back of the mice at a fixed injection volume of 10 ml/kg. When only one drug was used, saline was also administered at a different site from that of the drug. For observation of the antinociceptive effect of the drugs, the tail-pinch [21], tail-pressure [18], and acetic acid writhing [14] methods were used. These tests were conducted 30 minutes after drug administration. Statistical analysis was performed using Student's t-test. When the value of p was less than 0.05, the difference was considered as significant.

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TABLE 1

ANTINOCICEPTIVE EFFECTS OF MORPHINE-PENTAZOCINE COMBINATIONS WITH THE TAIL-PRESSURE TEST IN MICE

Drug	Dose	Response Threshold	
		Alone	Combined
	mg/kg, SC	mmHg	mmHg
Morphine	0.69	42.7 ± 23.5	102.0 ± 38.7
Pentazocine	4.75	38.7 ± 10.9	
Morphine	1.03	117.7 ± 48.9	295.7 ± 53.2*
Pentazocine	7.13	251.2 ± 46.3	
Morphine	1.38	212.0 ± 50.5	391.6 ± 6.0*
Pentazocine	9.50	333.0 ± 29.3	

Each value represents the mean and standard error for six mice.
*Significantly different from the value of morphine alone.

Experiment 1: Determination of ED50s for Morphine and Pentazocine by the Tail-Pinch Test in Mice

Four and three groups of six mice each (16.7 to 31.6 g) were used for each drug. Morphine and pentazocine were administered at doses ranging between 1 to 8 mg/kg and 24 to 54 mg/kg, respectively. Nociceptive stimulation was induced by pinching the tail by a 50 mm artery clip at a pinch-force of 500 g. Responding by the mouse within 2 seconds after application of the clip by either squeaking, turning the head, or biting the artery clip was regarded as the nociceptive response. The disappearance of these responses was regarded as evidence of the antinociceptive drug effect. The results of this test were shown as the % of animals tested which failed to respond within 2 seconds.

Four hours prior to drug administration a preliminary pinch test was conducted, and only the positively responding animals were used. ED50s were calculated by the Litchfield and Wilcoxon method [15].

Experiment 2: Interaction between Morphine and Pentazocine as Determined by the Tail-Pressure Test in Mice

Ten groups of six mice each (11.5 to 23.3 g) were used in this experiment. For observation of the nociceptive response, a sharp-edged plastic plate was placed on the root of the tail and pressure was gradually applied to the plate at a constant rate of increase by a motor-driven air-compressor. When the mice responded as described in Experiment 1, the pressure value at that point was recorded on a polygraph recorder. When either the mouse responded to the stimulation or the pressure reached to about 400 mmHg, the mouse was completely liberated from the stimulation. Immediately after the liberation, pressure was again applied to the tail of the mouse. This test procedure was repeated three times in succession at each test with the mean value being regarded as the threshold value. Only the mice that showed a reflex response to nociceptive stimulation at pressures below 70 mmHg in the preliminary test, conducted 4 hours prior to the experiment, were used.

Simultaneous administration of morphine and pentazocine was conducted at dose combinations of 0.69 and 4.75, 1.03 and 7.13, and 1.38 and 9.5 mg/kg, respectively. The results of morphine or pentazocine alone at these doses were also determined for comparison.

Experiment 3: Interaction between Morphine and Pentazocine as Determined the Acetic Acid Writhing Test

Ten groups of six mice each (21.5 to 29.4 g) were used in this experiment. For observation of the nociceptive response, the mice were treated intraperitoneally with 10 ml/kg of 0.6% of acetic acid 25 minutes after opioid administration. Each animal was then placed in a cylindrical transparent acrylic box 200 mm in diameter and 150 mm in height, and the number of writhing responses was counted for five minutes beginning 30 minutes after the drug administration, i.e., 5 minutes after the acetic acid administration.

In this experiment the dose combinations of morphine and pentazocine were 1.03 and 7.13, 1.38 and 9.5, 2.75 and 7.13 mg/kg, respectively. The effects of morphine or pentazocine alone at these doses were also determined for comparison.

Experiment 4: Interaction between Morphine and Pentazocine as Determined by the Tail-Pinch Test in Mice

The following two types of six experiments were conducted by the method described in Experiment 1. In each experiment nine groups of 10 mice each (15.5 to 29.8 g), as well as 23 mice (21.5 to 30.0 g) for vehicle control, were used.

Simultaneous administration of a fixed-dose of morphine with various doses of pentazocine. Pentazocine was administered at doses of 2.38, 4.75, 9.5, and 19.0 mg/kg simultaneously with morphine at 2.75 mg/kg and any antinociceptive effect was observed. The effect of morphine or pentazocine alone at these doses was also determined for comparison.

Simultaneous administration of a fixed-dose of pentazocine with various doses of morphine. Morphine was administered at doses of 0.34, 0.69, 1.38, and 2.75 mg/kg simultaneously with pentazocine at 19.0 mg/kg and any antinociceptive effect was observed. The effect of morphine or pentazocine alone at these doses was also determined for comparison.

TABLE 2

ANTINOCICEPTIVE EFFECTS OF MORPHINE-PENTAZOCINE COMBINATIONS WITH THE ACETIC ACID WRITHING TEST IN MICE

Drug	Dose	No. of Writhing Responses	
		Alone	Combined
	mg/kg, SC	per 5 min	per 5 min
Morphine	1.03	9.3 ± 0.6	2.2 ± 1.6*†
Pentazocine	7.13	9.7 ± 2.6‡	
Morphine	1.38	2.8 ± 1.0	1.2 ± 1.2†
Pentazocine	9.50	7.5 ± 1.7	
Morphine	2.75	0.0 ± 0.0	0.7 ± 0.5
Pentazocine	7.13	9.7 ± 2.6‡	

Each value represents the mean and standard error for six mice.
*Significantly different from the value of morphine alone.
†Significantly different from the value of pentazocine alone.
‡Result from the same experiment.

RESULTS

Experiment 1: ED50s for Morphine and Pentazocine Determined by the Tail-Pinch Test

The respective ED50s with 95% confidence limits for morphine and pentazocine in respect to antinociceptive effects were 2.75 (1.53–4.95) mg/kg, SC and 38.0 (22.49–64.22) mg/kg, SC.

Experiment 2: Interaction between Morphine and Pentazocine as Observed by the Tail-Pressure Test

The vehicle control mice, which were subcutaneously administered saline alone, responded at 39.5±4.9 (mean ±S.E.) mmHg of pressure. At a combination of morphine 0.69 and pentazocine 4.75 mg/kg, respectively equivalent to 1/4 and 1/8 of their ED50s as determined by the tail-pinch test, the pressure threshold was 102.0±38.7 mmHg which was about 2–3 times as great as that with either drug alone, but the difference was not statistically significant. In the cases of the combination of morphine and pentazocine at 1.03 and 7.13, or 1.38 and 9.50 mg/kg, the effects also tended to be greater than those with the respective drugs alone, but no statistical difference was observed in comparison with pentazocine alone (Table 1).

Experiment 3: Interaction between Morphine and Pentazocine as Observed by the Acetic Acid Writhing Test

The number of acetic acid-induced writhing responses was 19.3±0.6 (mean±S.E.) per five minutes in the vehicle control mice. During simultaneous administration of morphine and pentazocine at 1.03 and 7.13 mg/kg respectively, the antinociceptive effect was significantly greater than with the respective drugs alone. At a dose combination of 1.38 and 9.5 mg/kg, the effect also tended to be greater than with the respective drugs alone, but no statistical difference was observed in comparison with morphine alone (Table 2).

Experiment 4: Interaction between Morphine and Pentazocine as Observed by the Tail-Pinch Test

Simultaneous administration of a fixed-dose of morphine with various doses of pentazocine. In the vehicle control mice, the tail-pinch responses were not prevented at all. In this experiment morphine alone showed at 35% suppression of the response to the ED50 dose as estimated by the previous test, and simultaneous administration of pentazocine in the dose-range of 2.38 to 19.0 mg/kg always decreased the antinociceptive effect of morphine 2.75 mg/kg. As the dose of pentazocine increased, the combined effect decreased, and at 19.0 mg/kg the combined effect decreased to a level nearly equal to that of pentazocine alone (Fig. 1).

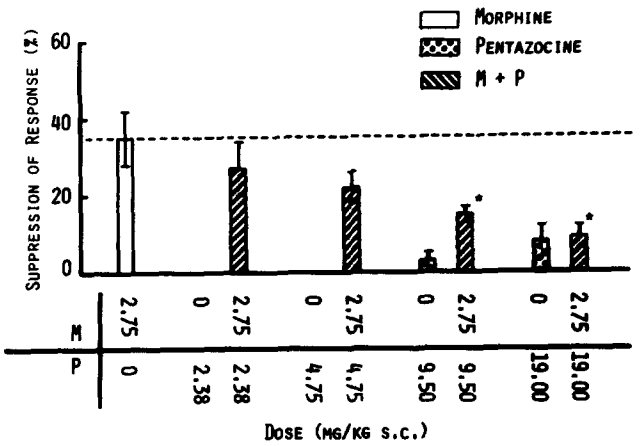

FIG. 1. Antinociceptive effects of morphine-pentazocine combinations with the tail-pinch test in mice [1]. Each value represents the mean and standard error for six experiments. M: morphine, P: pentazocine. *Significantly different from the value of morphine alone. Effect of 2.75 mg/kg of morphine was antagonized by administration of several doses of pentazocine.

Simultaneous administration of a fixed-dose of pentazocine with various doses of morphine. In this experiment pentazocine alone produced a 11.7% suppression of tail-pinch response after 19.0 mg/kg. During simultaneous administration of morphine in the dose range of 0.34 to 2.75 mg/kg with 19.0 mg/kg of pentazocine, the effect was always similar to that of pentazocine alone (Fig. 2). For example, 2.75 mg/kg of morphine suppressed the response by 43.3%, but

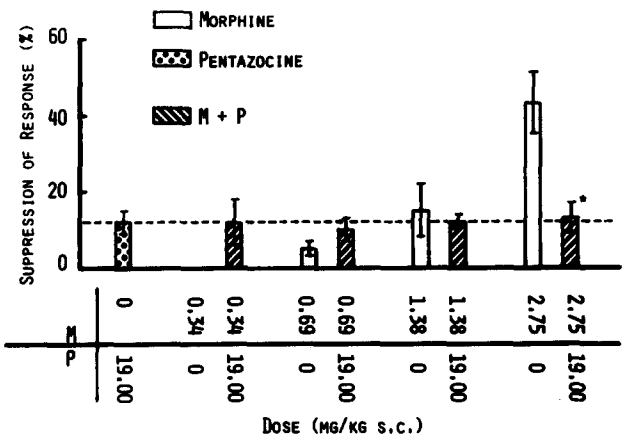

FIG. 2. Antinociceptive effects of morphine-pentazocine combinations with the tail-pinch test in mice [2]. Each value represents the mean and standard error for six experiments. M: morphine, P: pentazocine. *Significantly different from the value of morphine alone. During simultaneous administration of various doses of morphine and 19.0 mg/kg of pentazocine, the effects were always similar to that of pentazocine alone.

this dose of morphine combined with pentazocine 19.0 mg/kg showed only 13.3% suppression, or only slightly higher than that of pentazocine alone.

DISCUSSION

When administered alone, morphine and pentazocine suppressed the responses of mice to nociceptive stimuli in all tests. From comparison between doses of morphine and pentazocine used in these tests, the antinociceptive effects with morphine in these tests were greater than those with pentazocine. In the tail-pressure test, the antinociceptive effects produced by administration of all dose-combinations of morphine and pentazocine tended to be greater than those produced by either drug alone. In the case of a dose combination of morphine and pentazocine at 1.03 and 7.13 mg/kg, respectively, in the acetic acid writhing test, the antinociceptive effect was significantly greater than that produced by either drug alone. In the tail-pinch test, however, pentazocine dose-dependently decreased morphine's antinociceptive effects to a level similar to that with pentazocine alone at the dose combinations of pentazocine at 2.38 to 19.0 mg/kg along with morphine at 2.75 mg/kg. At the dose combinations of morphine from 0.34 to 2.75 mg/kg along with pentazocine at 19.0 mg/kg, the results were always similar to that of pentazocine alone.

The tail-pressure and acetic acid writhing tests showed no antagonism between morphine and pentazocine. The combination of both drugs rather showed synergism. On the other hand, all combinations of both drugs in the tail-pinch test showed only antagonism.

Concerning the mode of action of these drugs, the results with morphine and pentazocine in the acetic acid writhing test suggest that these drugs may act at least partially on the receptors of a common subtype [20]. Other investigators have also reported that these drugs interact competitively at a common receptor site [6, 8, 26]. Smits and Takemori observed that the slopes in the apparent pA_2 for pentazocine were found to be significantly different from morphine in the phenylquinone stretching test in mice, and suggested that these drugs inhibited stretching responses by interacting either with two different subtype receptors or with the same subtype receptors in a different manner [19]. Takemori *et al.* further examined the pA_2 values for these drugs and suggested that the interaction of analgesic receptors with morphine and pentazocine appeared to differ [22]. By introducing the newer concept of opioid receptors, the experiment of Gilbert and Martin in chronic spinal dog showed that morphine acted on the μ- and κ-receptor sites as an agonist, while pentazocine acted on the μ-receptor site as an antagonist and on the κ-receptor site as an agonist [7].

Based on these theories the results of the present study may be explained by the interaction of pentazocine with morphine at the μ-receptor site, and the independent agonistic action of pentazocine at the κ-receptor site. In the case of relatively low doses of morphine, the agonistic effects of morphine upon μ-receptors and pentazocine upon κ-receptors may produce synergistic analgesia. In the case of intermediate doses of morphine, the agonistic effects of morphine upon μ-receptors and of low doses of pentazocine upon κ-receptors may also produce synergistic analgesia. At these low doses of pentazocine, pentazocine may not be able to effectively compete with morphine at the μ-receptors to cause any significant antagonism of morphine-induced analgesia. In this case, however, relatively higher doses of pentazocine may compete with morphine at the μ-receptors and antagonize morphine-induced analgesia. In the case of relatively higher doses of morphine, both low and high doses of pentazocine may effectively compete with morphine at the μ-receptors and antagonize morphine-induced analgesia. Moreover, in this case, the agonistic effects of higher doses of pentazocine at the κ-receptors would still produce analgesia, but the amount of analgesia would be less than that produced by morphine alone or by combined doses of pentazocine and morphine that were synergistic.

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