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Original article

Antinociceptive Effect of Promethazine in Mice

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Abstract

Objective(s)
The present study was undertaken to investigate the nociception activity of promethazine, a tranquillizer devoid of hypnotic activity in mice.
Materials and Methods
Antinociception was evaluated, using the acetic acid-induced writhing, tail flick, hot plate and formalin pain tests.
Results
Promethazine (4 and 6 mg/kg) and acetylsalicylic acid (100 mg/kg) produced a significant inhibition of the second phase response in the formalin pain model ($P<0.05$) and the drug couldn't show an antinociceptive effect in the first phase. Morphine (10 mg/kg) inhibited both first and second phase response ($P<0.01$). Drug also showed a dose-dependent inhibition of acetic acid-induced abdominal writhes. The tail flick and hot plate latency weren't different from control ($P>0.05$) and administration of naloxone (0.1 mg/kg) couldn't block the antinociceptive effect of promethazine.
Conclusion
The data obtained suggest that antinociceptive effects of the promethazine may be mediated via peripheral and not central mechanisms.

Keywords: Antinociceptive, Formalin test, Hot plate, Promethazine, Tail flick, Writhing test

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Introduction

The management and treatment of pain is probably one of the most common and yet the most difficult aspects of medicinal practice. Analgesic therapy is currently dominated by two major classes of analgesic drugs; namely opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbances, renal damages (with NSAIDs drugs) and respiratory depression and possibly dependence (with opioids) (1, 2). It is obvious that the design of analgesic agents with fewer side effects is desirable. One of the ways to achieve this aim is, using other drugs that are not NSAIDs or opioids, but have analgesic effects such as some antihistamines. Histamine system plays an important role in central nociception (3). Animal and clinical data suggest that antihistamines may have efficacy in the management of pain. While many mechanisms of action have been proposed for the analgesic action of antihistamines, the exact mechanism is unknown (4). Promethazine is one of the phenothiazine derivatives and first-generation H_1 antagonists with anticholinergic effect which is administrated in treatment of marked sedation, emesis and prevention of motion sickness (5). The antiemetic properties of phenothiazines are mediated through inhibition of dopamine and muscarinic receptors (5). Sansone *et al* showed that H1 antagonists can potentiate the analgesic effect of opioids (6). H_1 and H_2 antagonists potentiate the antinociceptive effects of morphine and fentanyl (7). The analgesic action of pentazocine is affected in an inconsistent manner by H_2 antagonists (7). It seems that the potentiating effect of H-antagonists is related to the opioid muscarinic receptors (7). In other hand, there is relation between possible binding to the opioid receptors by antihistaminics and their facility in crossing the blood-brain barrier (8). Antihistamines like diphenylhydramine (H_1 antihistamine) have smoothly analgesia in Hot plate test but in combination with pentazocine, it can enhance the analgesic effect of pentazocine (9). In all studies, however, the antinociceptive effect of antihistamines were discussed but evaluation of promethazine in this goal and it's probable mechanism hasn't been reported. Based on these observations, in the present paper we have evaluated the antinociceptive effects of promethazine, using the writhing assay, tail flick, hot plate and formalin tests.

Materials and Methods

Drugs

The drugs used were promethazine (Exir Pharmaceutical Company, Lorestan, Iran), acetylsalicylic acid (Sigma Chemical Company, St. Louis, USA), morphine (Darou Pakhsh Pharmaceutical Company, Tehran, Iran), acetic acid (Sigma Chemical Company, St. Louis, USA) and naloxone (Darou Pakhsh Pharmaceutical Company, Tehran, Iran).

Animals

Male NMRI mice (25–35 g), from Laboratory Animal Centre of the School of Pharmacy, Kermanshah University of Medical Sciences, Iran, were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3 °C with a 12 hr light/dark cycle (light on from 06:00 to 18:00 hr). The ethical guidelines for the investigation of experimental animals were followed in all tests. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Analgesic activity

Mouse writhing assay

This was carried out according to the method described previously (10). Promethazine (1-6 mg/kg) or normal saline (10 ml/kg) were administered to mice before intraperitoneal injection of acetic acid (0.6% v/v in normal saline, 10 ml/kg). Acetylsalicylic acid (100 mg/kg, sc) was used as the reference drug. The numbers of writhes were counted for 15 min.

Formalin test

Formalin test was used as reported by Shibata *et al* (11) and Vianna *et al* (12). Twenty

microlitres of 1% formalin was injected subcutaneously into the right hind paw of mice. Pain responses were measured for 5 min (first phase) and 15-30 min (second phase) after formalin injection. Promethazine (1-6 mg/kg, ip) or morphine (10 mg/kg) and acetylsalicylic acid (100 mg/kg, sec) were administered 30 min before formalin injection. Control animals received the same volume of normal saline.

Tail flick assay

Tail-flick to radiant heat (Tail-Flick Apparatus Model P-162, Pouyaye Armaghan Co. Iran) was used to measure acute nociceptive responses in mice. The intensity of the thermal stimulus was adjusted to produce 3-4 sec latency in tail-flick response. The trial was automatically terminated at 12 sec if a response did not occur (cut off time). Measurement of threshold was made 30 min before and after administration of promethazine (1-6 mg/kg, ip) or morphine (10 mg/kg, sc). Normal saline (10 ml/kg) served as the control. The percentage of maximal possible antinociception (MPA) for each animal was calculated, using the formula: %MPA= [(Pre treatment–post treatment)/ (12−Pre treatment)]×100 (13). Naloxone (0.1 mg/kg) used as opioid antagonist 5 min before injection of the drug.

Hot plate test

The analgesic activity was evaluated with a thermostatically heated surface maintained at 55±1 ˚C. The reaction time was taken as the time period from the instant animal was put on the hot plate until the moment the animal licked its feet or jumped out. Each mouse was its own control; thus before treatment, its reaction time was determined twice at 10 min, interval. The mean of these two values was the reaction time before treatment (Tb). Thirty min after the treatment, the reaction time was again evaluated, but only once, this value represented the reaction time after treatment (Ta). For each group, averages of reaction times were then calculated, allowing the calculation of the percentage of variation by the ratio: $(T_a-T_b)\times100/T_b$ (14). Promethazine (1-6 mg/kg, ip) or morphine (10 mg/kg, sc) used for the treatment. Normal saline (10 ml/kg) served as the control and Naloxone (0.1 mg/kg) used as opioid antagonist 5 min before the treatment.

Statistical analysis

Results are expressed as mean±SEM. The one-way analysis of variance (ANOVA) followed by the Tukey's post-test was used to analyze the data. $P<0.05$ was the critical criterion for statistical significance.

Results

Mouse writhing

In control mice, the number of writhes during the 15 min test period was 69.2±2.3. The treatment of animals with promethazine (1-6 mg/kg) produced a significant and dose dependent inhibition of the control writhes (Table 1). The inhibition by 6 mg/kg was similar to that produced by 100 mg/kg acetylsalicylic acid (80.1 and 76.4%, respectively).

Formalin test

The drug demonstrated a dose-dependent relationship in second phase of formalin induced pain. Although there wasn't any significant inhibition by the drug compared to control, in the first phase (Table 2), however, all the doses significantly ($P<0.05$) inhibited the second phase, similar to acetylsalicylic acid (100 mg/kg). Morphine inhibited both first and second phase ($P<0.01$).

Table 1. Effect of promethazine on acetic acid-induced writhing in mice.

Group	Dose(mg/kg)	No. of writhings (per 15 min)	% Inhibition
Control	-	69.2±4.8	-
Promethazine	1	47.6±4.2	31.2
	2	35.9±1.8*	48.1
	4	26.4±2.7*	61.8
	6	17.2±1.5*	75.1
Acetylsalicylic acid	100	18.2±1.6*	73.6

Values are mean±SEM.* $P< 0.05$ vs. control (n=10).

Table 2. Effect of promethazine on formalin-induced pain.

Group	Dose (mg/kg)	0-5 min	% Inhibition	15-30 min	% Inhibition
Control	-	127.2±2.8	-	100.1±5.7	-
Promethazine	1	117.1±4.6	7.9	53.9±6.8*	46.1
	2	120.3±6.4	5.4	43.9±4.7*	56.1
	4	118.5±4.1	6.8	31.9±4.2*	68.1
	6	116.7±2.7	8.2	26.8±3.3**	73.2
Acetylsalicylic acid	100	155.3±5.3	-	29.4±7.6*	70.6
Morphine	10	22.1±1.2**	82.6	23.3±5.2**	76.8

Values are mean±SEM. * $P<0.05$, ** $P<0.01$ vs. control (n=10).

Tail flick

Table 3 shows the effect of the promethazine on tail flick response. Low doses of the drug couldn't change the reaction time compared to control ($P>0.05$) and the dose of 6 mg/kg have smooth antinociceptive effect. The effect of morphine (10 mg/kg) was significantly higher ($P<0.01$) than that produced by the highest dose of the drug. Naloxone (0.1 mg/kg) couldn't block the antinociceptive effect of promethazine.

Hot plate test

Table 4 shows the effect of promethazine on hot plate latency. Doses 1, 2 and 4 mg/kg of the drug couldn't change the reaction time compared to control ($P>0.05$) and the does of 6 mg/kg had smooth antinociceptive effect ($P<0.05$). Morphine (10 mg/kg) could increase the nociceptive inhibition percentage ($P<0.01$). Naloxone (0.1 mg/kg) couldn't block the antinociceptive effect of promethazine.

Discussion

As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs lacking these effects are being searched all over the world as alternatives to NSAIDs and opiates (15).

Table 3. Effect of promethazine on tail flick test.

Group	Dose (mg/kg)	Pre-treatment (sec)	Post-treatment (sec)	% Inhibition
Control	-	5.8±1.1	6.1±0.3	4.8
Promethazine	1	4.9±0.3	5.3±0.6	5.6
	2	6.2±0.7	6.6±0.1	6.8
	4	5.3±0.2	5.7±0.5	5.9
	6	6.1±1.1	6.7±0.8	10.1 *
Promethazine+Naloxone	6+0.1	6.4±0.4	7.1±1.2	12.5 *
Morphine	10	6.3±0.9	10.6±0.4	75.4* *
Morphine+Naloxone	10+0.1	5.9±0.3	6.2±0.1	4.9

Values are mean±SEM. * $P<0.05$, ** $P<0.01$ vs. control (n=10).

Table 4. Effect of promethazine on hot plate test.

Group	Dose (mg/kg)	T_b (sec)	T_a (sec)	% Inhibition
Control	-	4.9±2.1	5.2±0.3	6.1
Promethazine	1	5.1±1.1	5.4±0.9	5.8
	2	5.3±0.9	5.6±1.2	5.6
	4	6.0±0.7	6.4±0.5	6.6
	6	5.4±2.3	5.9±1.0	9.2 *
Promethazine+Naloxone	6+0.1	5.5±1.3	6.1±0.4	10.9 *
Morphine	10	6.1±0.5	10.6±0.6	73.7* *
Morphine+Naloxone	10+0.1	6.2±0.8	6.6±0.1	6.4

Values are mean±SEM. * $P<0.05$, ** $P<0.01$ vs. control. (n=10).

During this process, the analgesic activity of promethazine as antihistamine agent was studied and evaluated, using the abdominal writhing technique in mice and tail flick, hot plate and formalin pain tests, that results allowed appreciation of the mechanism of this activity. Data obtained from the present study indicate that promethazine inhibited only the second phase of formalin-induced pain, a model which is very useful for elucidating the mechanism of pain and analgesia (16). Drugs which act mainly centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripherally acting drugs, such as aspirin, only inhibit the late phase (17), as our paper shows these facts. According to this study, promethazine has peripheral analgesic effect. Thermal painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs (18). In the present study, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in tail-flick and hot plate tests, while promethazine failed to affect the response. Therefore, it seems that this drug had no central analgesic activity with spinal or supraspinal mechanisms. Promethazine inhibited acetic acid-induced writhing in mice, hence it can confirm that the analgesic effect of the drug is peripherally mediated. Many mechanisms have been proposed for the analgesic action of antihistamines and the exact mechanism is unknown and in some cases repugnant. For example Paalzow and Paalzow reported that low doses of promethazine (1.25-5 mg/kg, sc) dose-dependently facilitate nociception but in contrast, high doses (20-40 mg/kg, sc) induced an antinociceptive effect in the vocalization test in rats (19), these could be results of difference in method and procedure times that involve different neurotransmitters. However, the ability of promethazine, in this study for the following reasons confirms the peripheral and not central antinociceptive activities, first, suppress the abdominal writhes, second, inhibits the second phase of formalin induced pain, third, hasn't any effect on tail flick and hot plate latency and fourth, defeats the naloxone for blocking antinociceptive effect of promethazine.

Conclusion

It is concluded that promethazine possesses antinociceptive properties, which are probably mediated via peripheral mechanisms. The drug therefore, can be beneficial in managing of peripheral pain disorders.

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