Antinociceptive effects of meptazinol and its isomers on carrageenan-induced thermal hyperalgesia in rats

WANG Pei-Fen1, ZHANG Yu-Qiu1,3,*, QIU Zhui-Bai 2, ZHAO Zhi-Qi1

1Institute of Neurobiology, Fudan University, Shanghai 200433; 2School of Pharmacy, Fudan University, Shanghai 200032, China; and 3Shanghai Research Center of Acupuncture and Meridian, Shanghai 200032, China

Abstract: Using the latency of paw withdrawal (PWL) from a noxious thermal stimulus as a measure of hyperalgesia, the effects of i.p. injection of meptazinol and its isomers, 112824 and 112825, on carrageenan-induced thermal hyperalgesia were studied in awake carrageenan-inflamed rats. Peripheral inflammation was induced by intraplantar (i.pl.) injection of carrageenan (2 mg/100 µl) into one hindpaw in rats. Carrageenan produced marked inflammation (edema and erythema) and thermal hyperalgesia in the injected paws, which peaked at 3 h after injection and showed little change in magnitude for another 3 h.

Injection of 0.1 mg/kg meptazinol (i.p.) at 3 h after carrageenan had no effect on the PWLs of either inflamed or non-inflamed hindpaw during the next 100 min (P>0.05, n=8). At the dosage of 1 and 10 mg/kg, meptazinol produced marked anti-nociception and anti-hyperalgesia in non-inflamed and inflamed hindpaw, respectively (P<0.05, n=8). The prolonging effect of meptazinol on PWL in inflamed hindpaw was more potent than that in non-inflamed hindpaw. Pre-administration of 1.5 mg/kg naloxone significantly antagonized meptazinol-induced anti-nociception and anti-hyperalgesia. Intraperitoneal injection of an isomer of meptazinol, 112825 (1.5 mg/kg), but not 112824 (1 mg/kg), markedly increased the PWL of the non-inflamed hindpaw. Nevertheless, both the isomers produced similar anti-hyperalgesic effects to that of meptazinol (P<0.05, n=8), which was completely reversed by naloxone (1.5 mg/kg). The results suggest that meptazinol and its isomers have anti-nociceptive and anti-hyperalgesic properties with the former more potent. The effects are mainly mediated by mu opioid receptors. This study provides an important clue for extending clinical utilization of meptazinol and its isomers.

Key words: meptazinol; naloxone; carrageenan; hyperalgesia; nociception; rat
Meptazinol, m-(3-ethyl-l-methyl-hexahydro-1-H-azepin-3-yl) phenol hydrochloride, is a synthetic hexahydroazepine derivative with opioid agonist and antagonist properties\textsuperscript{1-4}. Receptor binding studies have shown that meptazinol is a specific mu-1 agonist with analgesic effect as 1/5~1/10 as the potency of morphine by intramuscular injection\textsuperscript{4,5-7}. Unlike morphine, the abuse potential of meptazinol seems relatively low and its central nervous system side effects, such as respiratory depression, drowsiness and dizziness, also appear to be less. Thus, meptazinol is an effective and safe anti-nociceptive agent for animals and induces analgesia in human\textsuperscript{5,8-10}. It has been reported that meptazinol produces anti-nociception in the physiological pain in the rat’s tail-flick test and mouse’s hot plate test\textsuperscript{11}. But, the role of this compound in the pathological pain has not been evaluated yet. If meptazinol produces a similar action in inflamed animals as in normal ones, it might be more useful for clinical therapy of painful conditions. An inflammatory pain model by intraplantar injection of carrageenan is extensively applied to the study of the pathological pain in the rat, which is characterized by similar clinical symptoms such as great edematous response, persistent hyperalgesia and allodynia\textsuperscript{12}. By means of the carrageenan-induced inflammatory pain model, the aim of the present study was to examine the analgesic effect of meptazinol and two of its isomers, 112824 and 112825, on carrageenan-induced inflammatory hyperalgesia.

1 MATERIAL AND METHODS

1.1 Animals. Experiments were performed on adult male Sprague-Dawley rats weighing 230~300 g (Grade Ⅱ, Certificate No.02-22-2). Rats were on a 12:12 h light-dark cycle and received food and water. Rats were supplied by the Experimental Animal Center of Fudan University. The treatment of the animals was in accordance with the guidelines of the International Association for the Study of Pain\textsuperscript{13}.

1.2 Drugs application. Peripheral inflammation was induced by intraplantar (i.pl.) injection of carrageenan (2 mg /100 μl of 0.9% normal saline (NS); λ-Carrageenan, Sigma) into one hindpaw in rats. Meptazinol, 112824, and 112825 were synthesized by School of Pharmacy, Fudan University. Drugs were dissolved in NS immediately before use. Three doses of meptazinol (0.1 mg/kg, 1 mg/kg, 10 mg/ kg), and a single dose of 112824 (1 mg/kg, approximate ED\textsubscript{50} dose) and 112825 (1.5 mg, approximate ED\textsubscript{50} dose) were applied intraperitoneally (i.p.) at 3 h after carrageenan.

1.3 Behavioral testing. The decrease in paw withdrawal latency (PWL) to noxious radiant heat was examined for evidence of thermal hyperalgesia in rats. Rats were placed in a clear plastic cage on a raised glass platform and allowed 30 min for adaptation. Noxious radiant heat was from a high-intensity light beam by means of a Model 33 Tail analgesia Meter (IITC/life Science Instruments, USA). Radiant heat was focused on the planar surface of a hind paw until the rat lifted its paw from the platform. The time from onset of radiant heat application to withdrawal of the rat’s paw was defined as the PWL. Bilateral paws were tested independently with a 10-min interval between trials. The heat was maintained at a constant intensity, which produced a stable withdrawal latency of approximately 8~10 s during the training period. A 20 s cut-off was imposed on the stimulus duration to prevent tissue damage\textsuperscript{14}. The rats that exhibited unstable PWL were discarded.

1.4 Data analysis. Data are presented as mean±SEM. Repeated measures ANOVA was used for overall effects, with the Newman-Keuls test for post-hoc analysis for differences between groups. P<0.05 was considered statistically significant.

2 RESULTS

2.1 Effects of i.p. administration of meptazinol on PWL. The radiant heat-evoked paw withdrawal responses were examined in bilateral hindpaws in 8 rats. There was no significant difference in paw withdrawal latencies (PWLs) to noxious thermal stimuli between the bilateral hindpaws (9.32±0.67 vs 9.48±0.54). While the baseline PWLs were stable for the period of 100 min, carrageenan (2 mg/100 μl) was intraplantarly injected into one hindpaw. Carrageenan produced marked inflammation (edema and erythema) and thermal hyperalgesia in the injected paws, which peaked at 3 h after injection and showed little change in magnitude for another 3 h. The contralateral, non-injected paw showed no obvious inflammatory response compared to baseline at all post-carrageenan time points.

Intraperitoneal (i.p.) injection of NS at 3 h after carrageenan had no effect on PWLs of the inflamed paw and non-inflamed paw during the next 100 min (Fig. 1). No significant difference was found between the PWLs before and after i.p. injection of NS (P>0.05, n=8).

The effect of i.p. injection of meptazinol at three different doses (0.1, 1, and 10 mg/kg) on the PWL of the inflamed paw was examined during the peak hyperalgesic response (3~5 h after carrageenan). As shown in Fig. 1,
0.1 mg/kg meptazinol had no effect on the PWLs of either inflamed or non-inflamed paw ($P>0.05$, compared with either the pre-drug value or the PWL obtained from NS controls, $n=8$). In both inflamed and non-inflamed paws, doses of 1 and 10 mg/kg meptazinol significantly increased the PWL ($P<0.05$, compared with either the pre-drug value or the PWL obtained from NS controls, $n=8$). The dose-response curves for meptazinol at 20 min, the time of their peak effects, are presented in Fig. 1C.

2.2 Effects of i.p. administration of isomers 112824 and 112825 on PWL

A single dose of 112824 (1 mg/kg) were applied intraperitoneally at 3 h after carrageenan. As shown in Fig. 2, 1 mg/kg 112824 markedly increased the PWL of inflamed hindpaw ($P<0.05$, compared with either the pre-drug value or the PWL obtained from NS controls, $n=8$), which is similar to that of meptazinol (1 mg/kg, approximate ED$_{50}$ dose) ($P>0.05$). However, in non-inflamed hindpaw, this dose of 112824 did not produce obvious change in PWL ($P>0.05$, compared with either the pre-drug value or the PWL obtained from the NS controls, $n=8$).

Similar to meptazinol (1 mg/kg), i.p. injection of 1.5 mg/kg 112825 produced anti-hyperalgesic and anti-nociceptive effects in both inflamed and non-inflamed hindpaws. As shown in Fig. 2, the increase in PWL was statistically significant as compared with either the pre-drug value or the PWL obtained from the NS controls, $n=8$).

Fig. 1. Effects of i.p. injection of meptazinol at different doses on PWLs of inflamed (A) and non-inflamed hindpaw (B). C: Dose-response curves of the effects of meptazinol on PWLs of inflamed and non-inflamed hindpaw. The effect of meptazanol on PWLs is expressed as percentage of control, which is plotted against the drug dose (mg/kg). *$P<0.05$ and **$P<0.01$ indicate significant differences from NS control. *$P<0.05$, **$P<0.01$ indicate significant differences from non-inflamed hindpaw. NS, normal saline; Mep, meptazinol; Carr, carrageenan.

Fig. 2. Effects of i.p. injection of meptazinol and its isomers 112824 and 112825 on PWLs of inflamed (A) and non-inflamed hindpaw (B). *$P<0.05$ and **$P<0.01$ indicate significant differences from NS control. NS, normal saline; Mep, meptazinol; Carr, carrageenan.
those obtained from the NS controls ($P<0.05$, $n=8$).

2.3 Effects of naloxone on the meptazinol-, isomers 112824-, or 112825-induced anti-nociception and anti-hyperalgesia

In order to assess the role of endogenous opioid receptors in systemic meptazinol- and its isomers-induced analgesia, naloxone (1.5 mg/kg), a non-selective opioid receptor antagonist, was systemically administered. The results showed that naloxone partly antagonized the anti-hyperalgesia and anti-nociception induced by meptazinol, but completely blocked the isomers 11284- and 11285-induced anti-hyperalgesia (Fig. 3, 4).

Fig. 3. Effects of pre-administration of naloxone on meptazinol-(A) and its isomers 112824-(B) and 112825-(C)induced inhibition on PWLs of inflamed hindpaw. *$P<0.05$ and **$P<0.01$ indicate significant differences from meptazinol and its isomers 112824 and 112825 alone. NS, normal saline; Mep, meptazinol; Carr, carrageenan.

Fig. 4. Effects of pre-administration of naloxone on meptazinol-(A) and its isomers 112824-(B) and 112825-(C)induced inhibition on PWLs of non-inflamed hindpaw. *$P<0.05$ and **$P<0.01$ indicate significant differences from meptazinol and its isomers 112824 and 112825 alone. NS, normal saline; Mep, meptazinol; Carr, carrageenan.

3 DISCUSSION

The previous studies demonstrated that systemic administration of meptazinol produced anti-nociception in tail-flick and hot plate test in naive mice and rats\cite{1,5,11}. The present results further provided evidence for the anti-nociception of meptazinol. In the present study, i.p. injec-
tion of meptazinol produced a potent and long-lasting anti-nociceptive effect on non-inflamed paw in a dose-dependent manner. Pre-administration of naloxone significantly, but not fully blocked meptazinol-induced anti-nociception, suggesting that meptazinol-induced antinociception might be mainly mediated by mu opioid receptors[3,5]. Given meptazinol-induced anti-nociception was not blocked by naloxone completely, an action on non-opiate mechanisms might be involved. It has been demonstrated that meptazinol, in contrast to opioid drugs in general, does not inhibit but potentiate contractions in guinea pig ileum bioassay. This effect could be antagonized by atropine[2,3]. Also, the antinociception of meptazinol in mice antagonized by both scopolamine and naloxone, suggesting that meptazinol might induce anti-nociception by a dual action on opiate and cholinergic mechanisms in mice[3,15]. Muscarinic receptor previously have been demonstrated to be present in the brain and spinal cord of human, cats, rats, and mice, and have been shown to mediate anti-nociception[16-19]. The recent study from our laboratory showed that systemically or intracerebroventricularly pre-administered atropine partly antagonized the inhibitory effect of meptazinol on nociceptive responses (unpublished data).

A new finding in this work is that meptazinol produced a dose-dependent attenuating effect on carrageenan-induced inflammatory hyperalgesia. The effect of meptazinol to prolong PWL in inflamed paw was more potent than that in non-inflamed paw. As mentioned above, meptazinol-induced anti-nociception is mainly mediated by mu-1 opioid receptor[4-6]. Substantial evidence indicates that the expression of mu opioid receptor in brain and spinal dorsal horn is markedly increased following peripheral inflammation[10,20], which could be attributed to the action of meptazinol at inflammatory states.

Another finding of the present study is that the isomers of meptazinol, 112824 and 112825, produced similar anti-hyperalgesia to meptazinol, suggesting the novel agents are probably used for therapy of inflammatory pain. Unlike meptazinol, pre-administration of naloxone completely blocked 112824- and 112825-induced anti-hyperalgesia. This anti-hyperalgesic effect appears to be solely mediated by opioid receptors. These two isomers might lose cholinergic, but keep opiate, properties of meptazinol. The significance of this alteration requires further studies.

In conclusion, the present study demonstrates that meptazinol and its isomers have anti-hyperalgesic and anti-nociceptive properties with the former more potent. These inhibitory effects are mainly mediated by mu-opioid receptors. This study has provided an important clue for extending clinical utilization of meptazinol and its isomers.

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**REFERENCES**


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