

TRAMADOL, A CENTRALLY ACTING OPIOID : ANTICONVULSANT EFFECT AGAINST MAXIMAL ELECTROSHOCK SEIZURE IN MICE

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Abstract : The present study was designed to investigate the pro- or anti-convulsant effect of tramadol using maximal electroshock (MES) test. An attempt was also made to determine the possible opioid receptor mechanism involved. MES seizures were induced through transauricular electrodes (60 mA, 0.2s) and the seizure severity was assessed by the duration of tonic hindlimb extensor phase. Intraperitoneal (ip) administration of tramadol resulted in a dose-dependent anticonvulsant action; the ED₅₀ for the effect was 33 mg/kg. The anti-MES effect of tramadol was antagonized by the low doses (0.05 and 0.1 mg/kg, sc) of MR 2266, a selective *kappa* receptor antagonist and also by the high doses (1.0 and 5.0 mg/kg, ip) but not the low doses (0.1 and 0.25 mg/kg) of naloxone. The results suggest that the anti-MES effect of tramadol is mediated by *kappa* receptors, since MR 2266 and naloxone (in high doses) are known to block these receptors.

Key words : tramadol naloxone MR 2266
kappa opioid receptors maximal electroshock seizure

INTRODUCTION

Tramadol, is an orally active, clinically effective, centrally acting opioid analgesic which has been reported to produce dose-related antinociception in mouse abdominal constriction, hot plate and tail flick tests (1, 2). The antinociceptive effect of tramadol is believed to be mediated *via* opioid (involving both *mu* and *kappa* receptors) as well as non-opioid (by inhibition of monoamine uptake) mechanisms (1, 2). In previous years, the pharmacology of opioids in various animal models of experimental convulsions has been extensively

investigated (3, 4). Both pro- and anticonvulsant actions of morphine, a *mu* receptor agonist have been demonstrated (3). Workers have reported no consistent effect of classic *kappa* agonists such as ethylketocyclazocine and ketocyclazocine in flurothyl seizure threshold test (4). However, other studies pointed out an anticonvulsant effect in electroshock seizure model with drugs having either *kappa* agonist or mixed *mu* and *kappa* agonistic activity (5-9). In the light of these developments focussing the implications of opioids in seizures, the present study was designed in which the tramadol was

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investigated for its pro- or anticonvulsant action using maximal electroshock seizure (MES) test, since there is no report in the literature on the seizure related effect of this compound. An attempt was also made to determine the possible opioid receptor mechanism involved.

METHODS

Animals

Albino mice of either sex (20–25 g) were used. Animals were housed in plastic cages at an ambient temperature of $25 \pm 2^\circ\text{C}$ and 45–55% relative humidity with a standard 12h light-dark cycle. They had free access to food and water and were acclimatised to their environment for at least one week before experimentation. Each experimental group consisted of a minimum of 6 animals. National Research Council's guidelines for the care and use of laboratory animals were followed throughout the experiments.

Drugs

Tramadol (Ambalal Sarabhai, Baroda, India), MR2266 (Boehringer Ingelheim, Germany) and naloxone (Sigma, USA) were used in the present study. Commercially available tramadol injection was diluted to the required volume with distilled water. Naloxone HCl was dissolved in distilled water. MR 2266 was made into solution with distilled water and few drops of 0.1 N HCl. Injection volume (10 ml/kg) was kept constant. Tramadol and naloxone were administered intraperitoneally (ip), whereas MR 2266 was administered by subcutaneous (sc) route.

Maximal electroshock (MES) convulsions : MES seizures (10) were induced by

using an electroconvulsometer (Techno, Lucknow). A 60 mA current was delivered transauricularly for 0.2s *via* small alligator clips attached to the pinna of each ear. This current intensity elicited complete tonic extension of the hindlimbs in control mice. For recording various parameters, mice were placed in a clear rectangular plastic cage with an open top, permitting full view of the animal's motor responses to seizure. Various phases of convulsions, *viz.* tonic flexion, extension, clonus of hindlimbs, stupor and mortality due to convulsions were timed in pilot studies (10). Since, in other parameters, except the duration of hind limb extension and the magnitude of mortality, there was no significant changes observed after different drugs, these were not considered for evaluation of drug effects.

Drug treatment schedule: Pilot studies were conducted examining the dose-response relationship of tramadol in mice. Each mouse received a single ip injection of tramadol. Thirty min after the drug administration (10–50 mg/kg), different groups of animals were subjected to MES seizure and the duration of hindlimb extensor phase and percent mortality were recorded. For the naloxone studies, mice were pretreated ip with 50 mg/kg of tramadol in separate groups and 20 min later, a dose of naloxone (0.1–5.0 mg/kg) was administered ip (5). The animals were then subjected to MES seizure 10 min later.

For the MR2266 studies, mice were concurrently administered with MR 2266 (0.05 and 0.1 mg/kg, sc) and tramadol (50 mg/kg, ip) in separate groups and 30 min later, the animals were subjected to MES seizure.

Statistics

The ED₅₀ value for tramadol was calculated using the method of Litchfield and Wilcoxon (11). Statistical significance was calculated by the Student's two-tailed 't' test with P < 0.05 as the level of significance.

RESULTS

MES test: The duration of tonic hindlimb extension for control mice was found to be 15.34 ± 0.93 (±SEM)s. Tramadol produced a dose-dependent decrease in the duration of hindlimb extensor phase. The maximum decrease was seen with a dose of 50 mg/kg

(50 mg/kg) was chosen for antagonist studies. Higher doses of naloxone (1.0 and 5.0 mg/kg) but not the lower doses (0.1 and 0.25 mg/kg) of the drug antagonized the anticonvulsant effect of tramadol. Besides, specific *kappa* receptor antagonist, MR 2266 (0.05 and 0.1 mg/kg) also antagonized the anticonvulsant effect of tramadol (Table I). The doses of naloxone and MR 2266 which reversed the effect of tramadol on protection of MES hindlimb extension also increased the mortality of animals due to MES convulsions. Alone neither naloxone nor MR 2266 in the doses used produced any change in the hindlimb extensor duration (Data not shown).

TABLE I : Effect of tramadol (TRM) on maximal electroshock (MES)-induced convulsions in mice: modification by naloxone (NLX) and MR2266.

S.No.	Treatment (mg/kg, ip or sc)†	Duration of extensor phase (s ± SEM)	% Mortality (/n)
1.	Control	15.34 ± 0.93	33 (2/6)
2.	TRM (10)	12.16 ± 0.59*	17 (1/6)
3.	TRM (25)	8.00 ± 0.74**	17 (1/6)
4.	TRM (50)	1.83 ± 1.67**	0 (0/6)
5.	TRM (50) + NLX (0.1)	2.33 ± 0.77	33 (2/6)
6.	TRM (50) + NLX (0.25)	5.50 ± 1.02	50 (3/6)
7.	TRM (50) + NLX (1)	11.00 ± 0.47***	50 (3/6)
8.	TRM (50) + NLX (5)	13.16 ± 0.83***	67 (4/6)
9.	TRM (50) + MR 2266 (0.05)†	10.50 ± 0.65***	33 (2/6)
10.	TRM (50) + MR 2266 (0.10)†	13.00 ± 0.74***	50 (3/6)

*P < 0.05; **P < 0.001 as compared to control group.

***P < 0.001 as compared to TRM (50 mg/kg, ip) group (n=6).

†MR2266 was injected by sc route.

tramadol (Table I). This dose of tramadol which protected the animals from tonic hindlimb extension also prevented the mortality due to seizure. The anticonvulsant ED₅₀ for tramadol was observed to be 33 (17.64 – 61.71, 95% confidence limits) mg/kg, ip.

Antagonist studies: A dose of tramadol giving maximum anticonvulsant effect

DISCUSSION

The results of the present study demonstrate that tramadol showed a protective effect against MES seizures in mice. This anticonvulsant effect was dose-dependent, antagonized by the high doses of naloxone and low doses of MR 2266.

Previous studies of preclinical pharmacology of tramadol have revealed that this drug is a modestly potent opioid which interacts with *mu*, *kappa* and *delta* opioid receptors, where it exhibits agonist effects (1). Due to the rapid and complete metabolism of tramadol in rodents, the effects seen in animal studies are principally, of the parent drug as well as of active metabolite, O-demethyl tramadol (1). This metabolite displays a preference for *mu* receptor, thus explaining why in rodents, but not in man tramadol shows a similarity to morphine (1). In this study tramadol has shown a significant anticonvulsant activity in MES test. Additionally, naloxone in higher doses (>1 mg/kg) but not in lower doses (0.1 and 0.25 mg/kg) completely antagonized the anticonvulsant effect of tramadol. This suggests that the mechanism of action for tramadol as anti-MES convulsant involves a *kappa* opioid receptor system. Antagonism of anticonvulsant effect of tramadol by MR2266, a selective *kappa* receptor antagonist further confirms the involvement of *kappa* opioid receptor system. In previous studies also such reversal of *kappa* agonist mediated anticonvulsant effect by low doses of MR2266 and high doses of naloxone has been observed, e.g. Tortella et al. reported a complete antagonism of anti-MES convulsion effect of U-50488, a selective *kappa* agonist by a high dose (10 mg/kg) but not by a low dose of naloxone (6). Frey observed complete antagonism of anti-electroshock effects of *kappa* agonist drug, nalbuphine, by MR 2266 and high dose (1 mg/kg) but not by a low dose of naloxone (7). Fischer et al. (8) demonstrated antagonism of anticonvulsant efficacy of another *kappa* agonist, U-54494A, against MES by a high dose of naloxone (3 mg/kg).

A recent study has also demonstrated an anti-MES convulsion effect of pentazocine and antagonism of this effect by high doses (1.0 and 5.0 mg/kg) but not by the low doses (0.1 and 0.25 mg/kg) of naloxone (9). These observations highlight the fact that naloxone in high doses blocks *kappa* opioid receptors and as a result antagonise the anti-electroshock effect of opioidergic compounds known to act on *kappa* receptors. Based on their studies and citing the work of others, it has been suggested by Tortella et al. (12) that dose of naloxone less than 1 mg/kg given peripherally and 10 µg centrally selectively antagonize *mu*-receptor mediated responses. Thus an inability of a low dose of naloxone to prevent the protective effect of tramadol against hindlimb extension show that the mechanism of action for tramadol as an anti-MES convulsant does not involve a *mu* opioid receptor system. Further, the results of complete blockade of anti-MES effect by MR 2266 and high doses of naloxone also indicate that non-opioid monoaminergic mechanism may not be involved in the anticonvulsant effect of tramadol as suggested for antinociceptive effect of this compound in earlier studies (1, 2) or monoaminergic mechanisms are evoked as postsynaptic events to opioid receptors.

The results of the present study thus support the observations of other workers (6-9) and indicate that the stimulation of *kappa* receptors in the brain results in protection of MES convulsions, and suggest that an endogenous *kappa* opioid system may be important in the regulation of cerebral excitability during seizure.

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REFERENCES

1. Rhoda Lee C, McTavish D, Sorkin EM. Tramadol: A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states. *Drugs* 1993; 46: 313-340.
2. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and non opioid components independently contribute to the mechanism of action of tramadol, an atypical opioid analgesic. *J Pharmacol Exp Ther* 1992; 260: 275-285.
3. Frenk H. Pro- and anticonvulsant actions of morphine and the endogenous opioids: Involvement and interactions of multiple opiate and non-opiate systems. *Brain Res Rev* 1983; 6: 197-210.
4. Cowan A, Geller EB, Adler MW. Classification of opioids on the basis of change in seizure threshold in rats. *Science* 1979; 206: 465-467.
5. Czuczwar SJ, Frey HH. Effect of morphine and morphine-like analgesics on susceptibility to seizures in mice. *Neuropharmacology* 1986; 25: 465-469.
6. Tortella FC, Robles L, Holaday JW. U 50488, a highly selective κ opioid: Anticonvulsant profile in rats. *J Pharmacol Exp Ther* 1986; 237: 49-53.
7. Frey HH. Effects of μ - and κ -opioid agonists on the electroconvulsive threshold in mice and antagonism by naloxone and MR 2266. *Pharmacol Toxicol* 1988; 62: 150-154.
8. Fischer W, Bodewei R, von Voigtlander PF, Müller M. Anti-convulsant and related effects of U-54494A in various seizure tests. *J Pharmacol Exp Ther* 1993; 267: 163-170.
9. Manocha A, Sharma KK, Mediratta PK. The anticonvulsant effect of pentazocine against maximal electroshock seizure in mice. *Indian J Pharmacol* 1997; 29: 194-197.
10. Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. *J Pharmacol Exp Ther* 1952; 106: 319-330.
11. Litchfield JT, Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 1949; 96: 99-113.
12. Tortella FC, Robles L, Mosberg HJ. Evidence for μ opioid receptor mediation of enkephalin-induced electroencephalographic seizures. *J Pharmacol Exp Ther* 1987; 240: 571-577.