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On the mechanism of anticonvulsant effect of tramadol in mice

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Abstract

The present study was conducted to examine the effects of tramadol, an atypical opioid on convulsive behaviour in maximal electroshock (MES) seizure test on mice. Moreover, an attempt was also made to investigate the role of possible receptor mechanisms involved. MES seizures were induced via transauricular electrodes (60 mA, 0.2 sec). Seizure severity was determined by (1) the duration of tonic hindlimb extensor (THE) phase and by (2) mortality due to electroconvulsions. Intraperitoneal (i.p.) administration of tramadol dose-dependently (10–50 mg/kg) decreased the duration of THE phase of MES. The anticonvulsant effect of tramadol was antagonized by the opioid antagonists, naloxone in high dose, and MR2266, a selective kappa antagonist but not by naltrindole, a delta opioid antagonist. Coadministration of either γ-aminobutyric acid (GABA)-ergic drugs (diazepam, GABA, muscimol and baclofen) or *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK801 with tramadol augmented the anticonvulsant effect of the latter drug. By contrast, flumazenil, a central benzodiazepine (BZD) receptor antagonist, counteracted the diazepam-induced facilitation of anti-MES effect of tramadol. Similarly, δ-aminovaleric acid (DAVA), a $GABA_B$ receptor antagonist, abolished the facilitatory effect of baclofen, a $GABA_B$ agonist on anti-MES action of tramadol. These BZD–GABAergic antagonists, flumazenil or DAVA, on their own also antagonized the anti-MES effect of tramadol administered alone. No significant effect on mortality was observed in any of the studied groups. Taken together, the current results have demonstrated a possible role for multitude of important neurotransmitter systems, i.e., opioid (kappa), $GABA_A$–BZD receptors system, $GABA_B$ receptors and NMDA channel involvement in the antielectroshock effect of tramadol in mice.

Keywords: Tramadol; Opioids; Kappa opioid receptors; Maximal electroshock seizures; γ-Aminobutyric acid; *N*-Methyl-D-aspartate

1. Introduction

There is sufficient evidence implicating the amino acids γ-aminobutyric acid (GABA), *N*-methyl-D-aspartate (NMDA), and opioids in the pathogenesis and/or amelioration of various neurological disorders including convulsions (Tortella, 1988; Feldman et al., 1997; Rogawski, 1998; Homayoun et al., 2002; Khavandgar et al., 2003; Schindler et al., 2004; Shafaroodi et al., 2004). Furthermore, many of the drugs that enhance $GABA_A$ergic neurotransmission in the brain have been shown to be clinically active anticonvulsants (Rogawski, 1998).

Tramadol hydrochloride ((±)-*trans*-2-[(dimethylamino) methyl]-1-(3-methoxy-phenyl)-cyclohexanol HCl) is a centrally acting and clinically effective analgesic (Dayer et al., 1994; Scott and Perry, 2000). It is a weak atypical opioid agonist that activates opioid receptors; has some selectivity for mu opioid receptors while it binds weakly to both kappa and delta receptors (Raffa et al., 1992; Scott and Perry, 2000). It is metabolized in the liver; the *O*-desmethyl (M1) metabolite of tramadol is pharmacologically active and has higher affinity for mu opioid receptors than the parent drug (Scott and Perry, 2000; Valle et al., 2000). Tramadol's low potential for dependence or abuse, together with its less respiratory depressant, minimal constipating or sedative effects, are some of the advantages of this atypical opioid (Miranda and Pinardi, 1998; Scott and Perry, 2000).

Tramadol produces antinociception in the acetylcholine-induced abdominal constriction, hot plate and tail-flick tests

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in mice and has shown antinociceptive activity in the air-induced abdominal constriction and hot plate tests in rats. Moreover, tramadol-induced antinociception was partly antagonized by naloxone in most of the antinociceptive paradigms in rodents (Raffa et al., 1992).

It has been demonstrated that opioids may show some of their behavioural effects in vivo by direct opioid receptor activation or indirectly by modulating GABA or NMDA receptors (Frenk, 1983; Atapour et al., 2000; Yajima et al., 2000; Hara et al., 2005).

Unfortunately, idiopathic seizures have stemmed from tramadol monotherapy in high doses in patients and even when tramadol is being used concomitantly with other drugs, although the incidence for such seizures is found to be very low (Budd and Langford, 1999; Gardner et al., 2000; Scott and Perry, 2000; Marquardt et al., 2005). An interesting study with *Xenopus* oocytes suggests that tramadol and its M1 metabolite at large concentrations inhibit GABA(A) receptors that might correlate with seizures (Hara et al., 2005).

To our knowledge, there is paucity of literature available that relates the role of tramadol on convulsions and the associated mechanism(s) involved. We have previously reported that tramadol shows anticonvulsant effects which are mediated by kappa opioid receptors in the maximal electroshock (MES) seizure paradigm in mice (Manocha et al., 1998b). Based on these preliminary findings, and in an attempt to further broaden the pharmacological effects of tramadol on convulsive behaviour, the current studies were intended to clarify the positive influence of tramadol, an atypical opioid (Raffa et al., 1992) on convulsive behaviour (i.e., anticonvulsant effects) in MES test in mice. The MES test as originally elaborated by Swinyard et al. (1952) assesses the ability of drugs to abolish electrically induced tonic hindlimb extension (THE) in animals. The MES test is an excellent animal model for identifying new antiepileptic drugs (AEDs) that block the seizure spread across the neural tissue and as such are likely to be effective for the management of generalized tonic–clonic seizures in humans (Löscher et al., 1991; White et al., 1998).

The current studies, apart from opioid receptors also determine the role of other possible receptor systems, like GABA–BZD and NMDA, which may be involved in the effect of tramadol on convulsive behaviour in mice in an attempt to better understand the receptor mechanisms underlying such behaviours.

2. Materials and methods

2.1. Animals

Albino Swiss mice of either sex (obtained from Central Animal Breeding House, AIIMS, Delhi) weighing from 20 to 25 g were used in these studies. The ratio of male and female mice in the control and drug-treated groups was kept same to avoid variation in responses due to sex differences. The animals were housed in plastic cages and were maintained on a 12:12-h light–dark (7:00 A.M. to 7:00 P.M.) schedule in a temperature-controlled (25±2 °C) animal room and relative humidity (about 45–55%). The animals had free access to standard rodent chow and water and were acclimatised to their environment for one week prior to experimentation. The animals were randomly distributed into different groups. Each experimental group comprised of a minimum of 10 animals. Mice were moved from the animal care facility to the testing laboratory room; each animal was caged separately after recording its body weight and was randomized to receive the treatments according to a random number table. They had identification marks identifying the dose level group and individual number. All experimental protocols were approved by the University college of medical sciences institutional review committee for animal subjects and experimental procedures were done according to the Guidelines for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH). All the behavioural studies were carried out between 10.00 A.M. and 6.00 P.M.

2.2. Drugs

Tramadol HCl (Cadila, Ahmedabad, India), naloxone HCl, naltrindole HCl, muscimol, δ-amino valeric acid (DAVA) (Sigma, USA), (−)-5,9 alpha-diethyl 2-(3-furyl-methyl)-2-hydroxy-6,7-benzomorphan (MR2266) (Boehringer Ingelheim, FRG), diazepam (Ranbaxy, Delhi, India), γ-aminobutyric acid (GABA) (BDH, Poole, Dorset, UK), baclofen (Ciba-Geigy, Switzerland), flumazenil (Ro15-1788) (F. Hoffmann La Roche, Basel, Switzerland), and (+)-5-methyl-10, 11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK801) (Merck Sharp and Dohme, West Point, PA, USA) were used in the present study. The drugs were used as their respective salts. Doses of the drug are reported as the actual amount of drug administered after correction for the salt content. Naloxone HCl, naltrindole HCl, GABA, muscimol, baclofen, DAVA and MK801 were dissolved in distilled water. MR2266 was made into solution with distilled H_2O aided by a few drops of 0.1 N HCl. Tramadol HCl injection (TRAMAZAC®) and diazepam injection (CALMPOSE®) were brought up to the required volume with d-H_2O before use. Flumazenil was suspended in d-H_2O with a few drops of Tween 80. Tramadol, naloxone, naltrindole and DAVA were injected intraperitoneally (i.p.) while MR2266, diazepam, GABA, muscimol, baclofen, flumazenil and MK801 were administered subcutaneously (s.c.) in the scruff of the neck of the animals. All drugs were freshly prepared prior to use and injection volume (10 ml/kg) was kept constant. The dosage selection, route of drug administration and time-scheduling of different compounds was based on preliminary experiments and pharmacokinetic considerations. Different doses of tramadol (10 or 50 mg/kg) were used for opioid antagonist experi-

ments and for other interaction studies, in an attempt to arrive at some clarified results and conclusions. Appropriate vehicle controls were used in all experiments. For each experimental series, separate controls were used to avoid data variation from day to day.

2.3. MES-induced convulsions

MES seizures were electrically induced by means of an electroconvulsometer (Techno Instruments, Lucknow, India). A 60 mA current was delivered transauricularly for 0.2 sec in mice via small alligator clips attached to the pinna of each ear. This current intensity elicited complete tonic hindlimb extension (THE) in control animals. For measuring various parameters, mice were placed in a clear rectangular plastic cage with an open top, permitting full view of the animals' motor responses to the seizures. In the preliminary study, different phases of convulsions, viz. tonic flexion, extension, clonus, stupor and mortality due to convulsions were recorded.

To evaluate the drug effect on seizure severity, the duration of THE and mortality due to convulsions were chosen as the parameters. Immediately after exposing the animals to MES treatment, recordings were done for convulsions and mortality. Furthermore, each animal was then individually observed for 2 h to study convulsive effects on general behaviour. Animals were monitored after 24 h to assess further mortality; however, animals did not exhibit mortality during this period. A compound is found to possess anticonvulsant property if it attenuates or abolishes the tonic hindlimb extensor (THE) phase of MES.

2.4. Treatment schedule of tramadol, naloxone, MR2266 and naltrindole in MES test

Data were collected on dose–response (10–50 mg/kg) relationship of tramadol on MES seizures in different groups of mice receiving a single i.p. injection of tramadol followed 30 min later by the MES seizure with duration of THE phase, mortality due to convulsions and general behaviour subsequently recorded. In the naloxone study, mice were pretreated with tramadol (50 mg/kg), received naloxone (0.1–5 mg/kg i.p.) 20 min later, and then exposed to the MES seizure 30 min after tramadol. Such a short pretreatment schedule of 10 min was used for naloxone in this study since it is a short acting opioid antagonist in both rodents and humans (Tallarida et al., 1978; Reisine and Pasternak, 1996). In the MR2266 study, mice were administered with MR2266 (0.05 and 0.1 mg/kg s.c.), a kappa opioid antagonist and tramadol (50 mg/kg, i.p.) concurrently at the same time by different routes (i.p. or s.c.) and were subjected to the MES seizure 30 min later. To study delta receptor involvement, mice received tramadol (50 mg/kg), followed 5 min later by naltrindole (0.25 mg/kg i.p.), a delta opioid antagonist and 25 min later by MES seizure. Tramadol, MR2266, and naltrindole treatment intervals, 25–30 min prior to MES were chosen on the basis of preliminary data and/or previous literature (Manocha et al., 1998b; Miranda and Pinardi, 1998; Scott and Perry, 2000). The per se effects of opioid antagonists on MES seizures were also measured.

2.5. Drug-interaction studies

2.5.1. Interaction between BZD–$GABA_A$ergic compounds and tramadol

The $GABA_A$ receptor agonists, GABA (100 and 200 mg/kg s.c.) and muscimol (0.5 and 1 mg/kg s.c.) were studied alone and/or in combination with tramadol (10 mg/kg i.p.) cotreatment 30 min prior to MES. A BZD agonist (diazepam: 2.5 mg/kg s.c., 30 min) and antagonist (flumazenil: 0.5 mg/kg s.c., 5 min) (Brogden and Goa, 1991) were administered alone and/or in combination with tramadol prior to MES.

2.5.2. Interaction between $GABA_B$ receptor compounds and tramadol

A $GABA_B$ receptor agonist (baclofen: 2.5 and 5 mg/kg s.c.) and antagonist (DAVA: 50 mg/kg i.p.) were studied alone and/or in combination with tramadol (10 mg/kg i.p.) and were injected 25–30 min prior to MES.

2.5.3. Interaction between glutamatergic drugs and tramadol

The effect of NMDA receptor antagonist MK801 (0.05 and 0.1 mg/kg s.c.) was studied alone and/or in combination with tramadol (10 mg/kg i.p.) 30 min prior to exposure to MES. In case of combination study, they were administered concurrently.

2.6. Statistical analysis

The duration of THE phase of MES convulsions, expressed as the arithmetic mean (±S.E.M.) was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's t test comparisons. A grouped Chi-square test was initially used to determine the overall differences in the mortality due to convulsions. If significant ($P<0.05$) effect was observed, individual differences were determined by single Chi-square test (Gupta, 1990).

3. Results

3.1. Effects of tramadol

The different doses of tramadol (10–50 mg/kg) produced straub's tail, hyperreactivity to sound and touch and drowsiness in mice when compared with vehicle-treated control animals. However, tramadol (50 mg/kg), besides the above behavioural symptoms, also produced occasional running, jumping and circling in mice.

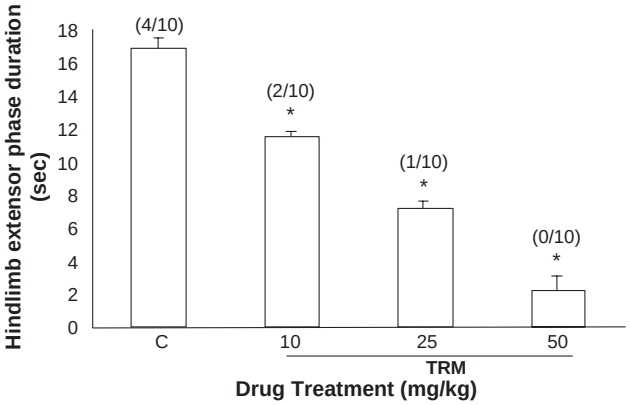

Fig. 1. Effects of tramadol (TRM) on maximal electroshock (MES)-induced convulsions in mice. Each bar represents mean values ± S.E.M. Bars (from the left) 2, 3, 4 vs. 1; $n=10$. *$P<0.001$ as compared to control (C) group (one-way ANOVA followed by Dunnett's t test) [$F(3,36)=42.84$, $P<0.01$]. Values in parentheses indicate mortality (grouped Chi-square test with Yates correction) [$\chi^2_{3df}=3.29$]. TRM is administered by the i.p. route.

MES induced tonic convulsions in all the animals with tramadol, dose-dependently (10–50 mg/kg) protecting the animals [ANOVA, $F(3,36)=42.84$; $P<0.01$]. Tonic hindlimb extension (THE) scores showed dose-dependent decreases following tramadol when compared to vehicle-treated control animals: 0 (16.90 ± 0.95 sec), 10 (11.50 ± 0.48 sec), 25 (7.20 ± 0.61 sec), and 50 mg/kg (2.20 ± 1.47 sec). Mortality decreased from 40% to 0% across tramadol doses (Fig. 1).

3.2. Interaction of tramadol with opioid receptor antagonists

Although opioid receptor antagonists naloxone at high doses (1 and 5 mg/kg), and MR2266 (0.05 and 0.1 mg/kg) significantly reversed ($P<0.001$) the protective effect of tramadol on MES-induced convulsions; naloxone at low doses (0.1 and 0.25 mg/kg), and naltrindole (0.25 mg/kg) did not show any significant reversal of tramadol effects. Furthermore, the mortality was not significantly altered in all the above groups. Post hoc analysis revealed that none of the three prototypical opioid receptor antagonists (i.e., naloxone, MR2266 or naltrindole) alone altered MES convulsions (Fig. 2).

3.3. Interaction between BZD–$GABA_A$ergic compounds and tramadol

As shown in Fig. 3, GABA (100 and 200 mg/kg), muscimol (0.5 and 1 mg/kg), and diazepam (2.5 mg/kg) alone suppressed tonic electroconvulsions in a dose-dependent fashion by significantly reducing ($P<0.001$) the duration of THE and nonsignificantly decreasing the mortality rate. Flumazenil treatment (0.5 mg/kg) significantly antagonized ($P<0.001$) diazepam's protective effects on MES convulsions.

Among diazepam, GABA or muscimol cotreatment with tramadol, these GABAergic drugs reliably augmented ($P<0.001$) tramadol's protective effects against MES convulsions. Furthermore, flumazenil cotreatment with tramadol significantly reversed ($P<0.001$) the anti-MES effect of tramadol as well as the diazepam-induced facilitation of tramadol effects (Fig. 3). However, the mortality was not significantly altered in any of the above groups (Fig. 3).

3.4. Interaction between $GABA_B$ receptor compounds and tramadol

As illustrated in Fig. 4, baclofen (2.5 and 5 mg/kg) per se significantly decreased ($P<0.001$) the THE duration

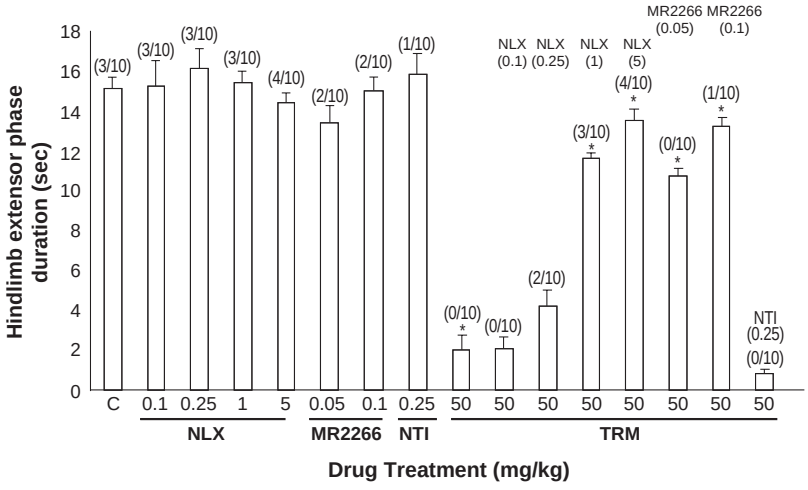

Fig. 2. Effects of naloxone (NLX), MR2266 and naltrindole (NTI) per se and on the anticonvulsant effect of tramadol (TRM) in the mouse maximal electroshock (MES)-induced convulsions test. Each bar represents mean values ± S.E.M. Bars (from the left) 2, 3, 4, 5, 6, 7, 8, 9 vs. 1; Bars 10, 11, 12, 13,14, 15, 16 vs. 9; $n=10$. *$P<0.001$ as compared with control (C) or per se effect of tramadol (one-way ANOVA followed by Dunnett's t test) [$F(15,144)=13.37$, $P<0.01$]. Values in parentheses indicate mortality (grouped Chi-square test with Yates correction) [$\chi^2_{15df}=10.08$]. TRM, NLX, NTI are given i.p. except MR2266 which is administered by the s.c. route.

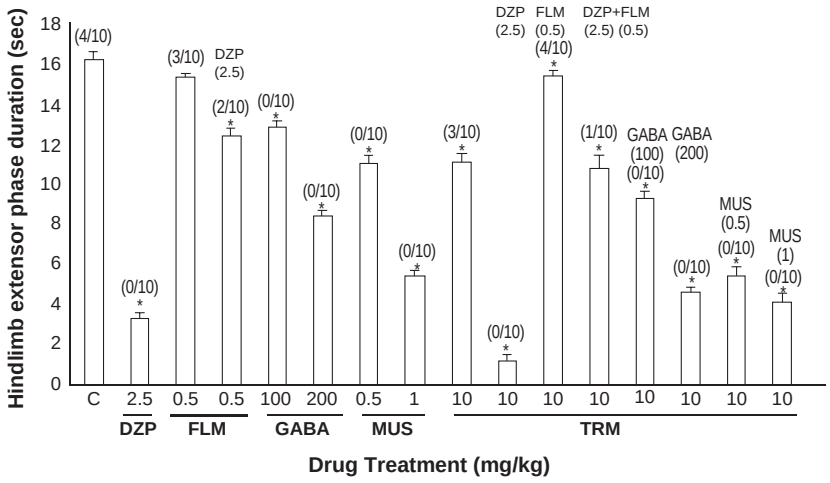

Fig. 3. Effect of benzodiazepine (BZD)–$GABA_A$ receptor acting drugs per se and on the anticonvulsant activity of tramadol (TRM) in maximal electroshock (MES)-induced convulsions in mice. Each bar represents mean values ± S.E.M. Bars (from the left) 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16 vs. 1; Bar 4 vs. 2; Bars 10, 11, 13, 14, 15, 16 vs. 9; Bar 12 vs. 10; $n=10$. *$P<0.001$ as compared with control (C) or per se effect of tramadol or diazepam or its combination (one-way ANOVA followed by Dunnett's t test) [$F(15,144)=123.45$, $P<0.01$]. Values in parentheses indicate mortality (grouped Chi-square test with Yates correction) [$\chi^2_{15df}=20.60$]. Diazepam (DZP), flumazenil (FLM), γ-aminobutyric acid (GABA), muscimol (MUS) are given s.c. excluding TRM which is administered by the i.p. route.

and nonsignificantly reduced the mortality incidence. Additionally, DAVA (50 mg/kg), a $GABA_B$ receptor blocker did not produce any effects on its own but significantly antagonized ($P<0.001$) the baclofen's protective effects.

Baclofen cotreatment with tramadol increased ($P<0.001$) the protective effect of tramadol on MES convulsions while DAVA (50 mg/kg) reliably antagonized the anticonvulsant effect of tramadol ($P<0.001$). Furthermore, in baclofen and tramadol cotreated animals, DAVA reversed ($P<0.001$) the facilitatory action of baclofen on the antielectroshock effect of tramadol. However, the mortality was not significantly affected in all these above groups. The results are shown in Fig. 4.

3.5. Interaction between glutamatergic drugs and tramadol

MK801 (0.05 and 0.1 mg/kg) per se demonstrated a significant protective effect ($P<0.001$) against MES-induced convulsions in comparisons to vehicle-treated control (Fig. 4). MK801 cotreatment with tramadol augmented ($P<0.001$) the protective effect of tramadol on MES convulsions and nonsignificantly reduced the mortality rate. The results are given in Fig. 4.

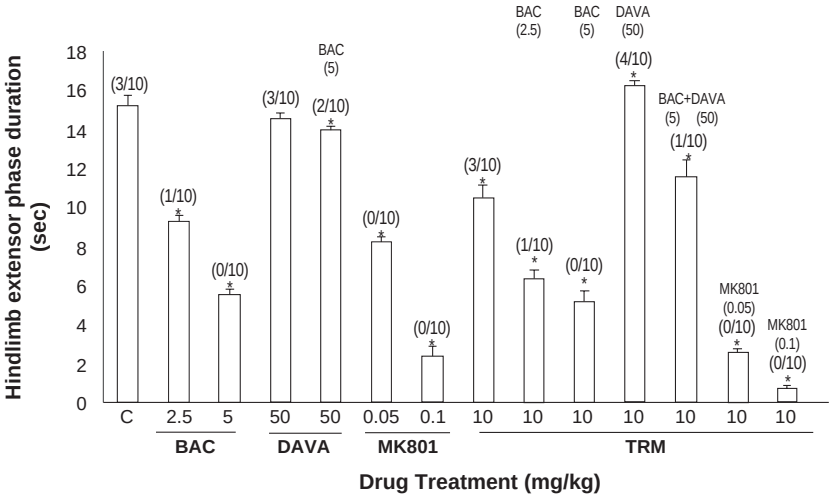

Fig. 4. Effects of $GABA_B$ergic drugs and MK801, an NMDA receptor antagonist per se and on the anticonvulsant effect of tramadol (TRM) in the mouse maximal electroshock (MES)-induced convulsions test. Each bar represents mean values ± S.E.M. Bars (from the left) 2, 3, 4, 6, 7, 8, 9, 10, 13, 14 vs. 1; Bar 5 vs. 3; Bars 9, 10, 11, 13, 14 vs. 8; Bar 12 vs. 10; $n=10$. *$P<0.001$ as compared with control (C) or per se effect of tramadol or baclofen or its combination (one-way ANOVA followed by Dunnett's t test) [$F(13,126)=88.10$, $P<0.01$]. Values in parentheses indicate mortality (grouped Chi-square test with Yates correction) [$\chi^2_{13df}=11.82$]. Baclofen (BAC), MK801 are given s.c. while TRM, δ-aminovaleric acid (DAVA) are administered by the i.p. route.

4. Discussion

The present behavioral study provides evidence for a role of the kappa opioid receptors in mediating the anticonvulsant-like behavioural effects of tramadol. Tramadol, a synthetic and centrally acting 'atypical' opioid (Raffa et al., 1992; Scott and Perry, 2000) was demonstrated to have anticonvulsant effects in the MES paradigm in mice. Furthermore, both tramadol and the classical anticonvulsant drug diazepam (Rogawski and Porter, 1990; Rang et al., 2003) produced qualitatively similar responses in the MES, the effects of tramadol could be attributable either to its direct action at the kappa opioid receptor or to an indirect consequence of GABA receptor modulation. The latter also seems likely because flumazenil, a specific central antagonist to BZD receptors (Brogden and Goa, 1991), attenuated the anticonvulsant behavioural response of tramadol. Consistent with this interpretation are the previous interesting findings which have shown kappa opioids to be active in several animal behavioural models of convulsions (Tortella et al., 1986, 1989; VonVoigtlander et al., 1987; De Sarro et al., 1993; Fischer et al., 1993; Yajima et al., 2000). Moreover, MR2266 is a more specific kappa opioid receptor antagonist (Laorden et al., 1991) while naltrindole effectively blocks delta opioid receptors (Reisine and Pasternak, 1996). This contrasts with that seen with non-selective opioid receptor antagonist, naloxone that is relatively a potent mu antagonist at low doses (Tallarida et al., 1978) while it preferentially blocks kappa receptors at high doses (Tortella et al., 1986; Reisine and Pasternak, 1996). The greater efficacy of MR2266 relative to naloxone and naltrindole in blocking the anticonvulsant effects of tramadol suggests that these effects were also mediated by the activation of kappa opioid receptors. This is in line with previous observations that nor-binaltorphimine, a selective kappa opioid antagonist and/or naloxone at high doses blocks kappa receptor-mediated behaviours induced by pentazocine, nalbuphine, and by more selective kappa opioid receptor agonists such as U-50488H and U-54494A in rodents (Tortella et al., 1986, 1989; Fischer et al., 1993; Manocha et al., 1997, 1998a).

Diazepam is an effective BZD receptor ligand that binds to the 'benzodiazepine receptor' on the $GABA_A$ receptor complex (GRC) and selectively potentiates the effects of GABA on $GABA_A$ receptors (Feldman et al., 1997; Rang et al., 2003). Both muscimol and GABA are selective agonists at $GABA_A$ receptors (Sieghart, 1992). In this current study, diazepam, muscimol, and GABA all produced anticonvulsant-like effects in the MES paradigm. The behavioural pattern, as manifested by a significant anticonvulsant behaviour, suggests that their response was more likely associated with effects on GABAergic transmission (i.e., $GABA_A$ receptor activation) as opposed to effects on the opioid receptor. Furthermore, the more selective central BZD receptor antagonist flumazenil alone did not produce any effects in the MES test but it antagonized the anticonvulsant effects of diazepam; it shows that BZD–$GABA_A$ receptors are probably involved in these diazepam effects.

Interestingly, an active dose of GABA, muscimol, or diazepam also augmented the anticonvulsant-like behaviour produced by tramadol. Based on these behavioural observations, the augmentation of protective effect of tramadol on MES seizures by GABAergic agents, like diazepam, GABA, and muscimol, suggests that $GABA_A$–BZD mechanisms may be participating in at least some of the tramadol's anti-MES effect. Furthermore, the reversal of diazepam's facilitatory effect on tramadol protection of MES convulsions by flumazenil indicates that the BZD site on BZD–$GABA_A$ receptor complex could possibly be involved in the action of tramadol in the brain. This suggestion also gets credence from observations, where flumazenil alone even attenuated the anti-MES seizure effect of tramadol when administered alone. Consistent with the above observations are the previous findings which have addressed kappa agonists (U-50488H, butorphanol) to be effective against $GABA_A$ receptor antagonist, pentylenetetrazole (PTZ) or bicuculline-induced convulsions in mice (Pircio et al., 1976; Yajima et al., 2000) and blockade of their anti-MES effect by flumazenil (Manocha et al., 2003a,b).

Baclofen is a selective agonist at $GABA_B$ sites (Bowery, 1993), by contrast δ-aminovaleric acid (DAVA) preferably antagonizes $GABA_B$-mediated responses (Schwarz et al., 1988). Baclofen was shown to produce anticonvulsant effects in the MES paradigm. The behavioural pattern, as manifested by a significant anticonvulsant behaviour, highlights that this particular response of baclofen was more likely associated with profound effects on GABAergic transmission (i.e., $GABA_B$ receptor activation) as opposed to effects on opioid receptor. DAVA when administered alone, did not produce any effects on MES, but it antagonized the anticonvulsant effects of baclofen, suggesting that the effects of baclofen are $GABA_B$ergic in origin. Furthermore, the augmentation of the anticonvulsant action of tramadol by baclofen and prevention of the anticonvulsant effect of tramadol alone and when administered in combination with baclofen by DAVA suggests that apart from $GABA_A$, $GABA_B$ receptors probably play a role in the antiseizurogenic effects of tramadol.

There is a growing evidence that the NMDA subtype of excitatory amino acid (EAA) receptors play an important role in epilepsy (Meldrum, 1992). MK801 is a non-competitive NMDA receptor antagonist that acts at a site within the ion channel of the NMDA receptor complex (Wong et al., 1986). In the present study, MK801 produced anticonvulsant-like effects in the mouse MES paradigm. Interestingly, MK801 also enhanced the anticonvulsant effects of tramadol on MES seizures, therefore suggesting that the anticonvulsant activity of tramadol is at least in part mediated at NMDA receptors. Consistent with these observations are the previous studies that highlight the fact that kappa opioids modulate glutamate-mediated excitatory

synaptic transmission in the central nervous system (CNS), both in the guinea pig and rat (Conner-Kerr and Terrian, 1993; Simmons et al., 1994; Cheng and Kojic, 1995) while tramadol and its M1 metabolite inhibit the NMDA receptors expressed in *Xenopus* oocytes (Hara et al., 2005). Additionally, there is sufficient evidence describing the neuroprotective effects of selective kappa opioids (U-50488H, U-54494A, GR 89696) in certain animal models of cerebral ischaemia (Birch et al., 1991) and NMDA-induced brain injury (Hudson et al., 1991). More importantly, the combination of NMDA receptor antagonists, with opioids, may lead to potentiation of opioid's clinical effect (Wiesenfeld-Hallin, 1998).

In conclusion, specifically the kappa opioid receptor subtype, alone or interdependent with the GABA and NMDA receptor, is likely to play a role in the mediation of anticonvulsant effects of tramadol. It appears that opioid receptors, such as kappa, BZD–$GABA_A$-coupled receptor system, $GABA_B$ receptors, and NMDA receptor channel probably play a role in the anti-MES response produced by tramadol. Also, the mouse MES paradigm used in the current study, was quite sensitive for opioidergic compounds but was not designed to reveal a residual role for opioid receptors in the effects of tramadol. These present data with tramadol provide an important information that the kappa opioid receptor may be a target for the development of anticonvulsants for grand mal seizures, and opioid compounds might be useful as adjuncts to conventional antiepileptic drugs (AEDs). Moreover, the shortcomings of the experiments are with the systemic route of drug administration in which it is quite difficult to discriminate the blood–brain barrier (BBB) penetrability from other variables (Oldendorf et al., 1972). The present findings can be replicated in an elaborated way to delineate specifically the roles for opioid receptors, and other complementary receptors in various convulsive models, may be better addressed and strengthened by receptor binding studies using some more selective opioid ligands.

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References

Atapour N, Kalantaripour TP, Nourpanah M, Niazi M. Chemical kindling and seizure susceptibility in morphine dependent rats. Eur Neuropsychopharmacol 2000;10:483–7.

Birch PJ, Rogers H, Hayes AG, Hayward NJ, Tyers MB, Scopes DIC, et al. Neuroprotective actions of GR89696, a highly potent and selective κ-opioid receptor agonist. Br J Pharmacol 1991;103:1819–23.

Bowery NG. $GABA_B$ receptor pharmacology. Annu Rev Pharmacol Toxicol 1993;33:109–47.

Brogden RN, Goa KL. Flumazenil: a reappraisal of its pharmacological properties and therapeutic efficacy as a benzodiazepine antagonist. Drugs 1991;42:1061–89.

Budd K, Langford R. Editorial: II. Tramadol revisited. Br J Anaesth 1999;82:493–5.

Cheng RG, Kojic L. κ-Opioid receptor agonists modulate excitatory transmission in substantia gelatinosa neurons of the rat spinal cord. J Neurosci 1995;15:6809–26.

Conner-Kerr TA, Terrian DM. Inhibition of glutamate release: a potential mechanism of action for the anticonvulsant U-54494A in the guinea pig hippocampus. Brain Res Bull 1993;31:573–80.

Dayer P, Collart L, Desmeules J. The pharmacology of tramadol. Drugs 1994;47:3–7.

De Sarro G, Trimarchi GR, Sinopoli S, Masuda Y, De Sarro A. Anticonvulsant effects of U-54494A and U-50488H in genetically epilepsy-prone rats and DBA/2 mice: a possible involvement of glycine/NMDA receptor complex. Gen Pharmacol 1993;24:439–47.

Feldman RS, Meyer JS, Quenzer LF. Principles of neuropsychopharmacology. Sunderland, MA: Sinauer; 1997.

Fischer W, Bodewei R, VonVoigtlander PF, Müller M. Anticonvulsant and related effects of U-54494A in various seizure tests. J Pharmacol Exp Ther 1993;267:163–70.

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.

Gardner JS, Blough D, Drinkard CR, Shatin D, Anderson G, Graham D, et al. Tramadol and seizures: a surveillance study in a managed care population. Pharmacotherapy 2000;20:1423–31.

Gupta SP. Statistical methods. 25th ed. India: Sultan Chand Publishers; 1990.

Hara K, Minami K, Sata T. The effects of tramadol and its metabolite on glycine, gamma-aminobutyric acid A, and *N*-methyl-D-aspartate receptors expressed in Xenopus oocytes. Anesth Analg 2005;100:1400–5.

Homayoun H, Khavandgar S, Dehpour AR. The involvement of endogenous opioids and nitricoxidergic pathway in the anticonvulsant effects of foot-shock stress in mice. Epilepsy Res 2002;49:131–42.

Hudson CJ, VonVoigtlander PF, Althaus JS, Scherch HM, Means ED. The kappa opioid-related anticonvulsants U-50488H and U-54494A attenuate *N*-methyl-D-aspartate induced brain injury in the neonatal rat. Brain Res 1991;564:261–7.

Khavandgar S, Homayoun H, Dehpour AR. Mediation of nitric oxide in inhibitory effect of morphine against electroshock-induced convulsions in mice. Pharmacol Biochem Behav 2003;74:795–801.

Laorden ML, Carrillo E, Puig MM. Prevention of hyperthermia-induced convulsions in immature rat by MR-2266, a kappa antagonist. Methods Find Exp Clin Pharmacol 1991;13:605–8.

Löscher W, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs: II. Maximal electroshock seizure models. Epilepsy Res 1991;8:79–94.

Manocha A, Sharma KK, Mediratta PK. The anticonvulsant effect of pentazocine against maximal electroshock seizure in mice. Indian J Pharmacol 1997;29:194–7.

Manocha A, Sharma KK, Mediratta PK. Anticonvulsant effect of nalbuphine on maximal electroshock seizure in mice. Indian J Pharmacol 1998a;30:306–10.

Manocha A, Sharma KK, Mediratta PK. Tramadol, a centrally acting opioid: anticonvulsant effect against maximal electroshock seizure in mice. Indian J Physiol Pharmacol 1998b;42:407–11.

Manocha A, Mediratta PK, Sharma KK. Studies on the anticonvulsant effect of U50488H on maximal electroshock seizure in mice. Pharmacol Biochem Behav 2003a;76:111–7.

Manocha A, Sharma KK, Mediratta PK. Possible mechanism involved in the anticonvulsant action of butorphanol in mice. Pharmacol Biochem Behav 2003b;74:343–50.

Marquardt KA, Alsop JA, Albertson TE. Tramadol exposures reported to statewide poison control system. Ann Pharmacother 2005;39:1039–44.
Meldrum BS. Excitatory amino acids in epilepsy and potential novel therapies. Epilepsy Res 1992;12:189–96.
Miranda HF, Pinardi G. Antinociception, tolerance, and physical dependence comparison between morphine and tramadol. Pharmacol Biochem Behav 1998;61:357–60.
Oldendorf WH, Hyman S, Braun L, Oldendorf SZ. Blood–brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. Science 1972;178:984–6.
Pircio AW, Gylys JA, Cavanagh RL, Buyniski JP, Bierwagen ME. The pharmacology of butorphanol, a 3,14-dihydroxymorphinan narcotic antagonist analgesic. Arch Int Pharmacodyn 1976;220:231–57.
Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 1992;260:275–85.
Rang HP, Dale MM, Ritter JM, Moore PK. Antiepileptic drugs. Pharmacology, 5th ed. Edinburgh: Churchill Livingstone; 2003. p. 550–61.
Reisine T, Pasternak G. Opioid analgesics and antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman and Gilman's the pharmacological basis of therapeutics, 9th ed. New York: McGraw-Hill; 1996. p. 521–55.
Rogawski MA. Mechanism-specific pathways for new antiepileptic drug discovery. In: French J, Leppik I, Dichter MA, editors. Antiepileptic drug development. Advances in Neurology, vol. 76. Philadelphia (PA): Lippincott-Raven Publishers; 1998. p. 11–27.
Rogawski MA, Porter RJ. Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev 1990;42:223–86.
Schindler CJ, Slamberova R, Vathy I. Cholera toxin B decreases bicuculline seizures in prenatally morphine- and saline-exposed male rats. Pharmacol Biochem Behav 2004;77:509–15.
Schwarz M, Klockgether T, Wüllner U, Turski L, Sontaq K-H. δ-Amino valeric acid antagonizes the pharmacological actions of baclofen in the central nervous system. Exp Brain Res 1988;70:618–26.
Scott LJ, Perry CM. Tramadol: a review of its use in perioperative pain. Drugs 2000;60:139–76.
Shafaroodi H, Samini M, Moezi L, Homayoun H, Sadeghipour H, Tavakoli S, et al. The interaction of cannabinoids and opioids on pentylenetetrazole-induced seizure threshold in mice. Neuropharmacology 2004;47:390–400.
Sieghart W. $GABA_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. Trends Pharmacol Sci 1992;13:446–50.
Simmons ML, Terman GW, Drake CT, Chavkin C. Inhibition of glutamate release by presynaptic κ_1-opioid receptors in the guinea pig dentate gyrus. J Neurophysiol 1994;72:1697–705.
Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 1952;106:319–30.
Tallarida RJ, Harakal C, Maslow J, Geller EB, Adler MW. The relationship between pharmacokinetics and pharmacodynamic action as applied to in vivo pA_2: application to the analgesic effect of morphine. J Pharmacol Exp Ther 1978;206:38–45.
Tortella FC. Endogenous opioid peptides and epilepsy: quieting the seizing brain? Trends Pharmacol Sci 1988;9:366–72.
Tortella FC, Robles L, Holaday JW. U50,488, a highly selective kappa opioid: anticonvulsant profile in rats. J Pharmacol Exp Ther 1986;237:49–53.
Tortella FC, Echevarria E, Lipkowski AW, Takemori AE, Portoghese PS, Holaday JW. Selective kappa antagonist properties of nor-binaltorphimine in the rat MES seizure model. Life Sci 1989;44:661–5.
Valle M, Garrido MJ, Pavón JM, Calvo R, Trocóniz IF. Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effects of main active metabolites of tramadol, (+)-*O*-desmethyltramadol and (−)-*O*-desmethyltramadol, in rats. J Pharmacol Exp Ther 2000;293:646–53.
VonVoigtlander PF, Hall ED, Camacho Ochoa M, Lewis RA, Triezenberg HJ. U-54494A: a unique anticonvulsant related to kappa opioid agonists. J Pharmacol Exp Ther 1987;243:542–7.
White HS, Wolf HH, Woodhead JH, Kupferberg HJ. The national institutes of health anticonvulsant drug development program: screening for efficacy. In: French J, Leppik I, Dichter MA, editors. Advances in neurology. Antiepileptic Drug Development, vol. 76. Philadelphia: Lippincott-Raven; 1998. p. 29–39.
Wiesenfeld-Hallin Z. Combined opioid–NMDA antagonist therapies. Drugs 1998;55:1–4.
Wong EHF, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL. The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. Proc Natl Acad Sci U S A 1986;83:7104–8.
Yajima Y, Narita M, Takahashi-Nakano Y, Misawa M, Nagase H, Mizoguchi H, et al. Effects of differential modulation of mu-, delta- and kappa-opioid systems on bicuculline-induced convulsions in the mouse. Brain Res 2000;862:120–6.