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# Studies on the anticonvulsant effect of U50488H on maximal electroshock seizure in mice

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**Abstract**

The present study was designed to investigate the effect of U50488H, a prototype non-peptide kappa opioid agonist on convulsive behaviour using a maximal electroshock (MES) seizure test in mice. An attempt was also made to explore the role of possible receptors involved. MES seizures were induced via transauricular electrodes (60 mA, 0.2 s). Seizure severity was evaluated by means of two parameters, i.e., (1) duration of tonic hindlimb extensor phase and (2) mortality due to convulsions. Intraperitoneal (ip) administration of U50488H dose dependently (5–20 mg/kg) decreased the hindlimb extensor phase of MES. The anticonvulsant effect of U50488H was attenuated by the general opioid antagonist, naloxone at a high dose, and by MR2266, a selective kappa antagonist, but not by naltrindole, a delta antagonist. Coadministration of γ-aminobutyric acid (GABA)ergic drugs (diazepam, GABA, muscimol, and baclofen) and the *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK801), with U50488H augmented the anticonvulsant effect of the latter drug in mice. On the other hand, flumazenil, a central benzodiazepine (BZD) receptor antagonist, reversed the protective effect of diazepam and similarly, δ-aminovaleric acid (DAVA), a $GABA_B$ receptor antagonist, blocked the protective effect of baclofen, a $GABA_B$ agonist on the anti-MES action of U50488H. These BZD–GABAergic antagonists, namely, flumazenil or DAVA, on their own also counteracted the anti-electroshock seizure effect of U50488H given alone. However, mortality was not significantly altered in any of the above animal groups. Taken together, the findings have shown a possible role for multitude of important neurotransmitter systems, i.e., opioid (kappa), NMDA channel, $GABA_A$–BZD-chloride channel complex, and $GABA_B$ receptors in the anticonvulsant action of U50488H.


*Keywords:* U50488H; Opioids; Convulsions; Maximal electroshock; γ-Aminobutyric acid; *N*-methyl-D-aspartate

## 1. Introduction

The epilepsies are the most common and frequently devastating family of neurological disorders that affects approximately 2.5 million people in the United States alone (McNamara, 2001; Löscher, 2002). There is a considerable body of evidence implicating a role for the amino acids, γ-aminobutyric acid (GABA), *N*-methyl-D-aspartate (NMDA), and opioids in the etiology and/or amelioration of a variety of neurological disorders including convulsive seizures (Olsen et al., 1984; Tortella, 1988; Simonato and Romualdi, 1996; Rogawski, 1998; Homayoun et al., 2002). $GABA_A$ receptor modulators are clinically effective anticonvulsants, and their ability to increase GABAergic transmission is a critical component of their therapeutic anticonvulsant activity (Rogawski, 1998).

Kappa opioids have received attention in seizure studies. The benzamide kappa analog, U54494A, inhibits tonic convulsions in the maximal electroshock (MES) test in mice (Fischer et al., 1993; Zhu et al., 1993) and has displayed equipotent anticonvulsant activity to the kappa opioid U50488H in an audiogenic convulsion paradigm in genetically epilepsy-prone DBA/2 mice (VonVoigtlander et al., 1987a; De Sarro et al., 1993). Further, U50488H also reduces tonic extension of both forelimbs and hindlimbs in a MES paradigm that reflects anticonvulsant activity in rats (Tortella et al., 1986, 1989). That particular anticonvulsant effect in rat was attenuated by the opioid antagonist, naloxone in high doses, or by norbinaltorphimine. However, intracerebroventricular (icv) administration of kappa agonists, including U50488H, induces characteristic "Popcorn" convulsions in mice. This convulsive effect of U50488H in mice was not

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susceptible to antagonism by naloxone or MR2266 (Bansinath et al., 1991).

It has been demonstrated that opioids may exert some of their behavioural effects in vivo by direct opioid receptor activation, or by indirect modulation of GABA or NMDA receptors (Frenk, 1983; Frey, 1988; De Sarro et al., 1993; Lauretti et al., 1994; Atapour et al., 2000; Broom et al., 2000; Yajima et al., 2000), whereas convulsions evoked by GABA antagonists (i.e., pentylenetetrazole or bicuculline) are not altered by kappa agonists (VonVoigtlander et al., 1987a; Fischer et al., 1993); the selective kappa-opioid receptor agonist U50488H produces a dose-dependent suppression of bicuculline-induced convulsions (Yajima et al., 2000).

In an attempt to broaden the pharmacological analysis of U50488H-induced effects on convulsive behaviour, the current studies examined the pro- or anticonvulsant effects of U50488H, a classical kappa opioid agonist (VonVoigtlander et al., 1983; Clark and Pasternak, 1988) on the MES test in mice. This test (Swinyard et al., 1952) is among the most widely used pharmacological models for assessing anticonvulsant activity by evaluating the ability of drugs to prevent electrically induced tonic hindlimb extension (THE) in animals. The MES test is an excellent animal model for the identification of new antiepileptic drugs (AEDs) that block seizure spread and as such are likely to be effective for the management of generalized tonic–clonic seizures (GTCS) in humans (White et al., 1998). In fact, the MES test using supra-threshold stimulation is probably the best validated of all seizure tests that predict drugs effective in GTCS in humans. Transauricular stimulation preferably activates the brainstem region that leads to elicitation of severe tonic convulsions in rodents (Löscher et al., 1991). Furthermore, to assess the possible role of kappa opioid receptors in the mediation of the convulsive behavioural effects of U50488H, we examined the effects of concurrent treatment with MR2266, a kappa receptor antagonist (VonVoigtlander et al., 1983), as well as the role of a number of other receptor systems including benzodiazepine (BZD)–GABAergic and NMDA involvement in the effect of U50488H on convulsive behaviour in mice.

## 2. Materials and methods

### 2.1. Animals

Albino Swiss mice of either sex (20–25 g) (procured from Central Animal Breeding House, AIIMS, Delhi) were used. The animals were housed in plastic cages at an ambient temperature of 25 ± 2 °C and 45–55% relative humidity and maintained on a 12:12 h light–dark (7:00 a.m. to 7:00 p.m.) cycle. Food and water were provided ad libitum and mice were acclimatized to their environment for at least 1 week before experimentation. The animals were randomly distributed into different groups of a minimum of 10 animals each. Each animal was weighed, caged separately, and had identification marks cryptically encoding the dose level and group. All experimental protocols were approved by the University College of Medical Sciences Institutional Review Committee for Animal Subjects and experiments were conducted according to the *Guidelines for the Care and Use of Laboratory Animals* as promulgated by the National Institutes of Health (NIH). All the experiments were performed between 10.00 a.m. and 6.00 p.m.

### 2.2. Drugs

Trans-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methanesulfonate (U50488H) (Sigma, USA), naloxone hydrochloride (Sigma), (−)-5,9 alpha-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan (MR2266) (Boehringer Ingelheim, FRG), naltrindole HCl (Sigma), diazepam (Ranbaxy, Delhi, India), GABA (BDH, Poole, Dorset, UK), muscimol (Sigma), baclofen (Ciba–Geigy, Switzerland), flumazenil (F. Hoffmann La Roche, Basel, Switzerland), δ-aminovaleric acid (DAVA) (Sigma), and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine) (Merck Sharp and Dohme, West Point, PA) 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. U50488H, naloxone hydrochloride, naltrindole HCl, GABA, muscimol, baclofen, DAVA, and dizocilpine (MK801) were dissolved in deionized water. MR2266 was made into solution with d-$H_2O$ aided by a few drops of 0.1 N HCl. Diazepam injection (CALMPOSE) was diluted to the required volume with d-$H_2O$ before use. Flumazenil was uniformly suspended in d-$H_2O$ with a few drops of Tween 80. U50488H, naloxone, naltrindole, and DAVA were administered intraperitoneally (ip) whereas MR2266, diazepam, GABA, muscimol, baclofen, flumazenil, and MK801 were injected subcutaneously (sc) in the scruff of the animal's neck. All drugs were freshly prepared before use, and injection volume (10 ml/kg) was kept constant. The selection of doses, route of administration, and time scheduling of different compounds were based on pilot experiments and pharmacokinetic considerations. Suitable vehicle controls were used in all experiments. For each series of experiments, separate controls were used to avoid data variation from day to day.

### 2.3. MES-induced convulsions

MES seizures were induced by an electroconvulsometer (Techno Instruments, Lucknow). A 60-mA current was delivered transauricularly for 0.2 s in mice by way of small alligator clips attached to each pinna (Swinyard et al., 1952). This current intensity elicited complete THE in control mice. Mice were placed in a clear rectangular plastic cage with an open top, permitting a full view of the animals' motor responses to seizure. After preliminary analysis of different convulsive parameters such as tonic flexion, ex-

tension, clonus, stupor, and mortality due to convulsions, the duration of THE and mortality due to convulsions were chosen as the parameters to evaluate drug effects on seizure severity. Immediately after MES treatment, recordings were done for convulsions and mortality. Further, 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; animals did not exhibit mortality during this period.

### 2.4. Treatment schedule of U50488H, naloxone, MR2266, and naltrindole in MES test

Data were obtained on dose–response (5–20 mg/kg) relationship of U50488H on MES seizures in different groups of mice receiving a single intraperitoneal injection of U50488H 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 U50488H (20 mg/kg), received naloxone (0, 0.1, and 1 mg/kg ip) 20 min later, and then exposed to the MES seizure 30 min after U50488H. In the MR2266 study, mice received MR2266 (0, 0.05, and 0.1 mg/kg sc) and U50488H (20 mg/kg) concurrently and were subjected to the MES seizure 30 min later. To study delta receptor involvement, mice received U50488H (20 mg/kg), followed 5 min later by naltrindole (0.25 mg/kg ip) and 25 min later by MES seizure. U50488H, naloxone, MR2266, and naltrindole treatment intervals prior to MES were chosen on the basis of preliminary data and/or previous literature (Tortella et al., 1986; Frey, 1988). Opioid antagonist effects on MES seizures were also measured.

### 2.5. Drug-interaction studies

#### 2.5.1. Interaction between BZD–$GABA_A$ergic compounds and U50488H

The $GABA_A$ receptor agonists, GABA (100 and 200 mg/kg sc), and muscimol (0.5 and 1 mg/kg sc) were studied alone and/or in combination with U50488H (5 mg/kg ip) cotreatment 30 min prior to MES. A BZD agonist (diazepam: 2.5 mg/kg sc, 30 min) and antagonist (flumazenil: 0.5 mg/kg sc, 5 min) (Brogden and Goa, 1991) were injected alone and/or in combination with U50488H prior to MES.

#### 2.5.2. Interaction between $GABA_B$ receptor compounds and U50488H

A $GABA_B$ receptor agonist (baclofen: 2.5 and 5 mg/kg sc) and antagonist (DAVA: 50 mg/kg ip) were studied alone and/or in combination with U50488H (5 mg/kg ip) and were administered 25–30 min prior to MES.

#### 2.5.3. Interaction between glutamatergic drugs and U50488H

The effect of NMDA receptor antagonist MK801 (0.05 and 0.1 mg/kg sc) was studied alone and/or in combination with U50488H (5 mg/kg ip) 30 min prior to exposure to MES.

### 2.6. Statistical analysis

The duration of THE phase of MES convulsions, expressed as the arithmetic mean ( ± S.E.M.) was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's comparisons. A grouped chi-squared test was initially conducted to determine overall differences in mortality due to convulsions. If a significant ( $P < .05$) effect was found, individual differences were determined by single chi-squared test (Gupta, 1990).

## 3. Results

### 3.1. Effects of U50488H

The different doses of U50488H (5–20 mg/kg) produced hyperreactivity to sound and touch as well as drowsiness in mice when compared to vehicle-treated control animals. However, a 20 mg/kg dose of U50488H also produced lack of movement and circling in mice. MES induced tonic convulsions in all animals with U50488H dose dependently

Table 1
Effect of naloxone (NLX), MR2266, and naltrindole (NTI) alone and on the anticonvulsant activity of U50488H in MES-induced convulsions in mice

| Group | Treatment (mg/kg, ip or sc) | Duration of hindlimb extensor phase (s) (mean ± S.E.M.)[a] | Mortality[b] |
|---|---|---|---|
| 1 | vehicle | 14.90 ± 0.28 | 4/10 |
| 2 | NLX (0.1) | 15.40 ± 3.44 | 3/10 |
| 3 | NLX (1) | 16.80 ± 1.18 | 4/10 |
| 4 | MR2266 (0.05)[c] | 14.60 ± 2.29 | 1/10 |
| 5 | MR2266 (0.1)[c] | 15.00 ± 1.69 | 1/10 |
| 6 | NTI (0.25) | 14.40 ± 2.75 | 1/10 |
| 7 | U50488H (20) | 3.60 ± 1.13*** | 0/10 |
| 8 | U50488H (20) + NLX (0.1) | 3.40 ± 1.16 | 0/10 |
| 9 | U50488H (20) + NLX (1) | 8.90 ± 0.31 * | 1/10 |
| 10 | U50488H (20) + MR2266 (0.05)[c] | 10.20 ± 0.13 ** | 0/10 |
| 11 | U50488H (20) + MR2266 (0.1)[c] | 12.30 ± 0.21*** | 1/10 |
| 12 | U50488H (20) + NTI (0.25) | 3.40 ± 1.18 $F(11,108) = 9.41$ ($P < .01$) | 0/10 $\chi^2_{11df} = 11.86$ |

Groups 2, 3, 4, 5, 6, 7 vs. 1; Groups 8, 9, 10, 11, 12 vs. 7; $n = 10$.

[a] One-way ANOVA followed by Dunnett's $t$ test.

[b] Grouped chi-squared test with Yates correction.

[c] Drugs administered by subcutaneous route.

* $P < .05$ as compared with control (vehicle) or effect of U50488H alone.

** $P < .01$ as compared with control (vehicle) or effect of U50488H alone.

*** $P < .001$ as compared with control (vehicle) or effect of U50488H alone.

(5–20 mg/kg) protecting the animals [$F(3,36)=30.88$; $P<.01$]. THE scores showed dose-dependent decreases following U50488H: 0 (16.5, ±1.2), 5 (9.3, ±0.8), 10 (6.6, ±0.8), and 20 (4.2, ±1.0 s) mg/kg. Mortality decreased from 30% to 0% across U50488H doses.

### 3.2. Interaction of U50488H with opioid receptor antagonists

Naloxone dose dependently and significantly reversed the protective effect of U50488H against MES-induced convulsions, whereas MR2266 was effective at both doses. In contrast, naltrindole failed to significantly reverse the U50488H effect. Mortality was not significantly altered in any of the above groups, and none of the opioid antagonists alone altered MES convulsions (Table 1).

### 3.3. Interaction between BZD–$GABA_A$ergic compounds and U50488H

As illustrated in Table 2, GABA (100 and 200 mg/kg), muscimol (0.5 and 1 mg/kg), and diazepam (2.5 mg/kg) alone offered protection against MES in a dose-dependent fashion by significantly reducing ($P<.001$) the duration of THE and nonsignificantly decreasing the mortality rate.

Table 2
Effect of BZD–$GABA_A$ receptor-acting drugs alone and on the anticonvulsant activity of U50488H in MES-induced convulsions in mice

| Group | Treatment (mg/kg, ip or sc) | Duration of hindlimb extensor phase (s) (mean ± S.E.M.)[a] | Mortality[b] |
|---|---|---|---|
| 1 | vehicle | 16.40 ± 0.16 | 3/10 |
| 2 | DZP (2.5)[c] | 4.40 ± 0.30 * | 0/10 |
| 3 | FLM (0.5)[c] | 15.60 ± 0.16 | 3/10 |
| 4 | DZP (2.5)[c] + FLM (0.5)[c] | 12.80 ± 0.20 * | 1/10 |
| 5 | GABA (100)[c] | 12.60 ± 0.30 * | 0/10 |
| 6 | GABA (200)[c] | 8.30 ± 0.15 * | 0/10 |
| 7 | MUS (0.5)[c] | 11.00 ± 0.33 * | 0/10 |
| 8 | MUS (1)[c] | 5.40 ± 0.16 * | 0/10 |
| 9 | U50488H (5) | 9.40 ± 0.30 * | 2/10 |
| 10 | U50488H (5) + DZP (2.5)[c] | 1.20 ± 0.33 * | 0/10 |
| 11 | U50488H (5) + FLM (0.5)[c] | 15.10 ± 0.28 * | 3/10 |
| 12 | U50488H (5) + DZP (2.5)[c] + FLM (0.5)[c] | 10.00 ± 0.82 * | 1/10 |
| 13 | U50488H (5) + GABA (100)[c] | 7.60 ± 0.67 * | 0/10 |
| 14 | U50488H (5) + GABA (200)[c] | 5.10 ± 0.46 * | 0/10 |
| 15 | U50488H (5) + MUS (0.5)[c] | 6.20 ± 0.13 * | 0/10 |
| 16 | U50488H (5) + MUS (1)[c] | 3.90 ± 0.53 * | 0/10 |
| | | $F(15,144)=143.60$ ($P<.01$) | $\chi^2_{15df}=13.69$ |

Groups 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16 vs. 1; Group 4 vs. 2; Groups 10, 11, 13, 14, 15, 16 vs. 9; Group 12 vs. 10; $n=10$. DZP, diazepam; FLM, flumazenil; GABA, γ-aminobutyric acid; MUS, muscimol.

[a] One-way ANOVA followed by Dunnett's $t$ test.

[b] Grouped chi-squared test with Yates correction.

[c] Drugs administered by subcutaneous route.

* $P<.001$ as compared with control (vehicle) or effect of U50488H or diazepam alone or its combination.

Table 3
Effect of $GABA_B$ergic drugs and NMDA receptor antagonist, MK801 alone and on the anticonvulsant activity of U50488H in MES-induced convulsions in mice

| Group | Treatment (mg/kg, ip or sc) | Duration of hindlimb extensor phase (s) (mean ± S.E.M.)[a] | Mortality[b] |
|---|---|---|---|
| 1 | vehicle | 15.70 ± 0.40 | 4/10 |
| 2 | BAC (2.5)[c] | 10.20 ± 0.33 * | 2/10 |
| 3 | BAC (5)[c] | 5.20 ± 0.33 * | 0/10 |
| 4 | DAVA (50) | 15.50 ± 0.43 | 3/10 |
| 5 | BAC (5)[c] + DAVA (50) | 14.10 ± 0.10 * | 1/10 |
| 6 | MK801 (0.05)[c] | 8.10 ± 0.10 * | 0/10 |
| 7 | MK801 (0.1)[c] | 1.50 ± 0.50 * | 0/10 |
| 8 | U50488H (5) | 9.10 ± 0.23 * | 2/10 |
| 9 | U50488H (5) + BAC (2.5)[c] | 5.70 ± 0.21 * | 1/10 |
| 10 | U50488H (5) + BAC (5)[c] | 3.30 ± 0.37 * | 0/10 |
| 11 | U50488H (5) + DAVA (50) | 16.30 ± 0.21 * | 4/10 |
| 12 | U50488H (5) + BAC (5)[c] + DAVA (50) | 11.80 ± 0.93 * | 2/10 |
| 13 | U50488H (5) + MK801 (0.05)[c] | 4.50 ± 0.17 * | 0/10 |
| 14 | U50488H (5) + MK801 (0.1)[c] | 1.20 ± 0.33 * $F(13,126)=190.08$ ($P<.01$) | 0/10 $\chi^2_{13df}=12.76$ |

Groups 2, 3, 4, 6, 7, 8, 9, 10, 13, 14 vs. 1; Group 5 vs. 3; Groups 9, 10, 11, 13, 14 vs. 8; Group 12 vs. 10; $n=10$. BAC, baclofen; DAVA, δ-aminovaleric acid.

[a] One-way ANOVA followed by Dunnett's $t$ test.

[b] Grouped chi-squared test with Yates correction.

[c] Drugs administered by subcutaneous route.

* $P<.001$ as compared with control (vehicle) or effect of U50488H or baclofen alone or its combination.

Flumazenil treatment significantly reversed ($P<.001$) diazepam's protective effects.

Among diazepam, GABA, or muscimol cotreatment with U50488H, these GABAergic drugs reliably augmented U50488H-induced protection against MES seizures. Flumazenil cotreatment with U50488H significantly ($P<.001$) attenuated the anti-MES effect of U50488H as well as the diazepam-induced facilitation of U50488H effects (Table 2). Mortality failed to be significantly altered in any of the above groups (Table 2).

### 3.4. Interaction between $GABA_B$ receptor compounds and U50488H

Table 3 summarizes baclofen's significant decreases in the duration of THE phase and nonsignificant reductions in mortality. DAVA failed to exert effects on its own but significantly ($P<.001$) reversed baclofen's protective effects.

Baclofen cotreatment with U50488H increased the protective effect of U50488H while DAVA (50 mg/kg) attenuated the anticonvulsant effect of U50488H ($P<.001$). In

baclofen and U50488H cotreated mice, DAVA reliably reversed ($P < .001$) the protective action of baclofen on the anticonvulsant effect of U50488H, but not mortality effects (Table 3).

### 3.5. Interaction between glutamatergic drugs and U50488H

MK801 alone produced a significant protection ($P < .001$) against MES-induced convulsions and augmented the protective effect of U50488H on MES seizures (Table 3).

## 4. Discussion

The current study provides evidence for a role of the kappa opioid receptors in mediating the anticonvulsant-like behavioural effects of U50488H. U50488H, a unique and selective kappa opioid (VonVoigtlander et al., 1983; Clark and Pasternak, 1988), was demonstrated to have anticonvulsant effect in the MES paradigm in mice. Although both U50488H and the established anticonvulsant drug diazepam (Rogawski and Porter, 1990) produced qualitatively similar responses in the MES, the former effects could be attributed either to its direct action at the kappa opioid receptor or to an indirect consequence of GABA receptor modulation. The latter seems likely because flumazenil, a central BZD receptor antagonist (Grecksch et al., 1983; Brogden and Goa, 1991), prevented the anticonvulsant behavioural response of U50488H. In line with these findings, U50488H, a synthetic benzeneacetamide, has been shown previously to be active in several behavioural models of convulsions in different species of animals (Tortella et al., 1986; VonVoigtlander et al., 1987a,b; De Sarro et al., 1993). Furthermore, U50488H in the periphery has an anticholinergic effect (Hayes et al., 1988) that could further explain the anticonvulsant efficacy of U50488H. The greater efficacy of MR2266 relative to naloxone and naltrindole in blocking the anticonvulsant effects of U50488H suggests that these effects were also mediated by the activation of kappa opioid receptors. This agrees with previous observations that nor-binaltorphimine and/or naloxone in high doses blocks kappa receptor-mediated behaviours induced by pentazocine, nalbuphine, U50488H, and U-54494A in rodents (Tortella et al., 1986, 1989; Fischer et al., 1993; Manocha et al., 1997, 1998), and blocks opioid receptor-mediated behaviours induced by U50488H and U-54494A in both genetically epilepsy-prone rats and in DBA/2 mice (De Sarro et al., 1993).

Diazepam is an effective BZD receptor ligand whereas both muscimol and GABA are selective agonists at $GABA_A$ receptors (Sieghart, 1992). Diazepam, muscimol, and GABA all produced anticonvulsant-like effects in the MES paradigm. The behavioural pattern, as expressed by a significant anticonvulsant behaviour, suggests that their response appeared to be mediated predominantly via GABAergic transmission (i.e., $GABA_A$ receptor activation) as opposed to effects on the opioid receptor. Additionally, flumazenil alone did not produce any effects in the MES, but it counteracted the anticonvulsive behaviour of diazepam; one may speculate that BZD–$GABA_A$ receptors are involved in these effects of diazepam.

More recently, U50488H has been found to be effective against bicuculline-induced convulsions (Yajima et al., 2000). A behaviourally active dose of GABA, muscimol, or diazepam also augmented the anticonvulsant behaviour produced by U50488H. Based on these behavioural observations, the augmentation of protective effect of U50488H 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 U50488H's anti-MES effect. Furthermore, the reversal of facilitatory effect of diazepam on U50488H protection of MES convulsions by flumazenil indicates that the BZD site on BZD–$GABA_A$ receptor complex could probably be involved in action of U50488H in the brain. This suggestion is substantiated by the current findings, where flumazenil on its own also attenuated the anti-electroshock seizure effect of U50488H when administered alone.

Baclofen is a potent $GABA_B$ receptor ligand (Bowery, 1993) while DAVA preferably antagonizes $GABA_B$-mediated responses (Schwarz et al., 1988). Baclofen was shown to produce anticonvulsant-like effects in the MES test. The behavioural pattern, as expressed by a significant anticonvulsant behaviour, indicates that this response of baclofen was more likely associated with profound effects on GABAergic transmission (i.e., $GABA_B$ receptor activation) as opposed to effects on the opioid receptor. DAVA alone produced no effects on the MES, but it antagonized the anticonvulsive behaviour of baclofen, further supporting that the effects of baclofen are $GABA_B$ergic in origin. Interestingly, the augmentation of the anti-seizurogenic action of U50488H by baclofen and the attenuation of the anticonvulsant effect of U50488H alone as well as when given in combination with baclofen by DAVA show that apart from $GABA_A$, $GABA_B$ receptors also play a role in the anti-MES effect of U50488H.

The role of the NMDA subtype of excitatory amino acid (EAA) receptors in convulsions is well documented (Meldrum, 1992). MK801 (dizocilpine) is an active noncompetitive antagonist at the NMDA subtype of EAA receptors that acts at a site within the ion channel of the NMDA receptor complex (Wong et al., 1986; McNamara et al., 1988). Interestingly, in this study, MK801 was shown to produce anticonvulsant-like effects in the MES test. Furthermore, MK801 enhanced the anticonvulsant effect of U50488H on MES seizures that suggests that the anticonvulsant effect of U50488H is at least in part also mediated at NMDA receptors. Consistent with the present data, studies of the guinea pig dentate gyrus (Wagner et al., 1992; Simmons et al., 1994), mossy fiber terminals in the hippocampus (Gannon and Terrian, 1991; Conner-Kerr and Terrian, 1993), rat substantia gelatinosa neurons of the spinal cord (Cheng and Kojic, 1995) suggest that kappa opioid receptor activation

modulates glutamate-mediated excitatory synaptic transmission in the CNS. Furthermore, Hudson et al. (1991) have shown that kappa opioid receptor agonists (i.e., U50488H or U-54494A) reduce NMDA-induced brain injury in the neonatal rat. The combination of NMDA receptor antagonists, particularly with opioids, may lead to enhancement of opioid's clinical effect (Wiesenfeld-Hallin, 1998).

In conclusion, the kappa opioid receptor subtype, alone or interdependent with the GABA and NMDA receptor, plays a pivotal role in the mediation of anticonvulsant effects of U50488H. The MES paradigm used was quite sensitive for opioid compounds. These data with U50488H provide an important information that the kappa opioid receptor may indeed be a target for the development of anticonvulsants for grand mal seizures, and opioid compounds might prove useful as adjuncts of conventional AEDs. Shortcomings of the experiments are with the systemic route of drug administration where it is difficult to separate the penetration of blood–brain barrier from other variables (Oldendorf et al., 1972). The present findings can be replicated comprehensively to delineate the specific roles for opioid receptors, and other complementary receptors in a variety of convulsive models may be better addressed by receptor binding studies using some more selective 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.

Bansinath M, Ramabadran K, Turndorf H, Shukla VK. Intracerebroventricular administration of κ-agonists induces convulsions in mice. Brain Res Bull 1991;27:75–9.

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.

Broom DC, Gno L, Coop A, Husbands SM, Lewis JW, Woods JH, et al. BU48: a novel buprenorphine analog that exhibits delta-opioid mediated convulsions but not delta-opioid-mediated antinociception in mice. J Pharmacol Exp Ther 2000;294:1195–200.

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.

Clark JA, Pasternak GW. U50,488: a kappa-selective agent with poor affinity for $mu_1$ opiate binding sites. Neuropharmacology 1988;27:331–2.

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.

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.

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. Brains Res Rev 1983;6:197–210.

Frey H-H. Effect of μ- and κ-opioid agonists on the electroconvulsive seizure threshold in mice and antagonism by naloxone and MR2266. Pharmacol Toxicol 1988;62:150–4.

Gannon RL, Terrian DM. U-50,488H inhibits dynorphin and glutamate release from guinea pig hippocampal mossy fiber terminals. Brain Res 1991;548:242–7.

Grecksch G, Prado de Carvalho L, Venault P, Chapouthier G, Rossier J. Convulsions induced by submaximal dose of pentylenetetrazol in mice are antagonized by the benzodiazepine antagonist Ro15-1788. Life Sci 1983;32:2579–84.

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

Hayes AG, Birch PP, Cavicchini E. Evidence that the kappa agonist U50488H has non-opioid actions. J Pharm Pharmacol 1988;40:718–20.

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.

Lauretti GR, Ahmad I, Pleuvry BJ. The activity of opioid analgesics in seizure models utilizing *N*-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole. Neuropharmacology 1994;33:155–60.

Löscher W. Current status and future directions in the pharmacotherapy of epilepsy. Trends Pharmacol Sci 2002;23:113–8.

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 1998;30:306–10.

McNamara JO. Drugs effective in the therapy of the epilepsies. In: Hardman JG, Limbird LE, Gilman AG, editors. Goodman & Gilman's the pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill; 2001. p. 521–47.

McNamara JO, Russell RD, Rigsbee L, Bonhaus DW. Anticonvulsant and antiepileptogenic actions of MK-801 in the kindling and electroshock models. Neuropharmacology 1988;27:563–8.

Meldrum BS. Excitatory amino acids in epilepsy and potential novel therapies. Epilepsy Res 1992;12:189–96.

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.

Olsen RW, Snowman AM, Lee R, Lomax P, Wamsley JK. Role of the γ-aminobutyric acid receptor-ionophore complex in seizure disorders. Ann Neurol 1984;16:S90–7.

Rogawski MA. Mechanism-specific pathways for new antiepileptic drug discovery. In: French J, Leppik I, Dichter MA, editors. Advances in neurology. Antiepileptic drug development, vol. 76. Philadelphia: Lippincott-Raven; 1998. p. 11–27.

Rogawski MA, Porter RJ. Antiepileptic drugs: pharmacological mecha-

nisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev 1990;42:223–86.

Schwarz M, Klockgether T, Wüllner U, Turski L, Sontaq K-H. δ-Aminovaleric acid antagonizes the pharmacological actions of baclofen in the central nervous system. Exp Brain Res 1988;70:618–26.

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 $\kappa_1$-opioid receptors in the guinea pig dentate gyrus. J Neurophysiol 1994;72:1697–705.

Simonato M, Romualdi P. Dynorphin and epilepsy. Prog Neurobiol 1996; 50:557–83.

Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 1952;106: 319–30.

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.

VonVoigtlander PF, Lahti RA, Ludens JH. U-50,488: a selective and structurally novel non-mu (kappa) opioid agonist. J Pharmacol Exp Ther 1983;224:7–12.

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 1987a;243:542–7.

VonVoigtlander PF, Ochoa MC, Lewis RA. Biochemical and functional interactions of a selective kappa opioid agonist with calcium. Adv Exp Med Biol 1987b;221:345–55.

Wagner JJ, Caudle RM, Chavkin C. κ-Opioids decrease excitatory transmission in the dentate gyrus of the guinea pig hippocampus. J Neurosci 1992;12:132–41.

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.

Zhu Y, Im W-B, Lewis RA, Althaus JS, Cazers AR, Nielsen JW, et al. Two metabolites of anticonvulsant U-54494A: their anticonvulsant activity and interaction with sodium channel. Brain Res 1993;606:50–5.