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Possible mechanism involved in the anticonvulsant action of butorphanol in mice

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Abstract

The study was designed to examine the effect of butorphanol, a classical opioid on convulsive behaviour using maximal electroshock (MES) test. An attempt was also made to investigate the role of possible receptor mechanisms involved. MES seizures were induced in mice via transauricular electrodes (60 mA, 0.2 s). Seizure severity was assessed by the duration of tonic hindlimb extensor phase and mortality due to convulsions. Intraperitoneal administration of butorphanol produced a dose-dependent (0.25–2 mg/kg) protection against hindlimb extensor phase. The anticonvulsant effect of butorphanol was antagonized by all the three opioid receptor antagonists (i.e., naloxone [mu], MR2266 [kappa], and naltrindole [delta], respectively). Coadministration of γ-aminobutyric acid (GABA)-ergic drugs (diazepam, GABA, muscimol, and baclofen) and *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK801), with butorphanol augmented the anticonvulsant action of the latter drug. In contrast, flumazenil, a central benzodiazepine (BZD) receptor antagonist, reversed the facilitatory effect of diazepam on the anti-MES effect of butorphanol. Similarly, δ-aminovaleric acid (DAVA), a $GABA_B$ receptor antagonist, antagonized the facilitatory effect of baclofen, a $GABA_B$ agonist on anti-MES action of butorphanol. These BZD–GABAergic antagonists, flumazenil or DAVA, per se also counteracted the anti-MES effect of butorphanol given alone. These data exemplify the benefits of using the MES test, which is sensitive to opioidergic compounds and distinguished convulsive behavioural changes associated with GABAergic and NMDAergic effects. Taken together, the results implicate a role for multitude of neurotransmitter systems, i.e., opioid (mu, kappa, delta), NMDA channel, BZD–$GABA_A$ chloride channel complex, and $GABA_B$ receptors in the anti-MES action of butorphanol.

Keywords: Butorphanol; Opioids; Convulsions; Maximal electroshock; γ-Aminobutyric acid; *N*-Methyl-D-aspartate

1. Introduction

The amino acids, γ-aminobutyric acid (GABA) and *N*-methyl-D-aspartate (NMDA), and opioids play an important role in the pathogenesis and/or treatment of various neurological disorders including convulsions (Tortella, 1988; Rogawski, 1998). $GABA_A$ receptor modulators are the most frequently prescribed class of drugs for the treatment of clinical convulsions (Rogawski, 1998). GABAergic compounds are believed to exert their anticonvulsant activity indirectly by stimulating the synthesis, modifying the deposition, or blocking the uptake of GABA by neurons and glia thereby resulting in an elevation of GABA concentrations in the brain that can act on critical postsynaptic $GABA_A$ receptors or directly by potentiating and activating postsynaptically located $GABA_A$ receptors (Feldman et al., 1997; Rogawski, 1998).

Opioids exert their biological actions by binding to three major subtypes of opioid receptors (mu, kappa, and delta). The opioid receptors are members of the G-protein-coupled receptor family with seven transmembrane domains (Uhl et al., 1994; Reisine and Pasternak, 1996). Opioid receptors are widely distributed in both the peripheral and central nervous systems, but significant densities are found in the neocortex, amygdala, hippocampus, nucleus accumbens, caudate–putamen, and spinal cord (Mansour et al., 1988; Dhawan et al., 1996). The opioid

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receptor is believed to have a putative role in the control of many physiological and behavioural responses, including nociception and post-ictal analgesia, cardiorespiratory function, feeding, cognitive behaviour, locomotion, thermoregulation, neuroendocrine functioning, and the occurrence of seizures (Dhawan et al., 1996; Reisine and Pasternak, 1996; Feldman et al., 1997; Coimbra et al., 2001a,b). The early indications that this receptor may be relevant to convulsive behaviour occurred when it was realized that morphine-like drugs excite hippocampal pyramidal neurons probably by inhibition of GABA release from inhibitory interneurons (Zieglgansberger et al., 1979; Reisine and Pasternak, 1996). It has been demonstrated that opioids may exert some of their behavioural effects in vivo by direct opioid receptor activation or indirectly by GABA or NMDA receptor modulation (Frenk, 1983; Frey, 1988; Lauretti et al., 1994; Atapour et al., 2000; Broom et al., 2000; Yajima et al., 2000). Indeed, a number of common behavioural and physiological effects are produced by both $GABA_A$ receptor agonists and opioids, including modulation of convulsive behaviour, learning, and memory, nociception, body temperature, cardiovascular function, endocrine secretions, and feeding behaviour (Mansour et al., 1988; Matsumoto, 1989; Paredes and Ågmo, 1992; Dhawan et al., 1996; Reisine and Pasternak, 1996).

There have been relatively few studies investigating the role of opioid receptors in animal models of behavioural convulsions (Frenk, 1983; Tortella et al., 1986; Fischer et al., 1993). The maximal electroshock test (MES) as originally described by Swinyard et al. (1952) is the most widely used pharmacological model for assessing anticonvulsant activity. The induction of an MES seizure with a supramaximal current delivered via pineal or corneal electrodes results in a behavioural seizure in mice and rats that is characterized by tonic extension of both forelimbs and hindlimbs. The tonic extension phase is followed by brief episodes of clonic activity of the forelimbs and hindlimbs that lead to a prolonged post-ictal period lasting several minutes. The MES evaluates 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 in humans (White et al., 1998).

A broad spectrum of anticonvulsant drugs selectively abolishes the THE in the MES. The traditional MES test is sensitive to the effects of opioidergic compounds (Tortella et al., 1986); however, it is unclear which particular receptor mechanisms are responsible for the mediation of their effect on convulsive behaviour. Therefore, the following series of studies were aimed at investigating the pro- or anticonvulsant effects of butorphanol, a classical opioid (Pircio et al., 1976; Gillis et al., 1995) in MES test. An attempt has also been made to determine a role of the possible receptor mechanisms involved in butorphanol's effect on convulsive behaviour in mice.

2. Materials and methods

2.1. Animals

Albino Swiss mice of either sex (20–25 g) (obtained from Central Animal Breeding House, AIIMS, Delhi) were used. The animals were kept 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 given ad libitum and mice were acclimatized to their environment for at least 1 week before experimentation. The animals were distributed into different groups on random basis. Each experimental group comprised of a minimum of 10 animals. 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 experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH). All the experiments were conducted between 10:00 a.m. and 6:00 p.m.

2.2. Drugs

Butorphanol tartrate (Sigma, USA), naloxone hydrochloride (Sigma), (−)-5,9 alpha-diethyl-2-(3-furyl methyl)-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. Butorphanol tartrate, naloxone hydrochloride, naltrindole HCl, GABA, muscimol, baclofen, DAVA, and dizocilpine (MK801) were dissolved in distilled water. MR2266 was made into solution with d-H_2O and 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. Butorphanol, naloxone, naltrindole, and DAVA were administered intraperitoneally whereas MR2266, diazepam, GABA, muscimol, baclofen, flumazenil, and MK801 were injected subcutaneously in the scruff of the animals. All drugs were freshly made before use

and injection volume (10 ml/kg) was kept constant. The dosage selection, route of administration, and time scheduling of different compounds was based on pilot experimentation and pharmacokinetic considerations. Suitable 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 induced by an electroconvulsometer (Techno Instruments, Lucknow). A current of 60 mA 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 evoked complete THE 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 animals' motor responses to seizure. 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. After MES, each animal was individually observed for 2 h for studying the convulsive behaviour and for 24 h for measuring mortality. A compound is known to possess anticonvulsant property if it attenuates or abolishes the extensor phase of MES.

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

Data were obtained on dose–response relationship of butorphanol on MES seizures. To this end, different groups of mice received a single intraperitoneal injection of the compound (0.25–2 mg/kg) and, after 30 min, animals were subjected to MES seizure and the duration of THE phase and mortality due to convulsions were recorded. The effect of butorphanol on the general behaviour of mice was also recorded. For the naloxone studies, after pretreating the mice with 2 mg/kg of butorphanol in separate groups, at 20 min, a dose of naloxone (0.1 and 1 mg/kg) was injected intraperitoneally; the animals were then exposed to MES seizure 10 min later. Such a short pretreatment schedule of 10 min was used for naloxone in this study because it is a short-acting mu antagonist in rodents and humans (Tallarida et al., 1978; Reisine and Pasternak, 1996). For the MR2266 studies, mice were concurrently administered MR2266 (0.05 and 0.1 mg/kg sc), a kappa opioid antagonist, and butorphanol (2 mg/kg) in separate groups and 30 min later, the animals were subjected to MES seizure. To study the contribution of delta opioid receptors, mice were pretreated with butorphanol (2 mg/kg) and at 5 min, an intraperitoneal dose of naltrindole (0.25 mg/kg), a delta opioid antagonist, was administered. The animals were then subjected to MES seizure 25 min later. Butorphanol, MR2266, and naltrindole were injected 25–30 min prior to MES as preliminary data indicated that this time period was adequate to allow for maximum or near-maximum drug effect. The per se effects of naloxone, MR2266, and naltrindole were also recorded. Butorphanol interacts with the kappa as well as the mu and delta opioid receptors (Horan and Ho, 1989).

2.5. Drug interaction studies

2.5.1. Interaction between $GABA_A$ and benzodiazepine (BZD) receptor ligands and butorphanol

$GABA_A$ 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 butorphanol (0.25 mg/kg ip). These agents were administered 30 min prior to MES. In the case of combination study, they were administered concurrently. BZD agonists, 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 butorphanol.

2.5.2. Interaction between $GABA_B$ receptor ligands and butorphanol

$GABA_B$ 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 butorphanol (0.25 mg/kg ip). These agents were administered 25–30 min prior to MES.

2.5.3. Interaction between glutamatergic ligands and butorphanol

The effect of NMDA receptor antagonist MK801 (0.05 and 0.1 mg/kg sc) was studied alone and/or in combination with butorphanol (0.25 mg/kg ip). Drugs were administered 30 min prior to exposure to MES. In the combination study, they were injected concurrently.

2.6. Statistical analysis

The duration of THE phase of MES convulsions expressed as the arithmetic mean ± S.E. was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t test. A grouped chi-square test was initially used to determine the overall differences in the mortality due to convulsions. If a significant effect was observed, individual differences were determined by single chi-square test (Gupta, 1990). $P < .05$ was considered as a statistically significant difference.

3. Results

3.1. Effects of butorphanol

The different doses of butorphanol (0.25–1 mg/kg) produced Straub's tail, periods of immobility, and hyperreactivity to sound and touch in mice when compared to

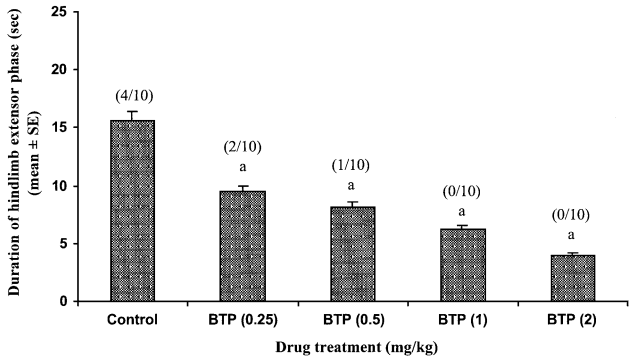

Fig. 1. Effect of butorphanol (BTP) on MES-induced convulsions in mice. BTP-treated groups: 0.25, 0.5, 1, and 2 mg/kg ip. [a]$P<.001$ as compared to control (vehicle) group, $n=10$ (one-way ANOVA followed by Dunnett's t test) [$F(4,45)=89.77$, $P<.01$]. Values in parentheses indicate mortality (grouped chi-square test with Yates correction) [$\chi^2(4)=5.02$].

vehicle-treated control animals. However, butorphanol in a dose of 2 mg/kg, besides the above behavioural symptoms, also produced drowsiness in mice.

MES induced tonic convulsions in all the animals. The endpoint, i.e., THE in vehicle-treated control was 15.60 ± 0.62 s. Pretreatment with butorphanol (0.25–2 mg/kg) offered a dose-dependent protection, the maximum decrease in THE observed being 4.00 ± 0.39 s [ANOVA, $F(4,45)=89.77$, $P<.01$] at 2 mg/kg dose (Fig. 1).

Table 1
Effect of naloxone (NLX), MR2266, and naltrindole (NTI) per se and on the anticonvulsant activity of butorphanol (BTP) in MES-induced convulsions in mice

Group	Treatment (mg/kg ip or sc)	Duration of hindlimb extensor phase (s) (mean ± S.E.)[a]	Mortality[b]
1	vehicle	15.80 ± 0.57	3/10
2	NLX (0.1)	14.80 ± 3.46	2/10
3	NLX (1)	15.60 ± 1.22	3/10
4	MR2266 (0.05)[c]	15.20 ± 2.19	1/10
5	MR2266 (0.1)[c]	14.80 ± 1.58	1/10
6	NTI (0.25)	15.70 ± 2.75	1/10
7	BTP (2)	3.40 ± 0.58***	0/10
8	BTP (2) + NLX (0.1)	4.80 ± 0.61	0/10
9	BTP (2) + NLX (1)	8.90 ± 0.23*	1/10
10	BTP (2) + MR2266 (0.05)[c]	9.00 ± 0.26*	0/10
11	BTP (2) + MR2266 (0.1)[c]	11.50 ± 0.62**	1/10
12	BTP (2) + NTI (0.25)	11.40 ± 0.16** $F(11,108)=7.70$, $P<.01$, $\chi^2(11)=5.46$	1/10

Groups 2–7 vs. Group 1; Groups 8–12 vs. Group 7, $n=10$.
[a] One-way ANOVA followed by Dunnett's t test.
[b] Grouped chi-square test with Yates correction.
[c] Drugs administered by subcutaneous route.
* $P<.05$ as compared with control (vehicle) or per se effect of butorphanol.
** $P<.01$ as compared with control (vehicle) or per se effect of butorphanol.
*** $P<.001$ as compared with control (vehicle) or per se effect of butorphanol.

3.2. Interaction of butorphanol with opioid receptor antagonists

Three opioid receptor ligands, naloxone (0.1 and 1 mg/kg), MR2266 (0.05 and 0.1 mg/kg), and naltrindole (0.25 mg/kg), all partially but significantly reversed the protective effect of butorphanol against MES-induced convulsions. However, the mortality was not significantly altered in all the above groups. In addition, post hoc analysis confirmed that none of the three prototypical opioid receptor antagonists had any effect per se against MES convulsions (Table 1).

3.3. Interaction between $GABA_A$ receptor ligands and butorphanol

GABA (100 and 200 mg/kg), muscimol (0.5 and 1 mg/kg), and diazepam (2.5 mg/kg) per se offered protection against MES in a dose-dependent fashion. There was a significant reduction ($P<.001$) in the duration of THE. Although, the mortality rate was reduced (0/10 vs. 4/10 in control). The difference was not statistically significant. Flumazenil treatment significantly reversed ($P<.001$) the protective effects of diazepam (Table 2).

Table 2
Effect of BZD–$GABA_A$ receptor acting drugs per se and on the anticonvulsant activity of butorphanol (BTP) in MES-induced convulsions in mice

Group	Treatment (mg/kg ip or sc)	Duration of hindlimb extensor phase (s) (mean ± S.E.)[a]	Mortality[b]
1	vehicle	16.00 ± 0.21	4/10
2	DZP (2.5)[c]	3.20 ± 0.32***	0/10
3	FLM (0.5)[c]	15.20 ± 0.13	3/10
4	DZP (2.5)[c] + FLM (0.5)[c]	12.60 ± 0.27***	1/10
5	GABA (100)[c]	13.00 ± 0.30***	0/10
6	GABA (200)[c]	8.20 ± 0.13***	0/10
7	MUS (0.5)[c]	10.80 ± 0.33***	0/10
8	MUS (1)[c]	5.40 ± 0.16***	0/10
9	BTP (0.25)	10.10 ± 0.16***	2/10
10	BTP (0.25) + DZP (2.5)[c]	1.40 ± 0.27***	0/10
11	BTP (0.25) + FLM (0.5)[c]	15.70 ± 0.21***	3/10
12	BTP (0.25) + DZP (2.5)[c] + FLM (0.5)[c]	10.50 ± 0.22***	1/10
13	BTP (0.25) + GABA (100)[c]	8.20 ± 0.33**	0/10
14	BTP (0.25) + GABA (200)[c]	6.10 ± 0.18***	0/10
15	BTP (0.25) + MUS (0.5)[c]	4.30 ± 0.93***	0/10
16	BTP (0.25) + MUS (1)[c]	3.50 ± 0.72*** $F(15,144)=158.75$, $P<.01$, $\chi^2(15)=17.73$	0/10

Groups 2, 3, 5–10, and 13–16 vs. Group 1; Group 4 vs. Group 2; Groups 10, 11, and 13–16 vs. Group 9; Group 12 vs. Group 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-square test with Yates correction.
[c] Drugs administered by subcutaneous route.
** $P<.01$ as compared with control (vehicle) or per se effect of butorphanol or diazepam or its combination.
*** $P<.001$ as compared with control (vehicle) or per se effect of butorphanol or diazepam or its combination.

When diazepam (2.5 mg/kg), GABA (100 and 200 mg/kg), or muscimol (0.5 and 1 mg/kg) was administered with butorphanol, these GABAergic drugs in their respective doses significantly augmented the protective effect of butorphanol against MES seizures. Flumazenil (0.5 mg/kg), when given along with butorphanol, significantly ($P<.001$) attenuated the anti-MES effect of the latter drug (Table 2). When animals previously treated with diazepam (2.5 mg/kg) and butorphanol were treated with flumazenil, there was a significant reversal ($P<.001$) of facilitatory effect of diazepam on the protective effect of butorphanol. However, the mortality was not significantly altered in all the above groups (Table 2).

3.4. Interaction between $GABA_B$ receptor ligands and butorphanol

Baclofen (2.5 and 5 mg/kg) per se significantly ($P<.001$) decreased the duration of THE phase. Although it also reduced the mortality incidences (1/10, 0/10) when compared to vehicle-treated control (3/10), the effect was not statistically significant. DAVA, a $GABA_B$ receptor blocker (50 mg/kg) did not produce any effect per se, but it significantly ($P<.001$) reversed the anti-MES effect of baclofen and increased the mortality incidence (2/10) (Table 3).

When baclofen (2.5 and 5 mg/kg) was administered with butorphanol (0.25 mg/kg), it increased ($P<.001$) the protective effect of butorphanol (Table 3) while DAVA (50 mg/kg) attenuated the anticonvulsant effect of butorphanol ($P<.001$). In animals pretreated with both baclofen (5 mg/kg) and butorphanol, DAVA significantly reversed ($P<.001$) the facilitatory action of baclofen on the anti-MES effect of butorphanol. However, the mortality was not significantly altered in all these groups (Table 3).

Table 3
Effect of $GABA_B$-ergic drugs and NMDA receptor antagonist, MK801 per se and on the anticonvulsant activity of butorphanol (BTP) in MES-induced convulsions in mice

Group	Treatment (mg/kg ip or sc)	Duration of hindlimb extensor phase (s) (mean ± S.E.)[a]	Mortality[b]
1	vehicle	14.60 ± 0.58	3/10
2	BAC (2.5)[c]	9.40 ± 0.27***	1/10
3	BAC (5)[c]	5.40 ± 0.27***	0/10
4	DAVA (50)	14.40 ± 0.16	3/10
5	BAC (5)[c] + DAVA (50)	14.10 ± 0.10***	2/10
6	MK801 (0.05)[c]	8.20 ± 0.13***	1/10
7	MK801 (0.1)[c]	2.00 ± 0.56***	0/10
8	BTP (0.25)	9.30 ± 0.52***	2/10
9	BTP (0.25) + BAC (2.5)[c]	5.20 ± 0.33***	0/10
10	BTP (0.25) + BAC (5)[c]	3.10 ± 0.18***	0/10
11	BTP (0.25) + DAVA (50)	16.10 ± 0.31***	4/10
12	BTP (0.25) + BAC (5)[c] + DAVA (50)	11.70 ± 0.96***	1/10
13	BTP (0.25) + MK801 (0.05)[c]	3.60 ± 0.16***	0/10
14	BTP (0.25) + MK801 (0.1)[c]	1.30 ± 0.30***	0/10
		$F(13,126)=154.14$, $P<0.01$, $\chi^2(13)=11.29$	

Groups 2–4, 6–10, 13, and 14 vs. Group 1; Group 5 vs. Group 3; Groups 9–11, 13, and 14 vs. Group 8; Group 12 vs. Group 10; $n=10$.
BAC, baclofen; DAVA, δ-aminovaleric acid.
[a] One-way ANOVA followed by Dunnett's t test.
[b] Grouped chi-square test with Yates correction.
[c] Drugs administered by subcutaneous route.
*** $P<.001$ as compared with control (vehicle) or per se effect of butorphanol or baclofen or its combination.

3.5. Interaction between glutamatergic ligands and butorphanol

MK801 (0.05 and 0.1 mg/kg) per se offered a significant protection ($P<.001$) against MES-induced convulsions as compared to vehicle-treated control (Table 3). When MK801 (0.05 and 0.1 mg/kg) was combined with butorphanol (0.25 mg/kg) in separate groups of mice, the protective effect of butorphanol in MES seizures was augmented by MK801, i.e., duration of THE was significantly reduced ($P<.001$) and there was no mortality as compared to mortality incidence (2/10) in the butorphanol (0.25 mg/kg)-treated group (Table 3).

4. Discussion

In this study, there is a direct evidence for a role of the opioid receptors in mediating the anticonvulsant-like behavioural effects of butorphanol. Butorphanol, a potent opioid receptor ligand (Pircio et al., 1976; Gillis et al., 1995) was demonstrated to have anticonvulsant effect in the MES paradigm in mice. Although butorphanol and established anticonvulsant drug diazepam (Johnston, 1996) produced qualitatively similar responses in the MES, the effects of butorphanol could be attributed either to its direct action at the opioid receptor or to an indirect consequence of GABA receptor modulation. The latter seems more likely because flumazenil, a central BZD receptor antagonist (Brogden and Goa, 1991), prevented the anticonvulsant behavioural response of butorphanol. Butorphanol, a totally synthetic morphinan, has been shown previously to be active in other animal behavioural models of convulsions (Pircio et al., 1976), but the specificity of its behavioural effects for the opioid receptor was not investigated using antagonism studies. The fact that low doses of naloxone, which reportedly block mu opioid receptors (Tallarida et al., 1978; Reisine and Pasternak, 1996), attenuated the anticonvulsant-like behavioural effects of butorphanol suggests that these effects were mediated by the activation of mu opioid receptors. Although naloxone in high doses is also a GABA receptor antagonist (Dingledine et al., 1978), the functional relevance of this effect is not known. Previous behavioural studies have shown that naloxone is effective at blocking opioid receptor-mediated behaviours induced by pentazocine, nalbuphine, and more selective kappa opioid receptor agonists such as U-50488H and U-54494A (Tortella et al., 1986; Fischer et al., 1993; Manocha et al., 1997; Manocha

et al., 1998). Like naloxone, small doses of both MR2266, a kappa opioid antagonist (Frey, 1988; Laorden et al., 1991), and naltrindole, a delta opioid receptor blocker (Reisine and Pasternak, 1996), also partially antagonized the anticonvulsant-like behavioural effects of butorphanol, which suggests that these effects were also mediated by the activation of kappa and delta receptors.

A number of studies demonstrated that butorphanol in vivo may produce behavioural effects by activating opioid receptors (Pircio et al., 1976; Gillis et al., 1995; Reisine and Pasternak, 1996). They and others, using different behavioural tests (Frenk, 1983; Tortella et al., 1986; Frey, 1988; Fischer et al., 1993; Atapour et al., 2000; Broom et al., 2000; Yajima et al., 2000), suggested that the activation of opioid receptors may be responsible in part for the anticonvulsant properties of opioidergic compounds. The common effects of multiple opioid receptor agonists including butorphanol, the effectiveness of the opioid receptor antagonists including naloxone (mu), MR2266 (kappa), and naltrindole (delta), and the ability to block behavioural responses of butorphanol by treatment with mu, kappa, and delta receptor antagonists support the idea that the activation of these opioid receptors was responsible in part for anticonvulsant-like behavioural responses in MES test.

Diazepam, an effective BZD receptor ligand, augments the neuronal inhibitory effects produced by stimulating various GABAergic pathways and is associated with an increased frequency of bursts of chloride channel openings. Further, extensive biochemical evidence suggests a close molecular association between specific BZD binding sites and GABA-regulated chloride channels (Johnston, 1996; Feldman et al., 1997). Both muscimol and GABA are direct $GABA_A$ receptor agonists (Johnston, 1996). In this study, diazepam, muscimol, and GABA were shown to produce anticonvulsant-like effects in the MES test. 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. Previous studies have also shown that selective GABAergic compounds produced potent anticonvulsant effects (Rogawski and Porter, 1990). The more selective central BZD receptor antagonist flumazenil per se produced no effects in the MES, but it counteracted the anticonvulsive behaviour of diazepam, further supporting that the effects of diazepam are BZD–GABAergic in origin.

Butorphanol has been found effective against pentylenetetrazole (PTZ)-induced convulsions (Pircio et al., 1976). These reports suggest that GABAergic system may be involved in the action of butorphanol, since PTZ interferes with GABA transmission and interacts with picrotoxinin binding site of BZD–$GABA_A$ receptor complex and inhibits its [^{35}S]-*t*-butylbicyclophosphorothionate's binding (Maksay and Ticku, 1985; Johnston, 1996). A behaviourally active dose of GABA, muscimol, or diazepam also increased the anticonvulsant behaviour produced by butorphanol. Since BZD agonists, like diazepam (Johnston, 1996), act on BZD sites believed to be part of a protein macromolecular complex that includes the large family of $GABA_A$ receptors and a Cl^- channel, diazepam treatment can impact GABAergic neurotransmission. Based on the foregoing discussion, the augmentation of protective effect of butorphanol on MES seizures by GABAergic agents, like diazepam, GABA, and muscimol, suggests that BZD–$GABA_A$ mechanisms may be participating in some of the butorphanol's anti-MES effect. Additionally, the reversal of facilitatory effect of diazepam on butorphanol protection of MES convulsions by flumazenil, a short-acting pure antagonist of the BZD recognition site of $GABA_A$ receptor, indicates that the BZD site on BZD–$GABA_A$ receptor complex could possibly be involved in action of butorphanol in the brain. This suggestion gets further credence from observations, where flumazenil per se also attenuated the anti-MES effect of butorphanol when given alone.

Baclofen is a potent $GABA_B$ receptor ligand (Bowery, 1993), whereas DAVA antagonizes $GABA_B$-mediated responses (Schwarz et al., 1988). DAVA has high affinity for $GABA_B$ receptor in comparison to $GABA_A$ sites. In fact, the augmentation of the antiseizurogenic action of butorphanol by baclofen and the attenuation of the anti-MES effect of butorphanol alone as well as when given in combination with baclofen by DAVA, a $GABA_B$ antagonist, show that besides $GABA_A$, $GABA_B$ receptors also play a role in the anti-MES effect of butorphanol. There is also growing evidence that the NMDA subtype of excitatory amino acid receptors play an important role in epilepsy (Meldrum, 1992). In fact, competitive and noncompetitive antagonists of NMDA receptor have demonstrated potent anticonvulsant activity in a wide range of seizure models. MK801 is a noncompetitive NMDA receptor antagonist that acts at a site within the ion channel of the NMDA receptor complex (Wong et al., 1986). In this study, MK801 enhanced the anticonvulsant effect of butorphanol on MES seizures, which indicates that the anti-MES effect of butorphanol is in part also mediated at the NMDA receptors. These data from the diazepam or baclofen/ butorphanol combination study are but one example of the utility of specifying active behaviours in the MES because otherwise, flumazenil's or DAVA's blockade of diazepam's or baclofen's anticonvulsant-like effects or perhaps the abolition of facilitatory actions of diazepam/baclofen on butorphanol's anticonvulsant effects, i.e., impact on THE of MES, could not be shown. More importantly, the combination of NMDA receptor antagonists particularly with opioids may lead to augmentation of opioid's clinical effect (Wiesenfeld-Hallin, 1998).

Several opioid receptor subtypes, alone or interdependent with the GABA and NMDA receptor, are likely to play a role in the mediation of anticonvulsant effects of butorphanol. Opioid receptors, such as mu, kappa, and delta, BZD–$GABA_A$-coupled receptor system, $GABA_B$ receptors, and NMDA receptor channel have been suggested to mediate

anticonvulsant responses to different drugs, which affect these receptors/channels. It appears that these same receptors/channels also play a role in the anti-MES response produced by butorphanol. Although the classical opioid receptor antagonist, naloxone, blocked anticonvulsant behavioural responses to butorphanol, the MES paradigm used was sensitive for opioidergic compounds but was not designed to reveal a residual role for opioid receptors in the effects of butorphanol. Nevertheless, results with butorphanol present an important evidence that the opioid receptor may indeed be a target for the development of anticonvulsants for grand mal seizures and opioidergic compounds may be used as adjuncts to conventional AEDs. The shortcoming of the experiments is in the systemic route of drug administration in which it is difficult to distinguish the penetration of blood–brain barrier from other variables (Oldendorf et al., 1972). The present findings can be replicated comprehensively in other various convulsive models followed by receptor binding studies using some more selective ligands.

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