ELSEVIER

European Journal of Pharmacology 377 (1999) 35–42

ejp

www.elsevier.nl/locate/ejphar

# Modification of naloxone-induced withdrawal signs by dextromethorphan in morphine-dependent mice

Davood Farzin

*Department of Pharmacology, Sari School of Medicine, Mazandaran University of Medical Sciences, 48168 Sari, Iran*

Received 28 January 1999; received in revised form 18 May 1999; accepted 26 May 1999

**Abstract**

In the present study the effect of dextromethorphan on naloxone-induced withdrawal signs in morphine-dependent mice was examined. In addition, the modulatory role of dopaminergic mechanisms upon the effect of dextromethorphan was investigated. Mice were rendered dependent on morphine by subcutaneous (s.c.) injections of morphine sulfate three times a day for 3 days, and withdrawal signs were induced by intraperitoneal (i.p.) administration of naloxone 2 h after the 10th injection of morphine sulfate on day 4. Dextromethorphan (20–50 mg/kg, i.p.) caused a significant decrease in withdrawal jumping, paw-shakes, grooming, burrows, writhing and diarrhea in morphine-dependent mice. The mixed dopamine $D_1/D_2$ receptor agonist apomorphine (0.5 and 1 mg/kg, s.c.) reduced the response induced by dextromethorphan. The effect of apomorphine was blocked by the dopamine $D_1$ receptor antagonist SCH 23390 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol maleate) (0.5 and 1 mg/kg, i.p.) but not by the dopamine $D_2$ receptor antagonist sulpiride (25 and 50 mg/kg, s.c.) nor the peripheral dopamine receptor antagonist domperidone (5 and 10 mg/kg, s.c.). These results suggest that the dopaminergic system(s) may in part mediate the suppressive action of the NMDA receptor antagonist dextromethorphan on naloxone-induced withdrawal signs in morphine-dependent mice. 

*Keywords:* Dextromethorphan; Morphine; Naloxone; Apomorphine; NMDA receptor antagonist; Withdrawal sign; (Mouse)

## 1. Introduction

Dextromethorphan is a well-known over-the-counter antitussive drug with over 42 years of clinical usage experience (Church et al., 1989; Tortella et al., 1989). It is a moderately potent but effective non-competitive NMDA receptor antagonist (Wong et al., 1988). Non-competitive NMDA receptor antagonists, such as dizocilpine maleate (MK-801) and dextrophan, a metabolite of dextromethorphan (Church et al., 1989) have anxiolytic, anticonvulsant (Clineschmidt et al., 1982) and analgesic effects (Elliott et al., 1995a,b). Koyuncuoğlu et al. (1974, 1976) reported that the endogenous NMDA receptor agonists aspartic or glutamic acids can antagonize some effects of morphine. Furthermore, treatment of mice with opioid receptor agonists has been reported to antagonize the effects of excitatory amino acid receptor agonists that preferably stimulate NMDA-type receptors of aspartatergic/glutamatergic system (Aanonsen and Wilcox, 1987). There is good evidence that after blockade of NMDA receptors with an antagonist such as MK-801, the development of morphine tolerance and dependence is reduced or prevented (Marek et al., 1991; Trujillo and Akil, 1991). Interactions between the glutamatergic and dopaminergic transmissions were also observed in relation to the development of morphine tolerance and dependence (Huang et al., 1997). There are cortical glutamatergic projections to extrapyramidal and limbic structures and high levels of NMDA receptors in these regions (Singh et al., 1992). Activation of NMDA receptors has been shown to be effective in stimulating the release of dopamine from the striatum and the limbic system (Imperato et al., 1990; Krebs et al., 1991; Jin and Fredholm, 1994). Recent reports also indicated that dopamine in the these regions plays a important role in opiate tolerance and dependence (Navarro et al., 1992; Martin et al., 1997). In vivo microdialysis studies show that μ-opioid receptor agonists increase extracellular dopamine levels in striatum and nucleus accumbens of the rat (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Kalivas and Stewart, 1991). A considerable amount of evidence also indicates that both acute and chronic administration of morphine increases dopamine synthesis (Urwyler and Tabakoff, 1981) and turnover (Guaza et al., 1980) in the rodents striatum and nucleus accumbens. The results of the above studies suggest that glutamate/aspartate–dopamine interactions at the several brain regions

0014-2999/99/$ - see front matter 
PII: S0014-2999(99)00396-9

may play a modulatory role upon the expression of opiate withdrawal in rodents. This hypothesis would predict that non-competitive NMDA receptor antagonist dextromethorphan should alleviate at least some of the signs of morphine abstinence and dopaminergic mechanisms have a modulatory role upon the effect of dextromethorphan. The purpose of the present study was to determine the effect of dextromethorphan on several parameters of withdrawal symptoms induced by naloxone in morphine-dependent mice and its possible interaction with dopaminergic mechanism(s).

## 2. Materials and method

### 2.1. Animals

All experiments were carried out on male Swiss–Webster mice from the Pasteur Institute (Iran), 20–25 g body weight. The animals were housed nine per plastic cage in an animal room maintained at $21 \pm 2$°C on a 12-h dark cycle. Food and water were available at all times except during the experiments. Each animal was used once only.

### 2.2. Induction of dependence

The mice were rendered dependent on morphine using the method previously described by Marshall and Grahame-Smith (1971). Morphine sulfate was injected subcutaneously (s.c.) three times daily at 09:30, 13:30 and 17:30 h on the following dosage schedule. The first three doses were 50, 50 and 75 mg/kg, respectively. The higher dose at the third daily injection was aimed to minimize any overnight withdrawal. Each of the dose was then increased by 25 mg $kg^{-1}$ $day^{-1}$. Morphine administration was carried out over a maximum of 3 days for all groups of mice. A dose of 50 mg/kg of morphine sulfate also was injected on the 4th day (2 h before naloxone injection). Hyperactivity and the Straub tail effect were seen after morphine injections.

### 2.3. Withdrawal

Groups of mice were tested for occurrence of withdrawal signs after their 10th injection of morphine on day 4. Two hours after their 10th injection of morphine (50 mg/kg), abstinence was precipitated by an intraperitoneal (i.p.) injection of naloxone (5 mg/kg), a dose producing approximately 50% withdrawal symptoms (Zarrindast and Farzin, 1996); then animals were placed individually into a cylindrical glass (25 cm in diameter, 40 cm height) and the withdrawal symptoms were recorded over a 30-min period.

### 2.4. Diarrhea

Each mouse was transferred to a cylindrical glass immediately following naloxone injection. The floor of each cylinder was lined with preweighed paper towelling to allow collection of wet and dry fecal matter, which was weighed 30 min after administering naloxone. Results are expressed as the weight in milligrams of fecal material defecated per 10 g body weight in 30 min.

### 2.5. Behaviour

Animals were also observed for 30 min during the collection of feces. The quantified signs of withdrawal were jumping (all feet off the floor), paw-shakes, grooming, burrows (escape digging) and writhing. The frequency of these behaviours (score per 30 min) was recorded for each animal and results are compared to the frequency in the appropriate control group.

### 2.6. Drugs

The following drugs were used: *R*(−)-apomorphine HCl (Research Biochemicals, USA), dextromethorphan HBr (Research Biochemicals), domperidone (Research Biochemicals), morphine sulfate (MacFarlan Smith, UK), naloxone HCl (Sigma, UK), SCH 23390 (*R*-(+)-8-chloro2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine7-ol maleate; Research Biochemicals), and sulpiride (Sigma). In all cases, the drug doses reported are for the base, except for morphine sulfate. The drugs were dissolved in saline, except for sulpiride and domperidone, which were dissolved in a drop of acetic acid and diluted with saline. The vehicle control in respective cases was acetic acid in saline. The drugs were given in a volume of 10 ml/kg and were prepared immediately before use. The dose of antagonists and pretreatment time were usually those used previously and shown to be pharmacologically active (Zarrindast and Abolfathi-Araghi, 1992; Zarrindast and Tabatabai, 1992; Zarrindast and Farzin, 1996; Zarrindast et al., 1996)

### 2.7. Statistical analysis

Comparisons between groups were made with a Newman–Keuls test following one-way analysis of variance (ANOVA). Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant.

## 3. Results

### 3.1. Effects of dextromethorphan on naloxone-induced withdrawal signs

In morphine-dependent mice, dextromethorphan (20–50 mg/kg, i.p.) given 30 min before naloxone (5 mg/kg, i.p.), produced dose-dependent reductions of withdrawal jumping ($F(4,47) = 12.22$, $p < 0.01$) ($ED_{50} = 24$ mg/kg),

paw-shakes ($F(4,43) = 8.16$, $p < 0.01$) ($ED_{50} = 26$ mg/kg), grooming ($F(4,40) = 8.11$, $p < 0.01$) ($ED_{50} = 29$ mg/kg), writhing ($F(4,40) = 3.77$, $p < 0.05$) ($ED_{50} = 25$ mg/kg), burrows ($F(4,40) = 22.02$, $p < 0.01$) ($ED_{50} = 23$ mg/kg) and diarrhea ($F(4,48) = 6.25$, $p < 0.01$) ($ED_{50} = 40$ mg/kg) (Fig. 1) (the $ED_{50}$ values obtained by regression analysis). The dose of 30 mg/kg of dextromethorphan was chosen for subsequent experiments because the mean of $ED_{50}$ values equalled approximately 28 mg/kg.

### 3.2. Effect of apomorphine on the suppressive action of dextromethorphan

In Fig. 2, it can be seen that the pretreatment of animals with mixed dopamine $D_1/D_2$ receptor agonist apomorphine (1 mg/kg, s.c., 30 min before naloxone) reversed the suppressive action exerted by dextromethorphan (30 mg/kg, i.p.) on naloxone-induced withdrawal jumping ($F(2,20) = 3.83$, $p < 0.04$), paw-shakes ($F(2,20) = 14.80$, $p < 0.0001$), grooming ($F(2,20) = 10.42$, $p < 0.001$), burrows ($F(2,20) = 7.03$, $p < 0.005$), writhing ($F(2,20) = 4.34$, $p < 0.03$). Pretreatment of animals with apomorphine also caused a reduction in the suppressive action of dextromethorphan on the total amount of fecal matter. The inhibitory effect of this drug became statistically significant at 0.5 and 1 mg/kg ($F(5,38) = 3.1$, $p < 0.02$) (Fig. 2). Withdrawal jumping ($F(2,20) = 0.12$, $p > 0.88$), paw-shakes ($F(2,20) = 0.38$, $p > 0.69$), grooming ($F(2,20) = 0.25$, $p > 0.78$), burrows ($F(2,20) = 0.82$, $p > 0.45$), writhing ($F(2,20) = 0.65$, $p > 0.53$) and diarrhea ($F(2,20) = 0.81$, $p > 0.46$) induced by naloxone in animals treated with apomorphine (0.5 and 1 mg/kg, s.c., 30 min before naloxone) alone were not significantly different from that of the saline controls (10 ml/kg, s.c.).

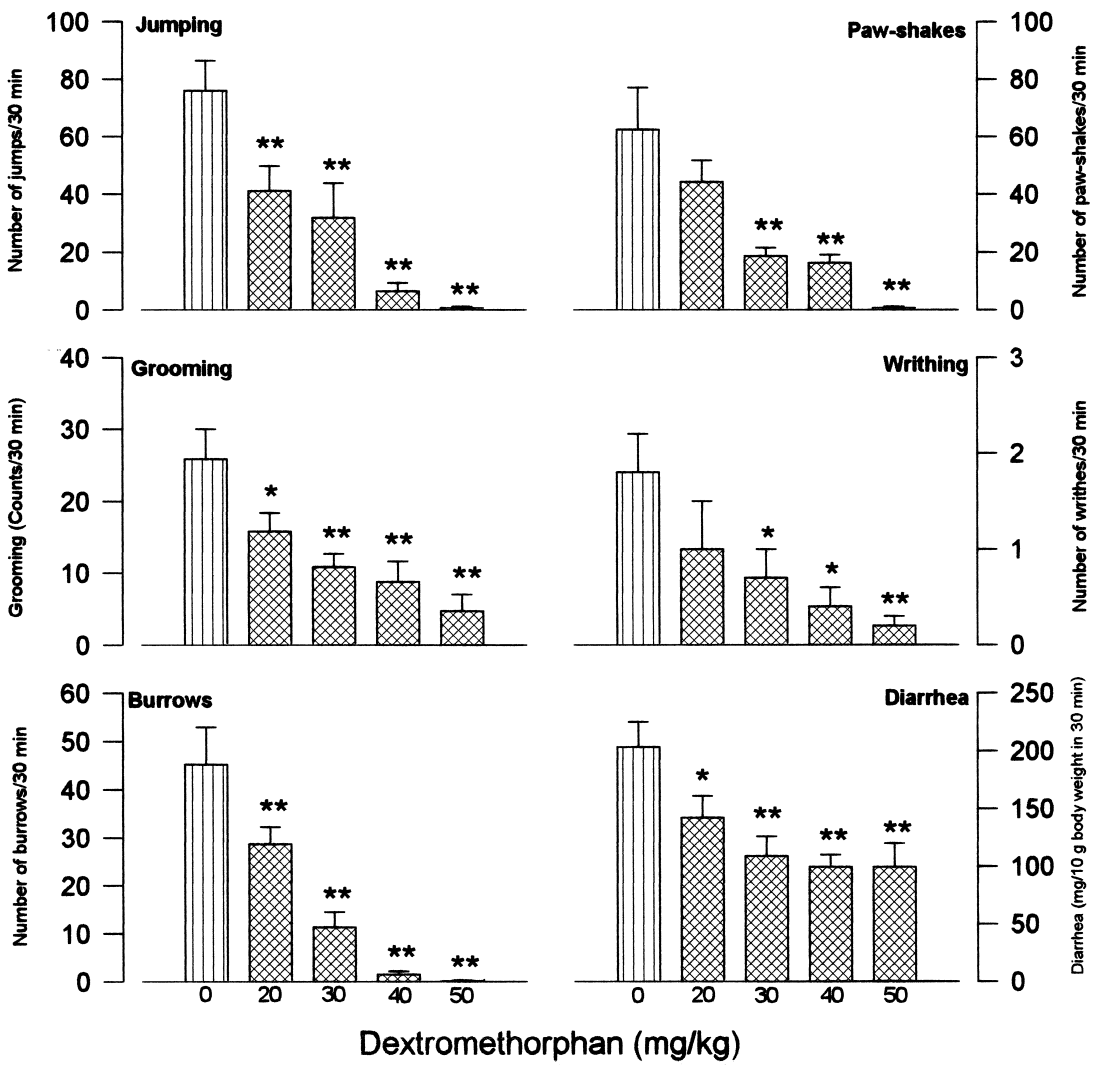


Fig. 1. Effect of dextromethorphan on naloxone-induced withdrawal signs in morphine-dependent mice. Morphine-dependent mice were injected i.p. with saline (10 ml/kg) and dextromethorphan (20–50 mg/kg). Saline or dextromethorphan were always given 30 min before induction of withdrawal by naloxone (5 mg/kg, i.p.). Jumping, paw-shakes, grooming, writhing and burrows were counted within 30 min and diarrhea was measured 30 min following naloxone. Results are expressed as mean + SEM ($n = 9$–15 mice/group). $^{*}p < 0.05$, $^{**}p < 0.01$, different from saline control groups.

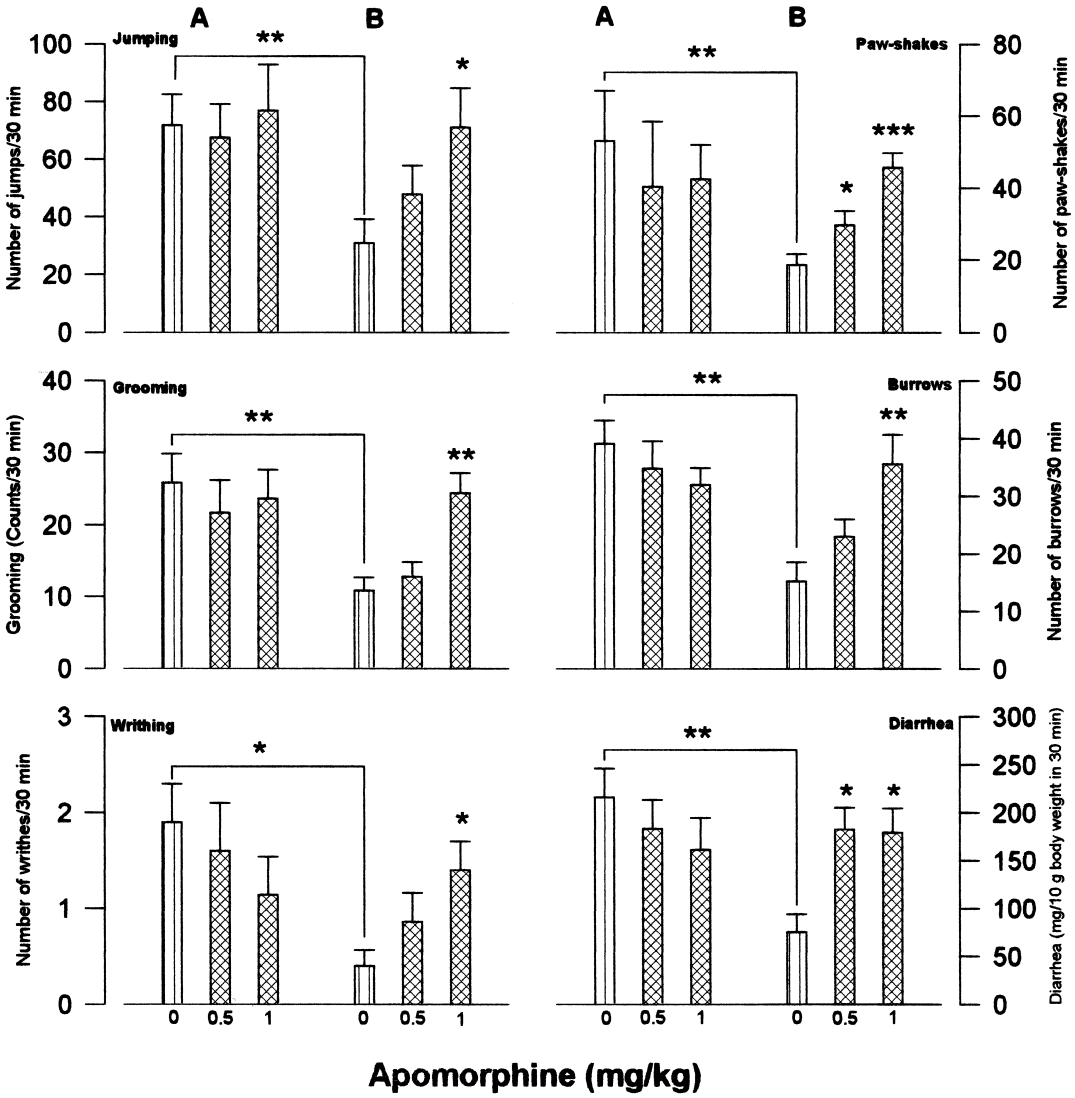


Fig. 2. Effect of apomorphine on withdrawal signs induced by naloxone (5 mg/kg, i.p.) in morphine-dependent mice, in the absence (A) or the presence (B) of dextromethorphan (30 mg/kg, i.p.). Results are expressed as mean + SEM ($n$ = 7–9 mice/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, different from saline control groups.

### *3.3. Effect of SCH 23390 on the withdrawal responses to co- administration of apomorphine plus dextromethorphan*

Pretreatment of animals with different doses of dopamine $D_1$ receptor antagonist SCH23390 (0.5 or 1 mg/kg, i.p., 45 min before naloxone) reversed the effect exerted by a cocktail of apomorphine (1 mg/kg, s.c., 30 min before naloxone) and dextromethorphan (30 mg/kg, i.p., 30 min before naloxone) on naloxone-induced withdrawal jumping ($F(8,56) = 8.24$, $p < 0.0001$), paw-shakes ($F(8,58) = 23.39$, $p < 0.0001$), grooming ($F(8,56) = 4.55$, $p < 0.0003$), burrows ($F(8,56) = 8.51$, $p < 0.0001$), writhing ($F(8,56) = 7.04$, $p < 0.0001$) and diarrhea ($F(8,56) = 5.64$, $p < 0.0001$) (Table 1). Pretreatment of animals with SCH 23390 (1 mg/kg) alone significantly decreased the jumping, paw-shakes and grooming responses induced by naloxone in morphine-dependent mice.

### *3.4. Effects of sulpiride and domperidone on the withdrawal responses to co-administration of apomorphine plus dextromethorphan*

Pretreatment of animals with different doses of dopamine $D_2$ receptor antagonist sulpiride (25 and 50 mg/kg, s.c., 45 min before naloxone) did not change the response exerted by apomorphine (1 mg/kg, i.p., 30 min before naloxone) plus dextromethorphan (30 mg/kg, i.p., 30 min before naloxone) on naloxone-induced withdrawal jumping ($F(2,18) = 0.17$, $p > 0.84$), paw-shakes ($F(2,20) = 0.40$, $p > 0.67$), grooming ($F(2,20) = 0.35$, $p > 0.71$), burrows ($F(2,20) = 1.13$, $p > 0.34$), writhing ($F(2,20) = 0.55$, $p > 0.58$) and diarrhea ($F(2,20) = 0.38$, $p > 0.69$) (Table 2). Treatment of animals with sulpiride (25 and 50 mg/kg, s.c.) alone did not have any significant effects on the withdrawal signs induce by naloxone (5 mg/kg, i.p.)

Table 1
Effect of the dopamine $D_1$ receptor antagonist SCH 23390 (SCH) alone or in combination with dextromethorphan (DEX) and a cocktail (C) of apomorphine and dextromethorphan on naloxone-induced withdrawal signs in morphine-dependent mice
Animals were injected saline (10 ml/kg), saline in combination with SCH 23390 (0.5 and 1 mg/kg), dextromethorphan (30 mg/kg) and a cocktail of apomorphine (1 mg/kg) and dextromethorphan (30 mg/kg) or SCH 23390 in combination with dextromethorphan and a cocktail of apomorphine and dextromethorphan. SCH 23390 were administered (i.p.) 45 min, apomorphine (s.c.) 30 min and dextromethorphan (i.p.) 30 min before naloxone (5 mg/kg, i.p.) injection. Results are expressed as mean ± SEM ($n$ = 7–9 mice/group).

| Treatment (mg/kg) | Signs | | | | | |
|---|---|---|---|---|---|---|
| | Jumps | Paw-shakes | Grooming | Burrows | Writhes | Diarrhea |
| Saline + saline | 68.7 ± 8.9 | 35.3 ± 3.6 | 25.9 ± 4 | 46.3 ± 7.2 | 2 ± 0.4 | 217 ± 30 |
| Saline + SCH 0.5 | 51.8 ± 8.5 | 28.8 ± 2.8 | 17.7 ± 3.4 | 49.7 ± 10 | 1.7 ± 0.4 | 193.5 ± 30 |
| Saline + SCH 1 | 26.1 ± 5.9[b] | 13.3 ± 1.7[c] | 11.4 ± 1.7[a] | 41.4 ± 10.2 | 2.3 ± 0.4 | 154 ± 41.5 |
| Saline + DEX 30 | 23.4 ± 7.7 | 16.7 ± 2.5 | 8.9 ± 1.8 | 13.7 ± 3.7 | 0.4 ± 0.2 | 67.5 ± 6.5 |
| SCH 0.5 + DEX 30 | 11.1 ± 3.3 | 8.1 ± 1.5 | 11.8 ± 2.1 | 11 ± 3 | 0.6 ± 0.3 | 84.3 ± 8.5 |
| SCH 1 + DEX 30 | 1.6 ± 0.9 | 0.6 ± 0.4[c] | 10.4 ± 6.3 | 8.7 ± 0.9 | 0.4 ± 0.2 | 118.4 ± 29 |
| Saline + C | 62.7 ± 16.1 | 39.6 ± 3 | 27.3 ± 2.4 | 39.3 ± 5 | 1.7 ± 0.3 | 177 ± 16.3 |
| SCH 0.5 + C | 28.8 ± 6.5[a] | 21.2 ± 3[c] | 14.4 ± 1.3[a] | 14.7 ± 2.5[a] | 0.8 ± 0.2 | 113.8 ± 9.2 |
| SCH 1 + C | 26.3 ± 4.7[a] | 13.6 ± 3.2[c] | 12.7 ± 1.5[a] | 9.7 ± 2[b] | 0.2 ± 0.2[a] | 66.8 ± 7.6[a] |

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, different from saline control groups.

in morphine-dependent mice as compared with vehicle-pretreated controls.

The naloxone-induced withdrawal jumping ($F(2,20) = 0.13$, $p > 0.88$), paw-shakes ($F(2,20) = 0.80$, $p > 0.46$), grooming ($F(2,20) = 1.05$, $p > 0.37$), burrows ($F(2,20) = 0.77$, $p > 0.48$), writhing ($F(2,20) = 0.13$, $p > 0.87$) and diarrhea ($F(2,20) = 0.21$, $p > 0.81$) in morphine-dependent mice treated with 5 and 10 mg/kg peripheral dopamine receptor antagonist domperidone (s.c., 35 min before naloxone) + a cocktail of apomorphine (1 mg/kg, i.p., 30 min before naloxone) and dextrometorphan (30 mg/kg, i.p., 30 min before naloxone) were not significantly different from that induced in animals treated with vehicle (10 ml/kg, s.c., 35 min before naloxone) + a cocktail of apomorphine and dextromethorphan (Table 2). Treatment of animals with domperidone (5 and 10 mg/kg,

Table 2
Effects of the dopamine $D_2$ receptor antagonist sulpiride (SUL) or peripheral dopamine receptor antagonist domperidone (DOM) alone or in combination with dextromethorphan (DEX) and a cocktail (C) of apomorphine and dextromethorphan on naloxone-induced withdrawal signs in morphine-dependent mice
Animals were injected vehicle (10 ml/kg), vehicle in combination with sulpiride (25 and 50 mg/kg), domperidone (5 and 10 mg/kg), dextromethorphan (30 mg/kg) and a cocktail of apomorphine (1 mg/kg) and dextromethorphan (30 mg/kg) or supiride and domperidone in combination with dextromethorphan and a cocktail of apomorphine and dextromethorphan. Sulpiride were administered (s.c.) 45 min, domperidone (s.c.) 35 min, apomorphine (s.c.) 30 min and dextromethorphan (i.p.) 30 min before naloxone (5 mg/kg, i.p.) injection. Results are expressed as mean ± SEM ($n$ = 7–9 mice/group).

| Treatment (mg/kg) | Signs | | | | | |
|---|---|---|---|---|---|---|
| | Jumps | Paw-shakes | Grooming | Burrows | Writhes | Diarrhea |
| Saline + vehicle | 83 ± 6.8 | 60.2 ± 13.2 | 25.7 ± 3.8 | 47.4 ± 7.2 | 1.9 ± 0.4 | 214.8 ± 30.5 |
| Vehicle + SUL 25 | 75.4 ± 12.6 | 56.3 ± 14.2 | 23.7 ± 4.1 | 38.6 ± 4.3 | 1.4 ± 0.4 | 197.4 ± 29.5 |
| Vehicle + SUL 50 | 66.3 ± 18.3 | 41.8 ± 11.8 | 20.3 ± 4.9 | 36.8 ± 3.8 | 1.4 ± 0.6 | 219 ± 30.7 |
| Vehicle + DEX 30 | 26.8 ± 5.5 | 12.6 ± 2.3 | 14 ± 3.2 | 10.4 ± 2.1 | 0.7 ± 0.2 | 122.3 ± 19.7 |
| SUL 25 + DEX 30 | 40.1 ± 8.3 | 14.4 ± 2.9 | 17.3 ± 3.5 | 11.7 ± 2.1 | 0.7 ± 0.3 | 137.4 ± 17.4 |
| SUL 50 + DEX 30 | 33.8 ± 10.4 | 9.8 ± 3.4 | 15.8 ± 4.5 | 9.6 ± 2.8 | 0.6 ± 0.3 | 132.6 ± 16 |
| Vehicle + C | 68.3 ± 16.9 | 52.8 ± 6.3 | 23.3 ± 3.1 | 32.1 ± 4.7 | 1.9 ± 0.4 | 197.4 ± 29.3 |
| SUL 25 + C | 76.7 ± 11.1 | 46.8 ± 8.6 | 22.3 ± 3.2 | 31.7 ± 4.7 | 2 ± 0.5 | 234.6 ± 29.8 |
| SUL 50 + C | 65 ± 14.7 | 42 ± 11.4 | 19.6 ± 3.6 | 22.1 ± 6.3 | 1.3 ± 0.6 | 207.4 ± 34 |
| Saline + vehicle | 62.9 ± 7.3 | 49 ± 7.5 | 20 ± 2.7 | 49.2 ± 5.3 | 2.2 ± 0.4 | 223.1 ± 20.6 |
| Vehicle + DOM 5 | 54.4 ± 8.8 | 42.6 ± 4.7 | 17.7 ± 3.2 | 45.1 ± 6.3 | 1.6 ± 0.4 | 227.8 ± 27.9 |
| Vehicle + DOM 10 | 53 ± 6.6 | 44.1 ± 7.5 | 18.7 ± 3.1 | 39 ± 5.6 | 1.7 ± 0.4 | 203.1 ± 23.1 |
| Vehicle + DEX 30 | 24.3 ± 3.8 | 17 ± 2 | 8.8 ± 1.5 | 16.1 ± 1.6 | 0.5 ± 0.2 | 140.9 ± 16.9 |
| DOM 5 + DEX 30 | 28.4 ± 6.4 | 20.7 ± 3.2 | 10.7 ± 1.9 | 12.4 ± 2.5 | 0.7 ± 0.3 | 129.1 ± 16.2 |
| DOM 10 + DEX 30 | 21 ± 4.6 | 18.6 ± 2.9 | 9 ± 1.3 | 14.6 ± 2.3 | 0.6 ± 0.3 | 139 ± 15.1 |
| Vehicle + C | 60.6 ± 5.5 | 44.4 ± 5.3 | 22.9 ± 3.1 | 29.4 ± 3.3 | 1.6 ± 0.3 | 183.4 ± 20.1 |
| DOM 5 + C | 64.8 ± 7.3 | 34.3 ± 5.9 | 17.6 ± 2.3 | 25.3 ± 3.6 | 1.4 ± 0.4 | 181.6 ± 16.5 |
| DOM 10 + C | 61 ± 6.6 | 38.8 ± 6.3 | 18.6 ± 2.9 | 24.3 ± 2.4 | 1.7 ± 0.4 | 197.3 ± 15 |

s.c., 35 min before naloxone) alone did not have any significant effect on the naloxone-induced withdrawal signs in morphine-dependent mice as compared with vehicle-pretreated controls.

## 4. Discussion

The purpose of this study was to determine the possible mechanism(s) of the acute administration of the NMDA receptor antagonist dextromethorphan on the naloxone-induced withdrawal signs alone and in combination with apomorphine. The main findings are as follows.

(a) The naloxone-induced withdrawal signs were significantly decreased by pretreatment with dextromethorphan.

(b) Apomorphine significantly antagonized the reduction of withdrawal signs induced by dextromethorphan.

(c) The inhibitory effect of apomorphine upon the suppressive action of dextromethorphan was blocked by the dopamine $D_1$ receptor antagonist SCH 23390 but not by dopamine $D_2$ receptor antagonist sulpiride nor the peripheral dopamine receptor antagonist domperidone.

The result of the present study support reports suggesting that NMDA receptor antagonists attenuate the development of morphine tolerance and dependence in rodents (Koyuncuoğlu et al., 1990, 1992). Recent researches have demonstrated the existence of an interaction between the glutamatergic and opioid systems for development of morphine tolerance and dependence (Cai et al., 1997; Martin et al., 1997). Several studies have also reported that morphine inhibits the enzymes producing aspartic and glutamic acids from asparagine and glutamine (Koyuncuoğlu et al., 1979; Bielarczyk et al., 1986). The production and release of aspartic/glutamic acids decrease during the development of morphine physical dependence that may lead to the upregulation and supersensitivity of NMDA receptors (Koyuncuoğlu et al., 1992). It has been reported that naloxone-induced withdrawal in morphine-dependent animals is associated with increased extracellular levels of glutamate within the pontine locus coeruleus, suggesting that glutamate my be involved in the expression of opioid withdrawal signs. (Aghajanian et al., 1994; Zhang et al., 1994; Feng et al., 1995). Direct intracerebroventricular injection of glutamate to the brains of morphine-dependent rats precipitated withdrawal signs in a dose-dependent manner (Tokuyama et al., 1996). Tokuyama et al. (1996) reported that the withdrawal signs elicited by glutamate are similar to those produced by naloxone. This leads to the hypothesis that dextromethorphan may antagonize the effect of glutamate in the anatomical site for mediating opioid abstinence and suppresses withdrawal signs by such a mechanism.

It has been proposed that repeated administration of opiates may lead to activation of the NMDA receptor through G-proteins associated with the μ-opioid receptor and/or other intracellular mechanisms (Mao et al., 1995, 1996). This opiate-related activation of NMDA receptors may initiate subsequent intracellular changes such as the activation of protein kinase C (Mao et al., 1994; Mayer et al., 1995). Many studies have reported that the activation of protein kinase C with a phorbol ester potentiate the desensitization of the μ-opioid receptor-induced current by regulating μ-opioid receptor coupling through a G-protein to an inwardly rectifying $K^+$ channel (Chen and Yu, 1994; Mao et al., 1996). Such NMDA receptor-mediated cellular and intracellular changes may lead to diminished responsiveness of the μ-opioid receptor to morphine. Dextromethorphan antagonizes the activation of NMDA receptor and thus stops the initiation of such intracellular cascades.

Chronic treatment with morphine causes an enhanced response to mixed dopamine $D_1/D_2$ receptor agonist apomorphine in rats (Ritzmann et al., 1982; Bhargava, 1983). The present data show that apomorphine antagonized the suppressive action of dextromethorphan thus suggesting an involvement of dopaminergic mechanisms in the attenuation of the naloxone-induced withdrawal signs by this non-competitive NMDA receptor antagonist. The subsequent point which emerges from this research concerns the inhibition of the apomorphine response upon the effect of dextromethorphan following administration of a dose ineffective by SCH 23390 but not by sulpiride nor domperidone. These results show the existence of an interaction between dextromethorphan and the central dopamine $D_1$ receptor mechanisms for the attenuation of naloxone-induced withdrawal signs. Both the striatum and nucleus accumbens may play a major role in opiate dependence (Navarro et al., 1992; Martin et al., 1997), and NMDA receptor stimulation is reported to induce dopamine release in these regions (Imperato et al., 1990; Jin and Fredholm, 1994). Alterations in the activity of striatum dopaminergic system have been observed during the development of morphine tolerance-dependence in rodents (Redmond and Krystal, 1984). Striatum contains a high density of both dopaminergic nerve terminals (Dray, 1979) and enkephalinergic neurons (Khatchaturian et al., 1985) with a high density of receptors for both neurotransmitters (Stoof and Kebabian, 1984; Tempel and Zukin, 1988). In mice, after chronic morphine exposure, the number of striatal dopamine $D_2$ receptors decreased with no changes in their affinity whereas the number and affinity of dopamine $D_1$ receptor remain unchanged (Navarro et al., 1992). In addition, in these animals, naloxone is unable to reverse the morphine-induced decrease in dopamine $D_2$ receptor activity. This decreased number of dopamine $D_2$ receptors is accompanied by a corresponding increase in cAMP concentrations, concordant with the well-known negative effect of dopamine $D_2$ receptors on adenylyl cyclase activity. It has been reported that SKF 38393, a dopamine $D_1$ receptor agonist exacerbated the withdrawal signs of chronically morphine-treated rats (Ben-Sreti et al., 1983; Verma and Kulkarni, 1995) and that the dopamine $D_2$ receptor agonist bromocriptine exert opposite influences

on the development of morphine tolerance and dependence (Verma and Kulkarni, 1995). Verma and Kulkarni (1995) reported that the administration of bromocriptine enhance the ability of non-competitive NMDA receptor antagonists to attenuate the development of morphine tolerance and dependence while SKF 38393 failed to do so. Thus, it was anticipated that when dopamine $D_1$ receptors were blocked by SCH 23390, apomorphine would stimulate dopamine $D_2$ receptors, thereby resulting in the potentiation of the suppressive action of dextromethorphan.

The present data show that administration of the dopamine $D_1$ receptor antagonist SCH 23390 itself reduced withdrawal signs. Although SCH 23390 is thought to be a selective dopamine $D_1$ receptor antagonist, it binds with high affinity to 5-$HT_2$ receptors in the brain (Bischoff et al., 1986) and antagonizes 5-$HT_2$ receptor activation both centrally and peripherally (Bijak and Smialowski, 1989). The administration of 5-$HT_2$ receptor antagonists attenuates naloxone-precipitated withdrawal (Neal and Sparber, 1986; Kleven and Sparber, 1989). This may account for the reduction of withdrawal jumping, paw-shakes and grooming by SCH 23390.

Interactions between sigma receptors and glutamatergic system were also observed in relation to the release of dopamine (Debonnel and De Montigny, 1996). Sigma-1 receptors in rat striatum regulate NMDA-stimulated dopamine release via a presynaptic mechanism (Gonzalez-Alvear and Werling, 1995). Since dextromethorphan has high affinity for sigma receptors and sigma receptors activity appeared to contribute to morphine withdrawal signs in morphine-dependent mice as haloperidol inhibited that naloxone-induced withdrawal while spiperon did not (Kreeger et al., 1995), it may be that dextromethorphan suppresses withdrawal signs by such a mechanism.

In summary, the present experiment demonstrate that dextromethorphan attenuates signs of naloxone-induced withdrawal syndrome in morphine-dependent mice. Furthermore, the results of this study show that apomorphine antagonizes the suppression of withdrawal signs produced by dextromethorphan. Since the dopamine $D_1$ receptor antagonist SCH 23390 but not dopamine $D_2$ receptor antagonist sulpiride nor peripheral dopamine receptor antagonist domperidone reverses the effect of apomorphine, it may be concluded that the central dopamine $D_1$ receptor mechanisms are involved in the modulation of the suppressive action of dextromethorphan on naloxone-induced withdrawal signs.

## References


Aanonsen, L.M., Wilcox, G.L., 1987. Nociceptive action of excitatory amino acids in the mouse: effect of spinally administered opioids, phencyclidine and sigma agonists. J. Pharmacol. Exp. Ther. 243, 9–19.

Aghajanian, G.K., Kogan, J.H., Moghaddam, B., 1994. Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus: an in vivo microdialysis study. Brain Res. 636, 126–130.

Ben-Sreti, M.M., Gonzalez, J.P., Sewell, R.D.E., 1983. The influence of selective dopaminergic and cholinergic agonists and antagonists on precipitated opiate abstinence. Alcohol Alcohol. 18, 353–357.

Bhargava, H.N., 1983. Binding of $^3$H-spiroperidol to striatal membranes of rats treated chronically with morphine: influence of Pro-Leu-Gly–$NH_2$ and cyclo (Leu-Gly). Neuropharmacology 22, 1357–1361.

Bielarczyk, H., Lysiak, W., Szutowicz, A., 1986. Synthesis of glutamate and aspartate in rat brain synaptosomes. Acta Biochem. Pol. 33, 239–251.

Bijak, M., Smialowski, A., 1989. Serotonin receptor blocking effect of SCH 23390. Pharmacol. Biochem. Behav. 32, 585–587.

Bischoff, S., Heinnch, M., Soontag, J.M., Krauss, J., 1986. The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-$HT_2$) receptors. Eur. J. Pharmacol. 129, 367–370.

Cai, Y.C., Ma, L., Fan, G.H., Zhao, J., Jiang, L.Z., Pei, G., 1997. Activation of *N*-methyl-D-aspartate receptor attenuates acute responsiveness of delta-opioid receptors. Mol. Pharmacol. 51, 583–587.

Chen, Y., Yu, L., 1994. Differential regulation by cAMP-dependent protein kinase and protein kinase C of the μ opioid receptor coupling to a G protein activated K channel. J. Biol. Chem. 269, 7839–7842.

Church, J., Jones, M.G., Davies, S.N., Lodge, D., 1989. Antitussive agents as *N*-methylaspartate antagonists: further studies. Can. J. Physiol. Pharmacol. 67, 561–567.

Clineschmidt, B.V., Martin, G.E., Bunting, P.R., Papp, N.L., 1982. Central sympathomimetic activity of (+)-5-methyl-10,11- dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties. Drug Dev. Res. 2, 135–145.

Debonnel, G., De Montigny, C., 1996. Modulation of NMDA and dopaminergic neurotransmissions by sigma ligands: possible implications for the treatment of psychiatric disorders. Life Sci. 58, 721–734.

Di Chiara, G., Imperato, A., 1988. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244, 1067–1080.

Dray, A., 1979. The striatum and substantia nigra: a commentary on their relationship. Neuroscience 4, 1405–1439.

Elliott, K., Brodsky, M., Hyanansky, A.D., Foley, K.M., Inturrisi, C.E., 1995a. Dextromethorphan shows efficacy in experimental pain (nociception) and opioid tolerance. Neurology 45, S66–S68.

Elliott, K., Brodsky, M., Hyanansky, A.D., Foley, K.M., Inturrisi, C.E., 1995b. Dextromethorphan suppresses both formalin-induced nociceptive behavior and the formalin-induced increase in spinal cord c-fos mRNA. Pain 61, 401–409.

Feng, Y.Z., Zhang, T., Rockhold, R.W., Ho, I.K., 1995. Increased locus coeruleus glutamate levels are associated with naloxone-precipitated withdrawal from butorphanol in the rat. Neurochem. Res. 20, 745–751.

Gonzalez-Alvear, G.M., Werling, L.L., 1995. $\sigma_1$ Receptors in rat striatum regulate NMDA-stimulated [$^3$H]dopamine release via a presynaptic mechanism. Eur. J. Pharmacol. 294, 713–719.

Guaza, C., Torrellas, A., Borell, S., Borell, J., 1980. The effects of acute and chronic administration of morphine on the turnover of brain and adrenal catecholamines in rats. Psychopharmacology 68, 43–49.

Huang, N.K., Tseng, C.J., Wong, C.S., Tung, C.S., 1997. Effects of acute and chronic morphine on DOPAC and glutamate at subcortical DA terminals in awake rats. Pharmacol. Biochem. Behav. 56, 363–371.

Imperato, A., Scrocco, M.G., Bacchi, S., Angelucci, L., 1990. NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. Eur. J. Pharmacol. 187, 555–556.

Jin, S., Fredholm, B.B., 1994. Role of NMDA, AMPA and kainate receptors in mediating glutamate- and 4-AP-induced dopamine and acetylcholine release from rat striatal slices. Neuropharmacology 331, 1039–1048.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.

Khatchaturian, H., Lewis, M.E., Schafer, M.K.H., Watson, S.J., 1985.

Anatomy of the CNS opioid systems. Trends Pharmacol. Sci. 7, 111–119.

Kleven, M.S., Sparber, S.B., 1989. Modification of the behavioral effects of 3-isobutyl-1-methylxanthine by serotonin in the expression of opiate withdrawal. Psychopharmacology 98, 231–235.

Koyuncuoğlu, H., Güngör, M., Sağduyu, H., Eroğlu, L., 1974. The antagonistic effects of aspartic acid on some effects of morphine on rats. Eur. J. Pharmacol. 27, 148–150.

Koyuncuoğlu, H., Güngör, M., Eroğlu, L., Sağduyu, H., 1976. The alterations of some effects of morphine in rats by glutamic acid and glycine. Med. Bull. Istanbul 9, 56–64.

Koyuncuoğlu, H., Keyer-Uysal, M., Berkman, K., Güngör, M., Genç, E., 1979. The relationship between morphine, aspartic acid and L-asparaginase in rats. Eur. J. Pharmacol. 60, 369–372.

Koyuncuoğlu, H., Güngör, M., Sağduyu, H., Aricioğlu, F., 1990. Suppression by ketamine and dextromethorphan of precipitated abstinence syndrome in rats. Pharmacol. Biochem. Behav. 35, 829–832.

Koyuncuoğlu, H., Dizdar, Y., Aricioğlu, F., Sayin, Ü., 1992. Effects of MK-801 on morphine physical dependence: attenuation and intensification. Pharmacol. Biochem. Behav. 43, 487–490.

Krebs, M.O., Desce, J.M., Kemel, M.L., Gauchy, C., Godeheu, G., Cheramy, A., Glowinski, J., 1991. Glutamate control of dopamine release in the rat striatum: evidence for presynaptic *N*-methyl-D-aspartate receptors on dopaminergic nerve terminals. J. Neurochem. 56, 81–85.

Kreeger, J.S., Yukhananov, R.Y., Larson, A.A., 1995. Altered *N*-methyl-D-aspartate (NMDA) activity in the mouse spinal cord following morphine is mediated by sigma activity. Brain Res. 672, 83–88.

Mao, J., Price, D.D., Mayer, D.J., 1994. Thermal hyperalgesia in association with the development of morphine tolerance in rats: roles of excitatory amino acid receptors and protein kinase C. J. Neurosci. 14, 2301–2312.

Mao, J., Price, D.D., Mayer, D.J., 1995. Mechanisms of hyperalgesia and opiate tolerance: a current view of their possible interactions. Pain 62, 259–274.

Mao, J., Price, D.D., Caruso, F.S., Mayer, D.J., 1996. Oral administration of dextromethorphan prevents the development of morphine tolerance and dependence in rats. Pain 67, 361–368.

Marek, P., Ben-Eliyahu, S., Gold, M., Liebeskind, J.C., 1991. Excitatory amino acid antagonists (kynurennic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res. 547, 77–81.

Marshall, I., Grahame-Smith, D.G., 1971. Evidence against a role of brain 5-hydroxytryptamine in the development of physical dependence upon morphine in mice. J. Pharmacol. Exp. Ther. 179, 634–641.

Martin, G., Nie, Z., Siggins, G.R., 1997. mu-Opioid receptors modulate NMDA receptor-mediated responses in nucleus accumbens neurons. J. Neurosci. 17, 11–22.

Mayer, D.J., Mao, J., Price, D.D., 1995. The development of morphine tolerance and dependence is associated with translocation of protein kinase C. Pain 61, 365–374.

Navarro, M., Fernández-Ruiz, J.J., Fodriguez De Fonseca, F., Hernández, M.L., Cebeira, M., Ramos, J.A., 1992. Modifications of striatal D2 dopaminergic postsynaptic sensitivity during development of morphine tolerance-dependence in mice. Pharmacol. Biochem. Behav. 43, 603–608.

Neal, B.S., Sparber, S.B., 1986. Mianserin attenuates naloxone-precipitated withdrawal in rats acutely or chronically dependent upon morphine. J. Pharmacol. Exp. Ther. 236, 157–165.

Redmond, D.E., Krystal, J.H., 1984. Multiple mechanisms of withdrawal from opioid drugs. Annu. Rev. Neurosci. 7, 443–478.

Ritzmann, R.F., Lee, J.M., Fields, J.Z., 1982. Modification of morphine-induced changes in striatal $^{3}$H-spiroperidol binding and stereotype behavior by cyclo (Leu-Gly). Life Sci. 30, 1573–1580.

Singh, N.A., Bush, L.G., Gibb, J.W., Hanson, G.R., 1992. Role of *N*-methyl-D-aspartate receptors in dopamine D1, but not D2, mediated changes in striatal and accumbens neurotensin systems. Brain Res. 571, 260–264.

Spanagel, R., Herz, A., Shippenberg, T.S., 1990. The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. J. Neurochem. 55, 1734–1740.

Stoof, J.C., Kebabian, J.W., 1984. Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci. 35, 2281–2296.

Tempel, A., Zukin, R.S., 1988. Neuroanatomical patterns of the mu-, delta- and kappa-opioid receptors of rat brain as determined by quantitative in vitro autoradiography. Proc. Natl. Acad. Sci. U.S.A. 84, 4308–4312.

Tokuyama, S., Wakabayashi, H., Ho, I.K., 1996. Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome. Eur. J. Pharmacol. 295, 123–129.

Tortella, F.C., Pellicano, M., Bowery, N.G., 1989. Dextromethorphan and neuromodulation: old drug coughs up new activities. Trends Pharmacol. Sci. 10, 501–507.

Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85–87.

Urwyler, S., Tabakoff, B., 1981. Stimulation of dopamine synthesis and release by morphine and D-A1A2 D-LEU5 enkephalin in the mouse striatum in vivo. Life Sci. 28, 2277–2286.

Verma, A., Kulkarni, S.K., 1995. Role of $D_1/D_2$ dopamine and *N*-methyl-D-aspartate (NMDA) receptors in morphine tolerance and dependence in mice. Eur. Neuropsychopharmacol. 5, 81–87.

Wong, B.Y., Coulter, D.A., Choi, D.W., Prince, D.A., 1988. Dextrophan and dextromethorphan, common antitussives, are antiepileptic and antagonize *N*-methyl-D-aspartate in brain slices. Neurosci. Lett. 85, 261–266.

Zarrindast, M.R., Abolfathi-Araghi, F., 1992. Effects of bupropion on core body temperature of mice. Psychopharmacology 106, 248–252.

Zarrindast, M.R., Farzin, D., 1996. Nicotine attenuates naloxone-induced jumping behaviour in morphine-dependent mice. Eur. J. Pharmacol. 298, 1–6.

Zarrindast, M.R., Tabatabai, S.A., 1992. Involvement of dopamine receptor subtypes in mouse thermoregulation. Psychopharmacolgy 107, 341–346.

Zarrindast, M.R., Nami, A.B., Farzin, D., 1996. Nicotine potentiates morphine antinociception: a possible cholinergic mechanism. Eur. Neuropsychopharmacol. 6, 127–133.

Zhang, T., Feng, Y.Z., Rockhold, R.W., Ho, I.K., 1994. Naloxone-precipitated morphine withdrawal increase pontine glutamate levels in the rat. Life Sci. 55, 25–31.