ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 203–207

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Effects of adrenoceptor agonists and antagonists on morphine-induced Straub tail in mice

Mohammad-Reza Zarrindast*, Banafsheh Ramezani-Tehrani, Maryam Ghadimi

Department of Pharmacology, Medical School, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran

Received 25 June 2001; received in revised form 17 October 2001; accepted 6 November 2001

Abstract

Straub-tail behavior was induced by subcutaneous injection of different doses (10–60 mg/kg) of morphine to mice. The maximum response was obtained with 20–40 mg/kg of the drug. The response induced by morphine (40 mg/kg) was decreased by intraperitoneal administration of different doses of clonidine (0.05–0.1 mg/kg). Pretreatment of animals with yohimbine (1–4 mg/kg ip) reversed the inhibitory action of clonidine. Yohimbine did not elicit any response by itself. Administration of prazosin (0.25, 0.5, and 1 mg/kg) reduced the morphine response. The combination of prazosin with yohimbine (1 mg/kg), but not with clonidine (0.05 mg/kg), caused a potentiated inhibition of the morphine effect. Phenylephrine (2–6 mg/kg ip) did not elicit any effect by itself and also did not alter the response induced by morphine or morphine plus clonidine. Dobutamine (2.5–10 mg/kg ip), atenolol (2.5–10 mg/kg ip), salbutamol (2.5–10 mg/kg ip), and propranolol (2.5–10 mg/kg ip) did not alter morphine-induced Straub-tail behavior in mice. In conclusion, activation of α_2-adrenergic pathways contributes to morphine-induced Straub tail, while α_1- and β_2-adrenergic may not be involved in this phenomenon. © 2002 Published by Elsevier Science Inc.

Keywords: Morphine adrenoceptor agonists; Adrenoceptor antagonists; Mice

1. Introduction

Opioids elicit their responses through different opioid receptor subtypes (Mann et al., 1988; Millan, 1990; Pleuvry, 1983; Traynor, 1989). Three opioid receptors, μ-, ∂-, and, κ-opioid receptors, have been cloned (Chen et al., 1993; Evans et al., 1992; Kieffer et al., 1992; Liang et al., 1995; Yasuda et al., 1993). At least two μ-receptor subtypes have also been proposed, i.e., μ_1- and μ_2-receptors (Hahn et al., 1982; Pasternak and Wood, 1986; Pasternak et al., 1980; Wolozin and Pasternak, 1981).

Morphine, a prototype agonist for the opioid receptors, produces different types of behavior. The drug induces analgesia and catalepsy through μ_1-opioid receptors, while μ_2-opioid receptors are involved in respiratory depression, physical dependence, gastrointestinal effects, and dopamine turnover (Pasternak, 1988). In addition, morphine has a range of other effects such as hypothermia (Zarrindast et al., 1994), nausea, vomiting, and constipation (Traynor, 1989). It contracts the sacrococcygeus muscle in mice, which results in erection of the tail (Straub tail) (Bilbey et al., 1960). The behavior may be used as a main determinant in the testing of opioid activity in mice (De Aceto et al., 1969), therefore it is important to clarify its mechanism(s). Straub tail, one of the classical effects of morphine, has been proposed to be mediated through the central μ_2-opioid receptor subtype (Nath et al., 1994). It has been suggested that σ-opioid receptors are not involved in the opioid agonist-induced Straub-tail reaction (Murray and Cowan, 1990) and κ-opioid receptor activation even reduces morphine-induced Straub tail (Narita et al., 1994). However, dopaminergic (Hasegawa et al., 1990b; Zarrindast et al., 1993), serotonergic (Adell et al., 1989; Hasegawa et al., 1990a), and nicotinergic mechainsms have also been shown to be involved in the behavior. The involvement of adrenergic system in the behavior has not been shown. There are several reports indicating interactions between opioid responses and adrenergic system (Gear et al., 1995; Ossipov et al., 1990; Paul and Tran, 1995). Cross-tolerance between

* Corresponding author. Tel.: +98-21-611-2802; fax: +98-21-640-4377/2569.

E-mail address: zarinmr@ams.ac.ir (M.-R. Zarrindast).

0091-3057/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
PII: S0091-3057(01)00749-3

the μ-opioid agonist morphine and adrenergic receptors has been also demonstrated (Kalso et al., 1993; Paul and Tran, 1995). There are also several reports indicating interactions between adrenoceptor mechanisms with opioid-induced muscular rigidity (Jerussi et al., 1987; Weinger et al., 1995) or opioid-induced Straub-tail behavior (Blumberg and Slovak, 1982; Kameyama et al., 1978); the exact mechanism(s) of interaction has not been evaluated. In the present study, an attempt has been made to determine the possible involvement of adrenoceptor mechanism(s) in the Straub-tail response induced by morphine in mice.

2. Method

2.1. Animals

Male albino mice (20–25 g) were used in these experiments. They were housed 10 per cage, in colony room 12/12 h light/dark cycle at 22±2 °C. The animals had free access to food and tap water except during the time of experiments. Each animal was used only once.

2.2. Straub-tail measurement

Mice were placed individually in a glass cylinder (30 cm wide, 50 cm long) and allowed to habituate for 30 min before drug injection. Immediately after drug administration, each animal was placed into the cylinder and the response was recorded by direct observation. The subject's behavior was sampled every 15 s and the total accumulated scores over the 30-min period was used to assess the response, except for Fig. 1, wherein the response of morphine was recorded every 15 min for 60 min. The Straub tail was graded according to modified numerical ratings (Kameyama et al., 1978) as follows: 0 = 0° (no response), 1 = 1–45°, 2 = 46–90° above the horizontal table. The test was initiated immediately after drug administration. Data are shown as the mean ± S.E.M. of at least nine mice.

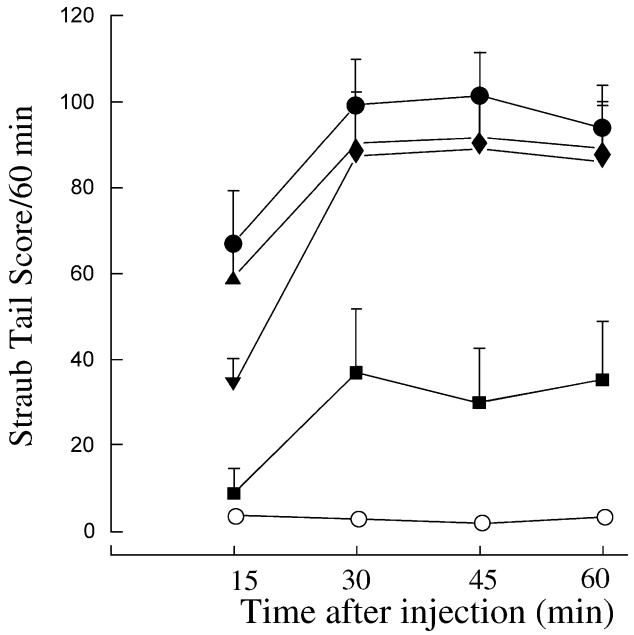

Fig. 1. Straub tail induced by morphine in mice. Animals were injected subcutaneously either with saline (○; 10 ml/kg) or with different doses of morphine 10 (■), 20 (▼), 40 (▲), and 60 (●) mg/kg. Total Straub-tail scores were recorded every 15 min after morphine administration for a period of 60 min. Each point is the mean ± S.E.M. of nine animals.

2.3. Drugs

The following drugs were used: morphine sulfate (MacFarlan, Smith England); propranolol (ICI, England); phenylephrine hydrochloride, prazosin hydrochloride, clonidine hydrochloride, and yohimbine (Sigma, Poole, England); dobutamine (Lilly, Germany); salbutamol and atenolol (Darupakhsh, Iran). All drugs were obtained as powders and were dissolved in 0.9% sterile physiological saline. Morphine was given subcutaneously and the other drugs were given intraperitoneally and in a volume of 10 ml/kg and were prepared immediately before use. The control groups received saline. Morphine was injected 15 min after the adrenoceptor agonists and 30 min after the antagonists.

2.4. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test were used to evaluate significance of the results obtained. Differences of $P < .05$ were considered significant.

3. Results

3.1. Effects of morphine on Straub tail in mice

Fig. 1 indicates the time-course effect of morphine. Different doses of morphine (10, 20, 40, and 60 mg/kg) were administered subcutaneously to mice, and Straub-tail scores were recorded for periods of 15 min at 15, 30, 45, and 60 min after the drug administration [Treatment: $F(4,40) = 12.5$, $P < .001$; Time: $F(3,120) = 50.5$, $P < .001$; Treatment × Time: $F(12,120) = 4.5$, $P < .001$]. Analysis showed that morphine induced Straub-tail behavior. The maximum response was obtained with 20–40 mg/kg of morphine at 30 min after the drug injection.

3.2. Effects of α-adrenoceptor agonists on morphine-induced Straub tail

Administration of the α_1-adrenoceptor agonist, phenylephrine (2, 4, and 6 mg/kg ip) or the α_2-adrenoceptor agonist, clonidine (0.075 mg/kg ip), 15 min prior to morphine (40 mg/kg sc) altered the morphine response [one-way ANOVA; $F(4,40) = 4.4$, $P < .01$]. Further analysis

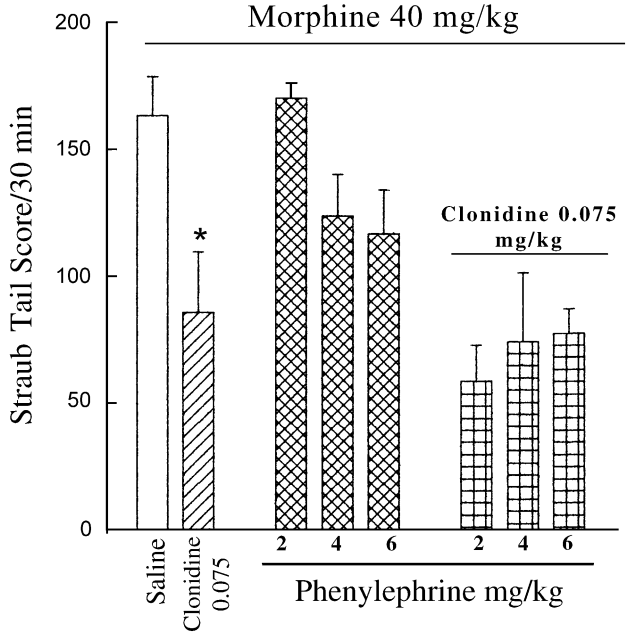

Fig. 2. Effect of α-adrenoceptor agonists on morphine-induced Straub tail in mice. Intraperitoneally, saline (10 ml/kg), clonidine (0.075 mg/kg), phenylephrine (2, 4, and 6 mg/kg), or clonidine plus phenylephrine were administered 15 min before morphine (40 mg/kg sc) injection. The morphine response was recorded for 30 min. Each point is the mean ± S.E.M. of nine animals. * $P<.05$, different from saline control group.

showed that clonidine, but not phenylephrine, reduced Straub tail induced by morphine. Combination of phenylephrine with clonidine did not alter the clonidine response [one-way ANOVA; $F(3,32)=0.32$, $P>.05$] (Fig. 2).

3.3. Effects of α-adrenoceptor antagonists with or without clonidine on morphine-induced Straub tail

Two-way ANOVA showed that the combination of the α_1-adrenoceptor antagonist, prazosin (0.25, 0.5, and 1 mg/kg) [$F(3,64)=38.4$, $P<.0001$] with yohimbine (1 mg/kg) [$F(1,64)=15.5$, $P<.001$] elicited interaction [$F(3,64)=5.3$, $P<.01$]. Further analysis indicates that the combinations of two drugs induced a higher inhibitory effect on Straub tail induced by morphine. Prazosin alone also decreased the morphine effect. The analysis showed that the same doses of prazosin [$F(3,64)=12.8$, $P<.0001$] in the combination with clonidine (0.01 mg/kg) [$F(1,64)=34.1$, $P<.0001$] did not show interaction [$F(3,64)=0.43$, $P>.05$] (Fig. 3).

It should be considered that the response of clonidine (0.1 mg/kg) was decreased by different doses of the α_2-adrenoceptor antagonist, yohimbine (1, 2, and 4 mg/kg) [one-way ANOVA; $F(3,32)=7.6$, $P<.001$]. These doses of yohimbine (1, 2, and 4 mg/kg ip) did not alter the morphine effect [one-way ANOVA; $F(3,32)=3.1$, $P>.05$] (data not shown).

3.4. Effects of α-adrenoceptor agonists and antagonists on morphine-induced Straub tail

One-way ANOVA showed that intraperitoneal administration of different doses of the β_1-adrenoceptor agonist, dobutamine (2.5, 5, and 10 mg/kg) [$F(3,32)=0.2$, $P>.05$], the β_1-adrenoceptor antagonist, atenolol (2.5, 5, and 10 mg/kg) [$F(3,32)=2.4$, $P>0.05$], the β_2-adrenoceptor agonist, salbutamol (2.5, 5, and 10 mg/kg) [$F(3,32)=1.5$, $P>.05$], or the nonselective β-adrenoceptor antagonist, propranolol (2.5, 5, and 10 mg/kg) [$F(3,32)=1.3$, $P>.05$] 15 min before morphine administration (40 mg/kg sc) did not alter the morphine response (Fig. 4).

4. Discussion

In the present study, the effects of adrenoceptor agonists and antagonists on morphine-induced Straub tail have been studied.

The present data indicated that morphine induced a dose-dependent Straub tail behavior, with a maximum response by 20–40 mg/kg 30 min after the drug injection. Adrenoceptor mechanisms have been implicated in Straub tail induced by morphine (Blumberg and Slovak, 1982; Kameyama et al.,

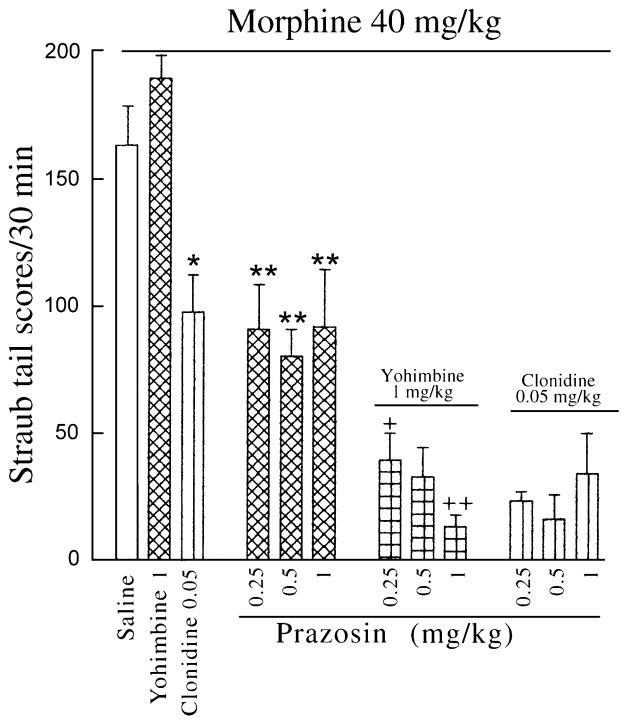

Fig. 3. Effect of prazosin, prazosin plus yohimbine, or prazosin plus clonidine on morphine-induced Straub tail. Saline (10 ml/kg ip), yohimbine (1 mg/kg ip), prazosin (0.25, 0.5, and 1 mg/kg) were injected 30 min and clonidine 15 min before morphine injection (40 mg/kg sc). Each point is mean ± S.E.M. of nine animals. * $P<.05$, ** $P<.01$ different from saline control group. $^{+}P<.05$, $^{++}P<.01$ different from respective yohimbine control group.

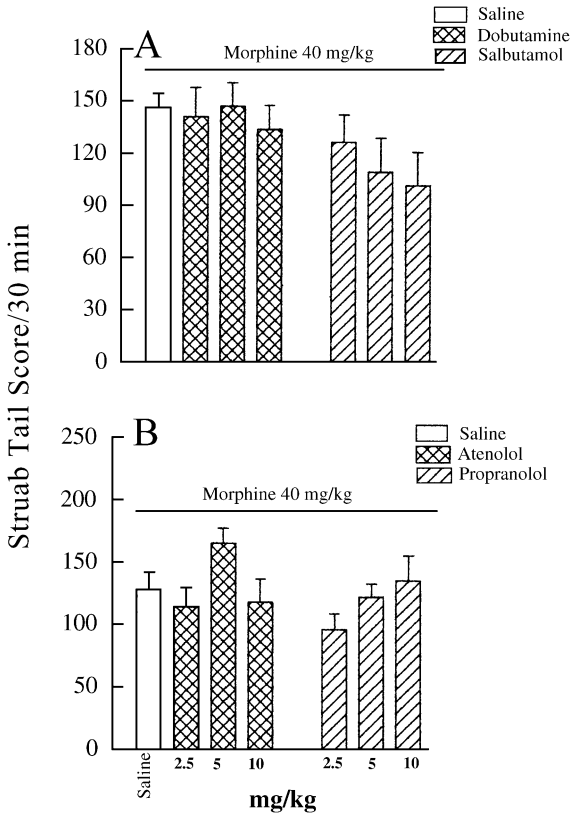

Fig. 4. Effect of β-adrenoceptor agonists on morphine-induced Straub tail. Animals were treated intraperitoneally either with saline or with different doses of dobutamine (2.5–10 mg/kg), atenolol (2.5–10 mg/kg), salbutamol (2.5–10 mg/kg), or propranolol (2.5–10 mg/kg) 15 min prior to morphine (40 mg/kg sc) injection. Straub-tail score was recorded for a period of 30 min. Each point is mean ± S.E.M. of nine animals.

1978), but interaction of clonidine with morphine-induced Straub tail has not been shown. The present results showed that Straub-tail episodes induced by morphine was decreased by the α_2-adrenoceptor agonist, clonidine, which may indicate involvement of adrenoceptor in the morphine-induced Straub-tail response. These results may be in agreement with others indicating an interaction between opioid receptor mechanism(s) and the adrenergic receptor system (Gear et al., 1995; Ossipov et al., 1990; Paul and Tran, 1995). The α_2-adrenoceptor antagonist, yohimbine, did not alter morphine-induced Straub tail by itself. Since the response induced by clonidine was decreased by yohimbine, but not by the α_1-adrenoceptor antagonist prazosin, it is concluded that α_2-adrenoceptor mechanisms are involved in the inhibition of morphine-induced Straub tail by clonidine. The drug has been shown to reverse the rigidity induced by morphine. Therefore, it is proposed that pharmacological reversal of opioid-induced rigidity is accomplished by a reduction of sympathetic tone which is selectively elicited by α_2-ader--aderenoceptor activation (see Jerussi et al., 1987). Therefore, the response of clonidine may be mediated through presynaptic receptor activation, and in turn, inhibition of noradrenaline release.

Our present data show that the α_1-adrenoceptor agonist, phenylephrine (2–6 mg/kg ip), neither altered morphine-induced Straub-tail response, nor altered the inhibition induced by clonidine. The drug also did not elicit any response by itself. However, prazosin (0.25–1 mg/kg) decreases the morphine effect. In regard to the ineffectiveness of phenylephrine and the potentiation between prazosin and yohimbine in blocking morphine effect, it may be postulated that α_2-adrenoceptors but not α_1-adrenoceptors, are involved in morphine-induced Straub tail. This can be supported since the existence of prazosin-sensitive α_2-adrenoceptors has been postulated. Such receptors have been characterized by molecular cloning studies and have been designated α_2B receptors (Harrison et al., 1991), but to date, they have not been functionally defined. Furthermore, the combination of yohimbine (1 mg/kg) with different doses of prazosin (0.25–1 mg/kg) induced a potentiated inhibition of the morphine response. One may conclude that either the response of prazosin is induced through postsynaptic α_2B receptors and/or α_1-adrenoceptor mechanism(s).

There are also reports indicating that both $5HT_2$ and α_1 receptors elicit a rise in Ca^{2+} (Tani et al., 1992). Morphine also elicits an increase in the intracellular free Ca^{2+} concentration (Jin et al., 1994). One explanation may be that increases in Ca^{2+} are involved in Straub tail and that prazosin inhibits morphine-induced Straub tail through a decrease in Ca^{2+} levels.

In the present study, neither the β-adrenoceptor agonists, dobutamine and salbutamol, nor the β-adrenoceptor antagonists, atenolol and propranolol, altered the morphine response. Therefore, β-adrenoceptor mechanisms are not involved in the morphine-induced Straub tail. This is the first study to explore the possible Straub tail. Furthermore, our data support the activation of α_2-adrenoceptors but not α_1-adrenoceptors as the probable mechanism involved in this effect, elucidating receptor mechanisms through which the adrenergic system contributes to the Straub-tail phenomenon.

References

Adell A, Sarna GS, Hutson PH, Curzon G. An in vivo dialysis and behavioural study of the release of 5-HT by *p*-chloroamphetamine in reserpine-treated rats. Br J Pharmacol 1989;97:206–12.

Bilbey DJL, Salem H, Grossman MH. The anatomical basis of the Straub phenomenon. Br J Pharmacol 1960;15:540–3.

Blumberg H, Slovak DM. Effects of yohimbine and naltrexone in counteractions of the morphine Straub tail and the amphetamine-potentiated morphine Straub tail in mice. Life Sci 1982;30:2115–21.

Chen Y, Mestek A, Liu J, Hurley J, Yu L. Molecular cloning and functional expression of μ-opioid receptor from rat brain. Mol Pharmacol 1993; 44:8–12.

De Aceto MD, Mckean DB, Pearl J. Effects of opiates and opiate antagonists on the Straub tail reaction in mice. Br J Pharmacol 1969;36: 239–55.

Evans CJ, Keith D, Morrison H, Magendzo K, Edwards R. Cloning of a delta receptor by functional expression. Science 1992;258:1952–5.

Gear RW, Gordon NC, Heler PH, Levine JD. Enhancement of morphine

analgesia by the alpha 2-adrenergic antagonist yohimbine. Neuroscience 1995;66:5–8.

Hahn EF, Carroll-Buatti M, Pasternik GW. Irreversible opiate agonists and antagonists: the 14-hydroxydihydromorphinone azines. J Neurosci 1982; 5:572–6.

Harrison JK, Pearson WR, Lynch KR. Molecular characterization of α-1 and α-2 adrenoceptors. Trends Pharmacol Sci 1991;12:62–7.

Hasegawa Y, Kurachi M, Okuyama S, Araki H, Otomo S. $5HT_3$ receptor antagonist inhibit the response of κ opioid receptors in the morphine-reduced Straub tail. Eur J Pharmacol 1990a;190:399–401.

Hasegawa Y, Kurachi M, Otomo S. Dopamine D_2 receptors and spinal cord excitation in mice. Eur J Pharmacol 1990b;184:207–12.

Jerussi TP, Capacchione JF, Benvenga MJ. Reversal of opioid-induced muscular rigidity in rats: evidence for alpha-2 adrenergic involvement. Pharmacol, Biochem Behav 1987;28:283–9.

Jin W, Lee NM, Loh HH, Thayer SA. Opioids mobilize calcium from inositol 1,4,5-triphosphate-sensitive stops in NG 108-15 cells. J Neurosci 1994; 14:1920–9.

Kalso EA, Sullivan AF, McQuay HJ, Dickenson AH, Roques BP. Cross-tolerance between mu opioid and alpha-2 adrenergic receptors, but not between mu and delta opioid receptors in the spinal cord of the rat. J Pharmacol Exp Ther 1993;265:551–8.

Kameyama T, Ukai M, Nabeshima T. Effects of catecholaminergic and tryptaminergic agents on the morphine-induced Straub tail reaction. Jpn J Pharmacol 1978;28:249–57.

Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG. The delta-opioid receptor: isolation of cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci USA 1992;89:12048–52.

Liang Y, Mestek A, Yu L, Carr LG. Cloning and characterization of the promoter region of the mouse μ-opioid receptor gene. Brain Res 1995; 679:82–8.

Mann PE, Arjune D, Romero M-T, Pasternak GW, Hahn EF, Bodnar RJ. Differential sensitivity of opioid-induced feeding to naloxone and naloxonazine. Psychopharmacology 1988;94:336–41.

Millan MJ. κ-Opioid receptors and analgesia. Trends Pharmacol Sci 1990; 11:70–6.

Murray CW, Cowan A. [D-Pen^2, D-Pen^5] enkephalin, the standard delta opioid agonist, induces morphine-like behaviors in mice. Psychopharmacology 1990;102:425–6.

Narita M, Suzuki T, Misawa M, Nagase H. Antagonism of the morphine-induced Straub-tail reaction by κ-opioid receptor activation in mice. Psychopharmacology 1994;110:254–6.

Nath C, Gupta MB, Patnaik GK, Dhawan KN. Morphine-induced Straub tail response: mediated by central μ_2-opioid receptor. Eur J Pharmacol 1994;263:203–5.

Ossipov MH, Harris S, Lloyd P, Messineo E. An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combination of clonidine and opiates. J Pharmacol Exp Ther 1990; 255:1107–16.

Pasternak GW. Multiple morphine and enkephalin receptors and relief of pain. J Am Med Assoc 1988;259:1362–7.

Pasternak GW, Wood PL. Multiple mu opiate receptors. Life Sci 1986; 38:1889–98.

Pasternak GW, Childers SR, Snyder SH. Opiate analgesia: evidence for mediation by a subpopulation of opiate receptors. Science 1980;208: 514–6.

Paul D, Tran JG. Differential cross-tolerance between analgesia produced by alpha 2-adrenoceptor agonists and receptor subtype selective opioid treatments. Eur J Pharmacol 1995;272:111–4.

Pleuvry BJ. An update on opioid receptors. Br J Anaesth 1983;55:143S–6S.

Tani A, Yoshihara Y, Mori K. Increase in cytoplasmic free Ca2+ elicited by noradrenaline and serotonin in cultured local interneurons of mouse olfactory bulb. Neuroscience 1992;49:193–9.

Traynor J. Subtypes of the κ-opioid receptor: fact or fiction? Trends Pharmacol Sci 1989;10:52–3.

Weinger MB, Chen DY, Lin T, Lau C, Koob GF, Smith NT. A role for CNS α-2 adrenergic receptors in opiate-induced muscle rigidity in the rat. Brain Res 1995;669:10–8.

Wolozin BL, Pasternak GW. Classification of multiple morphine and enkephalin binding sites in the central nervous system. Proc Natl Acad Sci USA 1981;78:6181–5.

Yasuda K, Raynor K, Kong H, Breder C, Takeda J, Reisine T, Bell GI. Cloning and functional comparison of κ and δ opioid receptors from mouse brain. Proc Natl Acad Sci USA 1993;90:3740–6736.

Zarrindast MR, Bayat A, Shafaghi B. Involvement of dopaminergic receptor subtypes in Straub tail behaviour in mice. Gen Pharmacol 1993; 24:127–30.

Zarrindast MR, Vahedy A, Heidari MR, Ghazi Khansari MR. On the mechanism(s) of morphine-induced hypothermia. J Psychopharmacol 1994; 8:222–6.