ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 189–193

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Potentiation of κ-opioid receptor agonist-induced analgesia and hypothermia by fluoxetine

Kumar V.S. Nemmani, Srinivas Gullapalli, Poduri Ramarao*

Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Phase-X, S.A.S. Nagar, 160 062 (Pb), India

Received 5 July 2000; received in revised form 31 January 2001; accepted 19 February 2001

Abstract

The effect of fluoxetine, a selective 5-HT reuptake inhibitor on the analgesic and hypothermic response of *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate (U-50,488H) and (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzo[*b*] thiophene-4-acetamide (PD 117302), κ-opioid receptor agonists, was determined in female Sprague–Dawley rats using the tail-flick method and telethermometer, respectively. Intraperitoneal injections of U-50,488H (U50) and PD 117302 (PD117) produced a dose-dependent analgesic and hypothermic response. Fluoxetine (10 mg/kg, ip) by itself did not produce an analgesic response. The analgesic response to U50 (10, 20, and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip) was potentiated by fluoxetine injected intraperitoneally 60 min prior to the injection of κ-opioid agonists. Similarly, the hypothermic response of U50 (20 and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip) was potentiated by fluoxetine. The results indicate that selective κ-opioid receptor agonists-induced analgesia and hypothermia is potentiated by fluoxetine, suggesting the role of extracellular 5-HT in the κ-opioid receptor-mediated analgesia and hypothermia.

Keywords: κ-opioid receptor agonist; U-50,488H; PD 117302; Analgesia; Hypothermia; Fluoxetine

1. Introduction

Opioids produce a number of pharmacological effects, which are mediated by μ-, δ-, and κ-opioid receptors. Recent development of the selective agonists *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate [U-50,488H (U50)], (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzo[*b*] thiophene-4-acetamide [PD 117302 (PD117)], and a selective antagonist, nor-binaltorphimine facilitated the characterization of subtypes of κ-opioid receptors and their pharmacological actions (Dhawan et al., 1996). The κ-opioid receptors mediate nociception, diuresis, feeding, thermoregulation, and neuroendocrine secretions (Dhawan et al., 1996). Several reports indicate that μ-opioid receptors mediate a hyperthermic response while the κ-opioid receptors mediate hypothermia (Geller et al., 1982, 1983).

Several studies indicate that 5-HT plays a role in morphine-induced analgesia. The role of 5-HT in the analgesic response mediated by κ-opioid receptors has been studied using different pharmacological interventions, e.g., depletion of 5-HT with reserpine, *p*-chlorophenylalanine (*p*-CPA), precursors of 5-HT (L-tryptophan), and selective 5-HT antagonists. Depletion of 5-HT by reserpine and *p*-CPA attenuated the analgesic response of U50 in mice (Von Voigtlander et al., 1984). The results indicate that the depletors of 5-HT attenuated the analgesic response of morphine slightly. In contrast, U50-induced analgesic activity was markedly inhibited by *p*-CPA, suggesting that a differential involvement of 5-HT in μ- and κ-opioid receptor-mediated analgesic response (Von Voigtlander et al., 1984). 5-HT receptor blockade has also been found to attenuate κ-opioid receptor-mediated analgesia (Ho and Takemori, 1989, 1990a). However, some reports indicate that 5-HT antagonists did not inhibit κ-opioid receptor-mediated analgesia (Linda et al., 1993; Milan and Colpaert, 1991). L-Tryptophan, the precursor of 5-HT, and L-tyrosine potentiated the analgesic response of U50 in mice (Barjavel et al., 1994). Fluoxetine, a selective 5-HT reuptake inhibitor, potentiates the analgesic response

* Corresponding author. Tel.: +91-172-214-692 ext. 2044; fax: +91-172-214-692-185. NIPER communication no: 60.

E-mail address: ramaraop@yahoo.com (P. Ramarao).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00537-8

of morphine without enhancing its affinity for opioid receptors or its discriminative stimulus property (Hynes et al., 1985). Clomipramine, a 5-HT reuptake inhibitor enhanced the analgesia induced by the spiradoline, a selective κ-opioid agonist (Kunihara et al., 1992). The κ-opioid agonists induce hypothermia (Adler and Geller, 1993). U50-induced hypothermia is potentiated by pretreatment with chlorpromazine (Adler and Geller, 1987). Despite all that is known about the role of 5-HT in morphine and κ-opioid receptor-mediated analgesia, little is known of its involvement in other κ-opioid receptor-mediated effects such as hypothermia. In order to investigate the role of 5-HT in κ-opioid receptor-mediated effects, the influence of fluoxetine, a selective 5-HT reuptake inhibitor on analgesia and hypothermia induced by U50 and PD117 was studied in rats.

2. Method

2.1. Animals

Female Sprague–Dawley rats weighing 175–200 g (Central Animal Facility, NIPER, India) were housed five to a cage in a room with controlled temperature (22 ± 1°C), humidity (50 ± 10%), and light (0600–1800 h). Food and water were made available ad libitum. All experiments were performed between 1000 and 1600 h to minimize diurnal variations.

2.2. Drugs

U-50 and PD 117 were gift samples from Pharmacia and Upjohn, Kalamazoo, MI, USA and Parke-Davis, Ann Arbor, MI, USA, respectively. Fluoxetine hydrochloride was a gift sample from Natco Pharma (I), Hyderabad, India. All doses refer to the salt forms of the drugs. All drugs were dissolved in distilled water and injected intraperitoneally in a volume of 5 ml/kg of body weight.

2.3. Apparatus and procedure

The analgesic response was determined by a modified tail-flick method as reported previously (D'Amour and Smith, 1941; Bhargava et al., 1989). Analgesia was measured at 0, 30, 60, 90, 150, and 180 min after intraperitoneal treatment with U50 or PD117. The rats were treated with either vehicle or fluoxetine (10 mg/kg, ip) 60 min prior to the administration of U50 or PD117. To prevent tissue damage, a cut-off time of 30 s was followed. The percent analgesic response at each time point was calculated according to the following formula: The percent analgesic effect = 100 × (test latency − control latency)/(cut-off time − control latency). From the percent analgesia vs. time plot, area under the curve was calculated using the trapezoidal method. The analgesic activity was expressed as the mean $AUC_{0-180\ min}$ ± S.E.M. Three doses of each of the κ-opioid agonists were used to determine the analgesic activity.

2.4. Temperature recording

The colonic temperature of rats injected with U50 (10, 20, and 40 mg/kg, ip) or PD117 (7.5, 15, and 22.5 mg/kg, ip) was measured using telethermometer as reported previously (Bhargava et al., 1989) in rats which were pretreated with either vehicle or fluoxetine (10 mg/kg, ip; 60 min prior). The colonic temperature of each rat was measured at 0, 30, 60, 90, 120, and 180 min after administration of U50 or PD117. The change in colonic temperature was calculated from the basal values and a graph was plotted as change in colonic temperature vs. time. The hypothermic activity was expressed as the mean $AUC_{0-180\ min}$ ± S.E.M.

2.5. Statistics

The analgesia and hypothermia induced by κ-opioid agonists in rats pretreated with vehicle or fluoxetine was compared using analysis of variance (ANOVA) followed by a post hoc multiple comparison test (Scheffe's *S* test). A value of $P < .05$ was considered to be significant.

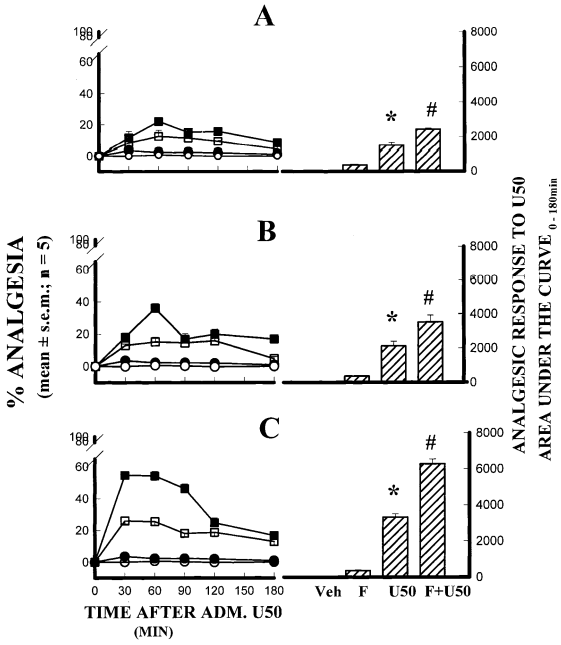

Fig. 1. Effect of fluoxetine (10 mg/kg, ip; 60 min prior) on the time-course of action and $AUC_{0-180\ min}$ of analgesic response of U50 (10, 20, and 40 mg/kg, ip) in rats. Results were expressed as mean ± S.E.M., $n = 5$. Veh. + U50 [□]; fluoxetine + U50 [■]; Veh. + Veh [○]; Veh + fluoxetine [●]. Panels A, B, and C represent 10, 20, and 40 mg/kg of U50, respectively. * $P < .05$ vs. vehicle-injected group; # $P < .05$ vs. U50-injected group.

3. Results

3.1. Effect of fluoxetine (10 mg/kg, ip) on the analgesic response to various doses of U50 and PD117

The effect of fluoxetine (10 mg/kg, ip; 60 min prior) on various doses of U50 (10, 20, and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip)-induced analgesia is shown in Figs. 1 and 2, respectively. In Fig. 1, Panels A, B, and C represent the 10-, 20-, and 40-mg/kg dose of U50, whereas in Fig. 2, Panels A, B, and C represent the 7.5-, 15-, and 22.5-mg/kg dose of PD117, respectively. U50 and PD117 produced dose-dependent analgesia in rat. The time-course for the analgesic response to various doses of U50 (10, 20, and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip) with or without the pretreatment of fluoxetine (10 mg/kg, ip; 60 min prior) clearly demonstrated that the pretreatment of fluoxetine potentiated the analgesic response induced by U50 and PD117 at all the dose levels. The data clearly shows that fluoxetine by itself did not modify the basal latencies, but at the same time, was capable of potentiating the analgesic response of rats injected either with U50 (10, 20, and 40 mg/kg, ip) or PD117 (7.5, 15, and 22.5 mg/kg, ip). Fluoxetine pretreatment potentiated the maximal response of U50 or PD117 at 30–60 min postinjections. The analgesic response gradually decreased thereafter.

3.2. Effect of fluoxetine (10 mg/kg, ip) on the hypothermic response to various doses of U50 and PD117

The effects of fluoxetine (10 mg/kg, ip; 60 min prior) on various doses of U50 (10, 20, and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip)-induced hypothermia is shown in Figs. 3 and 4, respectively. As shown in Figs. 3 and 4, dose-related changes in the hypothermic response were observed with U50 (10, 20, and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip). In the vehicle-treated group, stable colonic temperature was observed during the entire period of the study. Fluoxetine (10 mg/kg, ip) by itself did not produce hypothermia. On the other hand, pretreatment with fluoxetine potentiated the hypothermic response of U50 (20 and 40 mg/kg, ip) and PD117 (7.5, 15, and 22.5 mg/kg, ip). Fluoxetine pretreatment potentiated the maximal response of U50 and PD117, and the hypothermic response gradually decreased but was still present when the experiment was terminated 3 h after the treatment. All the animals recovered to normal at 24 h after the administration of κ-opioid

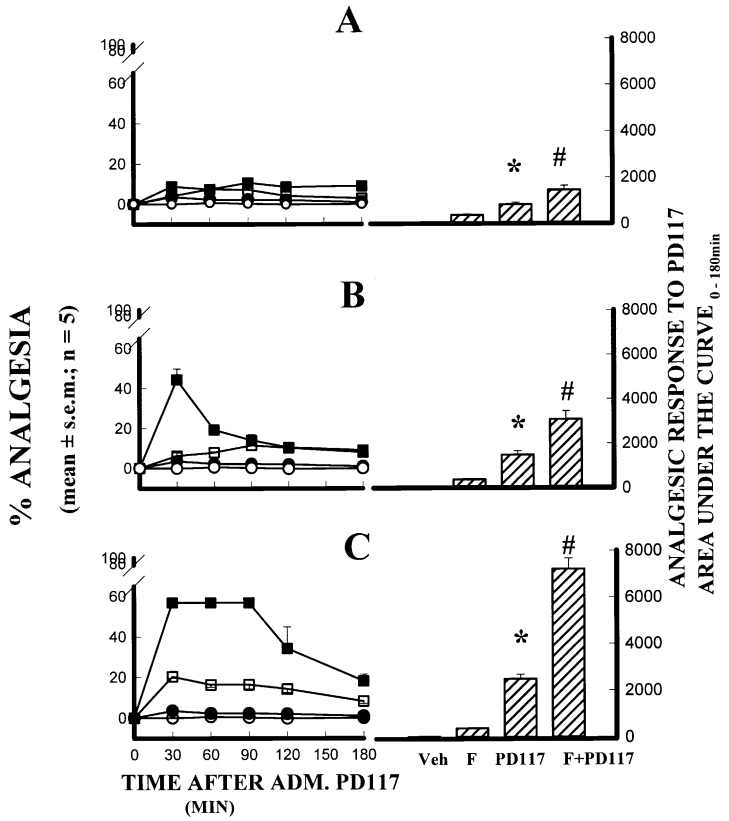

Fig. 2. Effect of fluoxetine (10 mg/kg, ip; 60 min prior) on the time-course of action and $AUC_{0-180\ min}$ of PD117 (7.5, 15, and 22.5 mg/kg, ip) analgesic response in rats. Results were expressed as mean ± S.E.M., $n = 5$. Veh. + PD117 [□]; fluoxetine + PD117 [■]; Veh. + Veh [○]; Veh + fluoxetine [●]. Panels A, B, and C represent 7.5, 15, and 22.5 mg/kg of PD117, respectively. * $P < .05$ vs. vehicle-injected group; # $P < .05$ vs. PD117-injected group.

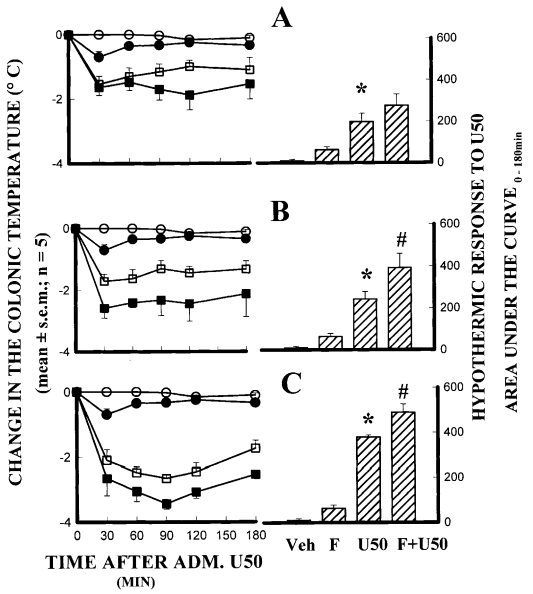

Fig. 3. Effect of fluoxetine (10 mg/kg, ip; 60 min prior) on the time-course of action and $AUC_{0-180\ min}$ of U50 (10, 20, and 40 mg/kg, ip) induced change in the colonic temperature in rats. Results were expressed as mean ± S.E.M., $n = 5$. Veh. + U50 [□]; fluoxetine + U50 [■]; Veh. + Veh [○]; Veh + fluoxetine [●]. Panels A, B, and C represent 10, 20, and 40 mg/kg of U50, respectively. * $P < .05$ vs. vehicle-injected group; # $P < .05$ vs. U50-injected group.

agonists. In the group that received fluoxetine and PD117 at 22.5 mg/kg dose, 40% mortality was observed within 90 min after administration of PD117, indicating the toxicity. Therefore, careful titration of the dose of PD117 is desirable when combining it with fluoxetine.

4. Discussion

In the present study, the selective κ-opioid agonists, U50 and PD117 produced dose-related changes of analgesia and hypothermia in rats. The data are consistent with previous reports (Bhargava et al., 1989; Clark et al., 1988). Fluoxetine by itself did not produce an analgesic response in rat, which is also in agreement with previous reports (Akunne and Solimin, 1994; Hynes et al., 1985). The present study clearly shows that fluoxetine potentiates the analgesic response of κ-opioid receptor-mediated analgesia. Administration of U50 releases 5-HT via a descending 5-HT system (Ho and Takemori, 1989). Additionally, with in vitro studies using brain slices and spinal cord synaptosomes, it was observed that U50 releases 5-HT (Ho and Takemori, 1990b; Passarelli and Costa, 1989). It has been reported that 5-HT_1 receptor antagonists (pindolol, methysergide, mianserine) and 5-HT_2 receptor antagonists (methysergide, mian-

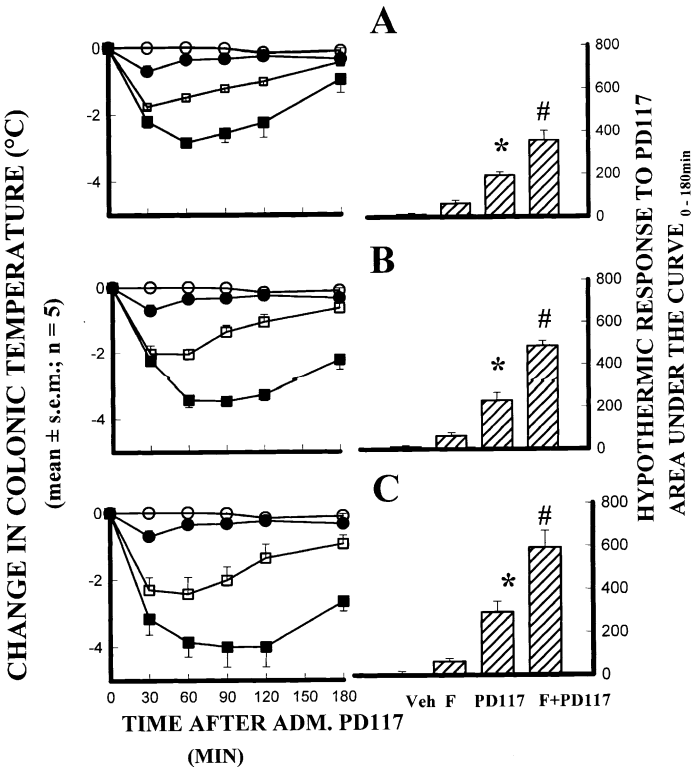

Fig. 4. Effect of fluoxetine (10 mg/kg, ip; 60 min prior) on the time-course of action and $AUC_{0-180\ min}$ of PD117 (7.5, 15, and 22.5 mg/kg, ip) induced change in the colonic temperature in rats. Results were expressed as mean ± S.E.M., $n = 5$. Veh. + PD117 [□]; fluoxetine + PD117 [■]; Veh. + Veh [○]; Veh + fluoxetine [●]. Panels A, B, and C represent 7.5, 15, and 22.5 mg/kg of PD117, respectively. * $P < .05$ vs. vehicle-injected group; # $P < .05$ vs. PD117-injected group.

serine, ketanserine, and pirenperone) antagonise the U50-induced analgesia suggesting that the released 5-HT acts on both 5-HT_1 and 5-HT_2 receptors (Ho and Takemori, 1989). The present observation of potentiation of analgesic response by fluoxetine might be due to increase in the levels of 5-HT at the extracellular sites by inhibiting reuptake of 5-HT.

Fluoxetine (10 mg/kg, ip) produced a short but significant hypothermia of 0.71 ± 0.18°C measured 90 min after fluoxetine administration. Our results are in agreement with Malone and Taylor (1998) who also reported a similar fall in body temperature. Fluoxetine administered 60 min prior to U50 or PD117 significantly potentiated the U50- or PD117-induced hypothermic response (Figs. 3 and 4). In keeping with the hypothesis that U50 and PD117 increase the release of 5-HT to produce hypothermia, the greater increase in extracellular 5-HT caused by fluoxetine may appear to potentiate the κ-opioid receptor-mediated hypothermia. Similarly, U50-induced hypothermia was potentiated by the pretreatment of chlorpromazine (Adler and Geller, 1987). This indicates that the biogenic amines play an important role in the κ-opioid receptor-mediated hypothermia. The exact mechanisms responsible for the potentiation of U50- and PD117-induced hypothermic response by fluoxetine observed in this study are not known. However, it may be due to an increase in the concentration of 5-HT at the extracellular sites. Further investigations are needed to characterize the exact nature and mechanisms involved in the potentiation of the hypothermic responses by fluoxetine.

In summary, fluoxetine enhanced analgesic and hypothermic actions of U50 and PD117 in rat. The differential degree of potentiation of the U50- and PD117-induced hypothermic effect by fluoxetine may reflect the subtype selectivity of these compounds for κ-opioid receptors. These results are of clinical importance because the κ-opioid agonists possess minimal abuse liability as compared to μ-opioid agonists such as morphine. Such combinations may be therapeutically beneficial, when used judiciously.

Acknowledgments

Council of Scientific and Industrial Research, New Delhi, India is gratefully acknowledged for grant of senior research fellowship to KVSN and SG. The authors are grateful to Pharmacia and Upjohn, USA, Parke-Davis, USA, and Natco Pharma, India for the supply of U-50,488H, PD 117302, and fluoxetine, respectively, as gift samples.

References

Adler MW, Geller EB. Hypothermia and poikilothermia induced by a κ-agonist opioid and a neuroleptic. Eur J Pharmacol 1987;140:233–7.

Adler MW, Geller EB. Physiological functions of opioids: temperature regulation. In: Herz A, editor. Opioids II. Heidelberg: Springer-Verlag, 1993. pp. 205–29.

Akunne HC, Solimin KF. 5-HT modulation of pain responsiveness in the aged rat. Pharmacol, Biochem Behav 1994;48:411–6.

Barjavel MJ, Thorat SN, Bhargava HN. Enhancement of a kappa-opioid receptor agonist-induced analgesia by L-tyrosine and L-tryptophan. Eur J Pharmacol 1994;258:173–8.

Bhargava HN, Gulati A, Ramarao P. Effect of chronic administration of U-50,488H on tolerance to its pharmacological actions and on multiple opioid receptors in rat brain regions and spinal cord. J Pharmacol Exp Ther 1989;259:21–7.

Clark CR, Halfpenney PR, Hill RG, Horwell DC, Hughes J, Jarvis TC, Rees DC, Schofield D. Highly selective kappa opioid analgesics, synthesis and structure–activity relationships of novel *N*-(2-amino-cyclohexyl)aryl-andaryloxy-acetamides. J Med Chem 1988;31:831–6.

D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74–9.

Dhawan BN, Cesselin F, Raghubir R, Reisine T, Bradley PB, Portoghese PS, Hamon M. International Union of Pharmacology: XII. Classification of opioids receptors. Pharmacol Rev 1996;48:567–92.

Geller EB, Hawks C, Tallarida RJ, Adler MW. Postulated thermoregulatory roles for different opiate receptors in rats. Life Sci 1982;31:2241–4.

Geller EB, Hawks C, Keinath SH, Tallarida RJ, Adler MW. Subclasses of opioids based on body temperature change in rats: acute subcutaneous administration. J Pharmacol Exp Ther 1983;225:391–8.

Ho BY, Takemori AE. Serotonergic involvement in the antinociceptive action of and development of tolerance to the kappa-opioid receptor agonist U-50,488H. J Pharmacol Exp Ther 1989;250:508–14.

Ho BY, Takemori AE. Attenuation to the antinociceptive action of the selective κ-opioid receptor agonist U50,488H by ICS-205-930. Eur J Pharmacol 1990a;178:371–3.

Ho BY, Takemori AE. Release by U-50 488H of [^{3}H] 5-HT from brain slices and spinal cord synaptosomes of U-50 488H — tolerant and non-tolerant mice. J Pharmacol Exp Ther 1990b;254:8–12.

Hynes MD, Lochner MA, Bemis KG, Hymson DL. Fluoxetine, a selective inhibitor of 5-HT uptake potentiates morphine analgesia without altering its discriminatory stimulus properties or affinity for opioid receptors. Life Sci 1985;36:2317–23.

Kunihara M, Ohyama M, Nakano M. Central monoaminergic mechanisms and analgesic activity of spiradoline mesylate, a κ-opioid receptor agonist in mice. Eur J Pharmacol 1992;214:111–8.

Linda AD, Kelly RP, Lin YP. Antinociceptive effects of the kappa opioid, U50,488H lack of modulation by 5-HT_2 antagonists. Psychopharmacology 1993;112:116–1120.

Malone DT, Taylor DA. Modulation of Δ^9-tetrahydrocannabinol-induced hypothermia by fluoxetine in the rat. Br J Pharmacol 1998;124:1419–24.

Milan MJ, Colpaert FC. 5-Hydroxytryptamine (5-$HT)_{1A}$ receptors and the tail-flick response: III. Structurally diverse 5-HT_{1A} partial agonists attenuate mu- but not kappa-opioid antinociception in mice and rats. J Pharmacol Exp Ther 1991;256:993–1001.

Passarelli F, Costa T. Mu and delta opioid receptors inhibit 5-HT release in rat hippocampus. J Pharmacol Exp Ther 1989;248:299–305.

Von Voigtlander PF, Lewis RA, Neff GL. Kappa opioid analgesia is dependent on serotonergic mechanisms. J Pharmacol Exp Ther 1984;231:270–4.