International Journal of Neuropsychopharmacology (2009), **12**, 1033–1044. Copyright © CINP 2009
doi:10.1017/S1461145709000236

ARTICLE

THEMED SECTION
Serotonin: New Aspects of its Functions in the Brain

CINP

Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression

Esther Berrocoso[1,2] and Juan-Antonio Mico[1,2]

[1] *Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Cádiz, Spain*
[2] *Ciber of Mental Health (CIBERSAM), ISCIII, Madrid, Spain*

Abstract

Although complete remission of symptoms is the goal of any depression treatment, many patients fail to attain or maintain a long-term, symptom-free status. The opioid system has been implicated in the aetiology of depression, and some preclinical and clinical data suggest that opioids possess a genuine antidepressant-like effect. This study aimed to investigate a potential antidepressant strategy combining different classes of monoaminergic compounds with the weak μ-opioid agonist codeine in the tail suspension test in mice, a paradigm aimed at screening potential antidepressants. The results showed that codeine produced an antidepressant-like effect when administered alone, that was effectively antagonized by the opioid antagonist naloxone. The combination of subeffective doses of codeine with the selective serotonin reuptake inhibitors (fluoxetine or citalopram) lead to an accentuated reduction in immobility time. In contrast, immobility time remained unchanged when codeine was combined with a noradrenaline reuptake inhibitor (desipramine) or with a noradrenaline/serotonin reuptake inhibitor (duloxetine). The immobility time also remained unchanged with the combination of subeffective doses of codeine plus (±)-tramadol (weak μ-opioid agonist with serotonin/noradrenaline reuptake inhibitor properties) or (−)-tramadol (noradrenaline reuptake inhibitor). Conversely, the combination with (+)-tramadol (μ-opioid agonist with serotonin reuptake inhibitor properties) produced a large decrease in the immobility time. All these combinations were without effects on motor behaviour in mice. These data support the hypothesis that a combination of classical serotonergic antidepressants and weak opioid receptor agonists may be a helpful new strategy in the treatment of refractory depression.

Received 31 August 2008; Reviewed 26 September 2008; Revised 11 February 2009; Accepted 12 February 2009; First published online 3 April 2009

Key words: Antidepressants, codeine, opioid, serotonin, tramadol.

Introduction

Depression is characterized by a wide range of debilitating emotional and physical symptoms that are thought to be mediated principally through the serotonergic and noradrenergic systems (Nutt, 2008). Although complete remission of symptoms is the goal of depression treatment, many patients fail to attain or maintain a long-term, symptom-free status. Recent findings (STAR*D Study) indicate that about 63% of patients with major depressive disorder fail to respond to suitable first-line monotherapy with a selective serotonin reuptake inhibitor (SSRI). Furthermore, when more treatment steps are required, lower acute remission rates, and higher relapse rates during the follow-up phase, are to be expected (Rush *et al*. 2006). These observations suggest that residual depressive symptoms predispose and portend a subsequent relapse in depression. This is especially relevant in long-term cases or when depression is comorbid with another illness (psychiatric or not). For these reasons, great interest has been shown in the discovery and development of treatment augmentation

Address for correspondence: E. Berrocoso, Ph.D., Department of Neuroscience, School of Medicine, Plaza Fragela 9, 11003 Cádiz, Spain.
Tel.: +34 956015224 *Fax*: +34 956015225
Email: esther.berrocoso@uca.es

strategies to improve the efficacy of antidepressants compounds.

Many residual symptoms are also physical in nature, pain being amongst these. Experiencing pain in depressive disorders can complicate diagnosis and impair treatment outcomes (Demyttenaere *et al*. 2006; Greco *et al*. 2004). In fact, recognizing and optimizing the management of pain that commonly co-exists with depression may be important in enhancing depression response and remission rates (Kroenke *et al*. 2008).

Opioid compounds, such as codeine, are mainly used for the treatment of pain. However, in addition to their analgesic effect, substantial evidence supports the theory that opioid system-enhancing agents have a genuine antidepressant effect (Jutkiewicz, 2006; Tejedor-Real *et al*. 1998). In this sense, early studies showed that chronic administration of the opioid antagonist naltrexone induces a depression-like syndrome, indicating a general role for opioid systems in depression (Hollister *et al*. 1981). Recent studies have shown that patients with severe depression coupled with anxiety display decreased serum β-endorphin levels (Darko *et al*. 1992; Djurovic *et al*. 1999) and decreased μ-opioid receptor availability (Kennedy *et al*. 2006). Furthermore, some clinical reports describe the effectiveness of the μ-opiate agonists, oxycodone and oxymorphone, and the partial agonist, buprenorphine in patients with refractory major depression (Bodkin *et al*. 1995; Stoll & Rueter, 1999). Regarding codeine, to the best of our knowledge there is no data about a putative clinical antidepressant effect. However, the data stated above suggests that endogenous opioid neurotransmission could be altered in patients diagnosed with major depressive disorder.

Considering that opioidergic and monoaminergic mechanisms extensively interact in the control of nociception, opioid compounds are frequently co-administrated with antidepressants to relieve pain (Knotkova & Pappagallo, 2007). However, this strategy has not yet been explored in depression. A remarkable example that an opioid + monoaminergic combination may be of utility in depression is (±)-tramadol, which is a weak agonist of μ-opioid receptors and, like antidepressants drugs, is able to inhibit the reuptake of serotonin (5-HT) and noradrenaline (NA). It is a widely used analgesic, placed on step 2 of the WHO's pain ladder, and interestingly, it has shown antidepressant properties, both clinically and pre-clinically (Rojas-Corrales *et al*. 1998, 2002; Shapira *et al*. 2001).

Bearing this in mind, we propose to explore a novel strategy in the treatment of experimental depression, combining opioid and monoaminergic compounds. We will attempt to take advantage of the existing knowledge of the effect of opioids and antidepressants in two closely linked illnesses, depression and pain. For this purpose, we have examined the effect of the combination of the weak opioid codeine, an analgesic placed on step 2 of the WHO's pain ladder, with antidepressant drugs in the tail suspension test in mice. The antidepressants studied were desipramine (NA reuptake inhibitor), fluoxetine and citalopram (SSRIs) and the dual antidepressant duloxetine (5-HT/NA reuptake inhibitor). Additionally, owing to its pharmacological profile, (±)-tramadol and its enantiomers were also explored in combination with codeine.

Method

Animals

Experiments were performed using albino male CD1 mice (25–30 g). All the animals were provided by the 'Servicio de Experimentación y Producción Animal' (SEPA) of the University of Cádiz. Animals were maintained under standard conditions: 12-h light/dark cycle (lights on 08:00 hours) with food and water available *ad libitum* and a constant temperature (21 ± 1 °C). All procedures and animal handling met the guidelines of European Community directive 86/609-EEC and Spanish Law (RD 1201/2005) regulating animal research. The experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation of the Faculty of Medicine at the University of Cádiz. Animals were housed in groups of 10 and a 7-d acclimatization period was allowed before the experiments. All mice were experimentally naive and used only once, and 10–14 animals were used per group. On each testing day, the mice were brought into the behaviour room 2 h prior to the test session in order to habituate them to the environment. The experiments were performed in a quiet room, during the light phase between 09:00 and 16:00 hours, by a single experimenter.

Drugs, treatment and experimental procedure

The following drugs were used in the study: codeine, desipramine (provided by Sigma-Aldrich-Quimica, Spain); fluoxetine and duloxetine (courtesy of Eli Lilly, USA), citalopram (courtesy of Grünental, Germany); (±)-tramadol, (−)-tramadol and (+)-tramadol (courtesy of Grünental-Andrómaco, Spain).

All drug solutions were prepared immediately before each trial and injected intraperitoneally in a volume of 10 ml/kg body weight 30 min before testing. They were dissolved in physiological saline (0.9%

NaCl) with the exception of duloxetine which was dissolved in distilled water. Control animals received saline (0.9% NaCl) only. The treatments were administered under blind conditions.

First, the effect of codeine, a weak opioid agonist, and the involvement of opioid receptors in this effect was studied in the tail suspension test. Second, dose–response studies were performed to assess the antidepressant-like effect of each antidepressant drug chosen to be combined with codeine in the tail suspension test. Next, the maximal non-significant doses were chosen for the interaction studies. Third, dose–response studies were performed to assess the antidepressant-like effect of the central acting analgesic (±)-tramadol and its enantiomers in the tail suspension test. Then, the maximal non-significant doses of (±)-tramadol and its enantiomers were co-administrated with a non-effective dose of codeine. Additionally, because the tail suspension test is a paradigm that evaluates the immobile/mobile behaviour of mice, muscular coordination and locomotor activity was assessed for codeine and for each pharmacological combination in order to explore the possible role of motor impairment in the results obtained in the tail suspension test. Test procedure and evaluation were carried out by a researcher who was blind to the treatment condition.

Asssessment of antidepressant-like activity (*tail suspension test*)

The tail suspension test has been chosen as a behavioural model predictive of the efficacy of antidepressant treatments (Perrault *et al*. 1992; Steru *et al*. 1985). The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Antidepressant medications reverse the immobility and promote the occurrence of escape-related behaviour.

The test performed was a modified version of that validated for NMRI mice by Steru and colleagues (Steru *et al*. 1985). Thirty minutes after injection, mice were individually suspended by the tail to a horizontal ring-stand bar (distance from floor was 20 cm) using adhesive tape (distance from tip of tail 2 cm). Typically, mice demonstrated several escape-oriented behaviours interspersed with temporally increasing bouts of immobility. A 6-min test session was videotaped. Videotapes were subsequently scored by a highly trained observer who was blind to the treatment. The parameter recorded was the number of seconds spent immobile.

Assessment of muscular coordination (*tightrope test*)

The tightrope test performed was a modified version of that validated by Ingram and colleagues (Ingram *et al*. 1981) for assessing balance and muscular coordination in mice. In this test the mouse is placed on the middle of a tightrope tied on each side to the rod of a chemical stand. The tightrope, which is 60 cm in length, is suspended above a mouse cage about 40 cm above its bedding of wood shavings. The trial scores positively when the mice reach the side poles at one end or spend 1 min suspended. Failure is when the animal falls from the rope and scores negatively. Each trial lasted 1 min. The data are shown as the percentage of mice positively completing the test.

Assessment of locomotor activity

The mice's spontaneous motor activity was measured by SMART (spontaneous motor activity recording and tracking) apparatus provided by Letica Scientific Instruments. A mouse was placed in a Plexiglas chamber ($22 \times 22 \times 20$ cm) and was allowed to explore freely. Its activity was monitored for 6 min, i.e. the same time used in the tail suspension test. Motor activity was assessed following the arbitrary units established by the SMART device.

Statistical analysis

Data were expressed as the mean ± S.E.M. of the parameter measured and were analysed using a one-way ANOVA followed by Dunnett's test in the dose–response studies. In the interaction study, data were analysed using a two-way ANOVA followed by Bonferroni's *post-hoc* test. The factors evaluated (between subjects) were: codeine treatment and naloxone/antidepressant/tramadol treatment. $p < 0.05$ was considered to be statistically significant.

Results

Effect of codeine and its interaction with opioid receptors

Dose–response studies were performed to assess the antidepressant-like effect of codeine in the tail suspension test (Fig. 1*a*). Codeine (10–40 mg/kg i.p) induced a significant decrease in immobility time in a dose-related manner (one-way ANOVA: $F_{3,36} = 3.44$, $p < 0.05$). Dunnett's *post-hoc* analysis indicated that codeine shows an antidepressant-like effect at 40 mg/kg ($p < 0.05$). Furthermore, codeine treatment did not affect either the muscular coordination in the tightrope

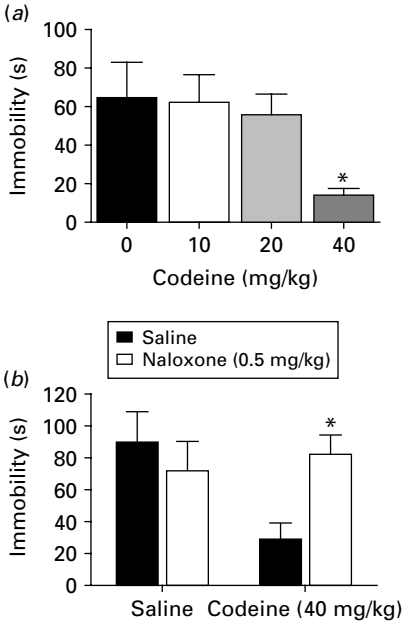

Fig. 1. Effect of codeine and its involvement with opioid receptors in the tail suspension test. (*a*) Codeine (10–40 mg/kg i.p.) significantly reduced the immobilitity time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$). (*b*) The opioid receptor antagonist naloxone (0.5 mg/kg s.c.) blocked the antidepressant-like effect displayed by codeine (40 mg/kg i.p.) (Bonferroni's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$). Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

test ($F_{2,28}=0.96$, n.s.) or locomotor activity ($F_{2,28}=0.79$, n.s.) (Table 1).

In order to explore the possible opioid contribution to the codeine antidepressant-like effect, an effective dose of codeine (40 mg/kg i.p.) was co-administered with the opioid receptor antagonist, naloxone (0.5 mg/kg s.c.) (Fig. 1*b*). A two-way ANOVA revealed no significant effect of codeine ($F_{1,35}=2.67$, n.s.) or naloxone treatment ($F_{1,35}=1.31$, n.s.), but there was a significant interaction between codeine × naloxone ($F_{1,35}=5.41$, $p<0.05$). Bonferroni's *post-hoc* test revealed that naloxone significantly blocked the codeine effect ($p<0.05$).

Table 1. Effect of codeine and the drug combinations in tightrope test and locomotor activity

	Tightrope test (%)	Activity
Saline	90.00 ± 10.00	2.78 ± 0.24
COD (20)	100.00 ± 0.00	2.48 ± 0.51
COD (40)	81.82 ± 12.20	2.81 ± 0.29
Saline	100.00 ± 0.00	2.51 ± 0.24
COD (20)	100.00 ± 0.00	2.85 ± 0.25
DMI (5)	100.00 ± 0.00	2.30 ± 0.18
COD + DMI	80.00 ± 13.33	2.39 ± 0.17
Saline	100.00 ± 0.00	2.72 ± 0.32
COD (20)	90.00 ± 10.00	2.03 ± 0.37
FIX (40)	50.00 ± 16.67*	0.76 ± 0.12***
COD + FLX	40.00 ± 16.33*	0.90 ± 0.12**
Saline	100.00 ± 0.00	2.74 ± 0.12
COD (20)	100.00 ± 0.00	2.45 ± 0.18
CIT (40)	90.00 ± 10.00	3.00 ± 0.34
COD + CIT	90.00 ± 10.00	3.39 ± 0.45
Saline	100.00 ± 0.00	2.51 ± 0.24
COD (20)	100.00 ± 0.00	2.85 ± 0.25
DLX(1.25)	80.00 ± 13.33	2.87 ± 0.20
COD + DLX	100.00 ± 0.00	4.22 ± 0.50**
Saline	80.00 ± 13.33	2.79 ± 0.39
COD (20)	90.00 ± 10.00	2.76 ± 0.24
(±)-TRM (16)	90.00 ± 10.00	2.53 ± 0.25
COD + (±)-TRM	100.00 ± 0.00	2.01 ± 0.35
Saline	80.00 ± 13.33	2.58 ± 0.42
COD (20)	90.00 ± 10.00	2.76 ± 0.24
(−)-TRM (32)	80.00 ± 13.33	1.93 ± 0.29
COD + (−)-TRM	90.00 ± 10.00	1.83 ± 0.34
Saline	100.00 ± 0.00	2.72 ± 0.32
COD (20)	90.00 ± 10.00	2.03 ± 0.37
(+)-TRM (16)	80.00 ± 13.33	2.43 ± 0.14
COD + (+)-TRM	90.00 ± 10.00	2.61 ± 0.24

COD, Codeine; DMI, desipramine; FLX, fluoxetine; CIT, citalopram; DLX, duloxetine; (±)-TRM, (±)-tramadol; (−)-TRM, (−)-tramadol; (+)-TRM; (+)-tramadol.
Data represent the mean ± S.E.M. of 10–11 animals per group. Drugs were intraperitoneally administered 30 min before the test. The values within parentheses represent the dose administered of each compound in mg/kg. Asterisks indicate a significant difference compared to saline (Bonferroni's *post-hoc* test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Effect of the combination: codeine + desipramine

Dose–response studies were performed to assess the antidepressant-like effect of the NA reuptake inhibitor desipramine, in the tail suspension test (Fig. 2*a*). Desipramine (5–20 mg/kg i.p.) induced a significant decrease in immobility time in a dose-related manner (one-way ANOVA: $F_{3,36}=6.70$, $p<0.01$). Dunnett's *post-hoc* analysis indicated that desipramine at 10 and 20 mg/kg significantly reduced the immobility time compared to saline-treated animals ($p<0.01$, respectively). Therefore, ineffective doses of both codeine (20 mg/kg i.p.) and desipramine (5 mg/kg i.p.) were co-administrated. Two-way ANOVA revealed no significant effect of codeine ($F_{1,36}=0.02$, n.s.), desipramine

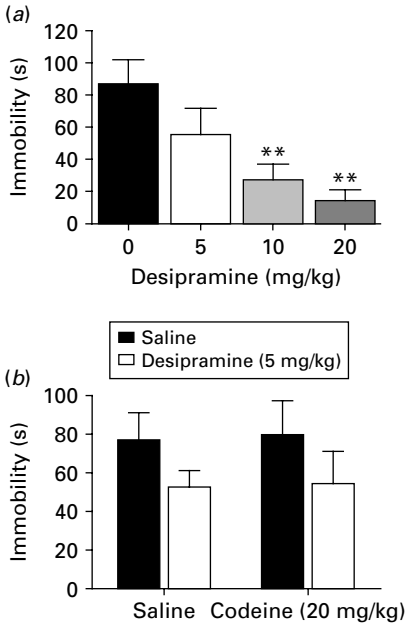

Fig. 2. Effect of the combination of the noradrenaline reuptake inhibitor desipramine + codeine in the tail suspension test. (*a*) Desipramine (5–20 mg/kg i.p.) significantly reduced the immobilitity time (Dunnett's *post-hoc* test) Asterisks indicate a significant difference compared to saline (** $p < 0.01$). (*b*) The combination of subeffective doses of desipramine (5 mg/kg i.p.) and codeine (20 mg/kg i.p.) did not significantly modify the immobility time. Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

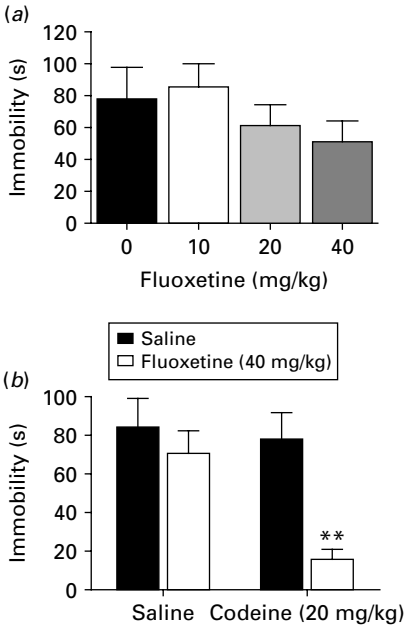

Fig. 3. Effect of the combination of the SSRI fluoxetine + codeine in the tail suspension test. (*a*) Fluoxetine (10–40 mg/kg i.p.) did not significantly modify the immobility time. (*b*) The combination of non-effective doses of fluoxetine (40 mg/kg i.p.) and codeine (20 mg/kg i.p.) significantly reduced the immobility time (Bonferroni's *post-hoc* test). Asterisks indicate a significant difference compared to saline (** $p < 0.01$). Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

($F_{1,36} = 2.95$, n.s.) or codeine × desipramine interaction ($F_{1,36} = 0.00$, n.s.) (Fig. 2*b*).

The combination of codeine (20 mg/kg i.p.) plus desipramine (5 mg/kg i.p.) did not affect the muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36} = 2.25$, n.s.), desipramine ($F_{1,36} = 2.25$, n.s.) or codeine × desipramine interaction ($F_{1,36} = 2.25$, n.s.). Similarly, locomotor activity was not affected (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36} = 0.98$, n.s.), desipramine ($F_{1,36} = 2.45$, n.s.) or codeine × desipramine interaction ($F_{1,36} = 0.36$, n.s.).

Effect of the combination: codeine + SSRI

Dose–response studies were performed to assess the antidepressant-like effect of the SSRI fluoxetine (Fig. 3*a*). Fluoxetine (10–40 mg i.p.) showed a tendency to decrease the immobility time, but no dose tested reached statistical significance compared to the control group ($F_{3,36} = 1.11$, n.s.). However, the co-administration of codeine (20 mg/kg i.p.) and a non-effective dose of fluoxetine (40 mg/kg i.p.) induced a significant antidepressant-like effect (Fig. 3*b*). Moreover, a two-way ANOVA demonstrated a significant effect of codeine ($F_{1,36} = 6.68$, $p < 0.05$), fluoxetine ($F_{1,36} = 10.39$, $p < 0.01$) and codeine × fluoxetine interaction ($F_{1,36} = 4.33$, $p < 0.05$). Subsequently, Bonferroni's *post-hoc* test showed that codeine significantly decreased the immobility time of fluoxetine ($p < 0.01$).

In the tightrope test, the administration of fluoxetine (40 mg/kg i.p.) reduced muscular coordination (Table 1). A two-way ANOVA revealed a significant effect of fluoxetine ($F_{1,36} = 15.52$, $p < 0.001$), but no significant effect of codeine treatment ($F_{1,36} = 0.62$, n.s.) or fluoxetine × codeine interaction ($F_{1,36} = 0.00$, n.s.). Subsequently, Bonferroni's *post-hoc* test showed that fluoxetine, both alone and administered in combination with codeine, significantly decreased muscular coordination ($p < 0.05$ respectively). Similar results were found in the locomotor activity test (Table 1). A two-way ANOVA revealed a significant effect of fluoxetine ($F_{1,36} = 34.98$, $p < 0.001$), but no significant effect was reached for codeine ($F_{1,36} = 1.10$, n.s.) or

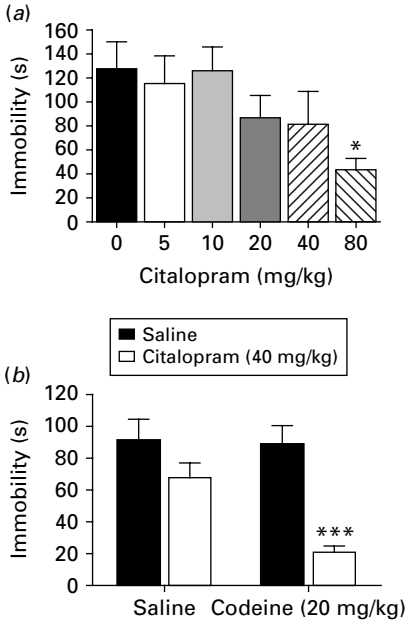

Fig. 4. Effect of the combination of the SSRI citalopram + codeine in the tail suspension test. (*a*) Citalopram (5–80 mg/kg i.p.) significantly reduced the immobility time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$). (*b*) The combination of subeffective doses of citalopram (40 mg/kg i.p.) and codeine (20 mg/kg i.p.) significantly reduced the immobility time (Bonferroni's *post-hoc* test). Asterisks indicate a significant difference compared to saline (*** $p<0.001$). Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10–14 animals per group.

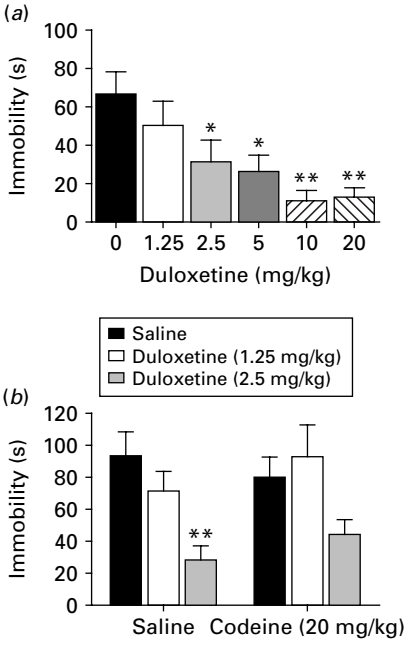

Fig. 5. Effect of the combination of noradrenaline/serotonin reuptake inhibitor duloxetine + codeine in the tail suspension test. (*a*) Duloxetine (1.25–20 mg/kg i.p.) significantly reduced the immobility time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$, ** $p<0.01$). (*b*) The combination of duloxetine (1.25 and 2.5 mg/kg i.p.) and codeine (20 mg/kg i.p.) did not significantly modify the immobility time. Asterisks indicate a significant difference compared to saline (** $p<0.01$). Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

fluoxetine × codeine interaction ($F_{1,36}=2.53$, n.s.). Subsequently, Bonferroni's *post-hoc* test showed that fluoxetine, both alone and administered in combination with codeine, significantly decreased locomotor activity ($p<0.01$, $p<0.001$ respectively).

Citalopram, a SSRI, was studied in the tail suspension test. Citalopram (5–80 mg/kg i.p) dose-dependently reduced immobility time (one-way ANOVA: $F_{5,54}=2.42$, $p<0.05$) (Fig. 4*a*). Dunnett's *post-hoc* analysis indicated that citalopram showed antidepressant-like effects at 80 mg/kg compared to the control group ($p<0.05$). As with fluoxetine, the combination of subeffective doses of both, codeine (20 mg/kg) and citalopram (40 mg/kg) provoked a significant reduction in the immobility time (Fig. 4*b*). A two-way ANOVA demonstrated a significant effect of codeine ($F_{1,52}=5.63$, $p<0.05$), citalopram ($F_{1,52}=19.46$, $p<0.001$) and codeine × citalopram interaction ($F_{1,52}=4.74$, $p<0.05$). Subsequently, Bonferroni's *post-hoc* test showed that codeine significantly decreased the immobility time of fluoxetine ($p<0.001$).

The combination of codeine (20 mg/kg i.p.) plus citalopram (40 mg/kg i.p.) did not affect muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=0.00$, n.s.), citalopram ($F_{1,36}=2.00$, n.s.) or codeine × citalopram interaction ($F_{1,36}=0.00$, n.s.). Similarly, neither codeine nor citalopram treatment, either alone or in combination, affected locomotor activity (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=0.03$, n.s.), citalopram ($F_{1,36}=4.03$, n.s.), or codeine × citalopram ($F_{1,36}=1.28$, n.s.).

Effect of the combination: codeine + duloxetine

Dose–response studies were performed to assess the antidepressant-like effect of the NA/5-HT reuptake inhibitor duloxetine (Fig. 5*a*). Duloxetine (1.25–20 mg/kg i.p) dose-dependently reduced immobility time (one-way ANOVA: $F_{5,54}=5.27$, $p<0.001$). Dunnett's *post-hoc* analysis indicated that duloxetine shows an antidepressant-like effect at 2.5, 5, 10 and 20 mg/kg

compared to the control group ($p<0.05$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively). As with desipramine, the combination of a non-effective dose of codeine at 20 mg/kg with duloxetine at 1.25 and 2.5 mg/kg did not reveal any significant change in immobility time (Fig. 5*b*). A two-way ANOVA revealed a significant effect of duloxetine treatment ($F_{2,54}=8.48$, $p<0.001$), but no significant effect of codeine ($F_{1,54}=0.52$, n.s.) or any codeine × duloxetine interaction ($F_{2,54}=0.94$, n.s.). Subsequently, and as expected, Bonferroni's *post-hoc* test showed that duloxetine, at 2.5 mg/kg, significantly decreased the immobility time.

Neither codeine (20 mg/kg i.p.) nor duloxetine (1.25 mg/kg i.p.) treatment, either alone or in combination, affected muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=2.25$, n.s.), duloxetine ($F_{1,36}=2.25$, n.s.), or codeine × duloxetine interaction ($F_{1,36}=2.25$, n.s.). However, codeine (20 mg/kg i.p.) co-administrated with duloxetine (1.25 mg/kg i.p.) increased locomotor activity (Table 1). A two-way ANOVA demonstrated a significant effect of codeine ($F_{1,36}=6.90$, $p<0.05$) and duloxetine ($F_{1,36}=7.30$, $p<0.05$) but not codeine × duloxetine ($F_{1,36}=2.48$, n.s.). Bonferroni's *post-hoc* comparisons revealed that the combination of codeine + duloxetine significantly increased motor activity ($p<0.01$).

Effect of the combination: codeine + (±)-tramadol and its enantiomers

First, dose-response studies were performed to assess the antidepressant-like effect of (±)-tramadol, the opioid with NA/5-HT reuptake-inhibiting properties (Fig. 6*a*). (±)-Tramadol (16–64 mg/kg i.p.) induced a significant decrease in immobility time in a dose-related manner (one-way ANOVA: $F_{3,36}=4.89$, $p<0.01$). Dunnett's *post-hoc* analysis indicated that (±)-tramadol at 32 and 64 mg/kg significantly decreased immobility time compared with saline-treated animals ($p<0.05$, $p<0.01$, respectively). The administration of non-effective doses of codeine (20 mg/kg i.p.) and (±)-tramadol (16 mg/kg i.p.) did not induce any significant effect in immobility time (Fig. 6*b*). A two-way ANOVA revealed no significant effect of codeine ($F_{1,36}=0.23$, n.s.), (±)-tramadol ($F_{1,36}=0.40$, n.s.) or codeine × (±)-tramadol ($F_{1,36}=0.01$, n.s.).

Neither codeine (20 mg/kg) nor (±)-tramadol (16 mg/kg) treatment, either alone or in combination, affected muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=1.06$, n.s.), (±)-tramadol ($F_{1,36}=1.06$, n.s.) or codeine × (±)-tramadol interaction ($F_{1,36}=0.00$, n.s.). In respect of locomotor activity (Table 1), a two-way ANOVA did not demonstrate any significant effect of codeine ($F_{1,36}=0.79$, n.s.), (±)-tramadol ($F_{1,36}=2.60$, n.s.) or codeine × (±)-tramadol ($F_{1,36}=0.62$, n.s.).

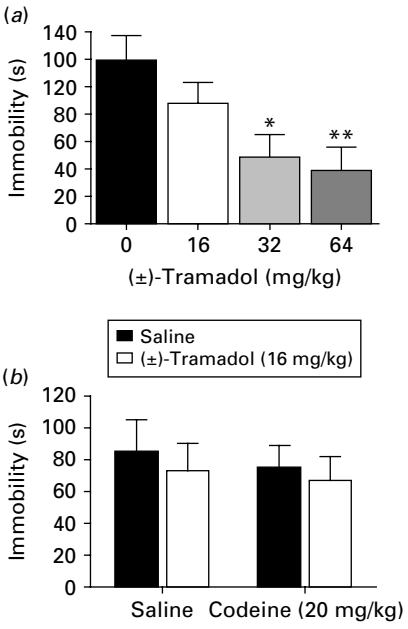

Fig. 6. Effect of the combination of (±)-tramadol + codeine in the tail suspension test. (*a*) (±)-tramadol (16–64 mg/kg i.p.) significantly reduced the immobility time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$, ** $p<0.01$). (*b*) The combination of subeffective doses of (±)-tramadol (16 mg/kg i.p.) and codeine (20 mg/kg i.p.) did not significantly modify the immobility time. Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

Second, dose–response studies were performed to assess the antidepressant-like effect of (−)-tramadol, the NA reuptake inhibitor (Fig. 7*a*). (−)-Tramadol (16–64 mg/kg i.p.) induced a significant decrease in immobility time in a dose-related manner (one-way ANOVA: $F_{3,36}=3.39$, $p<0.05$). Dunnett's *post-hoc* analysis indicated that (−)-tramadol at 64 mg/kg significantly decreased the immobility time compared to control ($p<0.05$). As with (±)-tramadol, the administration of codeine (20 mg/kg i.p.) and (−)-tramadol (32 mg/kg i.p.) did not show any significant effect (Fig. 7*b*). A two-way ANOVA revealed no significant effect of codeine ($F_{1,36}=0.01$, n.s.), (−)-tramadol ($F_{1,36}=0.15$, n.s.) or codeine × (−)-tramadol ($F_{1,36}=0.27$, n.s.).

Neither codeine (20 mg/kg) nor (−)-tramadol (32 mg/kg) treatment, either alone or in combination,

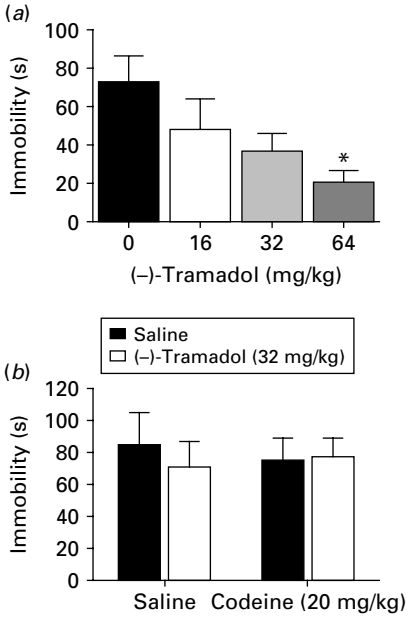

Fig. 7. Effect of the combination of (−)-tramadol + codeine in the tail suspension test. (*a*) (−)-tramadol (16–64 mg/kg i.p.) significantly reduced the immobility time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (* $p<0.05$). (*b*) The combination of subeffective doses of (−)-tramadol (32 mg/kg i.p.) and codeine (20 mg/kg i.p.) did not significantly modify the immobility time. Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

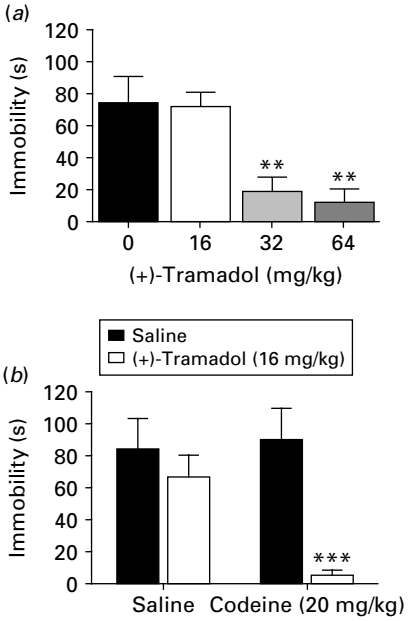

Fig. 8. Effect of the combination of (+)-tramadol + codeine in the tail suspension test. (*a*) (+)-tramadol (16–64 mg/kg i.p.) significantly reduced the immobility time (Dunnett's *post-hoc* test). Asterisks indicate a significant difference compared to saline (** $p<0.01$). (*b*) The combination of subeffective doses of (+)-tramadol (16 mg/kg i.p.) and codeine (20 mg/kgi.p.) significantly reduced the immobility time (Bonferroni's *post-hoc* test). Asterisks indicate a significant difference compared to saline (*** $p<0.001$). Drugs were administered 30 min before the test. Data represent the mean ± S.E.M. of 10 animals per group.

affected muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=0.72$, n.s.), (−)-tramadol ($F_{1,36}=0.00$, n.s.) or codeine × (−)-tramadol ($F_{1,36}=0.00$, n.s.). Regarding locomotor activity (Table 1), a two-way ANOVA demonstrated a significant effect of (−)-tramadol ($F_{1,36}=5.78$, $p<0.05$). However, neither codeine treatment ($F_{1,36}=0.01$, n.s.) nor codeine × (−)-tramadol ($F_{1,36}=0.18$, n.s.) showed a significant effect. Bonferroni's *post-hoc* comparisons did not reveal any statistically significant treatment effects.

Third, dose–response studies were performed to assess the antidepressant-like effect of (+)-tramadol, the opioid with 5-HT reuptake-inhibiting properties (Fig. 8*a*). (+)-Tramadol (16–64 mg/kg i.p.) induced a significant decrease in immobility time in a dose-related manner (one-way ANOVA: $F_{3,35}=9.23$, $p<0.001$). Dunnett's *post-hoc* analysis indicated that (+)-tramadol at 32 and 64 mg/kg significantly decreased immobility time compared to saline-treated animals ($p<0.01$ respectively). However, and in contrast to what occurred with its racemate and (−)-enantiomer, the combination of codeine (20 mg/kg i.p.) plus (+)-tramadol (16 mg/kg i.p.) induced a significant interaction of both treatments (Fig. 8*b*). A two-way ANOVA demonstrated a significant effect of (+)-tramadol ($F_{1,36}=11.62$, $p<0.01$) and codeine × (+)-tramadol ($F_{1,36}=5.09$, $p<0.05$), but no significant effect of codeine ($F_{1,36}=3.32$, n.s.). Subsequently, Bonferroni's *post-hoc* test showed that codeine significantly decreased the immobility time of (+)-tramadol ($p<0.001$).

The combination of (+)-tramadol + codeine did not affect the muscular coordination in the tightrope test (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=0.00$, n.s.), (+)-tramadol ($F_{1,36}=1.06$, n.s.) or codeine × (+)-tramadol interaction ($F_{1,36}=1.06$, n.s.). Similarly, neither codeine nor (+)-tramadol treatment, either alone or in combination, affected locomotor activity (Table 1). A two-way ANOVA showed no significant effect of codeine ($F_{1,36}=0.81$, n.s.), (+)-tramadol ($F_{1,36}=0.29$, n.s.) or codeine × (+)-tramadol ($F_{1,36}=2.37$, n.s.).

Discussion

The main finding of this study is that the association of opiates and 5-HT reuptake inhibitor compounds leads to the augmentation of the antidepressant-like effect in the tail suspension test in mice. Specifically, the combination of subeffective doses of codeine plus either fluoxetine, citalopram or (+)-tramadol provoked a robust reduction in immobility time. In contrast, the combination with NA reuptake inhibitors or mixed compounds did not modify immobility time. In addition, the data obtained in the tail suspension test does not seem to be due to any specific locomotor impairment caused by the drug combination.

Codeine is a widely used drug for pain suppression (Srinivasan *et al*. 1996) that has an affinity for μ-opioid receptors 200–3000 times less than morphine (Chen *et al*. 1991) and lacks affinity for NA or 5-HT reuptake sites (Raffa *et al*. 1993). This study demonstrates that codeine reduces immobility time in the tail suspension test. This effect is blocked by the opioid antagonist naloxone, suggesting the involvement of the opioid receptors in its antidepressant-like effect. Furthermore, in the present study the opiate analgesics with monoamine reuptake-inhibiting properties, (±)-tramadol and its enantiomers, also decreased immobility time. Interestingly, the opioid doses required to produce an antidepressant-like effect are higher than those needed to produce an analgesic effect, suggesting that the mechanisms underlying analgesic and antidepressant effects are different. Other remarkable evidence supporting this hypothesis is the case of tricyclic antidepressants which display their analgesic effect at lower doses than the antidepressant effect (Lynch, 2001). In conclusion, the tail suspension test, the most currently used model to assess antidepressant activity in genetically modified mice, appears to be a suitable model for the detection of the antidepressant-like effect of opioid compounds.

The present study supports the hypothesis that there is a pharmacological interaction by combining opioids with 5-HT reuptake inhibitors in the tail suspension test which does not seem to be affected by any locomotor impairment. In this sense, we have shown that the combination of non-effective doses of codeine + fluoxetine, one of the most prescribed antidepressant drugs, or codeine + citalopram (more selective than fluoxetine; Goodnick & Goldstein, 1998) produces a significant reduction in immobility time. In contrast, this potentiation does not occur in co-administration with either NA reuptake inhibitors (e.g. desipramine) or mixed reuptake inhibitors (e.g. duloxetine). We have chosen duloxetine as representative of the new-generation mixed reuptake inhibitors due to its well-balanced 5-HT/NA ratio and high monoamine reuptake inhibition potency compared to others, such as milnacipran and venlafaxine (Moret *et al*. 1985; Muth *et al*. 1986; Wong *et al*. 1993). Interestingly, the doses necessary to reach an antidepressant-like effect with SSRIs in this rodent strain are much higher than with other classes of antidepressants (noradenergic or dual). This is in agreement with previous literature regarding the poor effect of SSRIs in animal models of depression (Lucki *et al*. 2001; Petit-Demouliere *et al*. 2005). However, considering that many patients are resistant to SSRI treatment (Papakostas *et al*. 2008) the tail suspension test in this strain of mice could be considered as a model of refractory depression for SSRIs, being a valuable tool to explore pharmacological augmentation strategies as demonstrated in the present study with codeine.

The results obtained with the combination of codeine + (±)-tramadol or its enantiomers reinforce our hypothesis about opioid–5-HT cooperation. In fact, (±)-tramadol possesses both opioid and monoamine reuptake properties. Nevertheless, it is interesting to note that its potency in regard to opioids and monoamines is between that of its two enantiomers. Thus, the racemate compound binds weakly but effectively to opioid receptors and possesses 5-HT/NA inhibitor reuptake properties. Compared to their parent compound (+)-tramadol is more potent at binding to μ-opioid receptors and inhibiting the reuptake of 5-HT while (−)-tramadol is more potent at inhibiting the reuptake of NA (Raffa *et al*. 1993). Bearing in mind this pharmacological profile, the present study has shown that the co-administration of codeine with (+)-tramadol, similar to the effect displayed by SSRIs, significantly decreased immobility time. In contrast, (±)-tramadol and (−)-tramadol have not shown this effect.

Regarding the possible mechanisms implicated in these pharmacological interactions, a pharmacokinetic interaction seems unlikely since all the compounds tested act on CYP450 2D6. In fact, codeine, desipramine, fluoxetine, citalopram, duloxetine, tramadol and its enantiomers are metabolized via CYP450 2D6 (Brosen, 2004; Crone & Gabriel, 2004; Garrido *et al*. 2003; Sindrup & Brosen, 1995; Skinner *et al*. 2003) and all the antidepressant compounds tested are inhibitors of CYP2D6 (Brosen, 2004; Skinner *et al*. 2003). Consequently, a pharmacodynamic interaction appears more plausible. Indeed, substantial evidence supports the belief that impairment in the opioid

system underlies the pathophysiology of depression. This has been suggested, among other reasons, because μ-opioid receptors are densely distributed in several brain regions implicated in the response to stressors and emotionally salient stimuli. Furthermore, interestingly, it has been shown that there is such a pronounced reduction in μ-opioid receptor availability in the posterior thalamus and anterior cingulate cortex of patients with major depressive disorder that none responded to fluoxetine treatment (Kennedy *et al*. 2006). This would suggest a possible role for opioid therapy in refractory depression. Furthermore, in addition to this *per se* effect of μ-opioid agonists on these brain areas, a secondary mechanism has been suggested involving the serotonergic system. *In-vivo* microdialysis data have shown that systemic morphine administration in the dorsal raphe nucleus suppresses the GABAergic mediated inhibition of 5-HT release (Tao *et al*. 1996), which results in a disinhibition of serotonergic neurons and the release of an excess of central 5-HT in forebrain projection areas related to emotional integration, including the thalamus, nucleus accumbens, amygdala, frontal cortex, striatum, hypothalamus and ventral hippocampus (Tao & Auerbach, 1995). This would suggest that opiates indirectly stimulate 5-HT release in projection areas, in addition to their direct effect on opioid receptors. However, additional mechanisms could be implicated. There is some clinical and preclinical evidence showing how SSRI effects are augmented by dopamimetic compounds such as olanzapine (Shelton *et al*. 2001), bupropion (Lam *et al*. 2004) or methylphenidate (Weikop *et al*. 2007*b*). Their association will lead to an enhanced dopaminergic neurotransmission in corticolimbic areas (Weikop *et al*. 2007*a*, *b*). Interestingly, it has been shown that acute administration of morphine increases dopaminergic neurotransmission in the nucleus accumbens (Bassareo *et al*. 1996; Pothos *et al*. 1991). Therefore, dopaminergic neurotransmission augmentation could be contributing to the effect reported here. In summary, these actions (direct or indirect) in specific forebrain regions implicated in the response to emotional stress could be responsible for the augmentation of the antidepressant response in the tail suspension test and it would argue in favour of a possible pivotal role of opioid add-on therapies in refractory depression (Fichna *et al*. 2007).

In the field of pain, evidence indicates that opioids interact supraspinally with serotonergic neurons in the nucleus raphe magnus and periaqueductal grey matter, facilitating the descending antinociceptive pathway through the same mechanism described above (Millan, 2002). This fact could be interesting in the context of the comorbidity between depression and pain and it argues in favour of the combination of opioid and monoaminergic mechanisms acting in the somatic and emotional sphere.

Concerning the opioid–NA combination, our study shows that there is no cooperative interaction. It has been shown that opioids inhibit NA release in slices of hippocampus, cerebellum, cerebral cortex and preoptic area (Diez-Guerra *et al*. 1987; Hagan & Hughes, 1984; Peoples *et al*. 1991; Werling *et al*. 1987). Moreover, it has also been demonstrated that morphine alone has no effect on NA release, but it attenuated GABA-augmented NA release in the hypothalamus and abolished it in the cortex (Fiber & Etgen, 2001; Peoples *et al*. 1991). These data suggest that in brain areas related with mood regulation a blocking effect, rather than a synergistic one, would be more likely in the combination of codeine plus NA inhibitors in the tail suspension test. This could be the reason for the lack of interaction in mixed compounds. However, further studies will be necessary to explore the biochemical basis of opioid/5-HT/NA modulation in other paradigms of depression after long-term treatment.

In summary, in the present study we have shown that the combination of the weak opioid receptor agonist, codeine, and 5-HT reuptake inhibitors provokes a robust reduction in immobility time in the tail suspension test. This suggests that the combination of opioid and serotonergic mechanisms might be a new strategy for the development of antidepressant drugs for the treatment of refractory depression. Moreover, considering the analgesic properties of opioids they could act on pain when this symptom is present as a remaining symptom in depression.

Acknowledgements

We thank Mrs M. D. de Benito for her technical assistance and Mr A. Lawson for correcting the English in the manuscript. This study has been supported by the Spanish Ministry of Health, 'Instituto de Salud Carlos III, CIBERSAM CB (07/09/0033)', 'Fondo de Investigación Sanitaria (PI070687)' and 'Junta de Andalucía; Consejeria de Innovación, Ciencia y Empresa (CTS-510 and CTS-4303)'.

Statement of Interest

None.

References

Bassareo V, Tanda G, Petromilli P, Giua C, *et al.* (1996). Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. *Psychopharmacology* (*Berlin*) **124**, 293–299.

Bodkin JA, Zornberg GL, Lukas SE, Cole JO (1995). Buprenorphine treatment of refractory depression. *Journal of Clinical Psychopharmacology* **15**, 49–57.

Brosen K (2004). Some aspects of genetic polymorphism in the biotransformation of antidepressants. *Therapie* **59**, 5–12.

Chen ZR, Irvine RJ, Somogyi AA, Bochner F (1991). Mu receptor binding of some commonly used opioids and their metabolites. *Life Sciences* **48**, 2165–2171.

Crone CC, Gabriel GM (2004). Treatment of anxiety and depression in transplant patients: pharmacokinetic considerations. *Clinical Pharmacokinetics* **43**, 361–394.

Darko DF, Risch SC, Gillin JC, Golshan S (1992). Association of beta-endorphin with specific clinical symptoms of depression. *American Journal of Psychiatry* **149**, 1162–1167.

Demyttenaere K, Bonnewyn A, Bruffaerts R, Brugha T, *et al.* (2006). Comorbid painful physical symptoms and depression: prevalence, work loss, and help seeking. *Journal of Affective Disorders* **92**, 185–193.

Diez-Guerra FJ, Augood S, Emson PC, Dyer RG (1987). Opioid peptides inhibit the release of noradrenaline from slices of rat medial preoptic area. *Experimental Brain Research* **66**, 378–384.

Djurovic D, Milic-Askrabic J, Majkic-Singh N (1999). Serum beta-endorphin level in patients with depression on fluvoxamine. *Farmaco* **54**, 130–133.

Fiber JM, Etgen AM (2001). Modulation of GABA-augmented norepinephrine release in female rat brain slices by opioids and adenosine. *Neurochemical Research* **26**, 853–858.

Fichna J, Janecka A, Piestrzeniewicz M, Costentin do Rego JC (2007). Antidepressant-like effect of endomorphin-1 and endomorphin-2 in mice. *Neuropsychopharmacology* **32**, 813–821.

Garrido MJ, Sayar O, Segura C, Rapado J, *et al.* (2003). Pharmacokinetic/pharmacodynamic modeling of the antinociceptive effects of (+)-tramadol in the rat: role of cytochrome P450 2D activity. *Journal of Pharmacology and Experimental Therapeutics* **305**, 710–718.

Goodnick PJ, Goldstein BJ (1998). Selective serotonin reuptake inhibitors in affective disorders. I. Basic pharmacology. *Journal of Psychopharmacology* **12**, S5–20.

Greco T, Eckert G, Kroenke K (2004). The outcome of physical symptoms with treatment of depression. *Journal of General Internal Medicine* **19**, 813–818.

Hagan RM, Hughes IE (1984). Opioid receptor sub-types involved in the control of transmitter release in cortex of the brain of the rat. *Neuropharmacology* **23**, 491–495.

Hollister LE, Johnson K, Boukhabza D, Gillespie HK (1981). Aversive effects of naltrexone in subjects not dependent on opiates. *Drug and Alcohol Dependence* **8**, 37–41.

Ingram DK, London ED, Reynolds MA, Waller SB, *et al.* (1981). Differential effects of age on motor performance in two mouse strains. *Neurobiology of Aging* **2**, 221–227.

Jutkiewicz EM (2006). The antidepressant-like effects of delta-opioid receptor agonists. *Molecular Interventions* **6**, 162–169.

Kennedy SE, Koeppe RA, Young EA, Zubieta JK (2006). Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. *Archives of General Psychiatry* **63**, 1199–1208.

Knotkova H, Pappagallo M (2007). Adjuvants analgesics. *The Medicinal Clinics of North America* **91**, 113–124.

Kroenke K, Shen J, Oxman TE, Williams Jr. JW, *et al.* (2008). Impact of pain on the outcomes of depression treatment: results from the RESPECT trial. *Pain* **134**, 209–215.

Lam RW, Hossie H, Solomons K, Yatham LN (2004). Citalopram and bupropion-SR: combining versus switching in patients with treatment-resistant depression. *Journal of Clinical Psychiatry* **65**, 337–340.

Lucki I, Dalvi A, Mayorga AJ (2001). Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology* (*Berlin*) **155**, 315–322.

Lynch ME (2001). Antidepressants as analgesics: a review of randomized controlled trials. *Journal of Psychiatry & Neuroscience* **26**, 30–36.

Millan MJ (2002). Descending control of pain. *Progress in Neurobiology* **66**, 355–474.

Moret C, Charveron M, Finberg JP, Couzinier JP, *et al.* (1985). Biochemical profile of midalcipran (F 2207), 1-phenyl-1-diethyl-aminocarbonyl-2-aminomethyl-cyclopropane (Z) hydrochloride, a potential fourth generation antidepressant drug. *Neuropharmacology* **24**, 1211–1219.

Muth EA, Haskins JT, Moyer JA, Husbands GE, *et al.* (1986). Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochemical Pharmacology* **35**, 4493–4497.

Nutt DJ (2008). Relationship of neurotransmitters to the symptoms of major depressive disorder. *Journal of Clinical Psychiatry* **69** (Suppl. E1), 4–7.

Papakostas GI, Fava M, Thase ME (2008). Treatment of SSRI-resistant depression: a meta-analysis comparing within- versus across-class switches. *Biological Psychiatry* **63**, 699–704.

Peoples RW, Giridhar J, Isom GE (1991). Gamma-aminobutyric acidA (GABAA) receptor modulation of morphine inhibition of norepinephrine release. *Biochemical Pharmacology* **42** (Suppl.), S121–126.

Perrault G, Morel E, Zivkovic B, Sanger DJ (1992). Activity of litoxetine and other serotonin uptake inhibitors in the tail suspension test in mice. *Pharmacology Biochemistry and Behavior* **42**, 45–47.

Petit-Demouliere B, Chenu F, Bourin M (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology* (*Berlin*) **177**, 245–255.

Pothos E, Rada P, Mark GP, Hoebel BG (1991). Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. *Brain Research* **566**, 348–350.

Raffa RB, Friderichs E, Reimann W, Shank RP, *et al.* (1993). Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. *Journal of Pharmacology and Experimental Therapeutics* **267**, 331–340.

Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA (2002). Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. *Life Sciences* **72**, 143–152.

Rojas-Corrales MO, Gibert-Rahola J, Mico JA (1998). Tramadol induces antidepressant-type effects in mice. *Life Sciences* **63**, L175–180.

Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, *et al.* (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *American Journal of Psychiatry* **163**, 1905–1917.

Shapira NA, Verduin ML, DeGraw JD (2001). Treatment of refractory major depression with tramadol monotherapy. *Journal of Clinical Psychiatry* **62**, 205–206.

Shelton RC, Tollefson GD, Tohen M, Stahl S, *et al.* (2001). A novel augmentation strategy for treating resistant major depression. *American Journal of Psychiatry* **158**, 131–134.

Sindrup SH, Brosen K (1995). The pharmacogenetics of codeine hypoalgesia. *Pharmacogenetics* **5**, 335–346.

Skinner MH, Kuan HY, Pan A, Sathirakul K, *et al.* (2003). Duloxetine is both an inhibitor and a substrate of cytochrome P4502D6 in healthy volunteers. *Clinical and Pharmacology Therapeutics* **73**, 170–177.

Srinivasan V, Wielbo D, Simpkins J, Karlix J, *et al.* (1996). Analgesic and immunomodulatory effects of codeine and codeine 6-glucuronide. *Pharmaceutical Research* **13**, 296–300.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* (*Berlin*) **85**, 367–370.

Stoll AL, Rueter S (1999). Treatment augmentation with opiates in severe and refractory major depression. *American Journal of Psychiatry* **156**, 2017.

Tao R, Auerbach SB (1995). Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. *Neuroscience* **68**, 553–561.

Tao R, Ma Z, Auerbach SB (1996). Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. *British Journal of Pharmacology* **119**, 1375–1384.

Tejedor-Real P, Mico JA, Smadja C, Maldonado R, *et al.* (1998). Involvement of delta-opioid receptors in the effects induced by endogenous enkephalins on learned helplessness model. *European Journal of Pharmacology* **354**, 1–7.

Weikop P, Kehr J, Scheel-Kruger J (2007*a*). Reciprocal effects of combined administration of serotonin, noradrenaline and dopamine reuptake inhibitors on serotonin and dopamine levels in the rat prefrontal cortex: the role of 5-HT1A receptors. *Journal of Psychopharmacology* **21**, 795–804.

Weikop P, Yoshitake T, Kehr J (2007*b*). Differential effects of adjunctive methylphenidate and citalopram on extracellular levels of serotonin, noradrenaline and dopamine in the rat brain. *European Neuropsychopharmacology* **17**, 658–671.

Werling LL, Brown SR, Cox BM (1987). Opioid receptor regulation of the release of norepinephrine in brain. *Neuropharmacology* **26**, 987–996.

Wong DT, Bymaster FP, Mayle DA, Reid LR, *et al.* (1993). LY248686, a new inhibitor of serotonin and norepinephrine uptake. *Neuropsychopharmacology* **8**, 23–33.