British Journal of Pharmacology (2007) 151, 75–83
 0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Blockade of morphine-induced behavioral sensitization by a combination of amisulpride and RB101, comparison with classical opioid maintenance treatments

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Background and purpose: Maintenance treatments with methadone or buprenorphine are more or less efficient procedures for helping heroin addicts to stop or reduce drug abuse. Another approach to treat opiate dependence could be to target the endogenous opioid system by enhancing the effects of enkephalins by protecting them from enzymic degradation by the dual peptidase inhibitor RB101.
Experimental approach: As chronic treatment with the dopamine D2 antagonist amisulpride facilitates RB101-induced behavioral effects, we chose in this study to treat mice previously sensitized to the hyperlocomotor effects induced by morphine with a combination of amisulpride and RB101.
Key results: Expression of morphine-induced locomotor sensitization was abolished after combined treatment with amisulpride (20 mg.kg^{-1}, i.p.) and RB101 (80 mg.kg^{-1}, i.p.), whereas these drugs were not effective when used alone. We then compared these results with the effects of amisulpride combined with buprenorphine (0.1 mg.kg^{-1}, i.p.) or methadone (2.5 mg.kg^{-1}, i.p.) upon morphine-induced behavioral sensitization. Whereas the combination of amisulpride and buprenorphine partially blocked the expression of morphine sensitization, amisulpride + methadone was not effective in this paradigm.
Conclusions and implications: The combination of amisulpride + RB101 appears to be very efficient in blocking the expression of morphine-induced behavioral sensitization. This could reflect a reinstatement of a balance between the function of the dopamine and opioid systems and could represent a new approach in maintenance treatments for opiate addiction.
British Journal of Pharmacology (2007) **151,** 75–83. doi:10.1038/sj.bjp.0707195; published online 12 March 2007

Keywords: amisulpride; RB101; opiates; behavioral sensitization; locomotor activity; enkephalin

Abbreviations: APN, aminopeptidase N; NEP, neutral endopeptidase 24-11; RB101, *N*-(*R*,*S*)-2-benzyl-3((*S*)(2-amino-4-methylthio)butyl dithio)-1-oxopropyl]-L-phenylalanine benzyl ester

Introduction

Currently the major treatment modality for opiate addiction is methadone and buprenorphine maintenance. These treatments are based on the specific pharmacokinetic/pharmacodynamic properties of these ligands able to bind to the same targets that heroin, but with long-lasting effects (Garrido and Troconiz, 1999; Johnson *et al*., 2003). Numerous clinical studies have demonstrated their efficacy (Layson-Wolf *et al*., 2002; Sung and Conry, 2006). However, these pharmacotherapies also have limits (concomitant use of other drugs of abuse or misuse of the substitutes) (Uchtenhagen, 2003; Gonzalez *et al*., 2004), and the level of relapse unfortunately remains very high. These disadvantages could be due to the fact that opioid addiction induces deep brain alterations that should be normalized at both physiological and behavioral levels to achieve cessation of drug abuse. One way to reach this goal is to target the medication to a specific physiological system that is known to be significantly disturbed by chronic drug abuse.

Several studies have reported the central role of the endogenous opioid system in drug-addictive processes involved in opiate dependence (Van Ree *et al*., 1999; Mas Nieto *et al*., 2002). Thus, a novel and more physiological approach to the treatment of opiate dependence is to enhance the effects of the endogenous opioid peptides,

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Received 30 August 2006; revised 9 November 2006; accepted 19 December 2006; published online 12 March 2007

enkephalins, through inhibition of their catabolism (Roques and Noble, 1995; Noble and Roques, 2002). Enkephalins are very rapidly metabolized by two enzymes, neutral endopeptidase 24-11 (NEP) and aminopeptidase N (APN) (Roques *et al*., 1993). As both NEP and APN belong to the same group of metallopeptidases, dual peptidase inhibitors have been designed, such as RB101 (*N*-((*R*,*S*)-2-benzyl-3((*S*)(2-amino-4-methylthio)butyl dithio)-1-oxopropyl)-L-phenylalanine benzyl ester), able to completely protect enkephalins from enzymic degradation (Fournié-Zaluski *et al*., 1992).

We have recently shown that behavioral responses induced by RB101 in mice are facilitated by chronic treatment with the D2 dopamine antagonist amisulpride (Cordonnier *et al*., 2005). Moreover, disruption of the D2 dopamine receptor in mice increased preproenkephalin levels by about 50% (Baik *et al*., 1995) and no opiate-rewarding effects could be shown in D2 receptor knockout mice (Maldonado *et al*., 1997).

Thus, the goal of this study was to act on both dopaminergic and opioid systems and, particularly, to investigate whether a blockade of the dopaminergic system could potentiate the endogenous opioid system, leading to a more physiological 'opiate substitution' compared with exogenous opioid agonists.

Experiments were designed to evaluate the effectiveness of the combination of the D2 dopamine antagonist amisulpride with RB101 in blocking the expression of behavioral sensitization to morphine in mice. This behavioral sensitization is a consequence of neurochemical adaptations that occur following repeated administration of opioids, and more generally, drugs of abuse. This phenomenon is well-known to be long-lasting, which allows the treatment of animals for several days with drugs to investigate their potential to reduce or abolish the expression of morphine-induced behavioral sensitization. To mimic the situation encountered in opiate addicts, we have specifically investigated the efficacy of the combination of RB101 and amisulpride on the expression of the behavioral sensitization observed following a challenge injection of morphine in mice, already sensitized to the effects of the alkaloid.

Similar experiments were performed for comparison with the classical substitution treatments (methadone and buprenorphine), alone or in combination with amisulpride, to evaluate whether they could reduce the expression of behavioral sensitization in mice chronically treated with morphine.

Materials and methods

Animals

Male OF1 mice (20–22 g at the beginning of the experiments) (Charles River, Lyon, France) were used. Animals were housed in groups of 12 in a room with a 12 h alternating light/dark cycle and controlled temperature ($21 \pm 2^{\circ}C$). Food and water were available *ad libitum*. Animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) as well as French law, with the standard ethical guidelines and under the control of the Ethical Committee of the Faculty. Every effort was made to minimize the number of animals used and their discomfort.

Pharmacological treatments

The molecules used in this study have different pharmacokinetic/pharmacodynamic profiles and these were the basis of our treatment regimens. Amisulpride is well-known to dissociate very quickly from D2 receptors (Kapur and Seeman, 2001) and RB101 has a short-duration of action (Noble *et al*., 1992), so we chose to give twice daily injections of these two drugs. On the other hand, buprenorphine (Tzschentke, 2002) and methadone (Kreek and Vocci, 2002) are both long-acting, which justified only one daily injection of these two drugs.

The induction of behavioral sensitization to morphine in this study consists of one daily injection of morphine ($20\,mg\,kg^{-1}$, intraperitoneal (i.p.)) for 7 days, as described previously (Zhao *et al*., 2004).

Mice were first divided into two groups: those who received morphine ($20\,mg\,kg^{-1}$ i.p.), and those who received saline. They were injected once daily during 7 days (day 1 to day 7), in the beginning of the afternoon, and tested for locomotor activity on day 1 and day 7.

From day 8 to day 14, mice of each group were given amisulpride ($20\,mg\,kg^{-1}$), methadone ($2.5\,mg\,kg^{-1}$), buprenorphine ($0.1\,mg\,kg^{-1}$), RB101 ($80\,mg\,kg^{-1}$) or vehicle alone, or a combination of amisulpride with one of the three compounds (methadone, buprenorphine or RB101) or vehicle. Amisulpride was administered twice a day (1000–1800 hours) by i.p. injection, as was RB101. Methadone and buprenorphine were also injected ii.p., but only once a day in the morning. In all cases, the injection volume was $0.1\,ml.10\,g^{-1}$.

On day 15, all mice received a challenge injection of morphine ($10\,mg\,kg^{-1}$ i.p.). Immediately after the injection, all the animals were tested for locomotor activity during 60 min. No injections of amisulpride, methadone, buprenorphine or RB101 were given on this day (Table 1 and Figures 2–4).

In a second set of experiments, morphine-treated mice between day 1 and day 7 received on day 15 only, RB101 ($80\,mg\,kg^{-1}$ i.p.), buprenorphine ($0.1\,mg\,kg^{-1}$ i.p.), methadone ($2.5\,mg\,kg^{-1}$ i.p.), alone or in combination with amisulpride ($20\,mg\,kg^{-1}$ i.p.). From day 8 to day 14, all the animals received only saline ip. (Table 2 and Figure 5).

RB101, buprenorphine and methadone doses were chosen in agreement with preliminary studies in which the three drugs proved to be equieffective in three different behavioral tests (hot-plate test, forced swim test and locomotor activity) (L Cordonnier *et al*., personal communication).

Locomotor activity

The locomotor activity of mice was measured in an actimeter (Immetronic, Bordeaux, France) composed of eight cages of transparent plastic of equal size ($19 \times 11 \times 14\,cm^3$) under low illumination (<5 lux). One mouse was placed in each box to record its movements. Displacements were measured by photocell beams located across the long axis and above the

floor. Horizontal locomotor activity was expressed in scores (mean ± s.e.m.) as the total number of interruption of the photocell beams. The sessions lasted 60 min.

Statistical analysis

One-way analysis of variance (ANOVA) or repeated measures ANOVA were conducted using computer software (Statview, SAS institute Inc., Cary, NC, USA) for comparison across the experimental conditions. When a significant difference among the treatments was obtained, Newman–Keuls's *post hoc* test was applied to define which group contributed to these differences. Significance was accepted with $P<0.05$.

Materials

Amisulpride (Solian) was synthesized by Sanofi–Synthelabo (Bagneux, France) and solutions made in saline (0.9% sodium chloride, (NaCl)). RB101 was synthesized in the laboratory (Fournié-Zaluski *et al*., 1992) and dissolved in vehicle containing ethanol (10%), cremophor EL (10%) and distilled water (80%). Cremophor and methadone hydrogen chloride (HCl) were purchased from Sigma (Saint-Quentin Fallavier, France). Morphine HCl was purchased from Francopia (Gentilly, France). Buprenorphine was a generous gift from Schering-Plough (France). Methadone, morphine and buprenorphine were dissolved in saline.

Results

Behavioral sensitization to morphine

As shown in Figure 1a, morphine injections (20 mg kg^{-1} i.p.) on day 1 and day 7 induced an increased locomotor activity in mice. Moreover, the intermittent administration of morphine once daily for 7 days induced an enhancement of its locomotor effect between the first and last day of morphine treatment.

Locomotor activity of animals was also observed on day 15 with a challenge dose of morphine, after 8 days of abstinence. As shown in Figure 1b, mice given the repeated injections of saline and challenged with morphine on day 15 exhibited the same activity that mice which only received one injection of morphine (10 mg kg^{-1} i.p) on day 15 (referred respectively in Figure 1b as Sal-Mor and Mor). In contrast, the locomotor activity of the mice repeatedly treated with morphine, that is, morphine-sensitized mice (Mor-Mor), was significantly increased as compared with saline-treated mice given the challenge injection of morphine (Sal-Mor), showing the expression of a strong behavioral sensitization to morphine (Figure 1b).

Effect of amisulpride and/or RB101 treatment on the expression of behavioral sensitization induced by morphine

The groups of animals in this set of experiments are described in Table 1. As illustrated in Figure 2b, the challenge injection of morphine (10 mg kg^{-1} i.p.) on day 15 revealed an important increase of locomotor activity in mice treated previously with morphine from day 1 to day 7 (Group 2), as compared with saline-treated mice (Group 1). Saline-treated groups of mice which received amisulpride alone, RB101 alone or the combination of the two drugs from day 8 to day 14 had a locomotor activity not significantly different from the saline-treated group receiving a challenge dose of morphine on day 15 (Group 1) (data not shown).

Morphine-sensitized mice treated from day 8 to day 14 with RB101 (80 mg kg^{-1} i.p.) (Group 3) or amisulpride (20 mg kg^{-1} i.p.) (Group 4) alone showed a locomotor

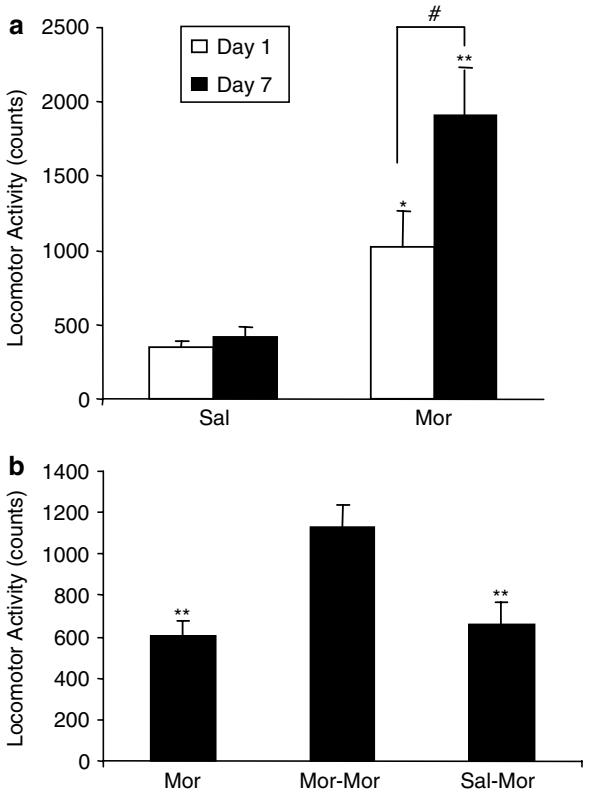

Figure 1 (**a**) Locomotor activity induced by saline (Sal) or morphine (Mor). Mice were injected with saline or morphine (20 mg kg^{-1} i.p.) once every day for 7 days. Locomotor activity was recorded for 60 min immediately after saline or morphine injection on day 1 and day 7. Each point represents the mean scores ± s.e.m. for $n=10$–11 per group. *$P<0.05$ compared with saline-treated group (day 1), **$P<0.01$ compared with saline-treated group (day 7), and $^{\#}P<0.05$ between the two morphine-treated groups (day 1 and day 7). ANOVA for repeated measures revealed a significant difference between day 1 and day 7 in morphine-treated animals ($F(1,16)=20.228$, $P=0.0004$ for treatment effect; $F(1,16)=17.934$, $P=0.0006$ for day effect; $F(1,16)=13.000$, $P=0.0024$ for interaction treatment-day). (**b**) Effect of a challenge injection of morphine (10 mg kg^{-1} i.p.) on day15 on locomotor activity of drug-naive mice or mice previously treated with morphine or saline from day1 to day7. Mice were divided into three groups : mice previously morphine-sensitized (Mor–Mor), mice treated with saline during the sensitization period (Sal–Mor), and mice receiving only the challenge injection of morphine (Mor). The challenge injection (morphine 10 mg kg^{-1} i.p.) was given on day 15, that is, 8 days after the cessation of morphine (20 mg kg^{-1} i.p.) repeated injections. Locomotor activity was recorded for 60 min immediately after morphine injection. Each point represents the mean scores ± s.e.m. for $n=9$–11 per group. **$P<0.01$ compared with Mor-Mor group. ANOVA, $F(2,27)=7.835$, $P=0.0021$.

Table 1 Pharmacological treatments to investigate the effects of RB101, methadone and buprenorphine alone or in combination with amisulpride on the expression of morphine-induced locomotor sensitization

	Day 1–7	*Day 8–14*	*Day 15*
Group 1	Sal	Vehicle	Mor
Group 2	Mor	Vehicle	Mor
Group 3	Mor	RB101 or Bup or Meth	Mor
Group 4	Mor	AMS	Mor
Group 5	Mor	AMS + RB101 or AMS + Bup or AMS + Meth	Mor

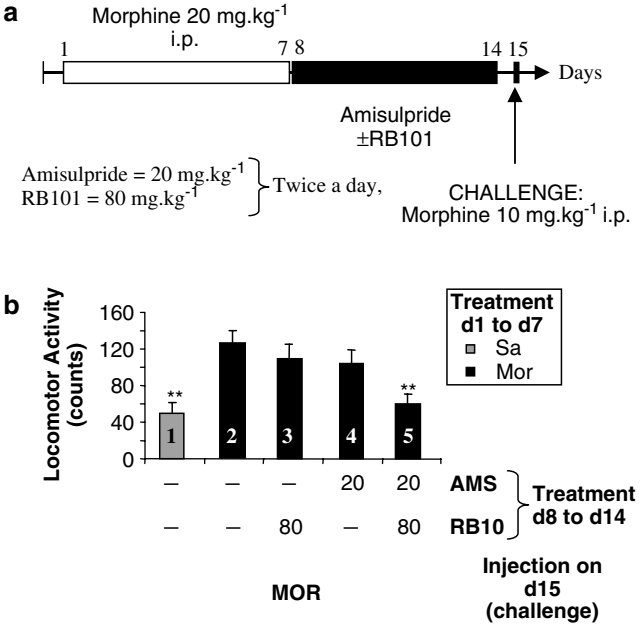

Figure 2 (**a**) Experimental design. Mice were given a daily injection of morphine (20 mg kg^{-1} i.p.) for 7 days. Then, they were given amisulpride (20 mg kg^{-1} i.p. twice a day) and/or RB101 (80 mg kg^{-1} i.p. twice a day) for 7 days. The challenge injection of morphine (10 mg kg^{-1} i.p.) was given on day 15. (**b**) Effect of a challenge injection of morphine (10 mg kg^{-1} i.p.) on day15 (Mor) on locomotor activity of mice previously treated with morphine or saline from day 1 to day 7, and with amisulpride (AMS) with or without RB101 from day8 to day 14 (treatment regimen described in panel a). Vertical bars represent the mean scores ± s.e.m. for $n = 14$–18 per group after 60 min of recording. $^{**}P < 0.01$ compared with the morphine-sensitized group without any treatment from day 8 to day 14. ANOVA global result, $F(8,141) = 9.626$, $P < 0.0001$.

activity similar to morphine-sensitized mice treated with vehicle (Group 2).

In contrast, the locomotor activity of morphine-sensitized mice treated with both amisulpride and RB101 (Group 5) from day 8 to day 14 was significantly decreased compared with morphine-sensitized mice, which only received vehicle injections during this period (Group 2). Moreover, no statistical difference was observed between this amisulpride + RB101 group (Group 5) compared with saline-treated

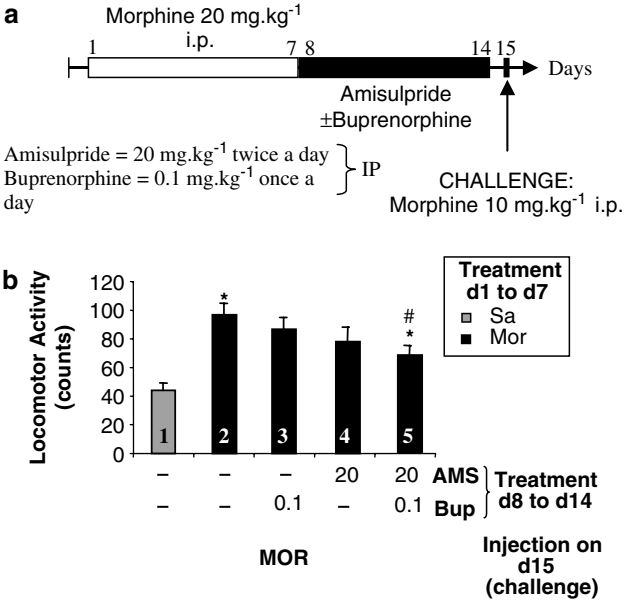

Figure 3 (**a**) Experimental design. Mice were given a daily injection of morphine (20 mg kg^{-1} i.p.) for 7 days. Then, they were given amisulpride (20 mg kg^{-1} i.p. twice a day) and/or buprenorphine (0.1 mg kg^{-1} i.p. once a day) for 7 days. The challenge injection of morphine (10 mg kg^{-1} i.p.) was given on day 15. (**b**) Effect of a challenge injection of morphine (10 mg kg^{-1} i.p.) on day15 (Mor) on locomotor activity of mice previously treated with morphine or saline from day 1 to 7, and with amisulpride (AMS) with or without buprenorphine (Bup) from day8 to day14 (treatment regimen described in panel a). Vertical bars represent the mean scores ± s.e.m. for $n = 19$–24 per group after 60 min of recording. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the morphine-challenged group, $^{\#}P < 0.05$ compared with the morphine-sensitized group. ANOVA global result, $F(8,159) = 12.305$, $P < 0.0001$.

mice, which received the morphine challenge injection (Group 1).

Effect of amisulpride and/or buprenorphine treatment on the expression of behavioral sensitization induced by morphine

As shown in Figure 3b, the challenge injection of morphine (10 mg kg^{-1} i.p.) on day 15 induced an important increase of locomotor activity in morphine-sensitized mice which only received vehicle injections between day 8 and day 14 (Group 2; see Table 1 for groups), compared with saline-treated mice receiving just the challenge injection of morphine (Group1). Injection of morphine on day 15 in saline-treated groups of mice which received amisulpride alone, buprenorphine alone or the combination of the two drugs from day 8 to day 14 had a locomotor activity not significantly different from the saline-treated group receiving a challenge dose of morphine on day 15 (data not shown).

Morphine-sensitized mice treated with buprenorphine (0.1 mg kg^{-1} i.p.) (Group 3) or amisulpride (20 mg kg^{-1} i.p.) (Group 4) alone between day 8 and day 14 showed a locomotor activity similar to morphine-sensitized mice (Group 2) following the challenge dose of morphine.

The locomotor activity of morphine-sensitized mice treated with both amisulpride and buprenorphine (Group

5) from day 8 to day 14 was significantly decreased compared with morphine-sensitized mice which only received vehicle injections during this period (Group 2). Nevertheless, an increase of the locomotor activity remains compared with saline-treated mice, which received the challenge injection of morphine (Group 1).

Effect of amisulpride and/or methadone treatment on the expression of behavioral sensitization induced by morphine

As illustrated in Figure 4b, the challenge injection of morphine (10 mg kg^{-1} i.p.) on day 15 revealed an important increase of locomotor activity in morphine-sensitized mice which received no other treatment between day 8 and day 14 (Group 2; see Table 1 for groups), compared with saline-treated mice receiving just the challenge injection of morphine (Group 1). Saline-treated groups of mice which received amisulpride alone, methadone alone or the combination of the two drugs had a locomotor activity not significantly different from the saline-treated group receiving a challenge dose of morphine on day 15 (data not shown).

Morphine-sensitized mice treated between day 8 and day 14 with methadone (2.5 mg kg^{-1} i.p.) alone (Group 3) exhibited a significantly higher locomotor activity compared with morphine-sensitized mice (Group 2), whereas morphine-sensitized mice only treated with amisulpride (20 mg kg^{-1} i.p.) (Group 4) showed a locomotor activity similar to morphine-sensitized mice (Group 2).

The locomotor activity of morphine-sensitized mice treated with both amisulpride and methadone between day 8 and day 14 (Group 5) was significantly decreased compared with morphine-sensitized mice treated with methadone only (Group 3), whereas no difference was observed with the morphine-sensitized mice that did only receive vehicle injections during this period (Group 2).

Effect of RB101, buprenorphine and methadone alone or in combination with amisulpride in morphine-treated mice

The treatments for these groups of animals are described in Table 2. As shown in Figure 5, administration of RB101 (80 mg kg^{-1}), buprenorphine (0.1 mg kg^{-1}) and methadone (2.5 mg kg^{-1}) on day 15 induced hyperlocomotor effects in morphine-treated mice (from day 1 to day 7). These effects were blocked by the co-administration of the dopamine antagonist, amisulpride (20 mg kg^{-1}). Moreover, sensitized responses were observed following RB101, buprenorphine or methadone in morphine-treated mice compared with saline-treated animals.

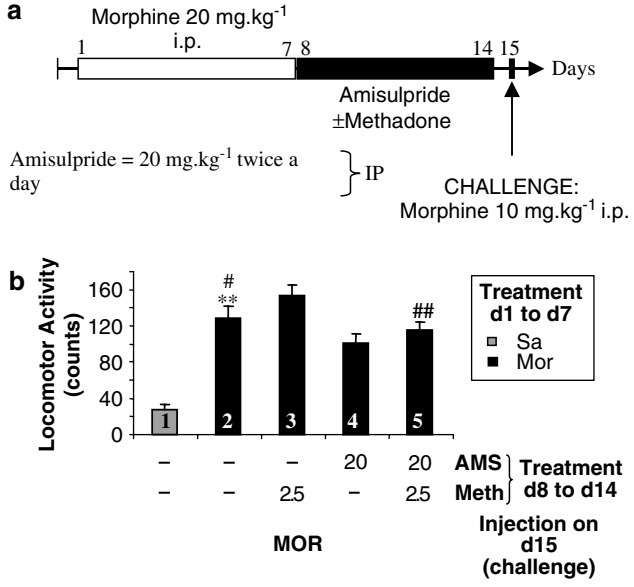

Figure 4 (**a**) Experimental design. Mice were given a daily injection of morphine (20 mg kg^{-1} i.p.) for 7 days. Then, they were given amisulpride (20 mg kg^{-1} i.p. twice a day) and/or methadone (2.5 mg kg^{-1} i.p. once a day) for 7 days. The challenge injection of morphine (10 mg kg^{-1} i.p.) was given on day 15. (**b**) Effect of a challenge injection of morphine (10 mg kg^{-1} i.p.) on day 15 (Mor) on locomotor activity of mice previously treated with morphine or saline from day 1 to day 7, and with amisulpride (AMS) with or without methadone (Meth) from day 8 to day 14 (treatment regimen described in panel a). Vertical bars represent the mean scores ± s.e.m. for $n = 18$–22 per group after 60 min of recording. $^{**}P < 0.01$ compared with the morphine-challenged group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the morphine-sensitized group treated with methadone. ANOVA global result, $F(8,169) = 37.019$, $P < 0.0001$.

Discussion

The major findings of the present study are as follows: (a) repeated administration of amisulpride, buprenorphine, methadone or RB101 alone were unable to suppress the expression of behavioral sensitization to morphine in the experimental conditions used; (b) repeated administration of the combination of buprenorphine + amisulpride or RB101 + amisulpride reduced or suppressed, respectively, the expression of behavioral sensitization to morphine. These results further support the well-known complexity of

Table 2 Pharmacological treatments to investigate the locomotor effects induced by RB101, methadone and buprenorphine alone or in combination with amisulpride in animals previously treated with saline or morphine

	Day 1–7	*Day 8–14*	*Day 15*
Group 1	Sal	Sal	Sal
Group 2	Sal	Sal	AMS
Group 3	Sal	Sal	RB101 or Bup or Meth
Group 4	Sal	Sal	AMS + RB101 or AMS + Bup or AMS + Meth
Group 5	Mor	Sal	Sal
Group 6	Mor	Sal	AMS
Group 7	Mor	Sal	RB101 or Bup or Meth
Group 8	Mor	Sal	AMS + RB101 or AMS + Bup or AMS + Meth

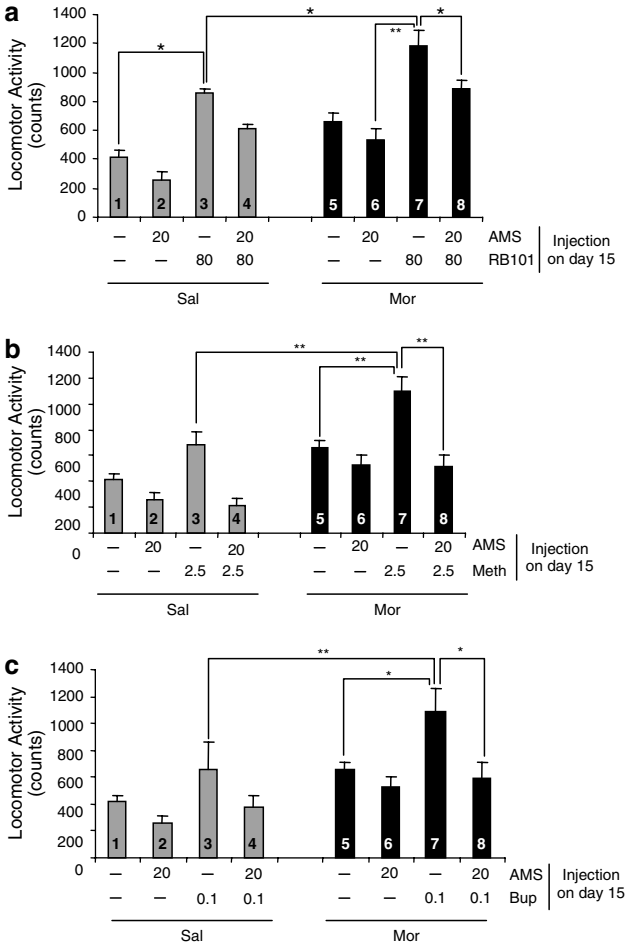

Figure 5 Effect of a challenge injection of RB101 (80 mg kg^{-1}) (**a**), methadone (Meth; 2.5 mg kg^{-1}) (**b**), buprenorphine (Bup; 0.1 mg kg^{-1}) (**c**) alone or in combination with amisulpride (AMS; 20 mg kg^{-1}) on day 15 on locomotor activity of mice previously treated with morphine or saline from day 1 to day 7. Vertical bars represent the mean scores ± s.e.m. for $n = 6$–9 per group after 60 min of recording. *$P < 0.05$, **$P < 0.01$.

the neurobiological consequences of repeated administration of drugs of abuse, suggesting that it is necessary to act simultaneously on different neuronal systems to reverse the sensitized phenotype. These systems must be those that undergo neuroadaptations after cessation of drug administration, ultimately leading to the development of persistent drug hyperresponsiveness.

Sensitization towards morphine in experimental animals was found to be long lasting. This is supported by our results showing that after cessation of morphine treatment for 1 week, an increase in locomotor activity was still observed following morphine administration. Similarly, administration of RB 101, methadone or buprenorphine only on day 15 in morphine-treated mice induced sensitized responses, which were blocked by the dopamine antagonist amisulpride (Figure 5). This sensitization was shown to involve opioid receptors associated with a hypersensitivity of the mesolimbic dopaminergic pathways. Thus, enhanced dopamine transmission in the mesoaccumbens is consistently associated with the expression of behavioral sensitization following repeated morphine administration (Kalivas and Duffy, 1987; Spanagel *et al*., 1993).

Consequently, simultaneous manipulations of both opioid and dopaminergic systems could be an effective mean to counteract the neural processes involved in opiate addiction and resulting in persistent features of drug abuse, such as compulsive drug-seeking behavior. Nevertheless, it could be counter-intuitive to try to prevent morphine-induced sensitization by other μ-opioid receptor agonists, such as the enkephalins. However, we have to keep in mind that even though both morphine and enkephalins bind to the same molecular target, the neural adaptations following repeated activation of μ-opioid receptors by these ligands are different (Whistler *et al*., 1999). This may explain the results obtained, showing that the combination of the dual inhibitor of enkephalin-degrading enzymes, RB101, and the D2 dopamine antagonist amisulpride was able to completely abolish the expression of behavioral sensitization to morphine (Figure 2b), whereas RB101 or amisulpride alone did not have any effect. It is important to emphasize that the effect observed with the combination of RB 101 + amisulpride depends on the treatment and not on the handling and injections, as all animals received i.p. injection (vehicle, RB 101, amilsulpride, or amisulpride + RB 101) in the same conditions during the 8–14 d period. Indeed, there is evidence showing that the ability of drugs to produce their pharmacological effects can be modulated by the environmental factors surrounding drug administration (Browman *et al*., 1998; Robinson *et al*., 1998). It is worth mentioning that amisulpride alone had no hypolocomotor effect on the animals that were not morphine-sensitized (data not shown), indicating that the dopaminergic antagonist, even used at high dose, did not induce sedative effects in mice, 24 h after its last injection. The effects observed with RB101 are certainly resulting from endogenous enkephalins and are not related to protection of other peptides from degradation. Probably for conformational reasons, other opioid peptides, such as β-endorphin or dynorphin, appear to be resistant to NEP and, to a lesser extent, to APN (Turner *et al*., 1987), wheeras other peptides that could modulate opioid system, including nociceptin or cholecystokinin are degraded by other peptidases (Migaud *et al*., 1996; Montiel *et al*., 1997).

Buprenorphine combined with amisulpride was less effective than the combination with RB101 in preventing the expression of behavioral sensitization to morphine (Figure 3b), wheeras both compounds were used at equiactive doses. Several hypotheses may explain these differences. It has been demonstrated that endogenous enkephalins have a high intrinsic efficacy (Noble and Roques, 1995), whereas buprenorphine is a partial agonist with a relatively low efficacy regarding the second messenger cascades (Traynor and Nahorski, 1995; Selley *et al*., 1997).

Moreover, we have previously demonstrated that chronic amisulpride treatment potentiates the effect of δ-opioid agonists (Cordonnier *et al*., 2005). This could explain the strong facilitating effects observed with the combination of RB101 + amisulpride, as enkephalins have high agonist potency toward δ-opioid receptors (Roques and Noble, 2004). In contrast, relatively low effects were observed with the combination of amisulpride + buprenorphine, as bupre-

norphine has no agonist activity at δ-opioid receptors (Toll *et al*., 1998; Lee *et al*., 1999; Romero *et al*., 1999; Huang *et al*., 2001). On the other hand, it has been shown that buprenorphine is a nociceptin (NOP) receptor agonist (Bloms-Funke *et al*., 2000) that could counteract the effects of μ-opioid receptors, as NOP receptors mediate antiopioid activity (Bloms-Funke *et al*., 2000).

Furthermore, the strong potency of the combination of RB101 + amisulpride may support the hypothesis that endogenous opioid peptides are key modulators of the 'hedonic homeostasis' (Kreek, 1997; Noble and Roques, 2002). Previously, we have shown that chronic amisulpride treatment facilitates enkephalin-induced behavioral responses (Cordonnier *et al*., 2005), in agreement with previous studies showing that chronic administration of neuroleptics was able to sensitize the enkephalinergic system (Maldonado *et al*., 1990). Moreover, chronic treatment with several neuroleptics has been shown to increase the synthesis of enkephalins or the preproenkephalin mRNA expression level in the rat striatum (Hong *et al*., 1978; Tang *et al*., 1983; Normand *et al*., 1987, 1988; Caboche *et al*., 1992; Jaber *et al*., 1994). Similar results were obtained following lesion of the mesolimbic dopaminergic system (Kalivas and Bronson, 1985) or by inactivating the D2 receptor in mice (Baik *et al*., 1995). In the same way, we have recently shown that chronic treatment of mice with amisulpride was able to increase preproenkephalin mRNA level in the striatum (L Cordonnier *et al*., unpublished results). Thus, it is possible that amisulpride may mediate its effects both through direct action on the dopaminergic pathway and through modulation of the endogenous opioid system. Both these actions may be synergistic resulting in a full reduction in expression of behavioral sensitization induced by morphine, when enkephalins are protected from their enzymatic degradation.

Regarding the results obtained with methadone, a cross-sensitization to morphine hyperlocomotor effects was observed. Moreover, even in combination with amisulpride, methadone did not prevent the expression of behavioral sensitization to morphine (Figure 4b). The differences between the results obtained with buprenorphine and methadone could result from different mechanisms of action on μ-opioid receptors. Thus, it has been demonstrated by site-directed mutagenesis that methadone and buprenorphine have different determinants for binding to the μ-receptor (Bot *et al*., 1998). Methadone also possesses glutamate receptor antagonist properties in addition to its μ-opioid agonist action (Gorman *et al*., 1997). These could contribute to the result obtained with this compound, even if the role of glutamatergic transmission in behavioral sensitization to opioids was not clearly established (Vanderschuren and Kalivas, 2000).

Moreover, in addition to a limited intrinsic efficacy, buprenorphine dissociates very slowly from μ-opioid receptors, compared with methadone. All these reasons may also explain why buprenorphine is described to produce less motivational effects and less physical dependence than methadone (Tzschentke, 2002).

The efficacy of the classical substitution treatments was not truly apparent in our study and this could be related to the paradigm itself. But in morphine self-administration studies, methadone and buprenorphine have also shown variable efficacy (Mello and Negus, 1996). This is compatible with established clinical data that show that existing maintenance treatments are only effective for a certain proportion of patients (mainly if persistent abuse is considered and not only retention or social improvement).

In summary, our study shows that single administration of RB101, methadone or buprenorphine on day 15 increased locomotor activity in saline- and morphine-treated mice, effects that were blocked, as expected, by the dopamine antagonist, amisulpride. The most important results were obtained in morphine-sensitized mice treated from day 8 to day 14 with the combination of amisulpride + RB101. Indeed, this combination appears to be very efficient in blocking the expression of morphine behavioral sensitization evaluated on day 15. This could be related to a reinstatement of a balanced function of the dopamine and opioid systems and could represent a new approach in maintenance treatments for opiate addiction.

The efficacy of the classical substitution treatment with buprenorphine seems to be slightly but significantly improved by combination with the dopaminergic antagonist amisulpride, whereas this compound was not able to improve the action of methadone, in this paradigm and in our experimental conditions. These results suggest that the combination of an opioid agonist and a dopamine antagonist could represent a possible way to optimize maintenance treatments. With regard to the results obtained in clinical studies, we are currently studying lower doses of methadone. Indeed, as methadone is a more powerful μ-agonist than buprenorphine, the use of smaller doses of methadone could be more relevant and might not induce cross-sensitization to the hyperlocomotor effect of morphine.

Acknowledgements

This work was supported by grants from Baron Edmond de Rothschild Chemical Dependency Institute of Beth Israel Medical Center (New York). We thank Pr MC Fournié-Zaluski and her team for the synthesis of RB101.

Conflict of interest

The authors state no conflict of interest.

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