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Original Paper

Effects of a *Rhodiola rosea* L. extract on acquisition and expression of morphine tolerance and dependence in mice

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Abstract

This study investigated the effect of *Rhodiola rosea* L. extract on acquisition and expression of morphine tolerance and dependence in mice. Therefore animals were injected with repeated administration of morphine (10 mg/kg, subcutaneous) twice daily for five or six days, in order to make them tolerant or dependent. *Rhodiola rosea* L. extract (0, 10, 15 and 20 mg/kg) was administered by the intragastric route 60 min prior to each morphine injection (for acquisition) or prior the last injection of morphine or naloxone on test day (for tolerance or dependence expression, respectively). Morphine tolerance was evaluated by testing its analgesic effect in the tail flick test at the 1st and 5th days. Morphine dependence was evaluated by counting the number of withdrawal signs (jumping, rearing, forepaw tremor, teeth chatter) after naloxone injection (5 mg/kg; intraperitoneal) on the test day (day 6). Results showed that *Rhodiola rosea* L. extract significantly reduced the expression of morphine tolerance, while it was ineffective in modulating its acquisition. Conversely, *Rhodiola rosea* L. extract significantly and dose-dependently attenuated both development and expression of morphine dependence after chronic or acute administration. These data suggest that *Rhodiola rosea* L. may have human therapeutic potential for treatment of opioid addiction.

Keywords

dependence, mice, morphine, *Rhodiola rosea*, tolerance, withdrawal

Introduction

Opioids have been used for millennia for their analgesic and rewarding properties, and morphine is still the most potent painkiller in clinical use today. Unfortunately, chronic opioid administration leads to the development of analgesic tolerance, and to the possible development of dependence and addiction (Ballantyne and Mao, 2003; Inturrisi, 2002; Mercadante, 1999). Indeed, the majority of therapeutically useful opiates, including morphine, elicit rewarding effects that have critical roles in drug abuse and the development of drug addiction, thus promoting the self-administration of these drugs both in human populations and in laboratory animals (Bryant et al., 2005; Kelley and Berridge, 2002). Chronic opioid exposure also generates physical dependence, which is revealed by the emergence of withdrawal symptoms upon treatment cessation (Inturrisi, 2002).

Morphine tolerance and dependence are complex phenomena that involve both neuronal and non-neuronal, i.e. glial, mechanisms. Activation of opioid receptors is a gateway to both the therapeutic and the adverse activities of opioids. Apart from the opioid system, some non-opioid brain systems have been shown to be involved in morphine addiction, including the noradrenergic, dopaminergic, serotoninergic and glutamatergic systems (Harrison et al., 1998; Milanes and Laorden, 2000; Noda and Nabeshima, 2004; Spanagel et al., 1990).

Considerable evidence suggests that mesolimbic dopaminergic projections from the ventral tegmental area to the nucleus accumbens have an important role in the rewarding effects of drug abuse, including those for morphine (Kelley and Berridge, 2002; Lingford-Hughes and Nutt, 2003). There is also increasing evidence that long-lasting neuroadaptation within these brain regions during the development of drug dependence contributes to the negative affective components of withdrawal, and thus underlies the persisting compulsive drug craving during and after abstinence (Koob, 2009; Koob and Le Moal, 1997; Lu et al., 2003).

Detoxification is often the first step in treating individuals with opioid dependence. The medications used to aid detoxification include opioid agonists, which allow a gradual removal of the narcotic from the brain, thereby reducing the intensity of the withdrawal symptoms. Other agents target the neuroadaptation underlying the physiological dependence, such as the *N*-methyl-d-aspartate (NMDA) glutamate receptor antagonists, and some can provide symptomatic relief by targeting specific withdrawal symptoms, such as anxiety.

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A novel approach for the treatment of adverse effects of drugs of abuse makes use of natural products, because of their efficacies and low toxicities (Kim et al., 1998; Lee et al., 2003; Sahraei et al., 2006). Several studies have highlighted the efficacies of medicinal herbs for the reduction of ethanol, nicotine and opioid dependence (Gupta and Rana, 2008; Lu et al., 2009). Recently, some preclinical studies have reported that *Hypericum perforatum* L., *Withania somnifera* Dunal, *Valeriana officinalis* L., *Matricaria chamomilla* L. and *Passiflora incarnata* L. have potential for treating morphine dependence, due to their antidepressant and anxiolytic properties (Dhawan, 2003; Feily and Abbasi, 2009; Kasture et al., 2009; Sharifzadeh et al., 2006). In addition, we have recently demonstrated the positive effects of a *Rhodiola rosea* L. (fam. Crassulaceae) extract in the prevention of nicotine withdrawal symptoms and to counter the development of nicotine dependence (Mattioli and Perfumi, 2009).

In the present study, we have evaluated the effects of a *R. rosea* extract (RHO) on the tolerance and dependence induced by morphine use, since it acts on the main central neurochemical systems that are involved in both addiction and behaviour. Indeed, *R. rosea* is a well-known traditional oriental medicine with adaptogenic, anxiolytic, antidepressive and anti-stress properties (Brown et al., 2002; Mattioli and Perfumi, 2007; Mattioli et al., 2009; Panossian and Wagner, 2005; Panossian et al., 1999; Perfumi and Mattioli, 2007). *R. rosea* appears to modulate the levels and activities of the biogenic monoamines, such as serotonin (5-HT), dopamine (DA) and noradrenaline (NA), and the opioid peptides, such as the beta-endorphins, in the nerve tracts that are mainly involved in the regulation of addiction, as well as of mood, anxiety and emotion (i.e. amygdala, hippocampus, hypothalamus and midbrain) (Kurkin and Zapesochnaya, 1986; Lazarova et al., 1986; Lishmanov et al., 1997; Maĭmeskulova et al., 1997; Saratikov et al., 1978; Stancheva and Mosharrof, 1987). *R. rosea* root extracts can also enhance the effects of these neurotransmitters in the brain, by increasing the permeability of the blood–brain barrier to precursors of dopamine and serotonin (Brown et al., 2002; Kelly, 2001).

Therefore, we have here determined whether pre-treatment with a hydroalcoholic root extract of *R. rosea* (RHO) can counter both the acquisition and the expression of tolerance and dependence induced by morphine use in mice.

Experimental procedures

Animals

Male CD-1 mice (Harlan SRC, Milan, Italy) weighing 25 g to 35 g were used. The mice were kept in a dedicated room, with a 12:12 h light/dark cycle (lights on at 09:00), a temperature of 20°C to 22°C, and a humidity of 45% to 55%. They were provided with free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). Each mouse was used in only one experimental session. All of the procedures were conducted in adherence to the European Community Directive for the Care and Use of Laboratory Animals. Ethical guidelines for the investigation of experimental pain in conscious animals were followed. The procedures were carried out according to the EEC ethical regulations for animal research (EEC Council 86/609; D.Lgs. 27/01/1992, No. 116), and tests have been approved by the local ethical committee.

Drugs

A dried hydroalcoholic extract from the roots of *R. rosea* (RHO) was used (provided by EPO S.r.l., Milan, Italy). The high-performance liquid chromatography (HPLC) analysis report showed a content of 3% total rosavins, expressed as rosavin and 1% salidroside. The ratio of rosavin to salidroside (3 : 1) is in line with published data (Abidov et al., 2003; Kurkin and Zapesochnaya, 1986). The extract was dissolved in absolute ethanol and diluted in tap water, to a final ethanol concentration of 1% v/v under all treatment conditions. RHO was administered orally (gavage; IG) at doses of 10, 15 and 20 mg/kg/10 ml. The same vehicle was administered to the control group.

Morphine hydrochloride (Salars S.p.a, Como, Italy) was dissolved in saline immediately before use, and administered subcutaneously (s.c.) at a dose of 10 mg/kg/10 ml. Naloxone hydrochloride (Sigma, St. Louis, MO, USA) was dissolved in water immediately before use, and administered intraperitoneally (i.p.) at a dose of 5 mg/kg/5 ml.

Induction of morphine tolerance

In accordance with Ren et al. (2004) and Abdel-Zaher et al. (2006), tolerance was induced in two groups of mice by the injection of morphine (10 mg/kg; s.c.), twice daily at 12-h intervals for five days. Tolerance was evaluated by testing the antinociceptive response to morphine in the tail flick test on the 5th day, 30 min after the last morphine injection. For the effects of RHO on the acquisition (development) of morphine tolerance, one group of mice was orally administered with 10, 15 and 20 mg/kg RHO, or vehicle alone, twice daily, 60 min before each morphine treatment.

To determine the effects of RHO on the expression phases of morphine tolerance, another group of morphine-treated mice received acute administration of RHO (10, 15 and 20 mg/kg) or vehicle, only 60 min before the last morphine injection on the test day (day 5). In addition, the effects of RHO alone on antinociception were examined in non-dependent mice. Animals received single or repeated administrations of different doses of RHO (10, 15 and 20 mg/kg; IG) or vehicle (10 ml/kg; IG).

The effects of RHO on development and expression of morphine tolerance were evaluated by testing the analgesic effects of morphine in the tail-flick test. Briefly, this consisted of irradiation of the lower third of the tail with an infrared source (Ugo Basile, Comerio, Italy). The basal pre-drug latency, which ranged from 2–3 s, was calculated as the mean of two trials performed with a 30 min interval. The mice then received RHO or the relevant vehicle 60 min before morphine or saline administration. The antinociceptive activity was evaluated on the 5th day, 30 min after the last morphine injection. A cut-off latency of 12 s was established to minimize tissue damage.

The antinociceptive effects are expressed as a percentages of the maximum possible effect (%MPE), according to the following formula: %MPE = (post-drug latency–baseline latency)/(cut-off value–baseline latency) × 100, where the post-drug latency was the tail-flick latency 30 min after the last morphine dose.

Induction of morphine dependence

To induce morphine dependence, naïve mice were treated with morphine (10 mg/kg; s.c.) twice daily at 12-h intervals for six days (Abdel-Zaher et al., 2006; Ren et al., 2004). Two hours after the last dose of morphine, the withdrawal syndrome (abstinence), as an index of morphine dependence, was precipitated by an i.p. injection of naloxone (5 mg/kg) (Abdel-Zaher et al., 2006; Ren et al., 2004). The combination of morphine with high dose naloxone on day 6 has been demonstrated to induce more severe symptoms, including autonomic signs, since naloxone precipitates dose-dependent withdrawal symptoms in animals acutely or chronically dependent upon morphine (Ren et al., 2004). Ten minutes before naloxone treatment, the mice were placed in a transparent acrylic cylinder (20 cm diameter, 35 cm high) to habituate them to the new environment. Immediately after the naloxone challenge, each mouse was again placed gently into the cylinder, and then monitored for 15 min for the occurrence of withdrawal symptoms (jumping, rearing, forepaw tremor, teeth chatter).

To examine the effects of RHO on morphine dependence, RHO was given at different doses (0, 10, 15 and 20 mg/kg; IG) to one group of mice chronically treated 1 h prior to each morphine injection (acquisition), or to another group of mice acutely treated before naloxone (expression) as described above. In addition, the effects of RHO alone on naloxone-induced jumping behaviour were examined in non-dependent mice, where they received single or repeated administration of different doses of RHO (10, 15 and 20 mg/kg; IG) or vehicle (10 ml/kg; IG). The assessment of naloxone-precipitated jumping behaviour after the administration of RHO has been described above.

Statistical analysis

The statistical analysis was performed using two-way split-plot analysis of variance (ANOVA), with treatment as the between-subject factor, and time as the within-subject factor, to analyse morphine tolerance; when appropriate, one-way ANOVA was used. The morphine dependence data were analysed by one-way ANOVA. The differences between groups were determined by Newman-Keuls post-hoc analysis. Statistical significance was set at $p < 0.05$, and the data are expressed as means ± S.E.M.

Results

Effects of RHO on expression of analgesic tolerance to morphine

The effects of RHO on the expression of tolerance to morphine-induced analgesia are shown in Figure 1.

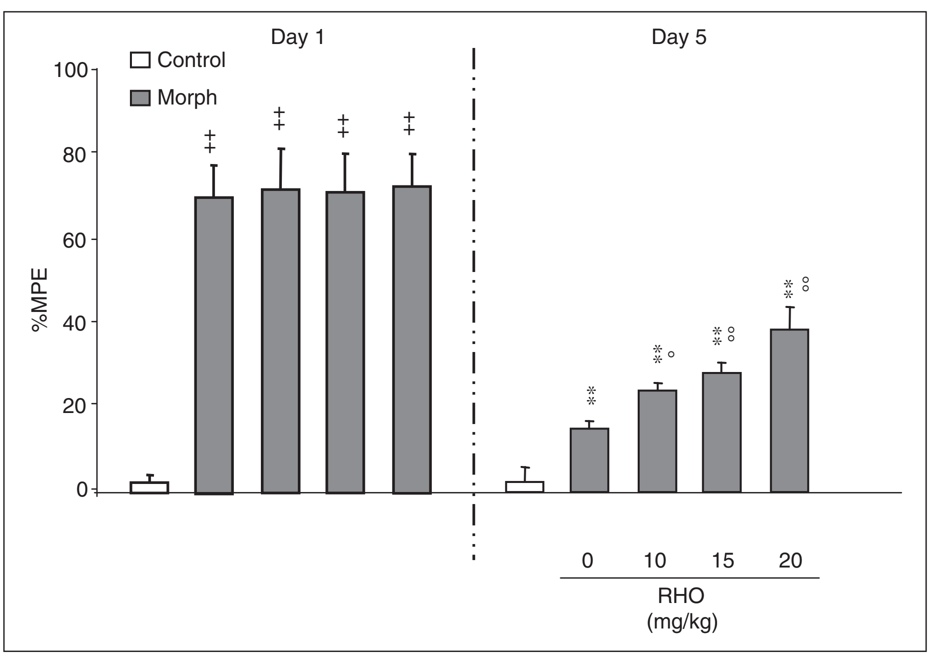

Figure 1. Effects of a single administration of RHO on the expression of tolerance to morphine-induced analgesia. Mice were treated twice daily for 5 days with either saline (Control; $n = 10$) or 10 mg/kg morphine (Morph; $n = 41$). RHO (0, 10, 15 and 20 mg/kg) was administered 1 h before the last morphine treatment. Morphine antinociceptive effect (% MPE) was assessed on day 1 and day 5, as indicated. Significant differences: $^{++}p < 0.01$, compared with control group; $^{**}p < 0.01$, compared with related-morphine group on day 1; $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$, compared with morphine group on day 5.

The overall analysis of variance revealed statistically significant treatment and time effects ($F(4,46) = 28.751$, $p < 0.001$; $F(1,4) = 94.892$, $p < 0.001$, respectively), and interaction time × treatment effects were seen for analgesia latency ($F(4,46) = 5.656$, $p < 0.001$).

The mice treated with morphine showed a maximal antinociceptive effect (%MPE) on day 1 ($p < 0.01$, between control and morphine groups). Repeated administration of 10 mg/kg morphine s.c. twice daily to morphine-treated mice induced a significant decrease in the analgesia latency in the tail-flick test, which resulted in a marked reduction on day 5, as compared with day 1 ($F(1,18) = 150.621$; $p < 0.001$) (Figure 1). Acute administration of RHO (10, 15 and 20 mg/kg) 60 min before morphine injection on the test day produced significant decreases in the expression of morphine tolerance in a dose-dependent manner, as compared with the morphine–vehicle group ($F(4,46) = 19.714$; $p < 0.001$). In particular, the post-hoc analysis revealed a statistically significant effect at all of the doses tested (RHO 10, $p < 0.05$; RHO 15, $p < 0.01$; RHO 20, $p < 0.001$) (Figure 1). Acute administration of RHO to non-dependent mice (Control) did not modify the analgesia latency in these mice ($p > 0.05$) (data not shown).

Effects of RHO pretreatment on development of analgesic tolerance to morphine

The effects of RHO on the development of tolerance to morphine-induced analgesia are shown in Figure 2. The overall analysis of variance revealed statistically significant treatment and time effects ($F(7,51) = 21.727$, $p < 0.001$, $F(1,7) = 9.833$, $p < 0.01$, respectively), and interaction time × treatment effects were seen for analgesia latency ($F(7,51) = 22.764$, $p < 0.001$). The mice treated with morphine showed a maximal antinociceptive effect (%MPE) on day 1 ($F(7,51) = 38.483$; $p < 0.001$). The post-hoc analysis revealed that acute co-administration of RHO with morphine did not modulate the analgesia at any of the doses tested on day 1, as compared with the morphine–vehicle group ($p > 0.05$). Repeated administration of 10 mg/kg morphine s.c. twice daily to the mice induced a significant decrease in the analgesia latency in the tail-flick test on day 5, seen as a 60% reduction ($F(7,51) = 9.248$; $p < 0.01$) (Figure 2). Pretreatment of the mice with 10, 15 and 20 mg/kg RHO 60 min before each morphine injection did not inhibit the development of tolerance to morphine analgesia at any of the doses tested ($p > 0.05$) (Figure 2). Finally, repeated administration of RHO to non-dependent mice (Control) did not modify the analgesia latency in these mice ($p > 0.05$) (Figure 2).

Effects of RHO on expression of morphine dependence

Figure 3 shows the effects of RHO on naloxone-precipitated withdrawal syndrome behaviour. Repeated administration of morphine produced physical dependence, as assessed by a characteristic set of behavioural responses, which included jumping ($F(1,18) = 67.397$; $p < 0.001$), rearing ($F(1,18) = 48.890$; $p < 0.001$), forepaw tremor ($F(1,18) = 20.404$; $p < 0.001$) and teeth chattering ($F(1,18) = 21.729$; $p < 0.001$)

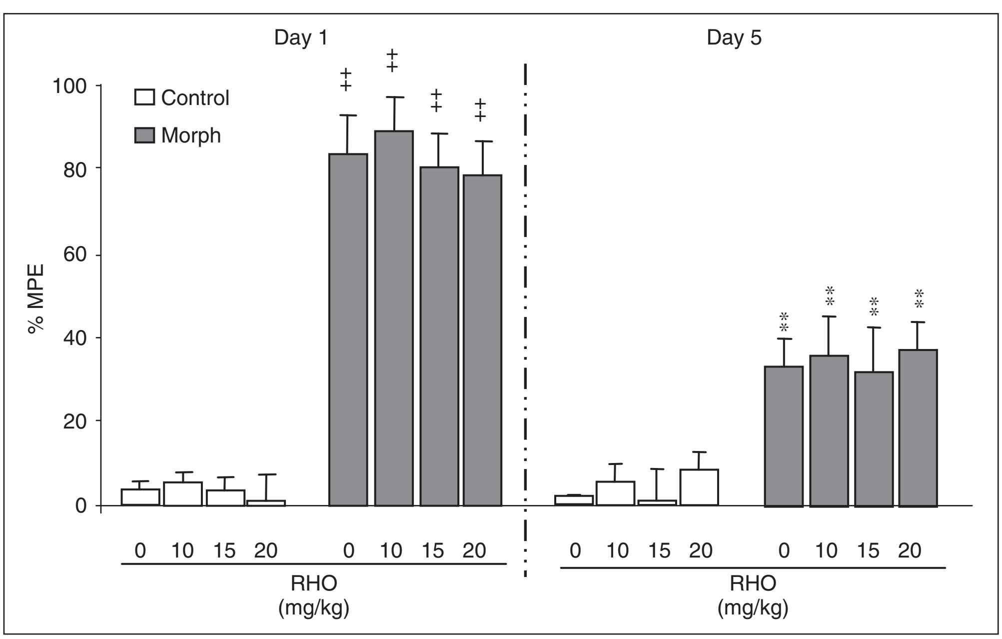

Figure 2. Effects of repeated administration of RHO on the development of tolerance to morphine-induced analgesia. Mice were treated twice daily for 5 days with either saline (Control; $n = 24$) or 10 mg/kg morphine (Morph; $n = 35$), with RHO (0, 10, 15 and 20 mg/kg) repeatedly administered 1 h before each morphine or saline treatment. Morphine antinociceptive effect (% MPE) was assessed on day 1 and day 5, as indicated. Significant differences: $^{++}p < 0.01$, compared with control group; $^{**}p < 0.01$, compared with related-morphine group on day 1.

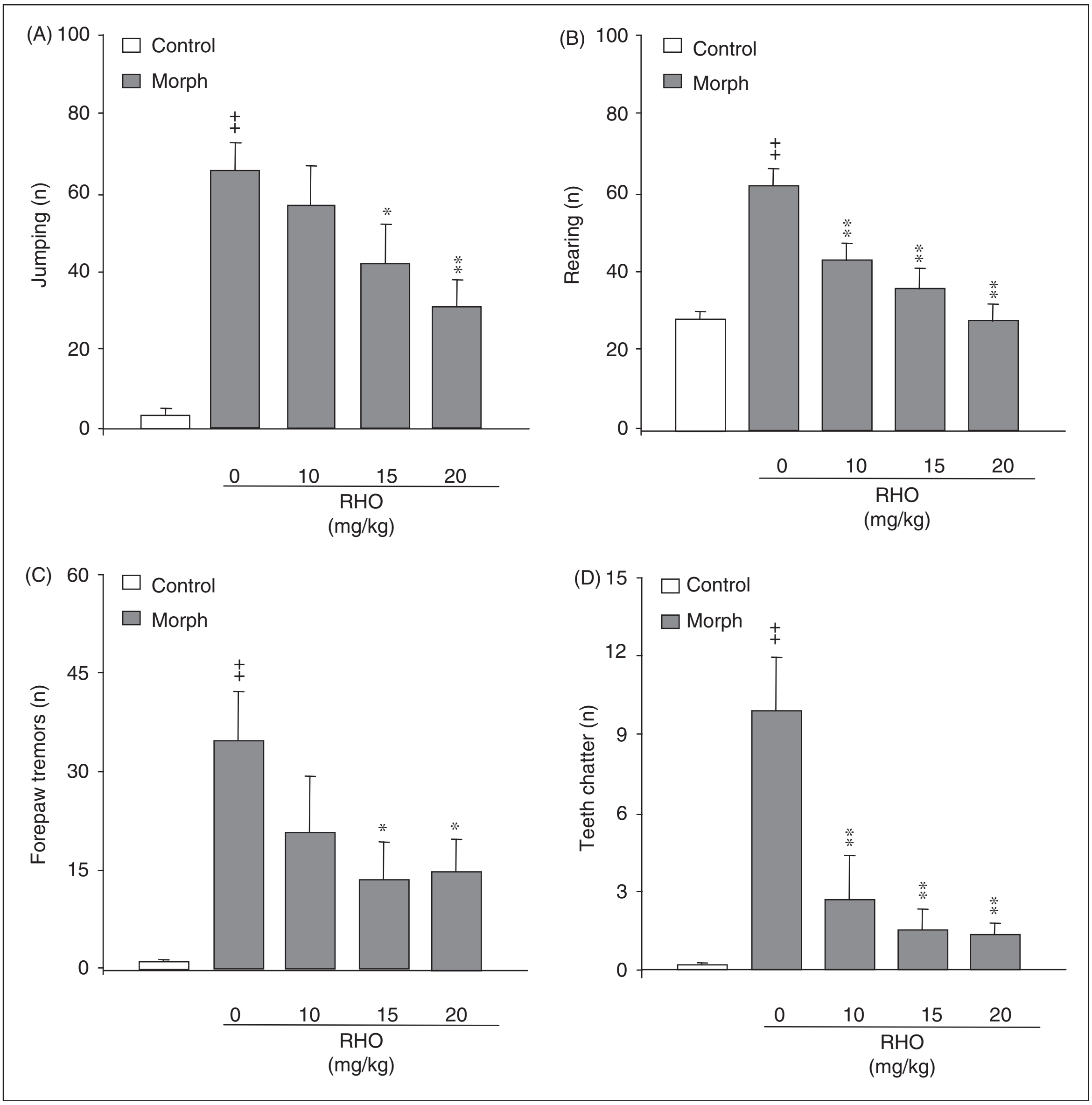

Figure 3. Effects of a single administration of RHO on the expression of morphine dependence. Mice were treated twice daily for five days with saline (Control; $n = 10$) or 10 mg/kg morphine (Morph; $n = 41$) treatment. On the sixth day, withdrawal syndromes were precipitated by injection of 5 mg/kg naloxone, 2 h after the last morphine injection. RHO (0, 10, 15 and 20 mg/kg) was administered 1 h before naloxone injection. The withdrawal symptoms are given as a measure of the frequency of these somatic signs: jumping (A), rearing (B), forepaw tremors (C), teeth chatter (D). Significant differences: $^{++}p < 0.01$, compared with control; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with morphine group.

following the naloxone challenge. As shown in Figure 3, acute administration of RHO 60 min prior to the naloxone injection significantly decreased the frequencies of the signs of withdrawal syndrome in a dose-dependent manner, as compared with frequencies of withdrawal manifestations of morphine-dependent mice treated with vehicle (jumping ($F(4,46) = 9.237$; $p < 0.001$), rearing ($F(4,46) = 11.672$; $p < 0.001$), forepaw tremor ($F(4,46) = 3.743$; $p < 0.01$) and teeth chattering ($F(4,46) = 9.800$; $p < 0.001$). No differences were seen for the control mice treated at all of the doses of RHO, as compared with vehicle ($p > 0.05$) (data not shown).

Effects of RHO pretreatment on development of morphine dependence

Figure 4 shows the effects of repeated co-administration of RHO with morphine on the naloxone-precipitated withdrawal symptoms. Following the naloxone challenge, the mice that received repeated administrations of morphine showed severe signs of withdrawal, as manifested by large significant increases in their jumping ($F(1,11) = 10.800$; $p < 0.01$), rearing ($F(1,11) = 12.528$; $p < 0.01$), forepaw tremor ($F(1,11) = 9.477$; $p < 0.01$) and teeth chatter

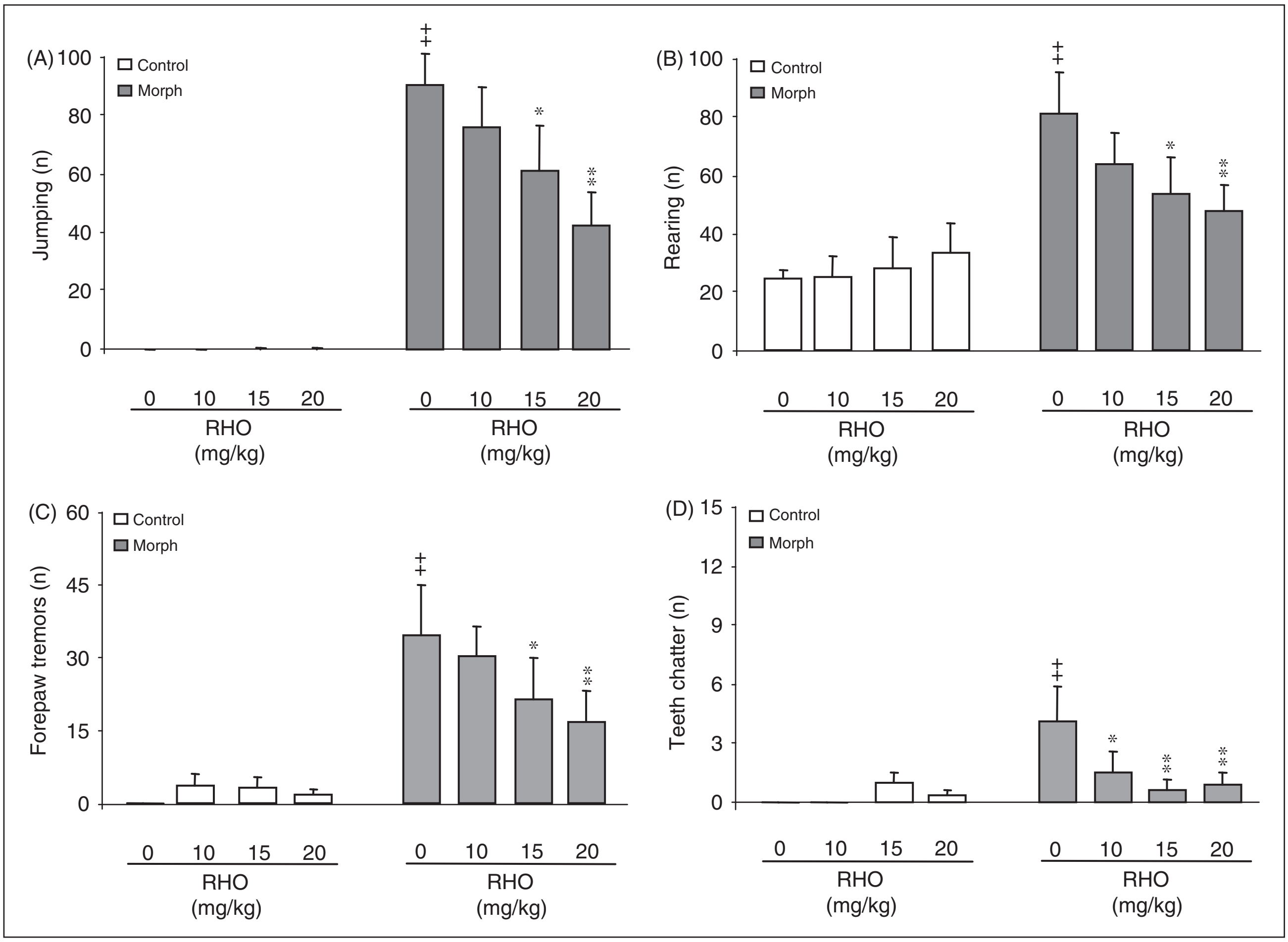

Figure 4. Effects of repeated administration of RHO on the development of morphine dependence. RHO (0, 10, 15 and 20 mg/kg) was repeatedly administered 1 h before every saline (Control; $n = 24$) or 10 mg/kg morphine (Morph; $n = 33$) treatment, twice daily for five days. On the sixth day, withdrawal syndromes were precipitated by injection of 5 mg/kg naloxone, 2 h after the last morphine injection. The withdrawal symptoms are given as a measure of the frequency of these somatic signs: jumping (A), rearing (B), forepaw tremors (C), teeth chatter (D). Significant differences: $^{++}p < 0.01$, compared with control; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with morphine group.

($F(1,11) = 53.698$; $p < 0.001$), as compared with the saline control group. Pretreatment of the mice with RHO 60 min before each morphine injection attenuated the development of these signs of withdrawal: jumping ($F(7,49) = 8.491$; $p < 0.001$), rearing ($F(7,49) = 2.567$; $p < 0.05$), forepaw tremor ($F(7,49) = 4.344$; $p < 0.001$) and teeth chatter ($F(7,49) = 10.282$; $p < 0.001$) (Figure 4). Indeed, the post-hoc analysis revealed statistically significant effects at the lowest RHO doses tested, of 10 and 15 mg/kg ($p < 0.05$), and a marked inhibition with the highest dose of 20 mg/kg, which reduced each of the naloxone-induced withdrawal symptoms by about 50% in the morphine-dependent mice ($p < 0.001$). The mice treated with only RHO (10, 15 and 20 mg/kg) did not show any significant differences in their withdrawal symptoms, as compared with the control group ($p > 0.05$) (Figure 4).

Discussion

The present study has evaluated the effects of RHO, a hydro-alcoholic extract of *R. rosea*, on both the acquisition and the expression of morphine tolerance and physical dependence in mice. The data demonstrate that administration of RHO attenuates the expression of morphine tolerance by increasing the antinociceptive response, but does not attenuate its acquisition. This lack of effect on tolerance acquisition might be due to the inability of RHO to counter the complex neuroadaptation involved in tolerance induction, including opioid receptor down-regulation and internalization (DuPen et al., 2007). At the same time, the mechanisms underlying opioid-induced tolerance are not completely understood.

Conversely, administration of RHO attenuates both the expression and the development of morphine dependence by reducing the naloxone-induced behavioural and vegetative withdrawal symptoms in a dose-dependent manner. Indeed, RHO was effective in reducing the incidence of withdrawal symptoms in morphine-dependent mice (jumping, rearing, forepaw tremor, teeth chatter). These data assume greater importance when it is considered that, historically, withdrawal symptoms were believed to have a major role in the relapse to drug-taking behaviour after drug abstinence (Koob et al., 1989).

The exact mechanisms underlying the ability of RHO to prevent dependence induced by morphine abuse are unclear. Multiple cellular and receptor adaptation processes are likely to take place under opioid treatment, and these have been relatively well documented. The involvement of limbic structures (nucleus accumbens, ventral tegmental areas and amygdala) and other related brain structures has been established in the mediation of the negative affective components of withdrawal with drugs of abuse and of the underlying the persistent compulsive drug craving following abstinence (Frenois et al., 2002; Koob and Le Moal, 1997; Lu et al., 2003; Nestler, 2001; Schulteis et al., 2000; Wise, 1988). Additionally, mesencephalic and autonomic brain areas (dopaminergic and noradrenergic nuclei, motor striatal areas, hypothalamic nuclei and periaqueductal grey) are preferentially involved in the expression of the overt somatic signs that appear during morphine withdrawal syndrome (Frenois et al., 2002).

R. rosea extracts appear to produce increases in the brain content of neurotransmitters such as NA, DA and 5-HT (Brown et al., 2002; Kelly, 2001), whereby deficits in these neurotransmitters have been shown to have roles in the expression of drug abstinence syndromes (Quattrocki et al., 2000). Thus, we would speculate that RHO reduces withdrawal symptoms and prevents dependence by enhancing the functional tone of these neurotransmitters in the same nerve tracts that are involved in both affective and somatic components of withdrawal, which would lead to the counteracting of long-lasting neuroadaptation (Kelly, 2001). Indeed, the ability of RHO to reduce both affective and somatic signs of nicotine withdrawal has been demonstrated recently (Mattioli and Perfumi, 2009).

Other potential neuronal processes underlying the effects of *R. rosea* extracts on morphine dependence include monoamine oxidase (MAO) inhibition. Recently Van Diermen et al. (2009) reported that *R. rosea* extracts exert their antidepressant activity by inhibiting MAOs A and B, two enzymes implicated in the degradation of biogenic amines. These include adrenaline, NA, DA and 5-HT, which are all closely involved in the reinforcing and rewarding effects of opioids (Blum et al., 2007; Cao et al., 2003; Kauhanen et al., 2000). Therefore, RHO might enhance monoaminergic neurotransmitter levels in synapses, and consequently promote the prevention of withdrawal syndrome.

Other possible neuronal processes underlying the effects of *R. rosea* extracts involve activation of the brain stress system. Previous evidence has shown that the function of corticotrophin-releasing factor (CRF), a 41-amino-acid neuropeptide, appears to be activated in the brain during drug withdrawal or exposure to drug cues (Basso et al., 1999; Sarnyai et al., 2001). CRF is not only considered to be an important neurotrophic factor for the initiation of behavioural responses to stress (Koob and Bloom, 1985; Vale et al., 1981), but it is also believed to act as a neurotransmitter for opioid addiction in the central nervous system, since the actions of opioids on the hypothalamic–pituitary–adrenal axis are believed to be mediated directly or indirectly by the release of CRF (Goeders, 1997; Pechnick, 1993; Sarnyai et al., 2001). Moreover, it has been reported that pretreatment with CRF antagonists can reduce several signs of opiate withdrawal (e.g. jumping, teeth chattering, writhing) (Heinrichs et al., 1995; Papaleo et al., 2007, 2008; Stinus et al., 2005). Previous studies have shown that RHO can reduce stress-induced and CRF-induced anorexia, probably by acting as a CRF antagonist (Mattioli and Perfumi, 2007; 2009; Olsson et al., 2009; Panossian and Wikman, 2009; Panossian et al., 2007; Shevtsov et al., 2003). Therefore, we can hypothesize that the *R. rosea* extract prevents both acquisition and expression of morphine dependence also by acting as a CRF antagonist.

The glutamatergic system is no less important, as it has an important role in morphine-induced neural and behavioural adaptation (Trujillo, 2000). Indeed, increased brain glutamate activity is related to the severity of the opioid withdrawal syndrome (Guo et al., 2009). Thus, glutamate antagonists have some beneficial effects on the severity of the signs of opioid withdrawal (Asl et al., 2008; Bisaga and Popik, 2000). In this context, it has been shown that salidroside, one of the main components of the *R. rosea* extract, increases Ca^{2+} release induced by glutamate and protects glutamate excitotoxic damage (Cao et al., 2006). In addition, there is increasing evidence that nitric oxide (NO) interacts with glutamate neurotransmission. Indeed, the activation of NMDA receptors is accompanied by increased formation of NO and increased NO release. Thus, NMDA modulation of opioid withdrawal might, at least in part, involve activation of NO synthesis (Uzbay and Oglesby, 2001). Glutamatergic inhibition and interaction with NO via NMDA receptors would be a more important approach for an explanation of the mechanisms of action of RHO.

Further explanation of the effects of RHO in morphine tolerance and dependence might involve effects on Hsp70 and Hsp72, the major inducible heat-shock proteins that protect the brain against the potentially harmful effects of morphine (Ammon-Treiber et al., 2004). Indeed, the hypothesis has recently been advanced that adaptogens such as *R. rosea* adapt the organism to stress conditions (i.e. morphine dependence) by regulating the 'stress-sensor' proteins Hsp70 and Hsp72, which have important roles as mediators of the stress response involved in the reparation of proteins during stress events (Panossian and Wikman, 2009; Panossian et al., 2009).

One of the most fascinating aspects of RHO is that it had absolutely no effects on physical and behavioural signs in naïve animals. Therefore, the use of RHO provides an opportunity to manipulate a system only when it is altered, as after chronic morphine exposure, without affecting it under normal physiological conditions (Barik and Wonnacott, 2009).

Several groups of biologically active substances have been identified in extracts of *R. rosea*, including organic acids, flavonoids, tannins and high amounts of phenolic compounds. In particular, the phenolic compounds include phenylpropane derivatives, such as the rosavins (rosavin, rosine, rosarin), which are specific components of *R. rosea*, and phenylethane derivatives, such as tyrosol and salidroside, which are present in all species of the *Rhodiola* genus and in a wide variety of species outside this genus (Kurkin and Zapesochnaya, 1986; Linh et al., 2000; Wang et al., 1992; Yoshikawa et al., 1996).

Therefore, it is far from established which compound(s) are actually responsible for the effects of RHO. However, even if it is not clear what is the active moiety that is responsible for the actions of *R. rosea* extracts, the compounds rosavin, rhodioloside and tyrosol that are present in this plant, and are structurally related to the catecholamines, might have important roles in coordinating and integrating the behavioural responses to addiction that are induced by this *R. rosea* extract (Panossian et al., 2008).

In conclusion, the positive effects of RHO in the expression of morphine tolerance and dependence, and its influence in the development of morphine dependence, demonstrate its human therapeutic potential and encourage the use of *R. rosea* extracts for treatment of opioid addiction. However, there remains the need for further studies to clarify the mechanisms of action and the active ingredients in *R. rosea* extracts that are responsible for the effects seen.

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