Psychopharmacology
DOI 10.1007/s00213-013-3351-y

ORIGINAL INVESTIGATION

Rhodiola rosea L. extract and its active compound salidroside antagonized both induction and reinstatement of nicotine place preference in mice

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Received: 26 June 2013 / Accepted: 28 October 2013

Abstract
Rationale Conventional pharmacological treatments for drug addiction aim to reduce three most important aspects: withdrawal syndrome, craving, and relapse. Pharmacological treatments currently available for the treatment of tobacco smoking are able to alleviate withdrawal symptoms but are not sufficiently effective in reducing craving and rarely effective to prevent relapse. *Rhodiola rosea* L., a well-known traditional oriental medicine with anxiolytic, antidepressive, antistress, and adaptogenic properties, has been recently shown to be effective in the prevention and treatment of nicotine-withdrawal symptoms.
Objectives The present study used the conditioned place preference (CPP) model to systematically investigate, in mice, the effects of a *R. rosea* L. extract (RHO) and its active compound salidroside (SDS), on the reinforcing properties of nicotine and their efficacy in the vulnerability to reinstatement.
Methods To study the effects on the rewarding properties of nicotine, RHO (10, 15, and 20 mg/kg) and SDS (0.2 mg/kg) were tested both in the acquisition and expression of CPP induced by nicotine injection (0.5 mg/kg). Moreover, the efficacy of RHO and SDS in preventing relapse induced by nicotine priming (0.1 mg/kg, s.c.) and by restraint stress was also evaluated.
Results Results showed the ability of RHO and salidroside to significantly reduce the rewarding properties of nicotine at all doses tested. RHO and SDS also suppressed both priming- and stress-induced reinstatement of CPP.
Conclusions The present study showed the positive effects of *R. rosea* L. in reducing rewarding properties and preventing relapse to nicotine and evidenced the important role of salidroside in the effects of the extract.

Keywords Addiction · Conditioned place preference · Nicotine · Reinstatement · *Rhodiola rosea* L. · Salidroside · Stress · Locomotor activity

Introduction

It is well established that tobacco use through cigarette smoking is the primary preventable cause of morbidity and mortality in modern society, and it is widely known that smoking causes a wide range of negative health consequences (Le Foll and Goldberg 2009; Tang and Dani 2009; Mdege and Chindove 2013). Even so, among smokers who attempt to quit without the help of a smoking cessation aid, fully 80 % relapse within the 1st year (Coleman et al. 2010; Gonzales et al. 2006).

In recent decades, several treatments have been developed to help people quit smoking. Treatment to help stop tobacco use and break dependence is effective, although long-term abstinence rates remain disappointingly low. Nicotine replacement therapy (NRT) (in the form of nicotine patch, nicotine gum, nicotine lozenge, nicotine inhaler, or nicotine nasal spray) or drugs for smoking cessation (bupropion sustained release and varenicline) are approved to treat tobacco dependence in most countries, and many national and professional society practice guidelines recommend their use (Hays and Ebbert 2010). Although each of the medications used to treat tobacco dependence has been rigorously tested for efficacy and safety, broader experience in clinical trials and in observational population-based studies suggests that adverse events associated with these medications are relatively common. Two of the medications (varenicline and bupropion) have come under increasing scrutiny because of reports of unexplained

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Published online: 22 November 2013

serious adverse events (SAEs), including behavior change, depression, self-injurious thoughts, and suicidal behavior (Hays and Ebbert 2010). A number of adverse effects are also commonly associated with NRT use, due to the pharmacological action of nicotine as well as the mode and site of the NRT application, although SAEs are rare (Rollema et al. 2007). Moreover, among the pharmacological treatments that have often been used to reduce withdrawal symptoms, few can reduce the drug craving, and they are also rarely effective in preventing relapse. In view of these limitations, interest has been growing in a treatment strategy that is aimed at reducing the three most important aspects: withdrawal syndrome, craving, and relapse.

Several studies have highlighted the efficacy of medicinal herbs in reducing drug dependence (Gupta and Rana 2008; Lee et al. 2003; Lu et al. 2009; Sahraei et al. 2006). In particular, some preclinical studies have reported that *Rhodiola rosea* L. extract (RHO) has a potent effect in the prevention and treatment of morphine dependence and reinstatement (Mattioli and Perfumi 2011a; Mattioli et al. 2012). These results suggested that RHO can reduce craving and vulnerability to relapse and might be an effective natural remedy for the treatment of opioid addiction (Mattioli et al. 2012). *Rhodiola* extracts were also efficacious in the prevention and treatment of nicotine dependence (Mannucci et al. 2012; Mattioli and Perfumi 2011b). *R. rosea* L. (family Crassulaceae) known as golden root or rosenroot, grows in arctic regions of Europe and Asia, and because of its anxiolytic, antidepressive, and antistress properties, it is one of the most popular plant adaptogens used today (Bystritsky et al. 2008; Darbinyan et al. 2007; Edwards et al. 2012; Mattioli and Perfumi 2007; Mattioli et al. 2009; Olsson et al. 2009; Panossian et al. 2010; Parisi et al. 2010; Perfumi and Mattioli 2007). *Rhodiola* rhizomes contain flavonoids, monoterpenes, triterpenes, phenolic acids, phenylethanol derivates (salidroside and tyrosol), and phenylpropanoid glycosides such as rosin, rosavin, and rosin specific to this plant (Ali et al. 2008; Ganzera et al. 2001). One of the most active constituents of rhizome is salidroside (SDS), a constituent with known therapeutic activity (Panossian and Wagner 2005; Panossian et al. 2010). *R. rosea* L. extract and salidroside appear to modulate the levels and activities of biogenic monoamines, such as serotonin (5-HT), dopamine (DA), and noradrenaline (NA). They also influence 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., the amygdala, hippocampus, hypothalamus, and midbrain) (Chen et al. 2009; Kelly 2001; Mannucci et al. 2012). It has been demonstrated that *R. rosea* L. and salidroside interact with the HPA system, particularly by inhibiting stress-induced secretion of cortisol (Olsson et al. 2009; Panossian et al. 2007, 2009), nitric oxide (Panossian et al. 2007, 2009), and heat shock protein 70 (Hsp70) molecules, which are involved in defense mechanisms that cope with stress and stress-induced disorders. Several studies indicate that stressors facilitate the initiation of smoking, decrease the motivation to quit, and increase the risk for relapse (Bruijnzeel 2012).

Therefore, based on these preclinical data, the present study was aimed to evaluate the efficacy of hydroalcoholic extract of *R. rosea* L. (RHO) and salidroside (SDS) in nicotine-positive reinforcement using the conditioned place preference (CPP) animal model, which is used to measure the appetitive value of natural and synthetic substances as well as to evaluate relapse to the abuse of drugs such as cocaine, opiates, alcohol, amphetamine, and nicotine (Biala and Budzynska 2006, 2008; Tzschentke 2007). According to recent studies, an unbiased assignment procedure and low doses of nicotine are effective for inducing CPP with nicotine in mice (Fattore et al. 2009; Kota et al. 2007, 2011). Taking into consideration these aspects, preliminary experiments examined several nicotine doses (0.1–1.0 mg/kg) to determine an effective nicotine dose to produce a CPP and to characterize nicotine-induced CPP in CD-1 mice. Nicotine-induced changes in locomotor activity also were examined and characterized in CD-1 mice (Rauhut et al. 2008). Once an effective nicotine dose was found and characterized, the effects of RHO and SDS on the establishment and expression of nicotine-induced CPP were determined. The effects of RHO and SDS on potential nicotine-induced changes in locomotor activity were also determined. Relapse is a major characteristic of drug addiction and the primary problem in the treatment of drug abuse (O'Brien 1997). Different types of stimuli can increase craving and subsequent vulnerability to relapse after detoxification. However, numerous preclinical and clinical studies have shown that the most important factor involved in drug-seeking behavior both in human addicts and in rodents is reexposure to the drug (priming) or to stressful conditions (Aguilar et al. 2009; Ribeiro Do Couto et al. 2003, 2006). Therefore, in the second set of experiments, we also used the model of CPP to assess the efficacy of RHO and SDS in the reinstatement of drug-seeking behavior induced by priming and by restraint stress.

Materials and methods

Animals

Male CD-1 mice (Harlan SRC, Milan, Italy) weighing 25 to 30 g were used. These mice were kept in a dedicated room, with a 12:12-h light/dark cycle (lights on at 0800 hours), temperature of 20 to 22 °C, and humidity of 45 to 55 %. They were provided free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). The mice were acclimatized to the housing conditions and handled for 7 days

before the start of the experiments. Animals were used only in one CPP experiment, and every effort was made to minimize animal suffering. All experiments were carried out according to the European Community Council Directive of 24 November 1986 (86/609/EEC).

Drugs

A dried hydroalcoholic extract from the roots of *R. rosea* L. (RHO) was used (provided by EPO S.r.l., Milan, Italy; lot number 1100179). The HPLC analysis report showed a content of 3 % total rosavins, expressed as rosavin, and 1 % salidroside (for chemical structures of main active compounds and HPLC fingerprint, see Mattioli et al. 2012). The extract and salidroside were dissolved in 1 % *v*/*v* ethanol solution and administered by gavage (intragastric (IG)) at doses of 10, 15, and 20 mg/kg/10 ml of RHO and 0.2 mg/kg/10 ml of SDS. The same vehicle (10 ml/kg of 1 % *v*/*v* ethanol solution) was administered to the control group. (−)-Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO, USA) was dissolved in saline immediately before use (pH adjusted to approximately 7.2 with NaOH 1 M) and subcutaneously (s.c.) administered at doses of 0.1, 0.3, 0.5, and 1.0 mg/kg (expressed as a free base).

Nicotine-induced CPP

An unbiased CPP paradigm was utilized in all studies (Kota et al. 2007, 2009, 2011; Sahraei et al. 2007). The animals' preference for the black compartment was 461±30 s, while that for the white side was 434±38 s. In addition, the drug and control compartments were randomly assigned for each animal. The place preference apparatus consisted of two distinct sides (20 cm×20 cm×20 cm), one black and one white, each with unique flooring. A partition separated the two sides with an opening that allowed access to either side of the chamber, and this partition could be closed for pairing days. In line with Kota et al. (2007), the CPP consisted of four distinct phases: handling habituation, preconditioning phase, conditioning phase, and postconditioning (test) phase. For all these phases, the mice were tested daily during the same time period.

Handling habituation

From Wednesday to Friday of the week, before the start of the place conditioning procedure, mice in the conditioned place preference studies were handled once a day for approximately 2 min each. Handling experience plays an important role in the ability of nicotine to produce CPP (Grabus et al. 2006; Kota et al. 2007).

Preconditioning phase

On day 1, animals were placed in the boxes with free access to all compartments for 15 min, and the time spent in each side was recorded.

Conditioning phase

On days 2–4, mice were injected with saline and confined to the saline-paired compartment for 20 min. At least 4 h later, the same mice were injected with nicotine and confined to the nicotine-paired side. Nicotine was given in the afternoon sessions to avoid confounding effects of acute nicotine withdrawal on the saline conditioning session (Kota et al. 2007; Smith et al. 2012).

Postconditioning phase

On day 5 (test day), no injection was given. Mice were allowed access to the entire apparatus for 15 min, and the time spent in each compartment during this 15-min session was recorded; data were expressed as the time spent on drug-paired side minus the time spent on saline-paired side. A positive number indicated a preference for the drug-paired side, while a negative number indicated an aversion to the drug-paired side. A number at or near 0 indicated no preference for either side.

Effects of different doses of nicotine in the induction of CPP

To evaluate the dose of nicotine able to induce CPP, different groups of mice ($n=11$ for each group) were injected with nicotine at different doses of 0.0, 0.1, 0.3, 0.5, and 1.0 mg/kg (s.c.) during the conditioning phase.

Effects of RHO and SDS on acquisition of nicotine-induced CPP

For acquisition studies, different groups of mice ($n=10$–11 for each group) were treated with RHO (10, 15, and 20 mg/kg, IG), SDS (0.2 mg/kg, IG), or its vehicle 60 min before each nicotine or saline injection during the conditioning phase, as described above.

Effects of RHO and SDS on expression of nicotine-induced CPP

To determine the effects of RHO and SDS on the expression of the reinforcing properties of nicotine, different groups of mice ($n=14$ for each group) were injected with 0, 10, 15, and 20 mg/kg of RHO and 0.2 mg/kg of SDS on the test day, 60 min prior to the postconditioning phase.

Extinction of nicotine-induced CPP

Mice were conditioned with nicotine for 3 days and tested for preference on the following day as described above. Mice were then evaluated for preference every 24 h in a drug-free state until no significant preference was observed.

Effects of RHO and SDS on drug-priming reinstatement of nicotine-induced CPP

To determine the effects of RHO and SDS on reinstatement of CPP, CPP was initially induced in mice (as described above). Then, mice were exposed to extinction sessions ($n=3$) to abolish the established CPP. One day after the last extinction trial, mice were returned to their home cages and tested again every 24 h until preference behavior was extinguished. Once preference was no longer evident, different groups of mice ($n=10$ for each group) received injections of RHO (0, 10, 15, and 20 mg/kg, IG) and SDS (0.2 mg/kg, IG), 60 min before a priming injection of a low dose of nicotine (0.1 mg/kg, s.c.) or saline. The treatment was performed in the colony room, which was different from the place where the previous conditioning injection was given (CPP room). The animals were reevaluated for preference under the same test day protocol (Kota et al. 2011; Jackson et al. 2013).

Effects of RHO and SDS on restraint stress reinstatement of nicotine-induced CPP

After the extinction was established (i.e., 3 days after CPP test), the animals were submitted to immobilization-induced stress for 30 min. Restraint is a powerful stressor that has been widely used in many studies (Leão et al. 2009; Lu et al. 2003; Ribeiro Do Couto et al. 2006). To induce restraint, the mice were allowed to pass into a cylindrical glass tube (4 cm in diameter, 10 cm in length, with holes 0.5 cm in diameter to allow respiration). Two test tubes of 0.5 cm diameter were then carefully introduced underneath the animal, thus reducing the diameter of the tube to 3 cm, so that was impossible for the animal to turn around (Leão et al. 2009; Ribeiro Do Couto et al. 2006). On the test day, the mice ($n=8$ for each group) were given RHO (0, 10, 15, and 20 mg/kg, IG) or SDS (0.2 mg/kg, IG) 60 min prior to the restraint stress. Immediately after the restraint, the reinstatement test was performed on the animals for 15 min.

Locomotor activity

Locomotor activity was measured by an open-field apparatus consisting of a square arena (43.2 cm×43.2 cm) equipped with two lines of 16 photocells to measure horizontal and vertical activity. The arena was lit by one red light lamp (25 W), and a white noise generator in the room produced an ambient background noise of ~70 dB. All data were recorded on a personal computer (MED-PC Open-Field Activity Software) in an adjacent control room. Animals were placed in the apparatus for 10 min of adaptation, and then, after injection of single or repeated injection of different doses of nicotine, RHO, SDS, or saline (as control), their locomotor activity was evaluated for a 30-min period.

Data analysis

The data are expressed as means ± standard error of the mean (SEM) of scores (i.e., the differences between post- and preconditioning time spent in the drug-paired compartment). The statistical analyses were performed using repeated measure analysis of variance (ANOVA), with the treatment and pretreatment (saline or nicotine conditions) as the between-subject variable and days as the within-subject variable, with different levels according to the experiment. Post hoc comparisons were carried out with Newman–Keuls test. Statistical significance was set at $p<0.05$.

Results

Effects of different doses of nicotine in the induction of CPP

The ability of various nicotine doses to produce a CPP in CD-1 mice is presented in Fig. 1. The ANOVA showed a significant effect of nicotine [$F_{(4, 49)}=7.847$; $p<0.01$]. The post hoc analysis revealed that repeated injections of nicotine at 0.3 and 0.5 mg/kg produced significant CPP ($p<0.05$ and $p<0.01$, respectively), whereas the highest dose of 1.0 mg/kg and the lowest dose of 0.1 mg/kg did not show any significant CPP or conditioned place aversion (CPA) ($p>0.05$). The saline treatment did not produce any significant effects in mice.

Effects of RHO and SDS on acquisition of nicotine-induced CPP

Figure 2 shows the effects of RHO and SDS on the acquisition of nicotine-induced CPP.

Repeated measures ANOVA revealed significant effects of pretreatment [$F_{(1, 115)}=5.214$; $p<0.05$], treatment [$F_{(4, 115)}=2.430$; $p<0.05$], and pretreatment × treatment [$F_{(4, 115)}=2.819$; $p<0.01$]. The post hoc analysis confirmed that repeated nicotine injections (0.5 mg/kg, s.c.) produced significant CPP ($p<0.01$), whereas the saline-treated mice did not show any significant conditioning or aversion. Repeated administration of RHO (10, 15, and 20 mg/kg, IG) and SDS (0.2 mg/kg, IG) 1 h before nicotine injection during the conditioning phase produced a significant decrease of the preference score between the drug-paired compartments in the post- and

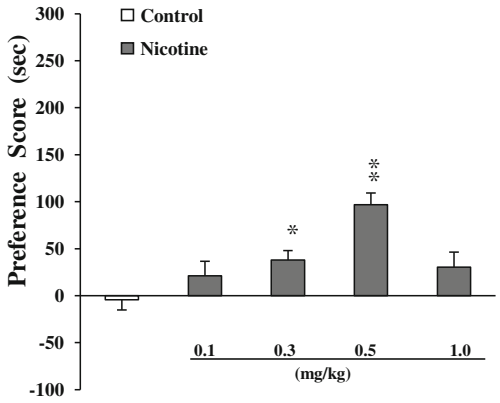

Fig. 1 Effects of different doses of nicotine in the induction of CPP. Mice were conditioned with different doses of nicotine (0.1, 0.3, 0.5, and 1.0 mg/kg, s.c.) and tested on test day. Results are expressed as the means ± SEM of preference score (seconds) of 11 mice. *$p<0.05$; **$p<0.01$, significant differences as compared to respective control group (*Control*); when not indicated, the differences were not statistically significant

preconditioning. The post hoc analysis revealed that RHO significantly decreased nicotine-induced CPP compared to the control group, both at the highest doses of 15 and 20 mg/kg ($p<0.001$) and also at the lowest dose of 10 mg/kg ($p<0.05$). The post hoc analysis confirmed a statistically significant effect of SDS ($p<0.001$). RHO and SDS per se did not produce any significant CPP or CPA, as compared with the saline control group ($p>0.05$).

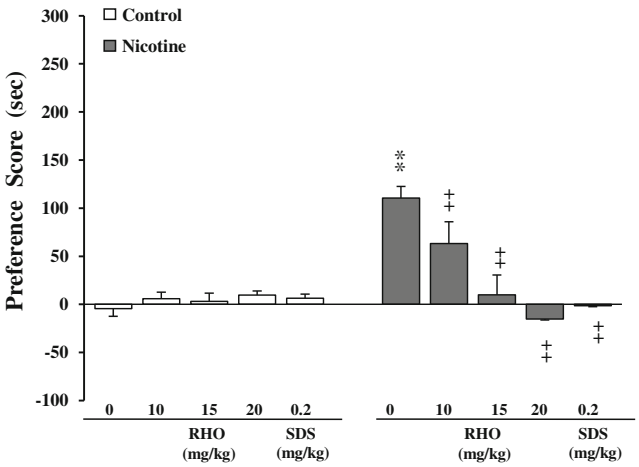

Fig. 2 Effects of RHO and SDS on acquisition of nicotine-induced CPP. Mice ($n=12$ and 13 for each group) were conditioned for two daily sessions for 3 days with 0.5 mg/kg nicotine. Sixty minutes before the treatment, each group received RHO (0, 10, 15, and 20 mg/kg, IG) and SDS (0.2 mg/kg, IG). On test day, they were placed into the apparatus and allowed to move freely for 20 min. Data are expressed as differences between the times spent in the compartment associated with drug in the post-CPP and the times spent in the compartment associated with drug in the pre-CPP. **$p<0.01$, significant difference as compared to respective control group (*Control*); $^{+}p<0.05$; $^{++}p<0.01$, significant difference as compared to the nicotine group (*Nicotine*); when not indicated, the differences were not statistically significant

Effects of RHO and SDS on expression of nicotine-induced CPP

Figure 3 shows the effects of RHO and SDS on the expression of nicotine-induced CPP. Repeated measures ANOVA revealed significant effects of pretreatment [$F_{(1,\ 120)}=36.264$; $p<0.001$], treatment [$F_{(4,\ 120)}=2.712$; $p<0.05$], and pretreatment × treatment [$F_{(4,\ 120)}=3.658$; $p<0.01$]. Data showed that single-dose administration of RHO (10, 15, and 20 mg/kg, IG) and SDS (0.2 mg/kg, IG) 1 h before the testing on test day (post-CPP) produced a significant decrease in the preference scores compared to nicotine-treated mice that received the vehicle. The post hoc analysis confirmed statistically significant effects at all of the RHO doses tested (10 mg/kg, $p<0.001$; 15 mg/kg, $p<0.05$; and 20 mg/kg, $p<0.001$) and at one SDS dose tested (0.2 mg/kg, $p<0.001$). The mice treated with only RHO and SDS did not show any CPP, compared with the saline control group ($p>0.05$) (data not shown).

Effects of RHO and SDS on drug-priming reinstatement of nicotine-induced CPP

The effects of RHO and SDS on the reinstatement of nicotine priming-induced CPP are shown in Fig. 4. Nicotine-induced preference was extinguished by day 8 in each group. On day 9, mice were given RHO and SDS 1 h before a priming injection of nicotine (0.1 mg/kg, s.c.). The priming injection of nicotine completely reinstated the extinguished nicotine-induced CPP as confirmed by the significant increased differences in the time spent in the previously nicotine-paired compartment when the mice were retested for CPP, compared to the time the mice spent in the same compartment after craving for nicotine was extinguished [$F_{(1,\ 9)}=29.291$; $p<0.01$]. Additionally, a significant difference was seen between post-CPP and reinstatement [$F_{(1,\ 9)}=6.750$; $p<0.01$]. The reinstatement of CPP induced by the nicotine priming injection was significantly reduced by pretreatment with RHO and SDS [$F_{(4,\ 45)}=9.176$; $p<0.001$]. Post hoc comparison indicated a significant effect at all doses of RHO ($p<0.01$) and at one SDS dose ($p<0.01$) which completely abolished the reinstatement of nicotine priming-induced CPP.

Effects of RHO and SDS on restraint stress reinstatement of nicotine-induced CPP

The effects of RHO and SDS on stress-induced reinstatement of nicotine-induced CPP are shown in Fig. 5. Restraint stress for 30 min completely reinstated the extinguished nicotine-induced CPP. Indeed, the preference score of nicotine-treated groups that underwent restraint stress increased, as confirmed by the observation that when mice were retested for CPP, they spent significantly more time in the nicotine-paired compartment compared to the time the mice spent in the same

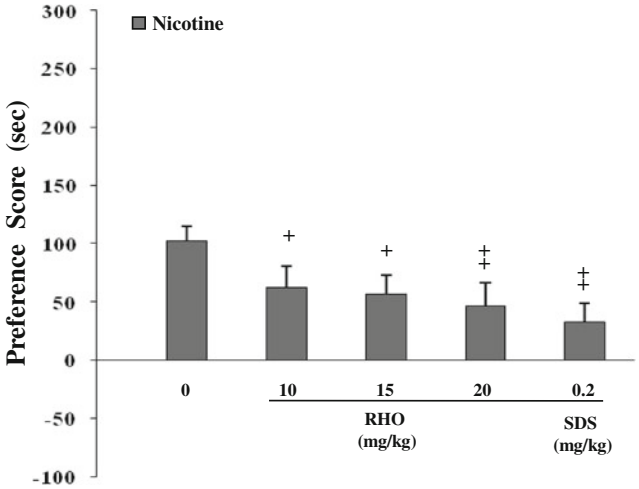

Fig. 3 Effects of RHO and SDS on expression of nicotine-induced CPP. Mice ($n=14$ for each group) were conditioned for two daily sessions for 3 days with 0.5 mg/kg nicotine. On test day, 60 min before the session, each group received RHO (0, 10, 15, and 20 mg/kg, IG) and SDS (0.2 mg/kg, IG), and they were placed into the apparatus and allowed to move freely for 20 min. Data are expressed as differences between the times spent in the compartment associated with drug in the post-CPP and the times spent in the compartment associated with drug in the pre-CPP. $^{+}p<0.05$; $^{++}p<0.01$, significant differences as compared to the nicotine group; when not indicated, the differences were not statistically significant

compartment after CPP for nicotine was extinguished [$F_{(1,\ 7)}=36.450$; $p<0.01$]. Additionally, a significant difference was

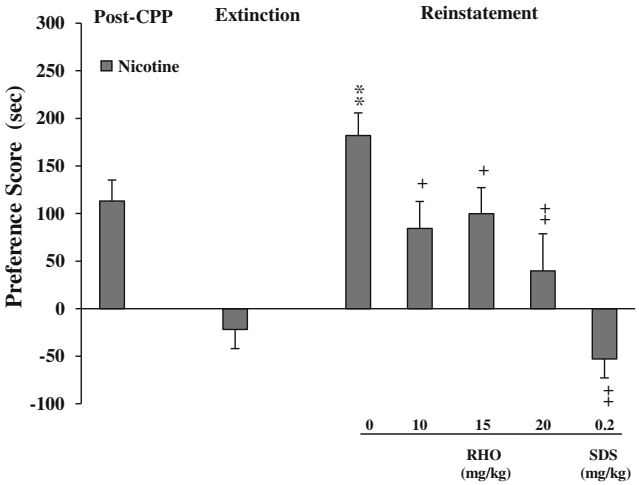

Fig. 4 Effects of RHO and SDS on drug-priming reinstatement of nicotine-induced CPP. After the extinction phase, mice ($n=10$ for each group) received different doses of RHO (0, 10, 15, and 20 mg/kg, IG) and one dose of SDS (0.2 mg/kg, IG) 60 min before the nicotine priming administration (0.1 mg/kg, s.c.). The effects of RHO and SDS on relapse to CPP were tested for 20 min after the priming administration. Data are expressed as differences between the times spent in the compartment associated with drug in the post-CPP and the times spent in the compartment associated with drug in the pre-CPP. $^{**}p<0.01$, significant difference as compared to the group treated with nicotine in post-CPP; $^{+}p<0.05$; $^{++}p<0.01$, significant differences as compared to the nicotine group; when not indicated, the differences were not statistically significant

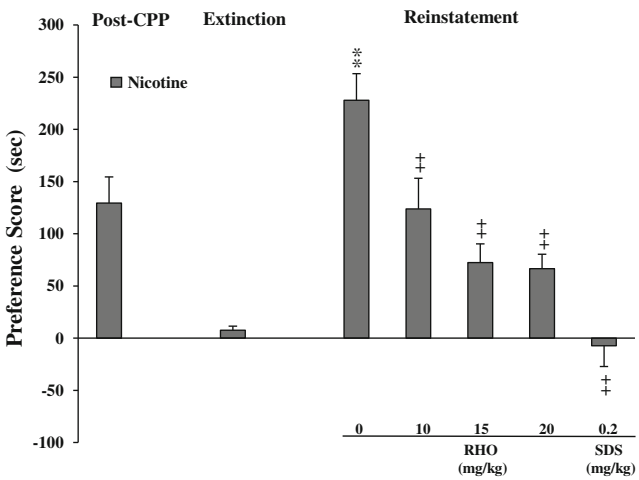

Fig. 5 Effects of RHO and SDS on restraint stress reinstatement of nicotine-induced CPP. After the extinction phase, mice ($n=8$ for each group) received different doses of RHO (0, 10, 15, and 20 mg/kg, IG) and one dose of SDS (0.2 mg/kg, IG), as indicated, 60 min before exposure to restraint stress by immobilization. The effects of RHO and SDS on relapse to CPP were tested immediately after 15 min of immobilization. Data are expressed as differences between the times spent in the compartment associated with nicotine in the post-CPP and the times spent in the compartment associated with nicotine in the pre-CPP. $^{**}p<0.01$, significant difference as compared to the group treated with nicotine in post-CPP; $^{+}p<0.05$; $^{++}p<0.01$, significant difference as compared to the nicotine group; when not indicated, the differences were not statistically significant

seen between post-CPP and reinstatement [$F_{(1,\ 7)}=18.250$; $p<0.01$]. Reinstatement of nicotine-induced CPP was completely abolished by pretreatment with RHO (10, 15, and 20 mg/kg) and SDS (0.2 mg/kg) [$F_{(4,\ 35)}=15.727$; $p<0.001$].

Effects of nicotine, RHO, and SDS on locomotor activity

In line with the finding of Rauhut et al. (2008) and Sahraei et al. (2007), the locomotor activity of the mice was significantly and dose-dependently reduced after a single administration of nicotine [$F_{(4,\ 49)}=3.243$; $p<0.05$]. Post hoc analysis revealed that while doses of 0.1, 0.3, and 0.5 mg/kg of nicotine did not alter locomotor activity (mean distance traveled ± SEM, 2,180±213, 2,237±191, and 2,340±218, respectively), mice once treated with the highest dose of nicotine were less active (1,250±190) than vehicle control mice (2,200±203) ($p<0.05$), suggesting that only this nicotine dose produced hypoactivity. Conversely, statistical analysis showed that repeated administration of the nicotine dose produced no changes in locomotor activity or hyperactivity (in other words, there was no sensitization). Indeed, the initially hypoactivity induced by the highest nicotine dose (1.0 mg/kg) was followed by the development of tolerance to the hypoactive effects after repeated nicotine administration (mean distance traveled, 2,288±224) [$F_{(4,\ 49)}=0.754$; $p>0.05$].

In addition, to verify whether RHO or SDS may affect motor activity in mice, 10, 15, and 20 mg/kg RHO and

0.2 mg/kg SDS were tested. Data showed that neither a single [$F_{(4, 55)}$=0.626; p>0.05] nor repeated administration [$F_{(4, 55)}$=0.840; p>0.05] of RHO and SDS changed the motor activity of the animals at all doses tested, thus confirming that both RHO and SDS per se are devoid of nonspecific motor effects. In fact, treated mice did not show any significantly different behavioral response compared with the control group; as with RHO, their mean score was no more than 3.0 % (RHO 10), 4.0 % (RHO 15), and 3.5 % (RHO 20) above the control scores, and with SDS, it was 2.0 % below the control score.

Finally, the effects of RHO and SDS on potential nicotine-induced changes in locomotor activity were determined. The results showed that RHO and SDS had no effect on nicotine-induced motor activity. Statistical analysis confirmed that none of the RHO and SDS doses co-administered with nicotine increased locomotor activity compared to nicotine mice; the mean distance traveled was 2,346±199 for nicotine, 2,355 ±215 for nicotine + 10 mg/kg RHO, 2,397±224 for nicotine + 15 mg/kg RHO, 2,374±194 for nicotine + 20 mg/kg RHO, and 2,298±195 for nicotine + 0.2 mg/kg SDS [$F_{(5, 67)}$=0.426; p>0.05]. Furthermore, none of the RHO + nicotine or SDS + nicotine mice were reliably more active than their RHO or SDS alone counterparts (p>0.5).

Discussion

Conventional pharmacological treatments for drug addiction aim to modulate or disrupt the effects of a drug at sites of action in the body by reducing three most important aspects: withdrawal syndrome, craving, and relapse. This treatment strategy has not yielded broadly effective medications for many drugs of abuse. Among the pharmacological treatments that have often been used to reduce withdrawal symptoms, few can reduce the drug craving, and they are also rarely effective in preventing relapse.

Recently, in mice, the effectiveness of RHO in the prevention and treatment of withdrawal syndrome, craving, and relapse to morphine was demonstrated (Mattioli and Perfumi 2011a; Mattioli et al. 2012). In addition, the same authors have shown that RHO is able to prevent and counteract also the nicotine withdrawal syndrome (Mattioli and Perfumi 2011b).

Therefore, in order to verify if *R. rosea* L. extract could constitute a new effective therapeutic strategy able of countering all the main aspects related to the treatment of nicotine addiction, the present study used the CPP model to systematically investigate, in mice, the effects of RHO and its active compound SDS, on the reinforcing properties of nicotine and their efficacy in the vulnerability to reinstatement induced by nicotine priming and restraint stress.

Several findings of interest have emerged. First, nicotine dose dependently produced a CPP or induced changes in locomotor activity in CD-1 mice. Second, *R. rosea* L. extract dose dependently attenuated both nicotine-induced CPP and reinstatement of nicotine-induced CPP without modifying the locomotor activity of the animals. Third, salidroside seems to play a primary role in the effect of *R. rosea* L. extract as it reduces both acquisition and expression of nicotine-induced CPP and prevents reinstatement induced by priming or stress.

The CPP animal model is widely used to evaluate the rewarding effects of abused drugs, one of the main aspects of craving, suggesting that CPP is a valid measure of craving (Littleton 2000; Liu et al. 2008; Grimm 2011; Martin-Fardon and Weiss 2013). Recently, the extinction and reinstatement phases of this paradigm have been used to assess relapse to drug seeking (Liu et al. 2008; Tzschentke 2007).

Often conflicting results have been reported for the CPP induced by nicotine. In fact, nicotine exerts opposite motivational effects, making it difficult to obtain a robust place preference comparable to that of the other drugs abused such as morphine or cocaine (Le Foll and Goldberg 2005a).

Several preclinical studies reported divergent observations; some asserted that nicotine was effective in inducing CPP, and others asserted that it was ineffective and that it induced CPAs. In addition, both positive and negative effects associated with nicotine appear to be influenced by environmental conditions and the context of the experiments, factors that may explain the difficulties in obtaining reliable results with nicotine-induced CPP (Caggiula et al. 2002; Le Foll and Goldberg 2009).

However, in line with the most recent literature in which unbiased assignment procedures and low doses of nicotine are effective in inducing nicotine-induced CPP, our results confirm that male CD-1 mice were responsive to the rewarding effects of a moderate nicotine dose (0.5 mg/kg). Also, it has been shown that mouse strains are differentially sensitive to the rewarding effects of nicotine (Fattore et al. 2009; Kota et al. 2007, 2009, 2011; Sahraei et al. 2007). For example, Grabus et al. (2006) found that moderate nicotine doses of 0.3 and 0.5 mg/kg produced a CPP in C57BL/6J and imprinting control region (ICR) mice, respectively. However, nicotine failed to produce a CPP in DBA/2J mice. The previous observation that a moderate nicotine dose (0.5 mg/kg) produced a CPP in ICR mice is particularly relevant for the current study, as CD-1 and ICR mice share a common genetic background. In addition, Rauhut et al. (2008) showed that nicotine dose dependently produced a CPP in CD-1 mice (Aldinger et al. 2009).

The goal of the present study was to evaluate the effect of RHO, a hydroalcoholic extract of *R. rosea* L., and its active compound salidroside on the different phases of conditioned place preference induced by nicotine. The doses of RHO tested in the present study were selected on the basis of earlier studies demonstrating that they were able to reduce nicotine and morphine withdrawal syndrome (Mattioli and Perfumi

2011a, b; Mannucci et al. 2012) and that they were devoid of any reinforcing aversive or specific effects (Mattioli et al. 2012). Moreover, at a similar range of doses, RHO counteracts craving and prevents relapse to morphine in mice CPP paradigm (Mattioli et al. 2012). A major finding of the present study is the ability of *R. rosea* extract to significantly reduce the reinforcing propriety of nicotine by preventing the development of nicotine-conditioned place preference and by countering its expression.

It is important to emphasize that, at the doses used, RHO administration did not cause a significant effect by itself during conditioning or on the test day ($p > 0.05$, data not shown).

The effect of RHO was dose dependent in the acquisition, but not in the expression phase. These results suggest that the different effects induced by repeated or acute administration of *R. rosea* extract might be due, at least in part, to different mechanisms that confer short- versus long-term motivational value to nicotine-paired stimuli (Forget et al. 2009). The hypothesis is that the development of a drug-induced conditioned place preference is likely to be correlated with the reinforcing effects of a drug of abuse, whereas the expression of CPP reflects how environmental stimuli previously associated with the effects of nicotine continue to influence behavior (Le Foll and Goldberg 2009; Tzschentke 2007).

The treatment with RHO before each administration of nicotine during the conditioning period was effective in preventing the acquisition of the CPP, thus demonstrating the direct influence of the extract on the reinforcing effects of nicotine and, mainly, on learning of the stimulus–reward association. The acute administration of RHO 1 h before the test decreased the expression of CPP induced by nicotine and, thus, was effective in reducing the influence of environmental factors in the development of CPP, in turn changing the ratio incentive motivation. This assumes a greater importance when one considers the critical role played by environmental stimuli in the reinforcing effects of nicotine (Caggiula et al. 2002; Le Foll and Goldberg 2005b, 2009). This ability of RHO to prevent the development and counteract the expression of CPP is extremely important, since the inhibition of CPP that results from a reduction in the reinforcing properties of drug abuse is believed to be a key element for reducing drug-seeking behavior and for limiting vulnerability to relapse, in humans and other animals (Aguilar et al. 2009; Tzschentke 2007).

Although positive reinforcement is the main factor in the acquisition of a drug habit, relapse is the overriding characteristic of addiction and the foremost challenge to the treatment of drug addiction. The results obtained in studies using CPP and self-administration versions of the reinstatement model endorse the idea that the neuronal and neurotransmission events that mediate reinstatement are not necessarily associated with and can differ from those that mediate drug reinforcement (Shalev et al. 2002). This may explain why many of the anti-craving drugs that are available today are not very effective in preventing relapse.

As with other drugs abused, nicotine priming and exposure to nicotine-associated stimuli or stressors can cause compulsive desire and trigger reinstatement of drug reward-related behavior in experimental animals and in humans (Biala et al. 2009, 2010; Caggiula et al. 2002; Chaudhri et al. 2006; Le Foll and Goldberg 2009). Numerous studies have examined the relapse of nicotine addiction through experiments using spontaneous recovery conditions, that is, cues associated with drug taking, drug priming, or stress-inducing stimuli, to reinstate previously extinguished nicotine-seeking behavior or nicotine conditioning (Biala et al. 2010; Fattore et al. 2009; Jackson et al. 2010; Kota et al. 2011; Leão et al. 2009; Rauhut et al. 2008).

Consistent with this literature, in the present study, both nicotine priming and restraint stress induced a reinstatement of the CPP that had been previously extinguished. A major finding of the study was that the acute treatment with RHO completely abolished the reinstatement of CPP induced by nicotine priming and stress (Leão et al. 2009; Jackson et al. 2010).

Finally, in the present study, we also evaluated the possible effect of SDS, one of the most studied active compounds of *R. rosea* L., on the development of nicotine-conditioned place preference, its expression, and its reinstatement induced by priming and stress, in order to define its role in the observed effects of the *R. rosea* L. extract. The results showed that the administration of the single active compound salidroside produced effects comparable to those obtained with the extract. In fact, administration of SDS alone neither changed the time spent in the drug-paired side in the place conditioning paradigm nor induced changes in locomotor activity. However, it counteracted or completely blocked rewarding proprieties and craving induced by nicotine. Therefore, we might confirm that salidroside plays an important role in the beneficial effect of *R. rosea* L extracts.

These results assume a greater importance when we consider that RHO and SDS have no effect on nicotine-induced motor activity, indicating the specificity of the extract and its active compound on nicotine-induced CPP and reinstatement of drug-seeking behavior.

Overall, the results of the present study highlight the effectiveness of this *R. rosea* L. extract in two important aspects of the treatment of addiction. Indeed, RHO was shown here to be effective in reducing both the reinforcing properties, a main aspect of craving, and the vulnerability to relapse that are induced by reexposure to drugs and restraint stress.

Looking to the future, further studies are required to investigate the exact mechanism of the extract action through additional biochemical studies on changes in brain neurotransmitters levels that are responsible for the effects observed.

Future steps should also be evaluated as to how other active compounds of *R. rosea* L affect nicotine addiction.

Finally, it is important to consider that medications that are effective in humans for improving smoking cessation rates generally appear effective in animal models in reducing intravenous nicotine self-administration, nicotine conditioning, nicotine withdrawal signs, and the behavioral effects related to nicotine-associated environmental stimuli, demonstrating a strong analogy between the responses of humans and experimental animals (Le Foll and Goldberg 2009).

In conclusion, the ability of RHO to counteract nicotine withdrawal syndrome (Mattioli and Perfumi 2011b) and its positive effects in the reduction of craving and prevention of relapse to nicotine emphasizes the therapeutic potential of *R. rosea* L. extracts, suggesting the usefulness of the plant or salidroside in helping former tobacco smokers to remain to be drug free and highlighting the potential of *R. rosea* L. extracts for treating the three most important aspects of nicotine addiction (i.e., withdrawal, craving, and relapse).

Acknowledgments The authors would like to thank Sheila Beatty for editing the English usage in the manuscript.

Conflict of interest The coauthors declare that they have no conflict of interest.

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