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Abstract

Rhodiola rosea L. is one of the most popular adaptogen and anti-stress plants in European and Asiatic traditional medicine. Its pharmacological properties appear to depend on its ability to modulate the activation of several components of the complex stress-response system. Exposure to both physical and psychological stress reduces feeding in rodents. The aim of this work was thus to determine whether in rats an hydroalcoholic *R. rosea* extract standardized in 3% rosavin and 1% salidroside (RHO) reverses hypophagia induced by (1) physical stress due to 60 min immobilization; (2) intracerebroventricular injection of corticotrophin-releasing factor (CRF, 0.2 µg/rat), the major mediator of stress responses in mammals; (3) intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS, 100 µg/kg); (4) intraperitoneal administration of fluoxetine (FLU, 8 mg/kg). The effect of the same doses of the plant extract was also tested in freely-feeding and in 20h food-deprived rats.

RHO was administered acutely by gavage to male Wistar rats 1 h before the experiments. The results show that at 15 and 20 mg/kg, RHO reversed the anorectic effects induced both by immobilization and by intracerebroventricular CRF injection. Moreover, at the same doses, RHO failed to reduce the anorectic effect induced both by LPS and FLU, and did not modify food intake in both freely-feeding and food-deprived rats. These findings strongly demonstrated that RHO is able selectively to attenuate stress-induced anorexia, providing functional evidence of claimed adaptogen and anti-stress properties of *Rhodiola rosea* L.

Keywords

Rhodiola rosea, adaptogens, restraint, CRF, anorexia, stress-induced anorexia, CRF-induced anorexia, LPS-induced anorexia, fluoxetine-induced anorexia

Introduction

It is well known that stress conditions caused by physiological and environmental factors can induce marked behavioural alterations due to a release of glucocorticoids and activation of a brain stress network (Dallman *et al.*, 2003). The neurobiologic mechanisms involved in such stress activation certainly involve activation of both the pituitary adrenal axis and the autonomic sympathetic system (Koob and Heinrichs, 1999). The activation of the hypothalamic–pituitary–adrenal axis by stress has long been known to involve the action of corticotrophin-releasing factor (CRF), that is a key regulator in the overall response of an organism to stress (Dunn and Berridge, 1990; De Souza and Grigoriadis, 1994; Koob and Heinrichs, 1999). CRF functions as a neurotransmitter in the central nervous system and has a critical role in coordinating the autonomic, electrophysiological and behavioural responses to stress (Heinrichs and Richard, 1999; Zorrilla and Koob, 2004). However, evidences suggest a neurotropic role for extrahypothalamic

CRF systems for mediating behavioural responses to stressors and its contribution to the behavioural state of stress in addition to the classic activation of adrenal steroids (Schulkin *et al.*, 1994). Particularly the locus coeruleus, the paraventricular nucleus of the hypothalamus, the bed nucleus of the stria terminalis and the central nucleus of the amygdala seem to be predominantly implicated in the behavioural actions of CRF (Erb and Stewart, 1999; Koob and Heinrichs, 1999). In addition to the effects on stress responses, hypersecretion of CRF in these brain areas may contribute to the symptomatology that is seen in neuropsychiatric disorders, such as depression, anxiety-related disorders and anorexia nervosa. In particular, a large body of evidence suggests a role for the endogenous brain CRF system in appetite regulation and in the aetiology of eating disorders (Heinrichs and Koob, 1992; Richard, 1993). In humans, the concentration of CRF is increased in the cerebrospinal fluid of patients suffering from anorexia nervosa, and a greater salivary cortisol response is induced by CRF in anorectic humans, compared to normal volunteers (Morley, 1987;

Gross *et al.*, 1994). When administered centrally in rats and mice, CRF and urocortin, a CRF-family neuropeptide, can induce anxiogenic-like behaviour and reduced food intake (Morley and Levine, 1982; Levine *et al.*, 1983; Heinrichs and Koob, 1992; Spina *et al.*, 1996; Hotta *et al.*, 1999; Koob and Heinrichs, 1999; Swiergiel and Dunn, 1999; Ciccocioppo *et al.*, 2001). Similarly, stress-induced anorexia can be reversed by treatment with selective CRF-receptor antagonists (De Souza, 1995; Koob and Heinrichs, 1999).

Some pharmacologically active plants that have been defined as 'adaptogens' are able to increase the ability of an organism to adapt to environmental stress factors and to avoid damage from such, mainly by regulating various elements of the stress system and by modulating stimulus-response coupling (Wagner *et al.*, 1994; Panossian *et al.*, 1999; Panossian, 2003; Panossian and Wagner, 2005).

Rhodiola rosea L. (fam. *Crassulaceae*), which is also known as 'golden root' or 'rose root', is one of the most important adaptogens, and it is a popular plant in traditional medicine in Eastern Europe and Asia, with a reputation for alleviating emotional, mental and physical disorders (German *et al.*, 1999; Spasov *et al.*, 2000; Shevtsov *et al.*, 2003; Panossian and Wagner, 2005). In traditional folk medicine, *R. rosea* is used to stimulate the nervous system, decrease depression, enhance work performance, longevity and resistance to high altitude sickness, and treat fatigue and symptoms of asthenia subsequent to intense physical and psychological stress (Kelly, 2001; Brown *et al.*, 2002; Zhu *et al.*, 2003). The adaptogenic and anti-stress properties of *R. rosea* have been attributed primarily to its ability to modulate the activation of several components of the stress-response systems, such as the sympatho-adrenal system (Lishmanov *et al.*, 1987; Panossian *et al.*, 1999; Panossian and Wagner, 2005) and the hypothalamic-pituitary-adrenal axis (Burchfield, 1979; Lishmanov *et al.*, 1987; Saratikov *et al.*, 1987; Panossian *et al.*, 1999; Panossian and Wagner, 2005). Moreover, *R. rosea* moderates the release of opioid peptides that occurs as part of the pituitary-adrenal axis response to stress (Lishmanov *et al.*, 1987; Lishmanov *et al.*, 1993).

Furthermore, the ability of *R. rosea* to increase the non-specific resistance in animals may be related to its ability to reduce the secretion of CRF, the major physiological mediator of stress (Lishmanov *et al.*, 1987; Maslova *et al.*, 1994).

With regard to the main components determining pharmacological characteristics of *R. rosea*, it is known that *R. rosea* roots contain a range of biologically active substances including organic acids, flavonoids, tannins and high amounts of phenolic compounds, particularly phenylpropane derivatives such as rosavins (rosavin, rosine, rosarin) which are specific components of *R. rosea*, and phenylethane derivatives, such as salidroside, which is contained in all species of the *Rhodiola* genus and in a wide variety of species outside the genus (Kurkin and Zapesochayna, 1986; Wang *et al.*, 1992; Yoshikawa *et al.*, 1996). According to revised 1989 Sovietic Pharmacopeia (Brown *et al.*, 2002) the extracts of *R. rosea* are standardized for both rosavins and salidroside. Rosavin, salidroside and additional phenolic compounds such as p-thyrosol and triandrin are thought to be critical for the

adaptogenic and anti-stress properties of the plant. Moreover *R. rosea* extracts used in several pre-clinical and clinical studies were standardized to minimum 3% rosavins and 0.8–1% salidroside (Darbinyan *et al.*, 2000; Kelly, 2001; Brown *et al.*, 2002; Abidov *et al.*, 2003; Panossian and Wagner, 2005).

Therefore, the purpose of the present study was to investigate whether an hydroalcoholic *R. rosea* L. extract standardized in 3% rosavin and 1% salidroside can prevent anorexia that is induced by different stress conditions, such as restraint-stress and intracerebroventricular injection of CRF.

In addition, to determine the selectivity of the effects of this *R. rosea* extract on stress-induced suppression of food intake, it was also tested in food-deprived rats in which anorexia was evoked by injection of two different anorectic agents: *E. coli* lipopolysaccharide, a pathogenic agent, and fluoxetine, a selective serotonin reuptake inhibitor. Finally we tested the effects of this *R. rosea* extract both in non-stressed food-deprived rats and in non-stressed freely-feeding rats.

Material and methods

Animals

Male Wistar rats (200–250 g; Harlan SRC, Milan, Italy) were individually housed in a room with an artificial 12:12h light/dark cycle (lights off at 7.00 PM), at constant temperature (20–22 °C) and humidity (45–55%). The rats were given free access to food pellets (4RF; Mucedola, Settimo Milanese, Italy) and tap water, except when otherwise detailed. All of the animals were handled once a day for 5 min during the first week after their arrival. Animals were used only once.

All of the procedures were conducted in adherence to the European Community Council Directive for Care and Use of Laboratory Animals (86/609/EEC).

Drugs

A dry hydroalcoholic extract from roots of *Rhodiola rosea* L. (RHO), provided by EPO S.r.l., Milan, Italy, was used. The HPLC analysis report showed a content of 3% total rosavins expressed as rosavin and 1% salidroside. The ratio of rosavin and salidroside (3:1) is in line with published data (Kurkin and Zapesochayna, 1986; Abidov *et al.*, 2003). The extract was dissolved in ethanol absolute and diluted in tap water in order to obtain a final ethanol concentration of 2% v/v in all treatment conditions. Then it was administered by intragastric administration (IG) at doses of 10, 15 and 20 mg/kg/10 ml. The same vehicle was administered to control group.

CRF (rat/human; RBI/SIGMA, Natick, MA, USA) was dissolved in sterile isotonic saline prior to intracerebroventricular injection (ICV) at the dose of 0.2 µg/µl/rat.

Lipopolysaccharide from *E. coli* (LPS; 0111:B4, No. L-2630; SIGMA, Milan, Italy) was dissolved in pyrogen-free isotonic saline and given by intraperitoneal injection (IP) at the dose of 100 µg/kg.

Fluoxetine HCl (FLU; SIGMA, Milan, Italy) was dissolved in sterile physiological saline and administered IP at the dose of 8 mg/kg/ml.

Surgical procedures

For intracranial surgery, the rats were anaesthetized by intramuscular injection of 100–150 µl/100 g body weight of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml). A guide cannula (outside diameter, 0.65 mm) for injections into the lateral cerebroventricle was stereotaxically implanted on left side and cemented to the skull (coordinates in mm with reference to bregma: AP -1.0; L 1.8; V 2.0) (Paxinos and Watson, 1998). The CRF was injected through a stainless-steel injector (internal diameter, 0.30 mm) that protruded 2.5 mm beyond the cannula tip. Experiments began 1 week after surgery. Before any behavioural experiments, the rats received two sham intracerebroventricular and intraperitoneal injections to familiarize them with the drug administration procedure. After completion of the experiments, the rats were sacrificed and the intracerebroventricular cannula placement was verified histologically.

Experimental procedure

All the experiments were performed at 10.00 AM, during the light phase of the cycle.

Effects of RHO on food intake in freely-feeding rats To evaluate the general effects of RHO on food intake, its effects were tested in freely-feeding rats. The animals ($n=44$) were divided into four groups that received intragastric administration of vehicle or RHO at 10, 15 and 20 mg/kg. Their food was temporarily removed and offered again 1 h later. Food consumption was determined 30, 60, 90 and 120 min, and 4, 6 and 24 h after RHO administration, by weighing of the food cups, with subtraction of the spillage from the total food intake. As the rats were not accustomed to eating at the time of study (3 h into light cycle), there was low food intake in the controls.

Effects of RHO on restraint stress-induced anorexia To evaluate the effects of RHO on food consumption under restraint-stress conditions, rats ($n=41$) were subjected to 20 h food deprivation, and then received intragastric administration of vehicle or RHO (10, 15 and 20 mg/kg). One hour afterwards, restraint stress was induced in the rats by being restrained in cylindrical Plexiglas tubes for 60 min, which has been reported to produce a marked inhibition of food intake (Ciccocioppo *et al.*, 2001). After the 60 min restraint, the rats were returned to their home cage, where food was made available *ad libitum*, and their food consumption was determined 30, 60, 90 and 120 min, and 4, 6 and 24 h later. Another group of deprived but non-stressed rats ($n=12$) served as a control for the effects of the restraint stress; the rats of this group were IG administered with vehicle and returned to their own cages without being subjected to restraint.

Effects of RHO on CRF-induced anorexia To determine the effects of RHO on CRF-induced anorexia, 20 h food-deprived rats

($n=32$) received intragastric administration of vehicle or RHO (10, 15 and 20 mg/kg), and 60 min later they received intracerebroventricular injections of 0.2 µg/rat CRF. This dose of CRF was chosen since it elicited an approximately 50% food reduction in food-deprived rats (Ciccocioppo *et al.*, 2001). The rats were given free access to food 20 min after the CRF injection and their food intake was determined 30, 60, 90 and 120 min, and 4, 6 and 24 h later. Another group of food-deprived rats ($n=8$) that were IG and ICV administered with RHO and CRF vehicles respectively, served as control for the effects of CRF.

Effects of RHO on LPS-induced anorexia To determine the selectivity of the anti-anorectic effect of RHO, a model of anorexia induced by LPS injection was used. Administration of low doses of LPS, a pathogenic agent, induces a moderate infection that is associated with a reduction in food consumption (Langhans *et al.*, 1989). Food-deprived rats ($n=31$) were injected intraperitoneally with 100 µg/kg LPS, and 4 h later they received intragastric administration of 10, 15 and 20 mg/kg RHO or its vehicle. Another group of food-deprived rats ($n=7$) received the respective vehicles and served as controls. Sixty minutes after RHO administration, the rats were offered access to food pellets, and their food consumption was determined 30, 60, 90 and 120 min, and 4, 6 and 24 h later.

Effects of RHO on fluoxetine-induced anorexia To evaluate better the selectivity of the anti-anorectic effect of RHO, in the present experiment we used a model of anorexia induced by FLU injection, a selective 5-HT reuptake inhibitors. Food-deprived rats ($n=29$) received intragastric administration of vehicle or RHO (10, 15 and 20 mg/kg), and 60 min later they were injected intraperitoneally with 8 mg/kg FLU (Currie *et al.*, 2004). The rats were given free access to food 30 min after the FLU injection and their food intake was determined 30, 60, 90 and 120 min, and 4, 6 and 24 h later. Another group of food-deprived rats ($n=7$) that were IG and IP administered with RHO and FLU vehicles respectively, served as control for the effects of FLU.

Effects of RHO on food intake in food-deprived rats To determine whether inhibition by RHO of the hypophagic effect of stress or of CRF are related to a direct orexigenic action, the effects of RHO were investigated on food consumption in food-deprived rats that had not been subjected to the stress conditions. For this purpose, 20 h food-deprived rats ($n=46$) received an intragastric administration of vehicle or RHO (10, 15 and 20 mg/kg). Another group of non-deprived rats ($n=7$) served as the control. Food was given 1 h after the administration, and its intake was recorded after 30, 60, 90 and 120 min, and 4, 6 and 24 h.

Statistical analysis

Data were analysed by two-way split-plot analysis of variance (with between-group comparisons for drug treatments and within-group comparisons for time). *Post hoc* comparisons were carried out by Newman-Keuls test. Statistical significance was set at $p < 0.05$.

Results

Effects of RHO on food intake in freely-feeding rats

As shown in Fig. 1, RHO administration did not modify the food consumption of the rats at any of the doses tested. The analysis of variance confirmed no significant effects of treatment on food intake ($F(3,40) = 1.509; p > 0.05$).

Effects of RHO on restraint stress-induced anorexia

The overall analysis of variance revealed a highly significant treatment effect for restraint-stress-induced anorexia ($F(4,48) = 59.843; p < 0.001$). This stress markedly reduced the food intake in the rats, as compared with the group that was not subjected to these stress conditions ($F(1,21) = 150.060; p < 0.001$) (Fig. 2A). The pre-treatment with RHO significantly reversed the anorectic effects of this restraint stress ($F(3,37) = 13.831; p < 0.001$). *Post hoc* comparisons show a significant inhibition of the effects of restraint stress ($p < 0.01$) following administration of 15 and 20 mg/kg RHO for up to 6h, but not for 10 mg/kg RHO ($p > 0.05$) (Fig. 2B).

Effects of RHO on CRF-induced anorexia

The overall analysis of variance revealed a highly significant treatment effect for CRF-induced anorexia ($F(4,35) = 3.940; p < 0.01$). As shown in Fig. 3A, the intracerebroventricular injection of 0.2 µg/rat CRF induced a marked reduction in food intake ($F(1,14) = 15.069; p < 0.01$). Pre-treatment with RHO completely reversed this effect of CRF ($F(3,28) = 3.151; p < 0.05$) at 30, 60 and 90 min after access to food. *Post hoc* analysis reveals a

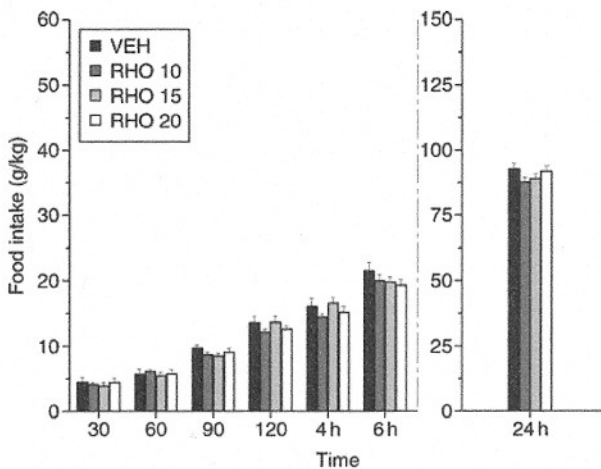


Figure 1 Effects of IG administration of vehicle (VEH) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg on cumulative food intake in freely-feeding rats. Data represent mean food intake (\pm SEM) of 10–12 rats. The differences in RHO treatment from the vehicle-treated rats are not statistically significant.

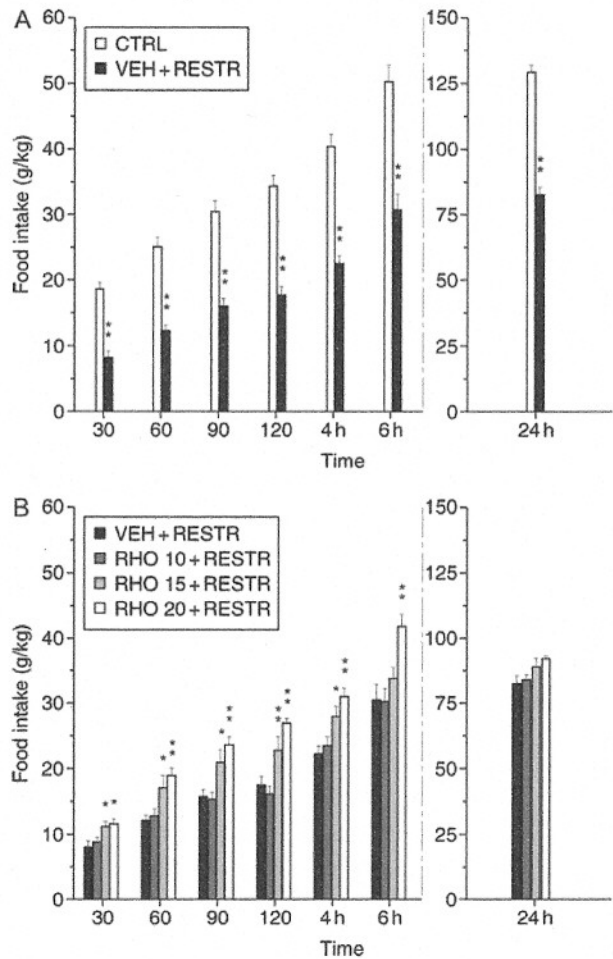


Figure 2 (A) Cumulative food intake in 20h food-deprived rats subjected to restraint stress (VEH + RESTR), as compared to a food-deprived group that was IG injected with RHO vehicle but not subjected to stress (CTRL). (B) Effects of IG administration of vehicle (VEH + RESTR) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg (RHO + RESTR) on restraint stress-induced anorexia. Data represent mean food intake (\pm SEM) of 9–12 rats. * $p < 0.05$, ** $p < 0.01$, significant differences from the relative vehicle-treated rats; where not indicated, the differences are not statistically significant.

statistically significant effect at the highest RHO doses tested, of 15 and 20 mg/kg ($p < 0.01$), but not at 10 mg/kg ($p > 0.05$) (Fig. 3B).

Effects of RHO on LPS-induced anorexia

As shown in Fig. 4A, intraperitoneal administration of LPS elicited a marked reduction in feeding ($F(1,13) = 8.764; p < 0.01$). Pre-treatment with RHO did not significantly modify this anorectic effect of LPS at any of the doses tested ($F(3,27) = 0.053; p > 0.05$) (Fig. 4B).

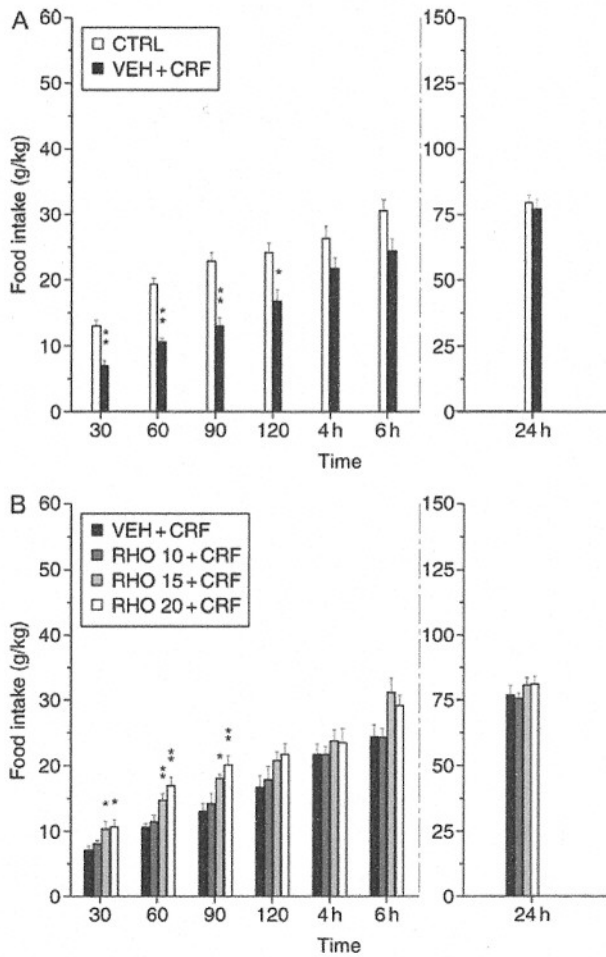


Figure 3 (A) Cumulative food intake in 20h food-deprived rats following ICV injection with 0.2 µg/ml CRF (VEH + CRF), as compared to a food-deprived control group receiving IG and ICV injections of RHO and CRF vehicle respectively (CTRL). (B) Effects of IG administration of vehicle (VEH + CRF) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg (RHO + CRF) on CRF-induced anorexia. Data represent mean food intake (\pm SEM) of eight rats. * $p < 0.05$, ** $p < 0.01$, statistically significant differences from the relative vehicle-treated rats; where not indicated, the differences are not statistically significant.

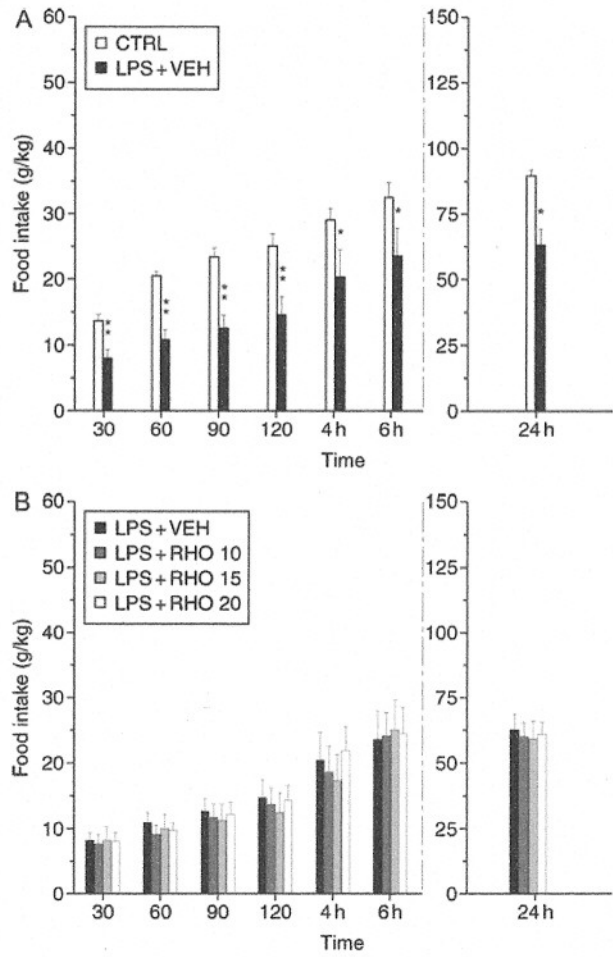


Figure 4 (A) Cumulative food intake in 20h food-deprived rats following IP injection with 100 µg/kg LPS (LPS + VEH), as compared to a food-deprived control group receiving IG and IP injections of RHO and LPS vehicle respectively (CTRL). (B) Effects of IG administration of vehicle (LPS + VEH) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg (LPS + RHO) on LPS-induced anorexia. Data represent mean food intake (\pm SEM) of seven to eight rats. * $p < 0.05$, ** $p < 0.01$, statistically significant differences from the relative vehicle-treated rats; where not indicated, the differences are not statistically significant.

Effects of RHO on fluoxetine-induced anorexia

As shown in Fig. 5A, intraperitoneal administration of FLU elicited a marked reduction in feeding ($F(1,12) = 62.909$; $p < 0.001$). Pre-treatment with RHO did not significantly modify this anorectic effect of FLU at any of the doses tested ($F(3,25) = 2.365$; $p > 0.05$) (Fig. 5B).

Effects of RHO on food intake in food-deprived rats

Food intake increased in 20h food-deprived rats, as compared with non-food-deprived rats ($F(1,20) = 329.720$; $p < 0.001$; data not shown). Pre-treatment with RHO (10, 15 and 20 mg/kg) had no significant effects on this food intake ($F(3,42) = 2.008$; $p > 0.05$) (Fig. 6).

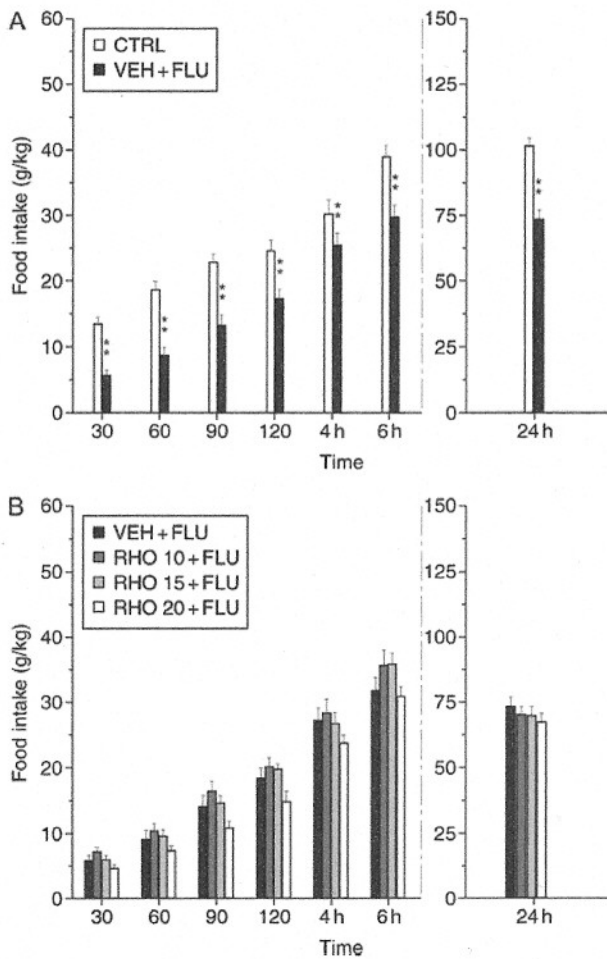


Figure 5 (A) Cumulative food intake in 20h food-deprived rats following IP injection with 8 mg/kg FLU (FLU + VEH), as compared to a food-deprived control group receiving IG and IP injections of RHO and FLU vehicle respectively (CTRL). (B) Effects of IG administration of vehicle (FLU + VEH) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg (LPS + RHO) on LPS-induced anorexia. Data represent mean food intake (\pm SEM) of seven to eight rats. $**p < 0.01$, statistically significant differences from the relative vehicle-treated rats; where not indicated, the differences are not statistically significant.

Discussion

The present study shows that this *R. rosea* L. extract effectively and dose-dependently reduces the marked anorexia induced in rats by exposure to different stressful conditions. Indeed, pre-treatment with RHO at doses of 15 and 20 mg/kg significantly reversed the anorectic effects following physical stress and central administration of CRF. These anti-anorectic effects appeared within 60 min

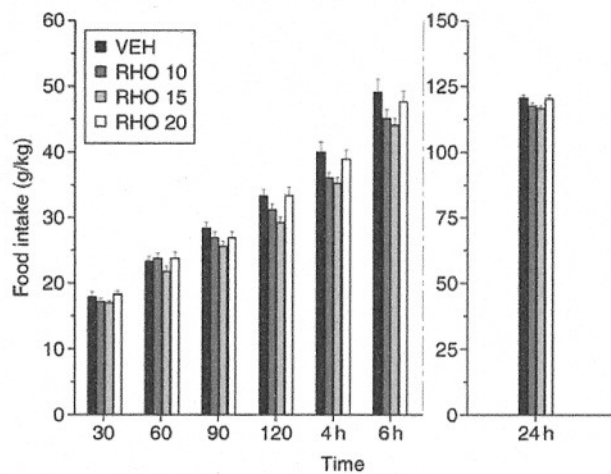


Figure 6 Effects of IG administration of vehicle (VEH) and the *R. rosea* L. extract (RHO) at 10, 15 and 20 mg/kg on cumulative food intake in 20h food-deprived rats. Data represent mean food intake (\pm SEM) of 10–12 rats. The differences in RHO treatment from the vehicle-treated rats are not statistically significant.

after a single oral administration of RHO, and lasted for up to 4–6 h. These data confirm the efficacy of single dose application of adaptogens in situations that require rapid responses to strain or stress conditions (Panossian and Wagner, 2005).

However, since the anorexia may be secondary to a self-sustaining cascade from the initial stress experience, it is unknown whether the anti-anorectic effect of the extract might be due to its long last pharmacological activity or whether the extract might only is active for a brief period (e.g. during the stressor application and into a brief post-stressor period) in order to prevent all the subsequent functional consequences. Appropriate studies in this regard could be necessary.

Conversely, the present study demonstrates that at the range of doses used, RHO does not increase feeding in freely-feeding rats or in non-stressed food-deprived rats. These findings suggest that the inhibitory effect of RHO on stress- and CRF-induced anorexia is not related to a non-specific orexigenic action of RHO.

On the other hand, oral administration of 15 and 20 mg/kg RHO failed to modify the anorectic effect induced both by intraperitoneal injection of *E. coli* LPS (Langhans *et al.*, 1989) and fluoxetine (Currie *et al.*, 2004). The absence of the effect of RHO in these models of anorexia, gives evidence of the selective anti-anorectic effect of RHO.

In fact LPS mediates anorexia through stimulating synthesis of different pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 and leptin (OB protein), tumour necrosis factor- α (TNF- α) and interferon (INF)- α , which potently reduce food intake, and are believed to be the major cytokines responsible for the anorexia that follows administration of LPS in mice and rats (reviewed in Plata-Salaman, 1991; Langhans and Hrupka, 1999; Plata-Salaman, 2001). Peripheral and brain mechanisms are also

involved in cytokine-induced feeding inhibition. Cytokines act on brain mechanisms involved in the control of feeding both directly (via neuronal mechanisms) or indirectly (via modulation of brain chemistry). Particularly IL-1 is a potent modulator of the hypothalamic areas, which contain neurotransmitter and neuropeptide systems closely associated with feeding control. For instance IL-1 induces the release of CRF, but this neuropeptide mediates only in part cytokines-induced anorexia, since the immunoneutralization of endogenous CRF in the brain reduces only weakly anorexia induced by IL-1 (Uehara *et al.*, 1989; Plata-Salaman, 1991). Consequently, even if *Rhodiola rosea* L. is able to block CRF activity in the brain this would not, however, be sufficient to block LPS-induced anorexia, since cytokine-induced feeding inhibition involves multiple mechanisms that involve interactions among multiple chemical and neuronal systems, besides the direct effect on gastrointestinal activities. Instead IL-1 also stimulates the release of catecholamines, histamine and serotonin strongly involved in feeding behaviours (Plata-Salaman, 1998; MohanKumar *et al.*, 1999).

Particularly, the serotonin system plays the major role in the control of feeding: in fact it is known that increases in serotonergic activity or stimulation of postsynaptic serotonergic receptors results in a decrease in food intake (Blundell, 1986). Several studies showed that both peripherally administered LPS and IL-1 α (Dunn, 1992a; Dunn, 1992b; Hrupka and Langhans, 2001) activate central serotonergic activity, and that the changes in serotonergic activity occur in a temporal manner that is consistent with its potential involvement in anorexia (Dunn, 1992a; MohanKumar *et al.*, 1999). Besides intraperitoneal injection of fluoxetine, a selective 5-HT reuptake inhibitor, produces a suppression of eating by reportedly increasing extracellular levels of endogenous 5-HT in forebrain nerve terminal regions via the blockade of the 5-HT transporter (Romero and Artigas, 1997; Trillat *et al.*, 1998; Currie *et al.*, 2004).

Moreover inhibition of 5-HT transmission via 8-OH-DPAT (5-HT_{1A} receptor agonist) consistently enhanced food intake both in LPS- and fluoxetine-treated rats (Hrupka and Langhans, 2001).

Together, these data suggest that the anti-anorectic effects of RHO are selective for stress- and CRF-induced anorexia, and that they may be related to the reported anti-stress properties of *R. rosea* L. (Kelly, 2001; Brown *et al.*, 2002; Zhu *et al.*, 2003).

Moreover, since it is known that CRF can also induce anxiogenic-like behaviour and, it is reported, the anxiolytic activity of the *Rhodiola rosea* L. extract (Brown *et al.*, 2002; Perfumi and Mattioli, 2006), the anorectic effect of the drug might be also related to its anxiolytic effect.

The exact mechanisms underlying these anti-anorectic effects of *R. rosea* remain unknown. However, acute stress, such as physical restraint, induces widespread activation of both the brain monoamine system and the CRF system (Dunn and Berridge, 1990; Gonzalez *et al.*, 1995; Koob and Heinrichs, 1999; Chaouloff, 2000; Ma and Morilak, 2005). Therefore, the anti-anorectic activities of *R. rosea* L. could be attributed to its ability to modulate the activation of several components of the stress-response system, such as the sympatho-adrenal system, which mainly controls the rapid response of an organism to an acute stressor (Lishmanov *et al.*, 1987; Panossian and Wagner, 2005).

The ability of *R. rosea* extract to revert stress-induced anorexia could also be related to its ability to reduce the secretion of CRF (Lishmanov *et al.*, 1987; Maslova *et al.*, 1994). As RHO administration blocks hypophagia induced by central administration of CRF, this suggests that RHO may exert its anti-anorectic and anti-stress effects by also acting as a CRF antagonist. Obviously this hypothesis may be directly determined by testing the whole extract or rather its main components at the CRF receptors in cellular assay.

The role of the CRF system in the modulation of ingestive behaviour is mediated by two different CRF receptor types, CRF-1 and CRF-2, which are both widely distributed in the brain (Heinrichs and Richard, 1999). Both CRF-1 and CRF-2 are involved in stress-induced inhibition of food intake, although they show different time courses of action: CRF-1 effects appear earlier than those of CRF-2 (Sekino *et al.*, 2004). Indeed, treatment with the non-selective CRF receptor agonist urocortin reduces food intake at both 0–1.5 h and 3–11 h, whereas CRF-1-deficient mice are refractory to the anorectic effects of urocortin during a period of 1.5 h following its injection, but not at 3–11 h (Bradbury *et al.*, 2000; Contarino *et al.*, 2000). Moreover, the inhibition of food intake induced by intracerebroventricular administration of urocortin 2, a selective CRF-2 agonist, does not appear until 6 h after treatment in freely-fed rats (Reyes *et al.*, 2001; Inoue *et al.*, 2003). Thus, as the anti-anorectic effects of RHO appear rapidly and last for 4–6 h at most, we speculate that it might exert its anti-anorectic effects by blocking brain CRF-1 receptors. To verify this hypothesis further studies using CRF-1 knockout mice could be useful.

The present study employed whole extract of *R. rosea*, which contains a range of biologically active substances; therefore, at present, we do not know which compounds are effectively responsible for the observed effects of RHO. However several studies have proposed that phenolic compounds, particularly phenylpropane and phenylethane derivatives, may represent major active principles for therapeutic activity of the plant (Kelly, 2001; Brown *et al.*, 2002). Since these compounds, such as rosavin, triandrin and salidroside, are structurally related to the catecholamines, they could play important roles in coordinating and integrating the behaviour responses to stress (Kurkin and Zapesochayna, 1986; Wang *et al.*, 1992; Yoshikawa *et al.*, 1996; Panossian and Wagner, 2005). However, further comparative studies using the main active components of this extract are necessary.

In conclusion, the present study provides original evidence that oral administration of *R. rosea* extract standardized in 3% rosavin and 1% salidroside results in a potent inhibition of the anorectic effects induced by CRF and stress, and provides functional evidence of claimed anti-stress properties of the plant.

Therefore, *Rhodiola rosea* L. may represent a promising pharmacological approach with important modulatory functions in mediating or regulating specific behaviour responses that are evoked by stress, as well as for stress-induced anorexia.

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