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β-Carboline harmine reverses the effects induced by stress on behaviour and citrate synthase activity in the rat prefrontal cortex

Abelaira HM, Réus GZ, Scaini G, Streck EL, Crippa JA, Quevedo J. β-Carboline harmine reverses the effects induced by stress on behaviour and citrate synthase activity in the rat prefrontal cortex.

Objectives: The present study was aimed at evaluating the effects of the administration of β-carboline harmine on behaviour and citrate synthase activity in the brain of rats exposed to chronic mild stress (CMS) procedure.
Methods: To this aim, after 40 days of exposure to CMS procedure, rats were treated with harmine (15 mg/kg/day) for 7 days, then memory, anhedonia and citrate synthase activity were assessed.
Result: Our findings demonstrated that stressed rats treated with saline increased the sucrose intake, and the stressed rats treated with harmine reversed this effect. Neither stress nor harmine treatment altered memory performance in rats. In addition, chronic stressful situations induced increase in citrate synthase activity in the prefrontal cortex, but not in the hippocampus and striatum. Treatment with harmine reversed the increase in citrate synthase activity in the prefrontal cortex.
Conclusion: These findings support the hypothesis that harmine could be involved in controlling the energy metabolism.

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Keywords: chronic mild stress, citrate synthase, depression, harmine

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Significant outcomes

- Effects of harmine on behaviour and citrate synthase activity.
- Harmine reversed the anhedonic behaviour induced by stress.
- Harmine decreased the citrate synthase activity in the rat's prefrontal cortex exposed to chronic mild stress (CMS).

Limitations

- We could have analysed the activity of other enzymes related to the Krebs cycle and we could have also assessed the activity of citrate synthase in other structures associated with depression, such as the amygdala and the nucleus accumbens.

Introduction

β-Carboline alkaloids, also known as harmala's alkaloids because they were first isolated from *Peganum harmala* (Zygophillaceae), are natural products that have been found in a number of medicinal plants, tobacco smoke, well-cooked foods (1,2) and endogenously in mammalian tissues (3,4). These alkaloids have a wide spectrum of pharmacological actions, including convulsive or anticonvulsive actions (5), tremorogenesis, an antioxidative action and immunomodulatory effects (6,7).

Recently, studies have reported that β-carboline harmine possesses antidepressant properties (8,9). In fact, harmine interacts with monoamine oxidase A and several cell-surface receptors, including serotonin receptor 2A (5-HT2A), which are involved in antidepressant pharmacotherapy (10). The results of the above studies suggest a possible importance of β-carbolines in the control of depressive states.

Some studies have appointed a role of β-carbolines in energy metabolism (11,12), which is involved in mitochondrial function. An enzyme that can be used to evaluate mitochondrial function is citrate synthase (13). Citrate synthase is a Krebs cycle enzyme that provides the electrons necessary for the mitochondrial respiratory chain. In addition, this enzyme is related to both depression and antidepressant actions (13). In fact, acute administration with the antipsychotic olanzapine and antidepressant fluoxetine increased citrate synthase activity in the brain areas studied (14). Abelaira et al. (15) also showed that imipramine increased the citrate synthase activity in the amygdala after acute treatment. Furthermore, there is also a relationship between mitochondria and monoamines, for example, a decrease was observed in the levels of serotonin in the brains of mice with multiple mitochondrial DNA deletions (16).

It has long been recognised that prolonged exposure to stress or excess glucocorticoids impairs memory function in both animal and human subjects (17–19). It is now also known that glucocorticoids have acute influences on cognition. Evidence from many different types of experiments indicates that glucocorticoids, released during or after emotionally arousing experiences, play a critical role in consolidating lasting memories. In contrast, elevated glucocorticoid levels shortly before retention testing impair retrieval of previously learned information (20,21).

Therefore, considering that habituation task and citrate synthase plays an important role in brain energy metabolism and that is probably involved in the pathophysiology of major depression, the objective of this study was to investigate the effects of the administration of harmine on citrate synthase activity within different cerebral areas of rats submitted to the CMS model.

Materials and methods

Animals

Male adult Wistar rats (60 days old) were obtained from the UNESC (Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil) breeding colony. They were housed five per cage, with food and water available *ad libitum* (except for the stressed group during the period when the stressor applied required no food or water) and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior recommendations for animal care and with approval by local ethics committee under protocol number 324/2008.

Drugs and treatment

Harmine was obtained from THC-Pharm/STI-Pharm (Frankfurt, Germany) and a dose of 15 mg/kg (22) was injected intraperitoneally over 7 days, following the CMS procedure. The treatments were administered in a volume of 1 ml/kg. To develop this study, we used 20 animals ($n = 10$ per group) separated in four groups, as follows: (1) non-stressed + saline; (2) non-stressed + harmine; (3) stressed + saline; and (4) stressed + harmine.

Experimental procedure

The CMS protocol was adapted from the procedure described by Gamaro et al. (23). The animals were divided into two groups: control and stressed. The control animals were kept undisturbed in their home cages during the 40 days of treatment, receiving only ordinary daily care with daily supports of food and water. The 40-day CMS paradigm was used for the animals in the stressed group. Individual stressors and the length of time they were applied each day were as follows: (i) 24 h of food deprivation; (ii) 24 h of water deprivation; (iii) 1–3 h of restraint as described later; (iv) 1.5–2 h of restraint at 4°C; (v) forced swimming during 10 or 15 min as described later; (vi) flashing light 120–210-min duration; and (vii) isolation (2–3 days).

Stressor stimuli were applied at different times every day to minimise its predictability. The restraint test was carried out by placing the animal in a 25×7 cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1 cm hole at the far end

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for breathing. The forced swimming test was carried out by placing the animal in a glass tank measuring 50 × 47 cm, with 30 cm of water at 23°C. Exposure to the flashing light was made by placing the animal in a 60 × 60 × 25 cm plywood box, divided into 16 cells of 15 × 15 × 25 cm with a frontal glass wall. A 40 W lamp, flashing at a frequency of 60 flashes/min, was used.

Sucrose intake (anhedonic behaviour)

After 40 days of treatment (stressors described above), the consumption of sweet food was measured to verify anhedonic behaviour. The animals were placed in a lit rectangular box (40 × 15 × 20 cm) with a glass ceiling, the floor and side walls being made of wood and divided into nine equal rectangles by black lines. Ten Froot Loops (Kellogg's® pellets of wheat and sucrose) were placed in one extremity of the box. Animals were submitted to five sessions of 3 min each, once a day, in order to become familiarised with this food (not its usual food supplied to the animals). After being habituated, the animals were exposed to two test sessions of 3 min each, where the number of ingested pellets was counted according to previous studies (24–26). This task was undertaken during the light cycle and the evaluation was realised by an observer with no knowledge of the groups. Results were noted when the animal ate 1, 1/2 or 1/4 part of the Froot Loops. The results of the test session were added and divided by one to obtain a single value. These evaluations were made since food deprivation, which is used in many behaviour tasks as a motivating stimulus (24).

Habituation to an open field

In the habituation to an open-field apparatus, animals were exposed twice, with a 24-h interval. The test was carried out in 60 × 40 cm (length and width) open-field surrounded by 50-cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided by black lines into 12 equal squares. In both sessions (training and testing sessions), animals were gently placed on the left rear square of the apparatus and allowed to explore freely for 5 min (27). Rats were treated 60 min prior after the training session, and the number of crossings of black lines and rearings were recorded in both sessions to evaluate the effects of drug treatment on the habituation to the novel environment.

Citrate synthase activity

After 40 days of CMS, the rats were killed and the skulls were removed. The prefrontal cortex, hippocampus and striatum were dissected and homogenised (1 : 10, w/v) in SETH buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). The homogenates were centrifuged at 800 × *g* for 10 min and the supernatants were kept at −80°C, until they were used for enzyme activity determination. Protein content was determined using the method described by Lowry et al. (28), using bovine serum albumin as standard. Citrate synthase activity was assayed according to the method described by Shepherd and Garland (29). The reaction mixture contained 100 mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,5'-di-thiobis-(2-nitrobenzoic acid), 0.1% Triton X-100 and 2–4 μg supernatant protein and was initiated with 100 μM oxaloacetate and monitored at 412 nm for 3 min at 25°C.

Statistical analysis

Data were analysed by one-way of analysis of variance (ANOVA), followed by Tukey's test, when F was significant and is expressed as mean ± SD. All analyses were performed using the statistical package for the social science software version 16.

Result

The stressed rats treated with saline increased the sucrose intake in rats exposed to CMS, and the treatment with harmine reversed the anhedonic effect in stressed rats ($F_{3–60} = 5.537$; $p = 0.006$) (Table 1).

In Figs 1a and b, there were no differences in the number of crossings and rearings between training and test session on stress and control groups, suggesting that rats were not habituated to the open-field apparatus ($F_{3–15} = 1.375$; $p > 0.05$).

As shown in Fig. 2, the stressed rats treated with saline significantly increased citrate synthase activity in the prefrontal cortex ($F_{3–15} = 3.825$; $p = 0.039$), and treatment with harmine reversed the increase of citrate synthase activity in this brain area. The CMS

Table 1. The effects of administration of harmine on sucrose intake in rats exposed to CMS model

	Mean	SEM
Control + saline	2.000	0.4225
Control + harmine	2.071	0.2973
Stress + saline	0.200*	0.2000
Stress + harmine	1.583#	0.3515

CMS, chronic mild stress.

*$p < 0.05$ versus control + saline; #$p < 0.05$ versus CMS + saline.

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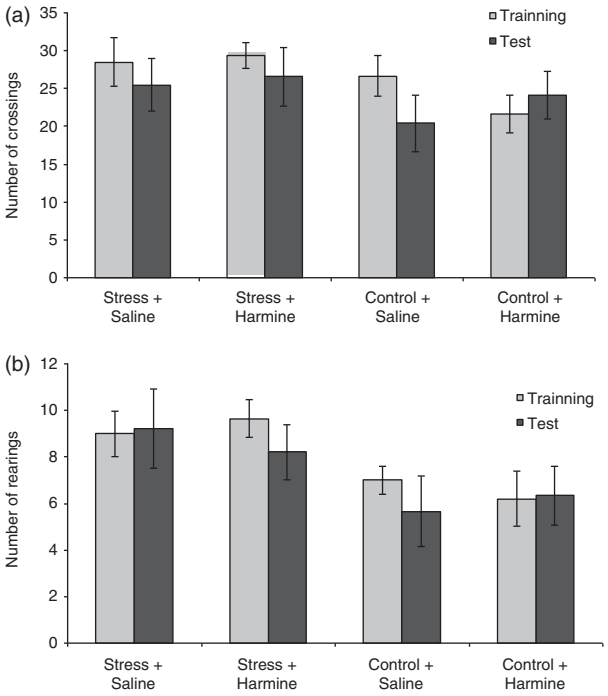

Fig. 1. Effects of the acute injection of harmine on the habituation to the open-field test. Normal rats were injected 60 min before the first exposure to the apparatus, and the number of crossing (a) or rearings (b) performed during training and test habituation trials were recorded. Data are mean ± SEM. $n = 12–15$ animals per group.

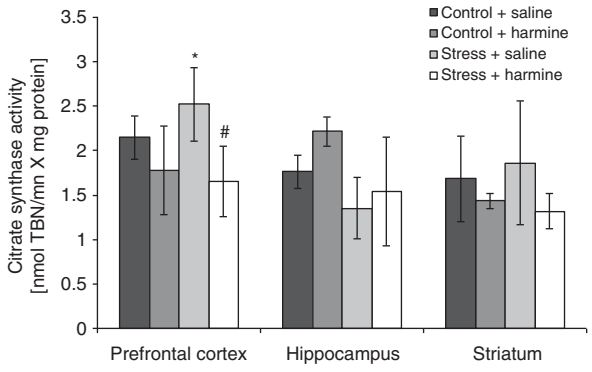

Fig. 2. Effects of chronic mild stress (CMS) on the citrate synthase activity in the prefrontal cortex, hippocampus and striatum of rats. Data were analysed by one-way analysis of variance (ANOVA), followed by Tukey's test, when F was significant. Values are expressed as nmol TNB/min/mg protein (means ± SD) of rats. $^{*}p < 0.05$ versus control + saline; $^{\#}p < 0.05$ versus CMS + saline, according to ANOVA, followed by Tukey's *post hoc* test.

procedure and treatment with harmine did not alter the activity of citrate synthase in the hippocampus ($F_{3–9} = 2.301$; $p = 0.177$) or striatum ($F_{3–15} = 1.264$; $p = 0.331$).

Discussion

The CMS test was developed in the late 1980s and is one of the most extensively investigated animal models of depression to date. It is noteworthy that rats subjected to CMS develop a wide spectrum of neurobiological alterations pertaining to the serotonergic system (30). Our results showed that the stressed rats treated with saline decreased the sucrose intake and the harmine treatment was able to reverse this effect. In accordance with available literature, the administration of harmine antagonised anhedonic behaviour assessed in CMS rats, without affecting the consumption of sweet food in non-stressed animals (30). In addition, studies from our group recently showed that acute treatment with harmine and imipramine decreased the immobility time of rats and increased both the climbing and swimming times of rats. Nevertheless, Farzin and Mansouri (9) demonstrated that treatment with harmane, norharmane and harmine dose dependently reduced the immobility time in mice. These results support the case that harmine has an antidepressant action in experimental studies.

Our results showed that there were no differences in the number of crossings and rearings between training and test session on stress and control groups, suggesting that rats were not habituated to the open-field apparatus. Moura et al. (31) showed that acute pretraining systemic administration of harmine improved novel recognition tested 1.5 h short-term memory after training in mice, and also there was no effect on long-term memory, suggesting that the animals were given pretraining injections, and both acquisition and early consolidation memory processes could have been affected. However, our results suggest that further experiments using behavioural parameters of memory are required to confirm that alkaloids exert cognitive enhancing action by facilitating processes specifically involved in memory formation. In addition, the finding that these alkaloids might induce memory enhancement needs to be extended to other memory tasks in future studies.

In addition, there is a strong body of evidence suggesting that dysfunction in brain metabolism is related to neuropsychiatry disorders, such as depression and bipolar disorder (13). Citrate synthase has been used as a quantitative enzyme marker for the presence of intact mitochondria. Some studies have already appointed to a role of β-carbolines in energy metabolism (11). Réus et al. (12) demonstrated that both the acute and chronic administration of harmine and imipramine increased creatine kinase activity and altered complex I, II and VI activities in the prefrontal cortex and in the striatum.

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Our findings showed that stressed rats treated with saline significantly increased citrate synthase activity in the prefrontal cortex, and that treatment with harmine reversed this increase. Very recently, we demonstrated that acute and chronic treatments with olanzapine and fluoxetine (alone or in combination) altered creatine kinase activity in the rat brain (14). Abelaira et al. (15) also showed that citrate synthase activity was increased in the amygdala with imipramine (30 mg/kg). Nevertheless, it was shown that the chronic administration of paroxetine increased citrate synthase activity in the prefrontal cortex, hippocampus, striatum and cerebral cortex of adult rats. Consistent with our findings, a previous study showed that acute treatment with the antidepressant tianeptine (10 and 15 mg/kg) decreased the level of citrate synthase activity in the prefrontal cortex (32), suggesting that this effect could be related, at least partly, with a desensitisation to the effects of repeated stress, or to an adaptation mechanism to these drugs.

In conclusion, present findings suggest that the anhedonic behaviour observed in rats subjected to chronic unpredictable stressful situations were reversed by treatment with harmine. In addition, this is the first study that has shown the effects of the administration of harmine on citrate synthase activity in stressed rats, and that these results may be related to the antidepressant-like effects of harmine, as previously related (11,22). Taken together, these findings support a rapid and robust reversion of anhedonic behaviour and physiological induced by chronic and unpredictable mild stress situations in rats. Finally, further studies are necessary to evaluate the mechanisms by which these effects occur.

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