

Original Research Article

The effects of crocin and safranal on the yawning induced by intracerebroventricular injection of histamine in rats

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Abstract

Objective: Crocin and safranal, as the major constituents of saffron, have many biological activities. This study investigated the effects of crocin and safranal on yawning response induced by intracerebroventricular (i.c.v.) injection of histamine in rats.

Materials and Methods: In ketamine/xylazine-anesthetized rats, a guide cannula was implanted in the right ventricle of the brain and yawning induced by i.c.v. injection of histamine. Crocin and safranal were intraperitoneally (i.p.) injected alone and before i.c.v. injection of histamine.

Results: Histamine at the doses of 10 and 20 µg/rat produced yawning. Mepyramine (a histamine H₁ receptor antagonist) 40 µg/rat significantly (p<0.05) prevented histamine (20 µg/rat)-induced yawning. Crocin (30 mg/kg) and safranal (1 mg/kg) significantly (p<0.05) increased histamine (10 µg/rat)-induced yawning. Crocin and safranal also induced yawning when injected before mepyramine plus histamine administration.

Conclusion: The results of the present study showed a yawning-inducing effect for central histamine, which was inhibited by mepyramine. Crocin and safranal increased histamine-induced yawning, and also produced yawning when the histamine action is blocked.

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Introduction

Yawning, as a common physiological event, occurs at low frequency in humans and animals (Walusinski, 2009). It is a brain-mediated physiological phenomenon in which a variety of neurotransmitters and neuropeptides including dopamine, excitatory amino acids, serotonin, nitric oxide, noradrenaline, -aminobutyric acid (GABA), adrenocorticotrophic hormone-related peptides, prolactin, urotensin, oxytocine and opioid peptides are involved

(Collins and Eguibar, 2010). Additional evidence suggests that brain histamine may also be involved in the induction of yawning (Tamaddonfard et al, 2008a).

Crocus sativus L., commonly known as saffron, is used in folk medicine as an antispasmodic, nerve sedative, expectorant, eupeptic, anticatarrhal, carminative, diaphoretic, stomachic, aphrodisiac and emmenagogue agent (Schmidt et al., 2007). Crocin and safranal are the major biologically active

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components of saffron (Rios et al., 1996). Pharmacological studies have demonstrated antiepileptic, anti-oxidative, anti-inflammatory, neuroprotective, memory improvement and anti-diabetic effects for crocin and safranal (Assimopolou et al., 2005; Nam et al., 2010; Gadrdoost et al., 2011; Boskabady et al., 2012; Tamaddonfard et al., 2012a; Tamaddonfard et al., 2013a, b, c).

Regarding the documents showing that crocin and safranal can influence histamine functions (Boskabady et al., 2012; Tamaddonfard et al., 2012b; Boskabady et al., 2011), the present study was designed to investigate the effects of intraperitoneal (i.p.) injection of crocin and safranal on the yawning induced by intracerebroventricular (i.c.v.) administration of histamine. We also examined yawning response of crocin and safranal after blocking histamine function with mepyramine, a histamine H₁ receptor antagonist.

Materials and Methods

Animals

Healthy adult male Wistar rats, weighing 250–280 g, were used in this study. Rats were kept in polyethylene cages with food and water available *ad libitum* in a laboratory with controlled ambient temperature ($22 \pm 0.5^\circ\text{C}$) and under a 12hr-12hr light-dark cycle (lights on 07:00). Experiments were carried out between 13:00 and 16:00. The experiment protocol was approved by the Laboratory Animal Care and Use Center of the Faculty of Veterinary Medicine of Urmia University, Urmia, Iran.

Chemicals

Chemicals used in this study included crocin (Fluka, Germany), safranal, (Sigma-Aldrich, USA), histamine dihydrochloride (Merck, Darmstadt, Germany) and mepyramine maleate (Sigma-Aldrich, USA). Crocin, histamine and mepyramine were dissolved in normal saline. Safranal

was dissolved in liquid paraffin (Amin and Hosseinzadeh, 2012; Tamaddonfard et al., 2013c).

Treatment groups

Forty eight rats were used in our experiments. Six rats were injected (i.c.v.) with normal saline and histamine (5, 10 and 20 $\mu\text{g}/\text{rat}$). Six were injected with mepyramine (40 $\mu\text{g}/\text{rat}$, i.c.v.) and mepyramine (40 $\mu\text{g}/\text{rat}$) plus histamine (20 $\mu\text{g}/\text{rat}$). Six rats were treated with normal saline and crocin (7.5, 15 and 30 mg/kg, i.p.). Six rats were injected (i.c.v.) of histamine at a dose of 10 $\mu\text{g}/\text{rat}$ after injection of crocin (30 mg/kg, i.p.). Six rats were injected with paraffin and safranal (0.25, 0.5 and 1 mg/kg, i.p.). Six rats were injected with histamine (10 $\mu\text{g}/\text{rat}$, i.c.v.) after injection of safranal (1 mg/kg, i.p.). To six rats, crocin (30 mg/kg, i.p.) was injected before injection of mepyramine (40 $\mu\text{g}/\text{rat}$, i.c.v.) plus histamine (20 $\mu\text{g}/\text{rat}$, i.c.v.). Safranal at a dose of 1 mg/kg (i.p.) was injected before injection of mepyramine (40 $\mu\text{g}/\text{rat}$, i.c.v.) plus histamine (20 $\mu\text{g}/\text{rat}$, i.c.v.) to six rats. Four-day- elapsed time was considered among injections. Crocin and safranal were injected (i.p.) 20 and 30 min and mepyramine was injected (i.c.v.) 5 min before injection (i.c.v.) of histamine.

Surgery

After a 15-day adaptation period, each rat was anesthetized with injection (i.p.) of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and then, placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The scalp was incised, and the bregma was exposed. A 23-gauge, 12-mm stainless-steel guide cannula was inserted in the right lateral ventricle of the brain. The tip of the cannula was aimed at the following coordinates: 0.8 mm posterior to the bregma, 2 mm lateral to the midline and 4 mm below the top of the skull (Paxinos and Watson, 1997; Tamaddonfard et al., 2008b). The cannula was then fixed to the skull using three

screws and dental acrylic. A 12-mm stylet was inserted into the cannula to keep it patent prior to injection. Animals were allowed a 10-day- recovery period before experiments were initiated.

Drug injection

For i.c.v. injections of histamine and mepyramin, a 28-gauge 12.5-mm injection needle was attached to a 30 cm polyethylene tube fitted to a 5 μ l Hamilton syringe. The volume of the chemical solutions to be injected into the lateral ventricle was 2 μ l and the injection was done over a period of 60 sec. Normal saline, paraffin, crocin and safranal were injected (i.p.) using a 25-gauge injection needle at a volume of 1 ml/kg.

Yawning

Yawning behavior was defined as a prolonged (more than 1 sec) wide opening of the mouth followed by a rapid closure (Collins, 2005). On the day of testing, rats were placed in clear plastic chambers (30 \times 25 \times 25 cm) and allowed to habituate to the chamber for a period of 30 min. Injections (i.p. and i.c.v.) were performed at the end of the habituation period, and the number of yawns was counted for a period of 45 min by blinded observers.

Cannula placement verification

During the surgery and before i.c.v. injections, a rise in the cerebrospinal fluid through the cannula was observed. For additional confirmation of the placement of the cannula in the lateral ventricle of the brain, at the end of experiments the rats were injected with 2 μ l methylene blue (i.c.v.), deeply anesthetized with a high dose of ether and decapitated. The brains were removed and placed in formaldehyde (10%) solution.

After 24 hr, the brains were sliced into 1 mm slices and the placement of the tip of the cannula and distribution of the dye in the lateral ventricle were visually controlled. Data from four rats with an

incorrect placement of the cannula were excluded from the analysis.

Statistical analysis

The data were analyzed using Graph Pad Prism software version 5. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's tests. All values are expressed as mean \pm SEM. Statistical significance was set at $p < 0.05$.

Results

As shown in Figures 1, 2 and 3, no significant differences were observed in the number of yawns obtained following i.c.v. injection of normal saline (1.2 ± 0.5) and i.p. injection of normal saline (1 ± 0.4) and paraffin (0.8 ± 0.5).

The number of yawns obtained after i.c.v. injection of normal saline was 1.2 ± 0.5 . Also, i.c.v. injection of histamine at a dose of 5 μ g/rat produced no significant yawning (3.7 ± 1.1), whereas at doses of 10 μ g/rat (8.5 ± 1.3) and 20 μ g/rat (12.7 ± 1.1) histamine produced significant yawning as compared to normal saline. Mepyramine (40 μ g/rat, i.c.v.) alone produced no significant yawning in comparison with i.c.v. injection of normal saline, whereas pretreatment with mepyramine (40 μ g/rat) before histamine (20 μ g/rat) significantly ($p < 0.05$) prevented histamine-induced yawning (Figure 1).

The number of yawns obtained after i.p. injection of normal saline and crocin at doses of 7.5, 15 and 30 mg/kg were 1 ± 0.4 , 1.2 ± 0.7 , 1.5 ± 0.7 and 1.8 ± 0.8 , respectively (Figure 2). No significant differences were observed among normal saline and crocin treated rats. Prior i.p. injection of crocin at a dose of 30 mg/kg significantly ($p < 0.05$) increased the number of yawns induced by i.c.v. injection of histamine 10 μ g/rat (Figure 2).

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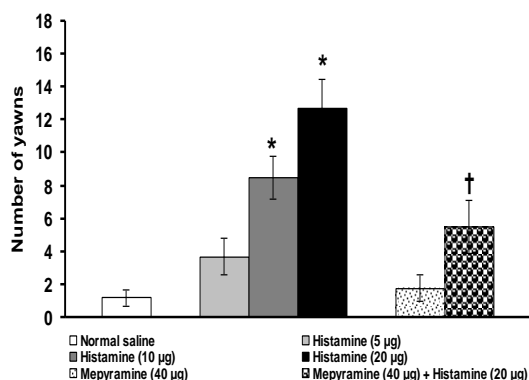


Figure 1. Yawning response after intracerebroventricular (i.c.v.) injection of normal saline, histamine, mepyramine and mepyramine before histamine. Values are expressed as mean \pm SEM (n = 6). * indicates a significant difference (p<0.05) as compared to normal saline. † indicates a significant difference (p<0.05) as compared to histamine (20 µg).

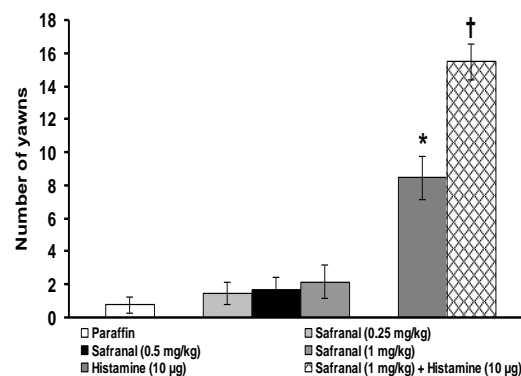


Figure 3. Yawning response after intraperitoneal (i.p.) injection of paraffin and safranal and intracerebroventricular (i.c.v.) injection of histamine and i.p. injection of safranal before i.c.v. injection of histamine. * indicates a significant difference (p<0.05) as compared to normal saline. † indicates a significant difference (p<0.05) as compared to histamine (10 µg).

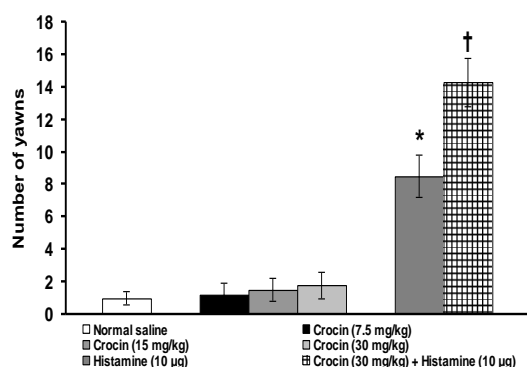


Figure 2. Yawning response after intraperitoneal (i.p.) injection of normal saline and crocin and intracerebroventricular (i.c.v.) injection of histamine and i.p. injection of crocin before i.c.v. injection of histamine. * indicates a significant difference (p<0.05) as compared to normal saline. † indicates a significant difference (p<0.05) as compared to histamine (10 µg).

The number of yawns obtained after i.p. injection of paraffin and safranal at doses of 0.25, 0.5 and 1 mg/kg were 0.8 ± 0.5 , 1.5 ± 0.7 , 1.7 ± 0.8 and 2.2 ± 1 , respectively (Figure 2). No significant differences were observed among paraffin and safranal treated rats. Prior i.p. injection of safranal at a dose of 1 mg/kg significantly (p<0.05) increased the number of yawns induced by i.c.v. injection of 10 µg/rat of histamine (Figure 3).

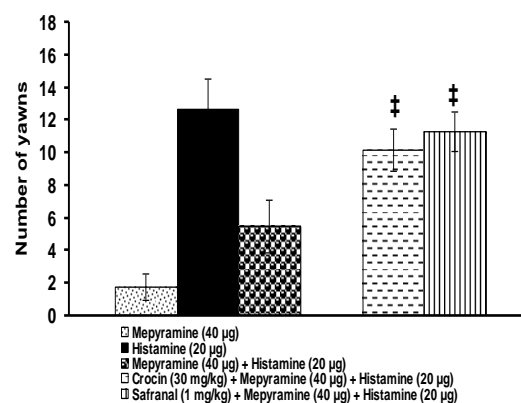


Figure 4. Yawning response after intraperitoneal (i.p.) injection of crocin and safranal before intracerebroventricular (i.c.v.) injection of mepyramine plus histamine. Values are expressed as means \pm SEM (n = 6). ‡ indicates a significant difference (p<0.05) as compared to mepyramine plus histamine.

Discussion

The results of the present study showed that o.k. injection of histamine produced yawning, which was prevented by mepyramine pretreatment. This means that brain histamine through its H₁ receptor is involved in yawning modulation. It has been reported that i.c.v. injection of chlorpheniramine (a histamine H₁ receptor antagonist) and ranitidine (a histamine H₂ receptor antagonist) inhibited yawning induced by central administration of histamine (Tamaddonfard *et al.*, 2008a). It was found that microinjection of histamine into the paraventricular nucleus (PVN) of the brain produces yawning-like response in the pentobarbital sodium-anesthetized rats. In addition, Seki *et al.* (2002) reported the involvement of histamine H₁ receptor in histamine-induced yawning. In this context, Seki *et al.* (2003) reported a suppressive effect for pirlamine (mepyramine) on the light-induced yawning response in rats.

Our results showed no yawning producing effect for crocin and safranal used alone. There are no reports showing the induction of yawning by these compounds. Natural substances such as curcumin also did not produce yawning when used alone (Tamaddonfard, 2013d).

The results of our study showed that crocin and safranal increased the number of yawning induced by histamine. In addition, after blocking the yawning producing effect of histamine by mepyramine, crocin and safranal produced yawning. These indicate that crocin and safranal may affect yawning by activation of histamine receptors. Histamine, through its H₁, H₂, H₃ and H₄ receptors, mediates many functions including allergy, acid secretion, memory and learning, pain and feeding behavior (Haas *et al.*, 2008). It seems that histamine H₁ and H₂ receptors are involved in yawning modulation of crocin and safranal. It was found that i.c.v. injection of cimetidine antagonized (+/-) N-n-propylnorapomorphine-induced stretching and yawning in rats (Ferrari and

Baggio, 1985). Moreover, histamine H₁ and H₂ receptor antagonists, chlorpheniramine and ranitidine, respectively, inhibited central histamine-induced yawning (Tamaddonfard *et al.*, 2008a). In the peripheral tissues, both crocin and safranal influence histamine H₁ receptor function. Also, i.p. injection of crocin suppressed histamine-induced local inflammation and increased the anti-inflammatory effect of chlorpheniramine (Tamaddonfard *et al.*, 2012). Moreover, the inhibitory effect of safranal and chlorpheniramine on the smooth muscle contraction induced by histamine in trachea chains was reported (Boskabady *et al.*, 2011). However, the role of other yawning-promoting mechanisms such as dopamine and acetylcholine systems needs to be considered in the effects of crocin and safranal on yawning. In this context, other active constituents in medicinal plants, such as curcumin of turmeric, used of multiple mechanisms in modulation of yawning (Tamaddonfard, 2013d).

In conclusion, the results of the present study indicated that central histaminergic system produced yawning, and mepyramine, a H₁ receptor antagonist, blocked histamine-induced yawning. Crocin and safranal increased the yawning induced by histamine. Also, when histamine action was blocked by mepyramine, crocin and safranal produced yawning.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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