

Modulation of Visceral Nociception, Inflammation and Gastric Mucosal Injury by Cinnarizine

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Abstract: The effect of cinnarizine, a drug used for the treatment of vertigo was assessed in animal models of visceral nociception, inflammation and gastric mucosal injury. Cinnarizine (1.25–20 mg/kg, s.c.) caused dose-dependent inhibition of the abdominal constrictions evoked by i.p. injection of acetic acid by 38.7–99.4%. This effect of cinnarizine (2.5 mg/kg) was unaffected by co-administration of the centrally acting dopamine D2 receptor antagonists, sulpiride, haloperidol or metoclopramide, the peripherally acting D2 receptor antagonist domperidone, but increased by the D2 receptor agonist bromocryptine and by the non-selective dopamine receptor antagonist chlorpromazine. The antinociception caused by cinnarizine was naloxone insensitive, but enhanced by propranolol, atropine and by yohimbine. The antinociceptive effect of cinnarizine was prevented by co-treatment with the adenosine receptor blocker theophylline or by the ATP-sensitive potassium channel (K_{ATP}) blocker glibenclamide. Cinnarizine at 2.5 mg/kg reversed the baclofen-induced antinociception. Cinnarizine at 2.5 mg/kg reduced immobility time in the Porsolt's forced-swimming test by 24%. Cinnarizine inhibited the paw oedema response to carrageenan and reduced gastric mucosal lesions caused by indomethacin in rats. It is suggested that cinnarizine exerts anti-inflammatory, antinociceptive and gastric protective properties. The mechanism by which cinnarizine modulates pain transmission is likely to involve adenosine receptors and K_{ATP} channels.

Keywords: Cinnarizine, visceral pain, inflammation, gastric mucosa, rat, mice.

Introduction

Cinnarizine (Stugeron R) is a calcium-channel blocker used in treatment of vertiginous disorders (Pianese et al. 2002) and migraine (Mansooreh et al. 2006). Among its most rare adverse effects are extrapyramidal symptoms and depression; these effects can persist during weeks, months or years after the withdrawal of the drug (Negrotti and Calzetti, 1997; Fabiani et al. 2004; Teive et al. 2004; Hirose, 2006) and can be explained by the inhibition of the passage of calcium in striatal neurons and a direct anti-dopaminergic features (Dopamine D1 and D2 receptor blockade) because of the similar chemical structure with neuroleptic drugs (Reiriz et al. 1994; Brucke et al. 1995) with more than 80% D2-receptor occupancy being required for drug-induced parkinsonism to appear (Hirose, 2006). Cinnarizine has been reported to possess anti-inflammatory and pain alleviating properties. Cinnarizine inhibited the ear oedema induced by croton oil or capsaicin in mice and reduced oedema induced in the rat hind paw by subplantar injection of carrageenan (Blazso et al. 1999). Intraperitoneal or intrathecal cinnarizine caused a dose-dependent antinociception in the rat tail-flick test (Rego et al. 1990). Cinnarizine administered via subcutaneous (Del Pozo et al. 1987) or intracerebroventricular route (Miranda et al. 1993) produced a dose-dependent antinociception in acetic acid writhing test in mice. This effect is naloxone insensitive (Miranda et al. 1993). Cinnarizine (20 mg/kg), inhibited 100% ethanol-induced lesion formation by 71% (Lozeva et al. 1994).

The present study aimed to investigate the effects of cinnarizine on visceral pain caused by intraperitoneal injection of acetic acid in mice, a model of visceral inflammatory pain (chemonociception) and to pharmacologically characterize and investigate the possible neural pathways involved in its analgesic effect. In addition, the behavioral effect of cinnarizine on locomotor activity and on immobility time in Porsolt's forced-swimming test, its effect on acute inflammation caused by subplantar carrageenan and the effects of the drug on gastric mucosal damage caused by indomethacin was studied.

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Materials and Methods

Animals

Sprague-Dawley strain rats weighing 120–130 g of body weight or Swiss male albino mice 20–22 g of body weight were used (National Research Centre, Cairo). Standard laboratory food and water were provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). Equal groups of 6 mice each were used in all experiments. The doses of cinnarizine used in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes (1964).

Drugs

Cinnarizine (Arab Drug Co., Cairo), guanethidine, propranolol hydrochloride, yohimbine hydrochloride, naloxone hydrochloride (Sigma, St. Louis, U.S.A.), bromocryptine (Novartis Pharma, Cairo), haloperidol, indomethacin (Kahira Pharm & Chem. IND Co., Cairo), glibenclamide (Hoechst Orient, Cairo), atropine sulphate, baclofen (Misr Pharm Co., Cairo), domperidone (Janssen-Cilag, Switz) were used. Analytical-grade glacial acetic acid (Sigma, St. Louis, U.S.A.) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use. Indomethacin was dissolved in a 5% solution of sodium bicarbonate.

Acetic acid-induced writhing

Separate groups of 6 mice each were administered vehicle or drug (1.5, 2.5, 5, 10 or 20 mg/kg, s.c.). After 30-min pretreatment interval, 0.6% acetic acid (0.2 ml/mice) was intraperitoneally (i.p.) administered (Koster et al. 1959). Each mouse was then placed in an individual clear plastic observational chamber, and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration. Further experiments were designed in an attempt to elucidate the mechanisms by which cinnarizine exerts its anti-nociceptive effect. The dose of 2.5 mg/kg of cinnarizine was selected to be used in the subsequent experiments.

Thus, the effect of co-administration of the alpha-2 adrenoreceptor antagonist yohimbine (5 mg/kg, i.p.), the beta adrenoreceptor antagonist,

propranolol (2 mg/kg, i.p.), the muscarinic acetylcholine receptor antagonist atropine (2 mg/kg, i.p.), the non-selective opioid receptor antagonist naloxone (5 mg/kg, i.p.), the non-selective adenosine receptor antagonist theophylline (20 mg/kg, i.p.), the GABA agonist baclofen (5 mg/kg, i.p.), and the potassium channel blocker glibenclamide (5 mg/kg, i.p.), indomethacin (5 mg/kg, i.p.) were examined on antinociception caused by cinnarizine.

Furthermore, the effect of the centrally acting dopamine D2 receptor antagonists, sulpiride (10 mg/kg, i.p.) and haloperidol (1.5 mg/kg, i.p.), the peripherally acting D2 receptor antagonist domperidone (10 mg/kg, i.p.) or D2 receptor agonist bromocryptine (3 mg/kg, i.p.), the D2 receptor antagonist metoclopramide (10 mg/kg) and the non-selective dopamine receptor antagonist chlorpromazine (3 mg/kg, i.p.) was examined. All drugs were administered 30 min prior to the abdominal constriction assay.

Rotarod testing

Motor performance was measured as the latency to fall from an accelerating rotarod located over plates connected to an automatic counter (Ugo Basile, Varese, Italy). Mice were trained to remain on a rotating rod for 2 min as the rod rotated toward the animal. After the 2-min training period, the mice were administered vehicle (saline) or drug and 30 min later placed on the rotating rod as it accelerated from 4 to 40 rpm over 5 min and the time that they could remain on the accelerating rod was noted (Millan et al. 1994). The cutoff time was 600 sec. The time was measured from the start of the acceleration period. The test was repeated 2 h after vehicle or drug injection. Six animals were used per dose and for the controls.

Porsolt's forced-swimming test

Each mouse was placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 22–23°C. The animal was forced to swim for 6 min and the duration of immobility was measured. The mouse was considered as immobile when it stopped struggling and moved only to remain floating in the water, keeping its head above water. The floating time, which is used as the measure of despair (Porsolt et al. 1977), was recorded after treatment after treatment with saline, cinnarizine (2.5, 5, 10 or 20 mg/kg, s.c.) or imipramine (15 mg/kg, s.c.).

Carageenan-induced paw oedema

Paw swelling was elicited by sub-plantar injection of 100 μ l of 1% sterile lambda carrageenan suspension in saline into the right hind paw (Winter et al. 1962). Contralateral paw received an equal volume of saline. The oedema component of inflammation was quantified by measuring the increase in paw volume (ml) with a plethysmometer (Ugo Basile, Milan, Italy) before carrageenan injection and at selected times thereafter. Oedema was expressed as a percentage of change from control (pre-drug) values. The effect cinnarizine (1.25, 2.5, 5, 10 or 20 mg/kg, s.c., 0.2 ml/rat, $n = 6$ / group) was studied. Cinnarizine was administered 30 min before the injection of the carrageenan suspension. The control groups received saline (0.2 ml/rat, $n = 6$ per group; s.c.).

Gastric ulcerogenic studies

Gastric mucosal damage was evoked by indomethacin (20 mg/kg, s.c.). Rats received either saline (0.2 ml/rat, s.c., $n = 6$) (control) or cinnarizine (2.5, 5 or 10 mg/kg, 0.2 ml/rat, s.c., $n = 6$ per group). Rats were killed 48 h later. Gastric mucosal lesions were scaled as described earlier (Mózsik et al. 1982).

Statistical analyses

Data are expressed as mean \pm S.E. The effects of different drugs used in the abdominal constriction assay are also expressed as percent inhibition (%) compared to the control value. Differences between vehicle (control) and treatment groups were determined by using one and two-way ANOVA followed by multiple comparison by the Tukey's honestly significant difference. A probability value less than 0.05 was considered statistically significant.

Results

Effect of cinnarizine on abdominal constrictions induced by acetic acid

Cinnarizine (1.25, 2.5, 5, 10 or 20 mg/kg, s.c.) caused dose-dependent inhibition of the abdominal constrictions evoked by i.p. injection of acetic acid by 38.7–99.4% (Fig. 1). This effect of cinnarizine (2.5 mg/kg) was unaffected by co-administration of the centrally acting dopamine D2 receptor antagonists, sulpiride, haloperidol or metoclopramide, the peripherally acting D2 receptor antagonist domperidone, but increased by the D2 receptor agonist bromocryptine and by the non-selective dopamine receptor antagonist chlorpromazine (Fig. 2).

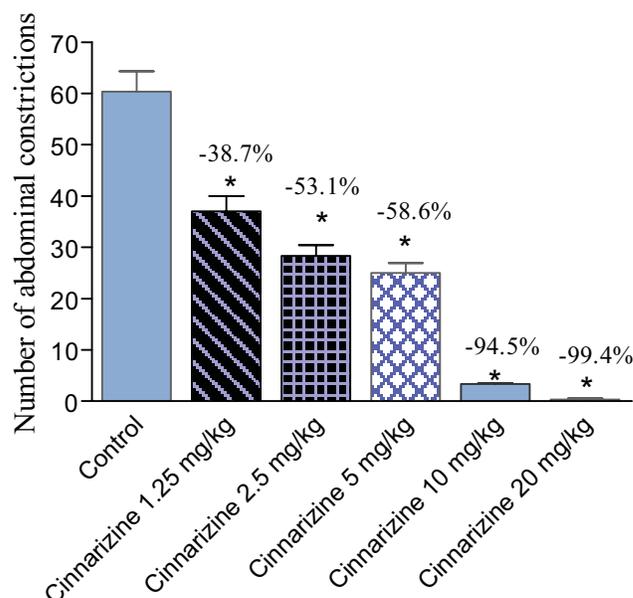


Figure 1. Effect of different doses of cinnarizine (1.25, 2.5, 5, 10 and 20 mg/kg) on abdominal constrictions caused by i.p. injection of dilute acetic acid in mice. Saline (control) or cinnarizine was s.c. administered 30 min prior to testing. Data are expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals are also shown. * $p < 0.05$ vs. control. Six mice were used per each group.

The antinociception caused by cinnarizine was unaffected by the opioid receptor antagonist naloxone, but enhanced by the beta-adrenergic antagonist propranolol, the muscarinic receptor antagonist atropine and by the alpha2-adrenergic antagonist yohimbine (Fig. 3).

The antinociceptive effect of cinnarizine was prevented by co-treatment with the adenosine receptor blocker theophylline (Fig. 4) and by K-channel blocker glibenclamide (Fig. 5). Cinnarizine at 2.5 mg/kg reversed the baclofen-induced antinociception (Fig. 5). Cinnarizine enhanced the antinociceptive effect of piracetam or vinpocetine (Fig. 6). When indomethacin (5 mg/kg, i.p.) was administered in combination with cinnarizine (2.5 mg/kg, s.c.), an additive effect was noted (Fig. 7).

Rotarod testing

Cinnarizine (1.5–20 mg/kg) did not produce any significant changes on the rotarod performances of the mice. There was no significant difference between the control group and cinnarizine-treated groups in the latency to fall (Table 1).

Effect of cinnarizine on immobility time in porsolt's forced-swimming test

Cinnarizine administered at 2.5 mg/kg reduced immobility time in the Porsolt's forced-swimming

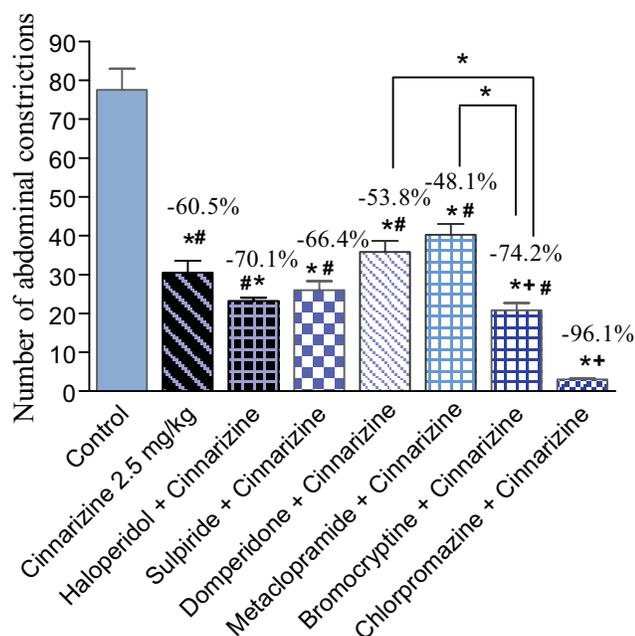


Figure 2. Effect of haloperidol (1.5 mg/kg, i.p.), sulpiride (10 mg/kg, i.p.), domperidone (10 mg/kg, i.p.), metoclopramide (10 mg/kg, i.p.), bromocriptine (3 mg/kg, i.p.) and chlorpromazine (3 mg/kg, i.p.) on antinociception caused by cinnarizine (2.5 mg/kg, s.c.) in the abdominal constriction assay in mice. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to control and between different groups as shown in the figure. The plus sign (+) indicates significant change from the cinnarizine alone (2.5 mg/kg)-treated group. The (#) sign indicates significant difference from the cinnarizine + chlorpromazine-treated group. Six mice were used per each group.

test by 24%, although higher doses of the drug failed to alter immobility time (Fig. 8).

Effect of cinnarizine on the carrageenan-induced paw oedema

Carrageenan injected into the rat hind paw elicited an inflammation (swelling and erythema) and a time-dependent increase in paw volume. In the control group, paw volume increased by $128.5 \pm 10.6\%$ at 4 h after injection of carrageenan. Cinnarizine at 1.25 or 2.5 administered s.c., 30 min prior to carrageenan had no significant effect on the paw oedema. Cinnarizine at 5, 10 and 20 mg/kg induced a dose-dependent inhibition of paw oedema response to carrageenan (100 μ l/paw) which was apparent within 1 h of carrageenan injection and with a maximal inhibitory effect of -22.7 , -29.9 and -43.4% , respectively (Fig. 9). The percentages of inhibition of the oedema response were -27.3 , -22.7 , -17.8 , -19.6% by 5 mg/kg cinnarizine; -23.6 , -28.8 , -29.9 , -21.2% by 10 mg/kg cinnarizine and -43.4 , -38.7 , -37 , -26.4% by 20 mg/kg cinnarizine at 1, 2, 3 and

Table 1. Assessment of motor coordination in cinnarizine-treated mice in the rotarod test.

Treatment	Rotarod latency (sec)
Saline	446.4 \pm 54.1
Cinnarizine 1.25 mg/kg	465.4 \pm 43.5
Cinnarizine 2.5 mg/kg	512.8 \pm 35.7
Cinnarizine 5.0 mg/kg	498.0 \pm 42.2
Cinnarizine 10.0 mg/kg	392.8 \pm 20.3
Cinnarizine 20.0 mg/kg	375.4 \pm 46.0

Data represent mean \pm S.E.

4 h post-carrageenan, respectively. Two-way ANOVA revealed a significant main effect for treatment ($F_{3, 84} = 6.9$; $P < 0.001$) and time ($F_{3, 85} = 64.2$; $P < 0.001$). Post-hoc analysis showed significant inhibition of oedema formation by 5, 10 or 20 mg/kg of cinnarizine at all time points in the test. Rats treated with cinnarizine at 20 mg/kg showed significantly less oedema than those given 2.5 or 5 mg/kg cinnarizine at 1, 2 and 3 h time points and than those treated with 1.25 mg/kg cinnarizine at all time points in the test.

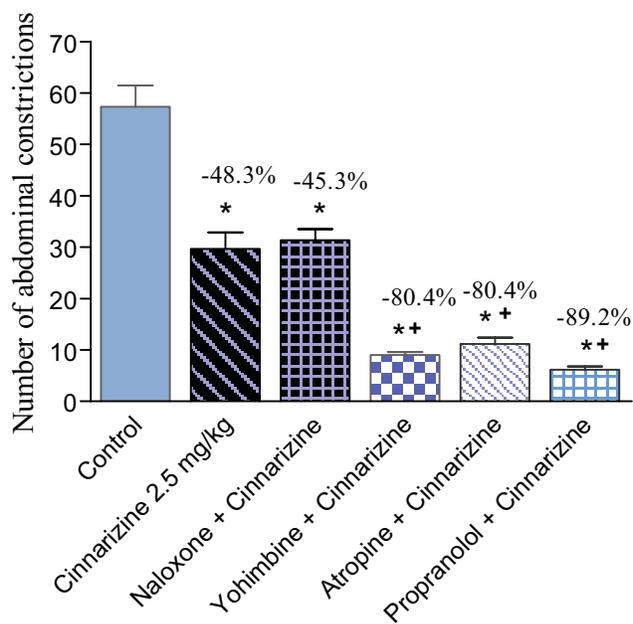


Figure 3. Effect of naloxone (5 mg/kg, i.p.), yohimbine (5 mg/kg, i.p.), atropine (2 mg/kg, i.p.) and propranolol (2 mg/kg, i.p.) on antinociception caused by cinnarizine (2.5 mg/kg, s.c.) in the abdominal constriction assay. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ vs. control and between different groups as shown in the figure. The plus sign (+) indicates significant difference from the cinnarizine + naloxone-treated group. Six mice were used per each group.

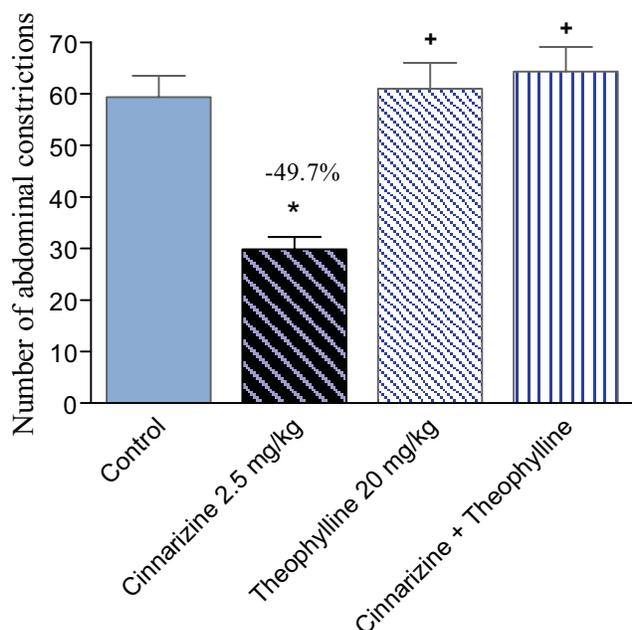


Figure 4. Effect of theophylline (20 mg/kg, i.p.) on antinociception caused by cinnarizine (2.5 mg/kg, s.c.) in the abdominal constriction assay. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. * $p < 0.05$ compared to control group. The plus sign (+) indicates significant difference from the cinnarizine alone-treated group. Six mice were used per each group.

Effect of cinnarizine on gastric mucosal lesions induced by indomethacin

In the indomethacin control group, the number and severity of gastric mucosal lesions were 5 ± 0.68 and 7 ± 1.0 , respectively. This was significantly reduced by co-administration of cinnarizine at 2.5, 5 or 10 mg/kg. It was noted however that the lower doses of the drug i.e. 2.5 or 5 mg/kg were more effective in inhibiting the development of gastric lesions than the higher dose of 10 mg/kg. Thus cinnarizine at doses of 2.5 or 5 mg/kg, reduced the number and severity of gastric mucosal lesions caused by indomethacin by 67 & 76% and by 68.6 & 74.4%. Cinnarizine at 10 mg/kg, reduced the number and severity of gastric lesions by 32 & 14.3% (Fig. 10).

Discussion

The present study provides evidence that cinnarizine exerts different effects on visceral pain, inflammation and on the development of gastric mucosal damage in mice and rat. Cinnarizine (1.25–20 mg/kg, s.c.) inhibited visceral pain evoked by i.p. acetic acid injection in mice. Cinnarizine at the doses used in the present study did not impair motor performance in the rota-rod test, thus

ruling out the confounding influence of a possible sedative effect. In man induction of extrapyramidal signs by cinnarizine has been reported, due to its antagonistic properties at dopamine D1 and D2 receptors (Fabiani et al. 2004; Teive et al. 2004). It is likely that higher doses are required in mice for cinnarizine to impair motor coordination significantly. In other studies, cinnarizine (75 and 200 mg/kg) antagonized the ethanol-induced impairment of locomotor activity on rota-rod test in mice (Czarnecka and Kubik-Bogucka, 1993). Cinnarizine induced no catalepsy in mice at the dose of 20 mg/kg, inducing only mild catalepsy at the doses of 60 and 180 mg/kg (Dall'igna, 2005).

Cinnarizine administered via subcutaneous (Del Pozo et al. 1987) or intracerebroventricular route (Miranda et al. 1993) produced a dose-dependent antinociception in acetic acid writhing test in mice. The writhing response to acetic acid is brought about by the release of prostacyclin synthesized by cyclo-oxygenase in the abdominal cavity of the mice (Berkenkopf and Weichman, 1988). It is reduced by cyclo-oxygenase inhibitors such as meloxicam or diclofenac (Santos et al. 1998), by

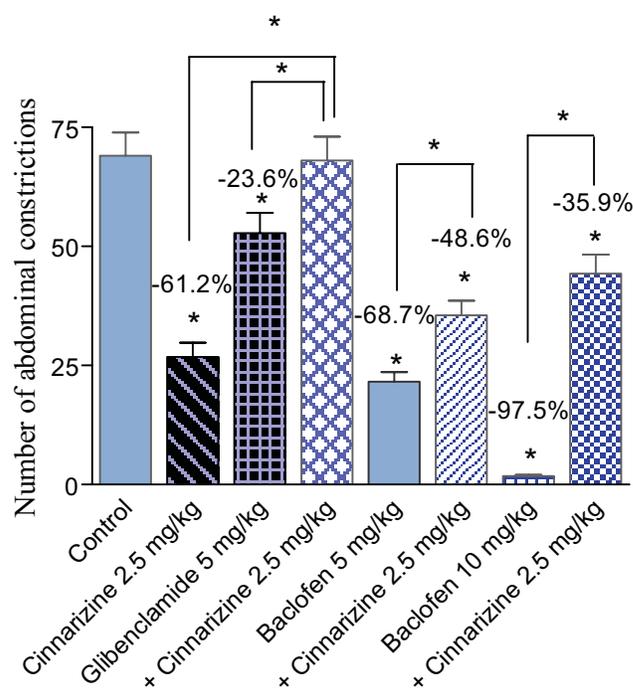


Figure 5. Effect of glibenclamide (5 mg/kg, i.p.) or baclofen (5 or 10 mg/kg, i.p.) on antinociception caused by cinnarizine (2.5 mg/kg, s.c.) in the abdominal constriction assay. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to control group and between different groups as shown in the figure. Six mice were used per each group.

morphine (Baamonde et al. 1989) and by antidepressant drugs (Singh et al. 2001). In the present study, an attempt was made to pharmacologically characterize and investigate the possible neural pathways involved in the analgesic effect of cinnarizine. The possible involvement of neurotransmitter systems, such as dopaminergic, opioid, purinergic, cholinergic, catecholaminergic, GABAergic, systems as well as ATP-gated potassium channels was evaluated. Cinnarizine possesses direct anti-dopaminergic features (Dopamine D1 and D2 receptor blockade) that are likely to contribute to the ability of this drug to cause extrapyramidal symptoms (Reiriz et al. 1994; Brucke et al. 1995). Dopamine D2 receptors are involved in modulation of nociceptive responses and dopamine D2-receptor antagonists e.g. sulpiride caused antinociception in different pain models (Ben-Sreti et al. 1983; Rooney and Sewell, 1989; Frussa-Filho et al. 1996). There is also an evidence of dopamine-mediated descending nociceptive inhibition of spinal neurons (Burkey et al. 1999). Therefore, the involvement of the dopamine receptors in antinociception induced by cinnarizine was investigated. The effect of cinnarizine was unaffected by co-administration of the centrally acting dopamine D2 receptor antagonists, sulpiride, haloperidol or

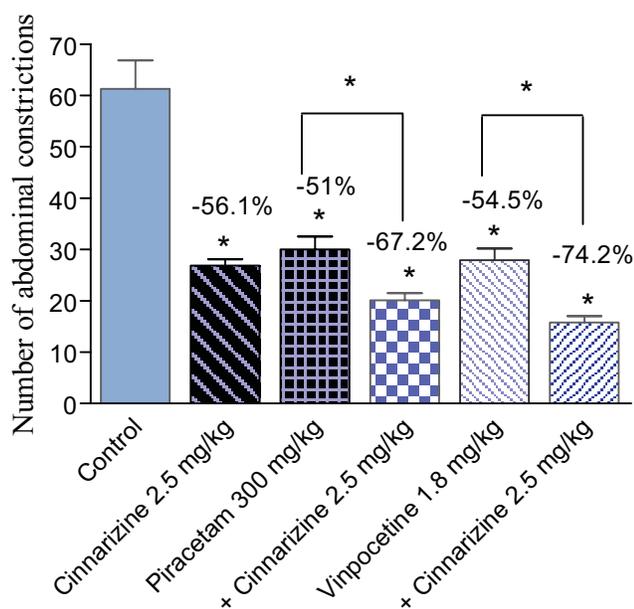


Figure 6. Effect of piracetam (300 mg/kg, i.p.) or vinpocetine (1.8 mg/kg, i.p.) on antinociception caused by cinnarizine (2.5 mg/kg, s.c.) in the abdominal constriction assay. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to control and between different groups as shown in the figure. Six mice were used per each group.

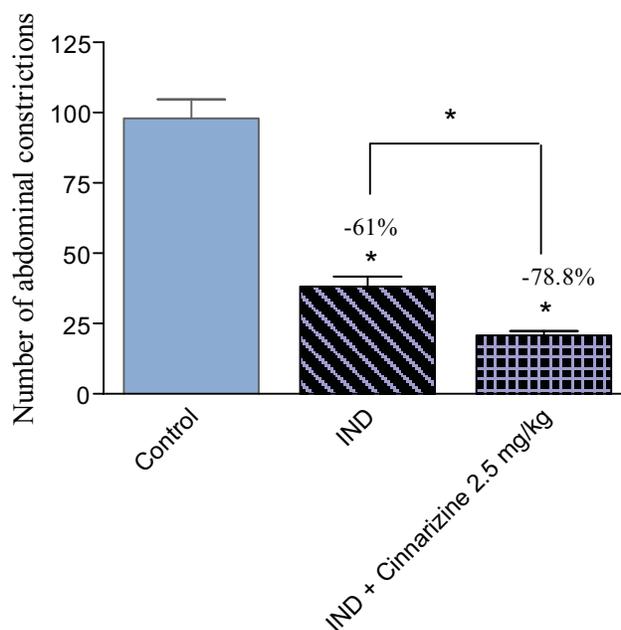


Figure 7. Effect of indomethacin (IND; 5 mg/kg, i.p.) or indomethacin (5 mg/kg, i.p.) + cinnarizine (2.5 mg/kg, s.c.) visceral pain in the abdominal constriction assay. Drugs or saline (control) were administered 30 min prior to testing. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to control and between indomethacin or indomethacin + cinnarizine as shown in the figure. Six mice were used per each group.

metoclopramide, the peripherally acting D2 receptor antagonist domperidone, but increased by the D2 receptor agonist bromocryptine and by the non-selective dopamine receptor antagonist chlorpromazine. These data do not suggest the involvement of dopamine D2 receptors in the visceral analgesic properties of cinnarizine. The antinociception caused by cinnarizine was in also unaffected by the opioid receptor antagonist naloxone, which is in agreement with earlier reports (Miranda et al. 1993).

The inhibition of adrenergic and cholinergic systems appears to facilitate cinnarizine-induced antinociception, since the co-administration of the beta-adrenergic antagonist propranolol, the muscarinic receptor antagonist atropine and the alpha2-adrenergic antagonist yohimbine rather enhanced the effect of cinnarizine observed in the present study. Most forms of pain arising from the gastrointestinal tract are mediated by activity in visceral afferent fibres running in sympathetic nerves (Cervero, 1988). Coeliac plexus block relieves visceral pain that is caused by carcinoma of the pancreas, stomach, gall bladder or liver (Brown et al. 1987; Eisenberg et al. 1995). Chemical sympathectomy attenuated visceral nociceptive responses

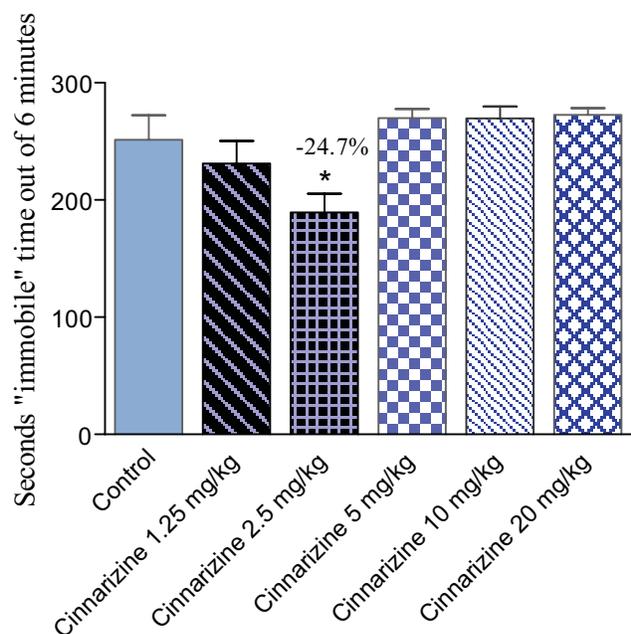


Figure 8. Effect of different doses of cinnarizine (1.25, 2.5, 5, 10 and 20 mg/kg) on the floating time in Porsolt's forced-swimming test in mice. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to saline control. Six mice were used per each group.

(Kalmari et al. 2001), while the adrenergic neurone blocker guanethidine reduced the number of abdominal constrictions induced by acetic acid in mice (Duarte et al. 1988). Beta adrenoreceptor antagonists e.g. propranolol and metoprolol reduced visceral pain caused by i.p. injection of acetic acid in rat (Korzeniewska-Rybicka and Plaznik, 2001).

The spinal cholinergic system and muscarinic receptors are also important for regulation of nociception. Spinally administered muscarinic receptor agonists can produce effective analgesia (Iwamoto and Marion, 1993). In the mouse acetic acid writhing test, M1-muscarinic agonists increased the pain threshold (Bartolini et al. 1992), while atropine, a cholinergic muscarinic antagonist caused hyperalgesia only when administered at high doses of 5 mg/kg (Ghelardini et al. 1990). In contrast, atropine administered at low doses of 1–100 μ g/kg, resulted in analgesia, which might have been due to amplification of cholinergic transmission by a selective blockade of presynaptic muscarinic autoreceptors (Ghelardini et al. 1990). In the present study, atropine administered ip at 2 mg/kg increased the analgesic effect of cinnarizine, thereby, suggesting an interaction at the muscarinic receptors.

Adenosine is an endogenous purine nucleoside that functions as an extracellular signalling molecule. It is released locally at sites of cellular

trauma, and interacts with specific cell-surface purinergic receptors near its site of release to exert its effects. Adenosine acts as an inhibitory neuromodulator in the central and peripheral nervous system (Kowaluk, 1998; Sawynok, 1999). Blockade of adenosine receptors by theophylline, a non-selective adenosine receptor antagonist at A_1 and A_2 receptors, was shown to induce hyperalgesia (Paalzow, 1994). Adenosine A_1 receptor agonists are effective antinociceptive agents in neuropathic and inflammatory pain (Curros-Criado and Herrero, 2005) and mice lacking the adenosine A_1 receptor are hyperalgesic (Wu et al. 2005). In the present study the antinociceptive effect of cinnarizine was prevented by co-treatment with the adenosine receptor blocker theophylline, suggesting that cinnarizine antinociception involves adenosine receptors.

Adenosine triphosphate (ATP)-sensitive K^+ channels (K_{ATP}) play an important role in the mechanisms of pain modulation (Asano et al. 2000; Han et al. 2004; Rodrigues et al. 2004). Intrathecal administration of K_{ATP} channel openers produces antinociception (Asano et al. 2000). They can also contribute to the sensitization of primary afferents observed in gastrointestinal pain states (Cervero and Laird, 2003). In the present study, antinociception induced by cinnarizine was prevented by the administration of glibenclamide, a blocker of K_{ATP} channel. This may suggest that this antinociceptive effect of cinnarizine may also rely on ATP-gated potassium channels.

In the present study also the administration of cinnarizine reversed the baclofen—induced antinociception. Baclofen, a prototypical agonist for $GABA_B$ receptors, alters nociception at the level of the spinal cord by acting on $GABA_B$ receptors located on primary afferent terminals and is known to produce analgesia in man and animals (Dirig and Yaksh, 1995; Hara et al. 2004). Cinnarizine inhibits the reuptake of GABA by sections of the rat brain cortex (Mirzozian et al. 1998), which is likely to account for the observed effect of cinnarizine on the baclofen-antinociception.

In the present study, the effect of cinnarizine on immobility time in Porsolt's forced-swimming test, a commonly used tool for screening of potential antidepressants (Porsolt et al. 1977) was examined. Only at the dose of 2.5 mg/kg, did cinnarizine reduce immobility time by 24%, although higher doses of the drug were without effect. Other researches reported a decrease of immobility time by 5 mg/kg of cinnarizine (Sushma et al. 2004).

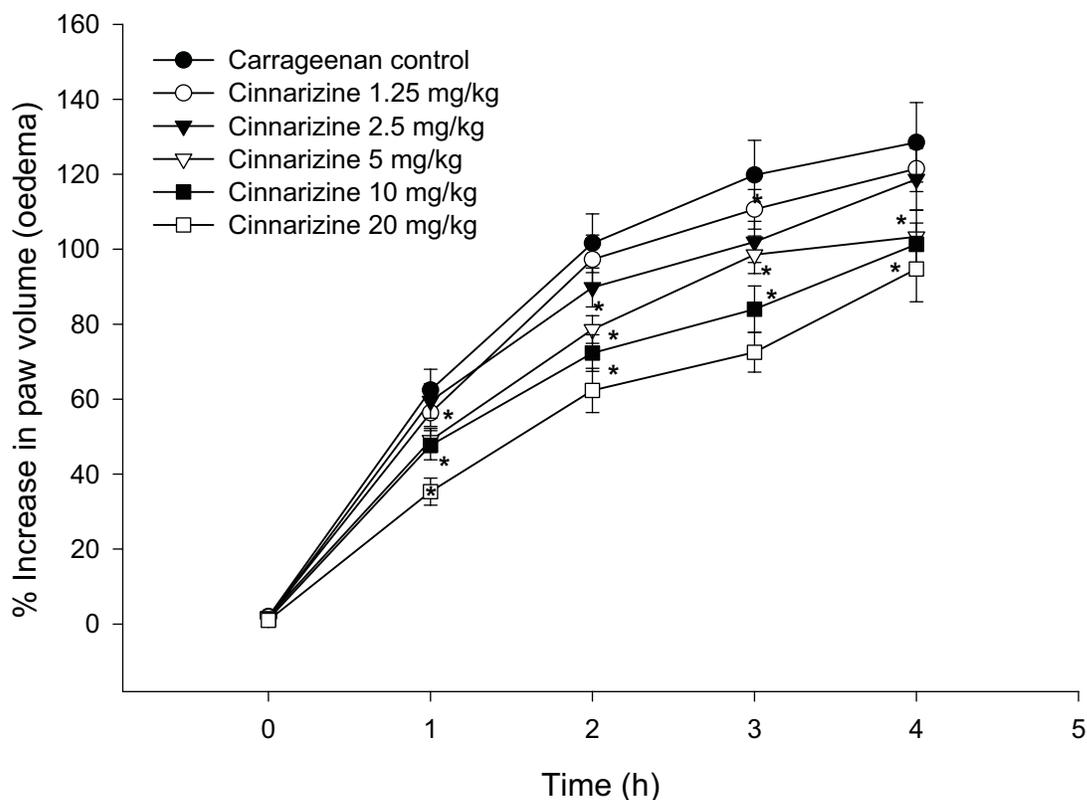


Figure 9. Effect of different doses of cinnarizine (1.25, 2.5, 5, 10 and 20 mg/kg) on the paw oedema caused by sub-plantar injection of carrageenan in rats. Data expressed as mean \pm S.E. Percent inhibition (%) compared to the control animals is shown. * $p < 0.05$ compared to saline control. Six rats were used per each group.

Dopamine is implicated in the symptoms of depression (Willner, 1995; Brunswick et al. 2003; Remy et al. 2005) and dopamine re-uptake inhibitors, bupropion and nomifensine reduce immobility in the forced swimming test by activation of D1 and D2 receptors (Yamada et al. 2004). Cinnarizine, however, exerts direct anti-dopaminergic effects (Dopamine D1 and D2 receptor blockade) (Reiriz et al. 1994; Brucke et al. 1995). It is worthy to mention that cinnarizine also displays inhibitory activity on catecholamine uptake in storage vesicles (Terland and Flatmark, 1999) which could be involved at least in part in the observed decrease in immobility time by the drug.

In paw oedema caused by carrageenan, cinnarizine at doses of 5–20 mg/kg, produced a dose-dependent and marked inhibition of paw oedema response to carrageenan. Cinnarizine in low doses failed to reduce the inflammatory response. This result is in accordance to what has been reported previously of the anti-inflammatory property of cinnarizine (Blazso et al. 1999).

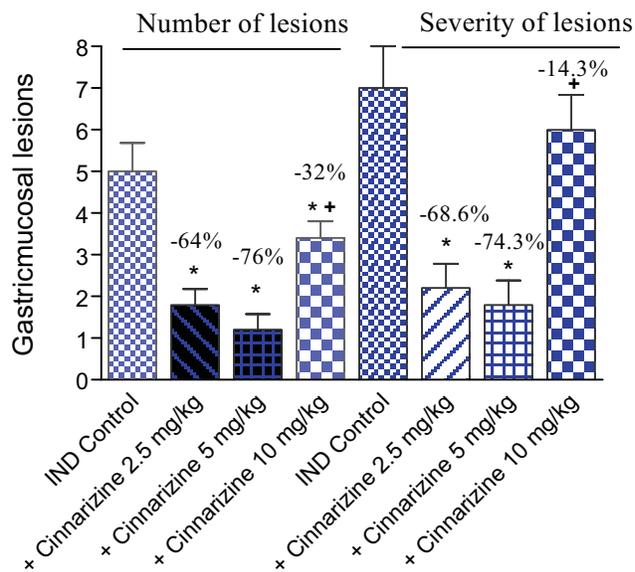


Figure 10. Effect of cinnarizine (2.5, 5 and 10 mg/kg) on the number and severity of gastric lesions caused by s.c. indomethacin (20 mg/kg) in rats. Data expressed as mean \pm S.E. * $p < 0.05$ compared to saline control. The plus sign (+) indicates significant difference from the 2.5 mg/kg cinnarizine group. Six rats were used per each group.

The effect of cinnarizine on gastric mucosa was also examined in the present study. Gastric lesions induced by indomethacin were reduced dose-dependently by co-administration of cinnarizine, although it was noted that this effect was more evident with lower doses of 2.5, 5 mg/kg. Studies indicated that cold/restraint stress- and ethanol-induced lesions was decreased by the administration of cinnarizine, possibly due to decrease in the elevated histamine content by the drug (Marazova et al. 1993; Lozeva et al. 1994).

Cinnarizine has a complex the complex mechanism of action. In addition to a calcium channel blocking activity and antihistaminic properties, binding to both H1 and H2 receptors (Nagai et al. 1986; Nguyen et al. 2001), the drug displayed dopamine D1 and D2 receptor blocking effects as well as inhibitory effects on the reuptake of GABA (Mirzoian et al. 1998) and on catecholamine uptake (Terland and Flatmark, 1999). The antihistaminic or catecholamine reuptake blocking properties might be involved in the antioedema effect observed in the present study. The beneficial effect of cinnarizine on gastric lesions can be attributed to inhibition of gastric acid secretion (Bouclier and Spedding, 1985), to its vasodilator properties (Izumo et al. 1999), leading to an increase in gastric mucosal blood flow or to its antihistaminic properties (Nagai et al. 1986; Nguyen et al. 2001).

In summary the present study confirms and extends previous studies suggesting anti-inflammatory, antinociceptive and gastric protective properties for cinnarizine. The study indicates that mechanism by which cinnarizine modulates pain transmission is likely to involve adenosine receptors and ATP-gated potassium channels. The study in addition shows that cinnarizine inhibits GABA-mediated antinociception.

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