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Interaction of angiotensin II and adenosine A_1 and A_{2A} receptor ligands on the writhing test in mice

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Abstract

The effects of adenosine A_1 and A_{2A} receptor agonists and antagonists administered intraperitoneally (ip) and their interaction with angiotensin II (Ang II) administered intracerebroventricularly (icv) were studied in mice using the acetic acid-induced abdominal constriction test. Ang II (0.1 μg/mouse) induced antinociception in this model. The adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA; 0.05, 0.25 and 0.5 mg/kg) also showed a well-developed antinociceptive effect. Ang II (0.1 μg/mouse) administered 5 min before CPA (0.25 mg/kg) decreased the number of writhes, i.e., it enhanced the antinociceptive effect of CPA. Losartan, an AT_1 receptor antagonist (25 μg/mouse icv), enhanced the antinociceptive effect of CPA, while the AT_2 receptor antagonist 1-[-4-(dimethylamino)-3-methylphenylmethyl]-5-diphenylacetyl)-4,5,6,7-tetrahydro 1*H*-4-imidazol [4,5*c*]pyridine-6 carboxylic acid, ditrifluoroacetate, dihydrate (PD 123319; 10 μg/mouse) had less effect. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX; 0.1 mg/kg), an adenosine A_1 receptor antagonist, exhibited a pronociceptive effect and did not change the antinociceptive effect of Ang II. The adenosine A_{2A} receptor agonist PD-125944 (DPMA; 0.1, 0.5 and 1 mg/kg) showed pronounced antinociceptive effect. Ang II (0.1 μg/mouse) did not significantly influence the antinociceptive effect of DPMA (0.1 mg/kg). The A_{2A} receptor antagonist 3,7-dimethyl-1-propargilxanthine (DMPX; 0.1 mg/kg) had no effect on the number of writhes and did not influence the effect of Ang II. These data indicate that the antinociceptive effect of Ang II interacts with that produced by adenosine A_1 receptor agonist. © 2002 Published by Elsevier Science Inc.

Keywords: Writhing test; Mice; Angiotensin II; Adenosine A_1 receptor subtype; Adenosine A_{2A} receptor subtype

1. Introduction

An antinociceptive effect of angiotensin II (Ang II) has recently been demonstrated in the acetic acid-induced abdominal constriction test (Georgieva and Georgiev, 1999). Ang II peptide analogue sarmesin and the AT_2 receptor subtype antagonist 1-[-4-(dimethylamino)-3-methylphenylmethyl]-5-diphenylacetyl)-4,5,6,7-tetrahydro 1*H*-4-imidazol [4,5*c*]pyridine-6 carboxylic acid, ditrifluoroacetate, dihydrate (PD 123319) attenuated this effect (Georgieva and Georgiev, 1999). There is evidence for the participation of the renin–angiotensin system (RAS) in stress-induced analgesia (Haulica et al., 1986), increase in latencies to thermal stimuli (Toma et al., 1997) and attenuation of morphine-induced analgesia (Kaneko et al., 1985, Han et al., 2000). Ang II administered intrathecally (it) induce a short-term antinociceptive effects in the tail-flick test through an endogenous opioid mechanism and activation of the AT_1 receptor subtype in the rat spinal cord (Toma et al., 1997). Quantitative autoradiography studies reveal high concentrations of Ang II receptor binding sites in the midbrain periaqueductal gray, where endogenous opioids produce analgesia, as well as in the thalamus, which serves as a point of termination for afferent information concerned with pain (Wright and Harding, 1992). Using immunocytochemistry, it has been shown that the same brain regions display moderate to high amounts of immunoreactivity to a conjugated form of an adenosine derivative (Braas et al., 1986). The antinociceptive actions of adenosine and adenosine analogues in animal models have been known for a longer time — since the early mid-1970s. The endogenous compound adenosine has various modulatory effects in the peripheral and central nervous systems, mediated through specific cell surface-associated receptors (Sollevi, 1997). Adenosine receptors are localized primarily on neurons postsynaptic to primary afferents and descending

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projections within the dorsal horn, but some receptors are present on central terminals of primary afferent neurons (Sawynok, 1998).

At peripheral nerve terminals in rodents, adenosine A_1 receptor activation produces antinociception by decreasing, while adenosine A_2 receptor activation produces pronociceptive properties by increasing, cyclic AMP levels in the sensory nerve terminal. In the spinal cord, adenosine A_1 receptor activation produces antinociceptive properties in acute nociceptive, inflammatory and neurophatic pain tests (Sawynok, 1998).

Demonstrating the rank order potencies of adenosine agonists (DeLander and Hopkins, 1987) supported the involvement of adenosine A_2 receptors in the spinal mechanisms of nociception. The selective adenosine A_{2A} receptor agonist CGS 21680 has exhibited efficacy in inflammatory and neurophatic pain tests, but this usually occurred in higher doses and could thus still reflect adenosine A_1 receptor activation (Sawynok, 1998).

There are data indicating that the interaction between Ang II and adenosine receptors can occur. Thus, peripherally administered adenosine increases local vascular production of Ang II, which selectively attenuates the adenosine A_2 receptor-mediated reduction in renal and mesenteric vascular resistance (Smits et al., 1993). Adenosine may modulate cardiovascular responses of Ang II and III. Thus, microinjection of an adenosine A_1 receptor antagonist into the area postrema attenuated the depressor and bradycardic effects of Ang II and III, while an Ang III peptide antagonist significantly altered the cardiovascular effects of adenosine in the area postrema (Lin et al., 1995). Ang II and adenosine interact also in the regulation of seizure susceptibility in pentylenetetrazole seizure threshold and kindling in mice (Georgiev and Tchekalarova, 1998, Tchekalarova and Georgiev, 1999). Long-term theophylline treatment selectively changes the effects of Ang II and adenosine agonists on the pentylenetetrazole seizure threshold mainly through angiotensin AT_1 and adenosine A_1 receptor subtypes (Tchekalarova et al., 2000).

The objective of the present study was to investigate whether mechanisms could interact with the antinociceptive effect of Ang II and, conversely, whether Ang II receptors could influence the antinociceptive effects of adenosine agonists.

2. Method

2.1. Animals

The experiments were carried out on male albino mice ICR strain (18–20 g) bred in an air-conditioned room at a temperature of 24 ± 1 °C with food and water available ad libitum except during the experiments. All tests were conducted between 09:00 and 12:00 h.

2.2. Drugs

Acetic acid (diluted with distilled water to a concentration of 1%) was administered intraperitoneally (ip) in the volume of 0.1 ml/10 g/b.wt. Ang II (Ciba-Geigy Pharmaceuticals), losartan (DuP 753; 2-*n*-butyl-4-chloro-5 hydroxymethyl-1-[2-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazol potassium salt; AT_1 receptor antagonist; generously supplied by Dr. R.D. Smith, Du Pont Merck, Wilmington, DE) and PD 123319 (AT_2 receptor antagonist; kindly presented as a gift from Parke-Davis, Ann Arbor, MI) were dissolved in saline and administered intracerebroventricularly (icv) using an injection volume of 2 μl at a rate of 1 μl/30 s. The injections were given free hand directly into the right cerebral ventricle of conscious mice (Haley and McCormick, 1957). The injection coordinates were 3 mm caudal to the right coronary suture and 2.5 mm lateral to the midline into a depth of 3 mm from the scalp. N^6-cyclopentyladenosine (CPA; adenosine A_1 receptor agonist, RBI), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; adenosine A_1 receptor antagonist, RBI), PD-125944 (DPMA; adenosine A_{2A} receptor agonist, RBI) and 3,7-dimethyl-1-propargilxanthine (DMPX; adenosine A_{2A} receptor antagonist, RBI) were dissolved in saline and injected intraperitoneally in a volume of 1 ml/kg. The equivalent volume of vehicle was administered to the control groups. Each group consisted of 10–12 mice.

2.3. Acetic acid-induced abdominal constriction test

The mice were placed in individual cages and the number of abdominal constrictions (writhes) of each mouse was counted at 5-min intervals for 30 min. CPA and DPMA were injected 5 min, Ang II 10 min, losartan 15 min, DPCPX and DMPX 20 min and PD 123319 30 min before acetic acid. Counting of abdominal constrictions started immediately after injection of acetic acid. The mice with decreased number of writhes were considered protected by the test agent (Collier et al., 1968). All experimental procedures were carried out in accordance with the institutional guidance and the general recommendations of the National Institute of Health Guide regarding the care and use of animals for scientific purposes.

2.4. Statistical analysis

The data were analysed by a multifactor analysis of variance (one-way ANOVA), followed by the Duncan test for comparison of differences at $P < .05$.

3. Results

CPA at a dose of 0.05 mg/kg significantly decreased the number of abdominal constrictions at 0–5-, 5–10- and 15–20-min intervals after acetic acid injection. At a dose of

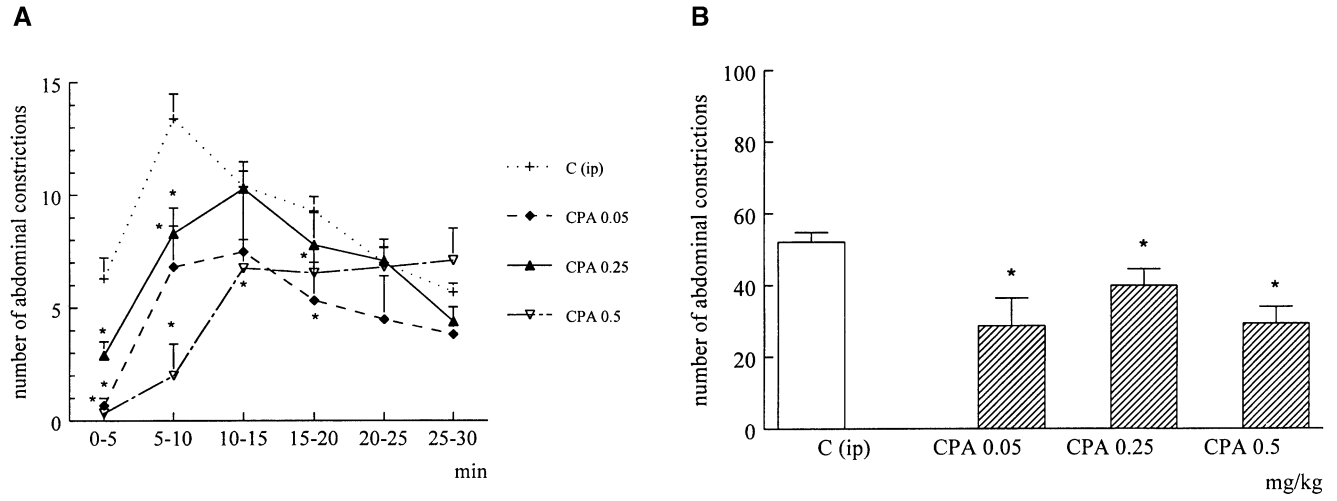

Fig. 1. Time course for the effect of intraperitoneal treatment of animals (male mice) with CPA in mg/kg b.wt. (A). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 5 min (at each 5-min interval) for the period of observation (30 min). Cumulative data for the effect of intraperitoneal treatment of animals (male mice) with CPA in mg/kg b.wt. (B). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 30 min. The control (C) values are from animals injected intraperitoneally with the vehicle ($n = 12$). * $P < .05$ vs. controls.

0.25 mg/kg, it significantly decreased the writhes at 0–5- and 5–10-min intervals after acetic acid injection, and at a dose of 0.5 mg/kg, it significantly decreased the writhes from 0 to 20 min after acetic acid injection (Fig. 1A). Cumulative data for the whole 30-min period displayed that CPA at the three doses used significantly decreased the number of writhes (Fig. 1B).

Time course showed that DPMA at a dose of 0.1 mg/kg significantly decreased the number of writhes during the whole period of observation. At a dose of 0.5 mg/kg, it significantly decreased the number of writhes from 0 to 20 min, and at a dose of 1 mg/kg, it decreased the number of writhes only from 0 to 10 min (Fig. 2A). Cumulative data of DPMA for the 30-min period emphasized its significant antinociceptive effect at the three doses used (Fig. 2B).

Ang II (0.1 μg/mouse) significantly decreased the number of writhes during almost the whole 30-min period of observation. The dose of Ang II, as well as doses of its receptor antagonists in subsequent experiments, were selected based on results from previous experiments carried out in the same test (Georgieva and Georgiev, 1999). Ang II (0.1 μg/mouse) injected 5 min before CPA (0.25 mg/kg) significantly increased the antinociceptive effect of CPA (Fig. 3A).

Ang II (0.1 μg/mouse) did not significantly influence the antinociceptive effect of DPMA at a dose of 0.1 mg/kg (Fig. 3B).

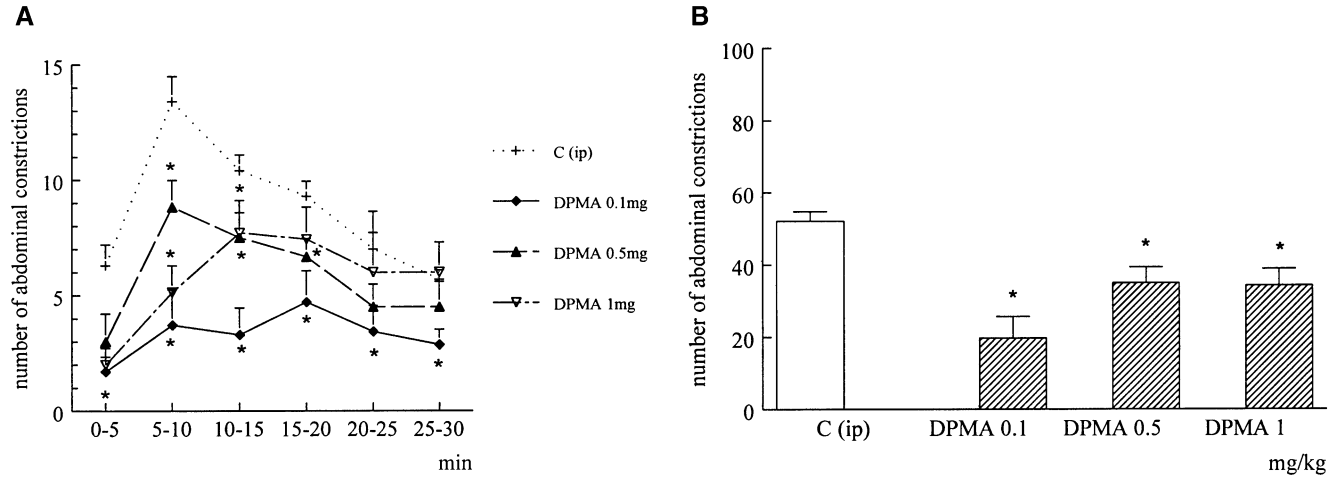

Fig. 2. Time course for the effect of intraperitoneal treatment of animals (male mice) with DPMA in mg/kg b.wt. (A). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 5 min (at each 5-min interval) for the period of observation (30 min). Cumulative data for the effect of intraperitoneal treatment of animals (male mice) with DPMA in mg/kg b.wt. (B). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 30 min. The control (C) values are from animals injected intraperitoneally with the vehicle ($n = 12$). * $P < .05$ vs. controls.

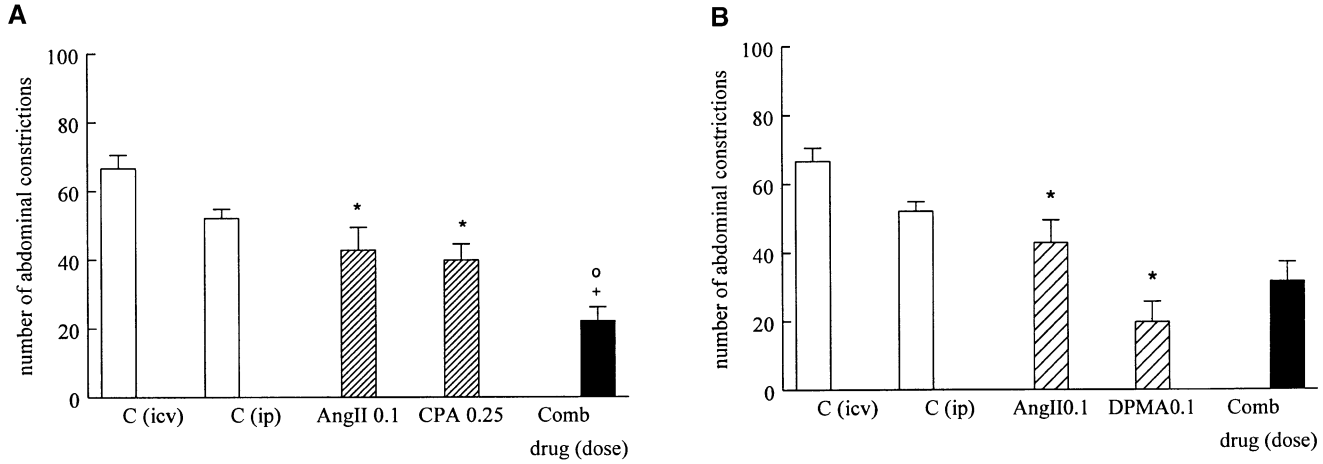

Fig. 3. Cumulative data for the combination (Comb) of intracerebroventricularly administered Ang II (0.1μg/mouse) 5 min before CPA 0.25 mg/kg b.wt. (A). Cumulative data for the combination (Comb) of intracerebroventricularly administered Ang II (0.1 μg/mouse) 5 min before DPMA 0.1 mg/kg b.wt. (B). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 30 min. The control (C) values are from animals injected intraperitoneally or intracerebroventricularly with the vehicle ($n = 12$). * $P < .05$ vs. controls, ° $P < .05$ vs. Ang II, $^{+}P < .05$ vs. CPA or DPMA.

DPCPX (0.1 mg/kg) significantly increased the number of writhes and showed a pronociceptive effect, and the dose of 1 mg/kg was ineffective. DMPX (0.1 and 1 mg/kg) significantly increased the number of writhes at 10–15-min intervals (time course is not showed). Cumulative data showed that changes induced by DMPX at doses of 0.05, 0.1 and 1 mg/kg were not significant vs. controls for the 30-min period of observation (Fig. 4A). DPCPX (0.1 mg/kg) and DMPX (0.1 mg/kg) administered 10 min before Ang II (0.1 μg/mouse) did not influence the antinociceptive effect of Ang II (Fig. 4B).

Losartan (25 μg/mouse) administered 10 min before CPA (0.25 mg/kg) significantly increased the antinociceptive effect of CPA (Fig. 5A).

PD 123319 (10 μg/mouse) injected 25 min before CPA (0.25 mg/kg) decreased the number of writhes only at 0–5-min interval as compared to CPA (Fig. 5B).

4. Discussion

In the present study, we established an antinociceptive effect of the adenosine A_1 and A_{2A} receptor agonists CPA and DPMA on acetic acid-induced abdominal constriction test. The data are in some accordance with similar data showing that adenosine agonists produced a dose-related inhibition of acetylcholine-induced writhing in mice (Herrick-Davis et al., 1989). There is substantial evidence that

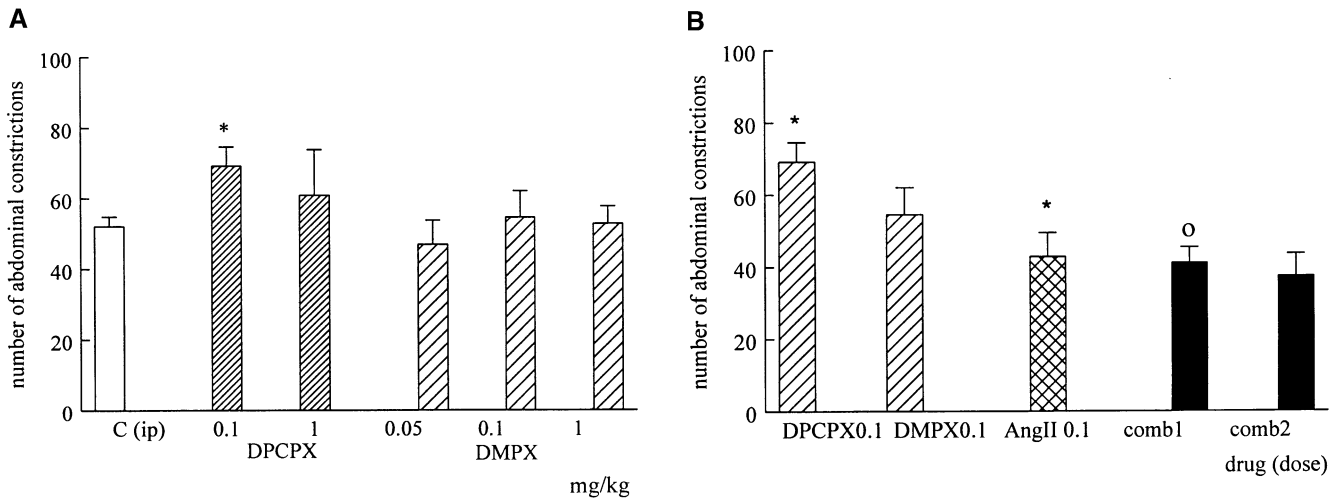

Fig. 4. Cumulative data for the effect of intraperitoneal treatment of animals (male mice) with DPCPX and DMPX in mg/kg b.wt. (A). The number of abdominal constrictions (mean ± S.E.M.) was summarised for 30 min. The control (C) values are from animals injected intraperitoneally with the vehicle ($n = 12$). * $P < .05$ vs. controls. Cumulative data for the combination (Comb1) of intraperitoneally administered DPCPX (0.1 mg/kg) 10 min before Ang II (0.1μg/mouse) and for the combination (Comb2) of intraperitoneally administered DMPX (0.1 mg/kg) 10 min before Ang II are summarised for 30 min. The control values are from animals injected intraperitoneally or intracerebroventricularly with the vehicle ($n = 12$). * $P < .05$ vs. controls, ° $P < .05$ vs. DPCPX.

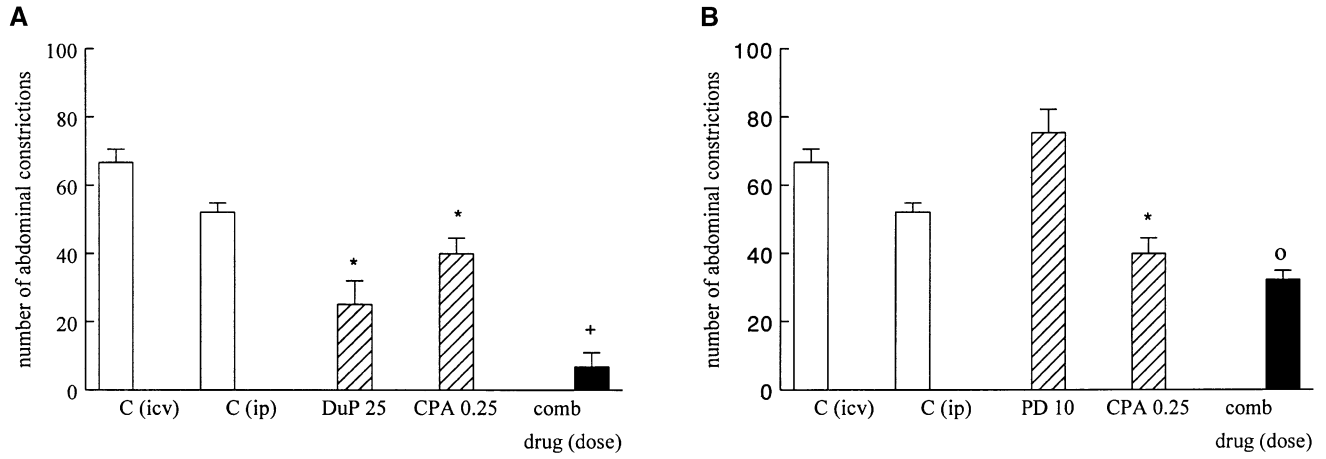

Fig. 5. Cumulative data for the combination (Comb) of intraperitoneal treatment of animals (male mice) with losartan (DuP 25 μg/mouse) 10 min before CPA at a dose of 25 mg/kg (A) and for the combination (Comb) of intracerebroventricular treatment with PD 123319 (10 μg/kg) 25 min before CPA at a dose of 25 mg/kg (B). The treatment of abdominal constrictions (mean ± S.E.M.) was summarised for 30 min. The control (C) values are from animals injected intraperitoneally or intracerebroventricularly with the vehicle ($n = 12$). * $P < .05$ vs. controls, ° $P < .05$ vs. PD 123319, $^{+}P < .05$ vs. CPA.

adenosine agonists inhibit spinal sensory transmission related to nociception by acting at adenosine A_1 receptors (Nakamura et al., 1997). The present data showed that pretreatment with Ang II provoked augmentation of antinociception of CPA. The effect of CPA was increased after supraspinal administration of the AT_1 receptor antagonist losartan but not by the AT_2 receptor antagonist PD 123319, which suggests modulation of CPA antinociceptive effects only by AT_2 receptor subtype. In previous studies, we suggested that the antinociceptive effect of Ang II is related to AT_2 receptor subtype activation (Georgieva and Georgiev, 1999). The adenosine A_1 selective receptor antagonist DPCPX given alone revealed a pronociceptive effect but did not influence the effect of Ang II, which indicates that that adenosine A_1 receptors do not play a direct role in the antinociceptive effect of Ang II. The antinociceptive effect of the A_{2A} selective receptor agonist DPMA in writhing test in the present study needs to be noted. While more recent studies continue to emphasize the involvement of adenosine A_1 receptors in spinal antinociception (Poon and Sawynok, 1998), there are a number of observations, which suggest an involvement of A_{2A} receptor in this action. Thus, the selective adenosine A_{2A} receptor agonist CGS 21680 injected intrathecally inhibited the tail-flick response in mice, the effect of which was reduced dose dependently by intrathecal pretreatment with A_{2A} selective antagonist DMPX (Suh et al., 1997). If interpreting the present results, one needs to consider the participation of possible mechanisms. AT_1, AT_2 and adenosine A_1 receptors are coupled to Gi proteins (Bottari et al., 1993, DeGasparo et al., 2000, Fredholm et al., 1994). These receptors can induce a variety of different cellular responses, inducing inhibition of adenylyl cyclase, stimulation of phospholipase C, generation of Ca^{2+} and protein kinase C signal (Bottari et al., 1993, DeGasparo et al., 2000, Fredholm et al., 1994). One other mechanism, which could contribute to the influence of Ang II in the present experiments, might be related with Ang II-induced inhibition of voltage-gated calcium currents in N-type Ca^{2+} channel, which could be realised via adenosine A_1 receptor activation (Shapiro et al., 1994). Note also that the antinociceptive effect of adenosine A1 receptor might occur as part of a neuroprotective action (DeMendoça et al., 2000). The decrease in body temperature produced by adenosine analogues (DeMendoça et al., 2000) might also play a complementary role of their antinociceptive effect.

In conclusion, Ang II may increase the antinociceptive effect of A_1 receptor agonist CPA on writhing test in mice perhaps through the AT_2 receptor subtype, while it has no influence on the antinociception of A_{2A} receptor agonist DPMA. In general, the effects of Ang II as well as of adenosine A_1 receptor ligands on nociception might be jointly modulated.

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